

**ABSTRACTS FROM THE
12TH INTERNATIONAL ISSX MEETING**

**28–31 July 2019
Portland, Oregon, USA**



12th International ISSX Meeting 28–31 July 2019

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SC1.1 - THE NEXT FRONTIER IN ADME RESEARCH: PREDICTING AND VERIFYING TISSUE DRUG CONCENTRATIONS USING A PROTEOMICS AND PET IMAGING APPROACH**Jashvant D. Unadkat***University of Washington, USA*

In the drug development process, predicting CYP metabolic clearance of drugs using recombinant and human liver microsomes (HLMs) has been relatively successful. However, for several reasons predicting non-CYP and transporter-based clearance of drugs remains a challenge. First, the rate-determining step in the clearance of a drug must be identified – is it metabolism or transport or both. If transport is rate-determining, using recombinant enzymes or HLMs for predicting the *in vivo* clearance of drugs (IVIVE) will not be successful. Vice-versa is also true, that is using transporter-expressing cell lines will not help predict metabolic clearance of drugs. If both processes are rate-determining, both need to be included in IVIVE. Second, quantification of transporters and non-CYP enzymes in various ADME tissues is needed. Such data are beginning to be available. For IVIVE of transporter-mediated clearance, the mechanism of transport, plasma membrane vs. intracellular expression of transporters, both *in vitro* and *in vivo*, should be considered. For example, the membrane potential difference, which drives OCT2 transport, appears to be different in cell lines vs. kidney epithelial cells. Third, significant challenges remain in predicting *in vivo* efflux transport activity, especially in the liver (e.g. MRP2) and kidneys. These challenges/gaps will be discussed.

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SC1.2 - ADVANCING PREDICTIONS OF TISSUE AND INTRACELLULAR DRUG CONCENTRATIONS USING *IN VITRO* AND PBPK MODELING APPROACHES**Yingying Guo***Eli Lilly and Company, USA*

Knowledge of unbound tissue drug concentrations is important to the understanding of efficacy, toxicity and drug-drug interactions especially for drugs with transporter-mediated drug disposition. This presentation will examine the state-of-the-art on predictions of tissue and intracellular drug concentrations based on the recent ITC3 whitepaper (1). Critical overview will be provided for the *in vitro* methods that determines intracellular and subcellular drug concentrations. Selected examples will be used to illustrate the utility of preclinical, clinical and pharmacodynamic data to support physiologically based pharmacokinetic (PBPK) modeling to understand disconnects between systemic and tissue drug exposure. Current best practices and practical strategies will also be discussed for selecting methods to estimate or predict tissue and intracellular concentrations and then apply to PBPK modeling for human pharmacokinetic, efficacy and safety assessment in drug development.

Reference:

1. Guo Y, Chu X, Parrott NJ, Brouwer KLR, Hsu V, Nagar S, et al. Advancing Predictions of Tissue and Intracellular Drug Concentrations Using In Vitro, Imaging and Physiologically Based Pharmacokinetic Modeling Approaches. *Clinical Pharmacology and Therapeutics*. 2018;104(5):865-89..

SC1.3 - INFLUENCE OF TRANSPORTER POLYMORPHISMS ON DRUG DISPOSITION AND RESPONSE: A PERSPECTIVE FROM THE INTERNATIONAL TRANSPORTER CONSORTIUM**Sook Wah Yee***University of California San Francisco, USA*

Membrane transporters are critical determinants of drug disposition, toxicity and response. They are becoming increasingly important in drug development both as drug targets and sites of clinical drug-drug interactions, DDIs. In this short course, I will focus on transporters in the Solute Carrier and ATP Binding Cassette Superfamilies, that have common and reduced function polymorphisms which are considered highly influenced to drug response and disposition by the International Transporter Consortium (see *Clinical Pharmacology and Therapeutics*, 104, 803-817, 2018). Examples will include highly important polymorphisms in OATP1B1 and BCRP, as well as OCT1, that was recently highlighted by the International Transporter Consortium as a transporter with important polymorphisms that should be considered in drug development programs. Finally, I will highlight commonly used databases for research relevant to membrane transporters.

SC1.4 - CAN BSEP INHIBITION TESTING IN DRUG DISCOVERY AND DEVELOPMENT REDUCE LIVER INJURY RISK? AN INTERNATIONAL TRANSPORTER CONSORTIUM PERSPECTIVE**Gerry Kenna**

Safer Medicines Trust, United Kingdom

The Bile Salt Export Pump (BSEP) is a protein located in the apical plasma membrane domain of hepatocytes, which mediates ATP-dependent vectorial efflux of bile acids into bile. Inhibition of BSEP activity has emerged as one of several important mechanisms by which idiosyncratic drug induced liver injury (DILI) may arise in humans, but not in the animal species used in nonclinical drug safety studies. Recently, the International Transporter Consortium (ITC) has recommended that proactive evaluation and understanding of BSEP inhibition can aid internal decision making on DILI risk in drug discovery and development and has provided pragmatic guidance on how this can be undertaken (see: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6220754>). The objective is to reduce and manage DILI risk. The reasons for these recommendations will be presented. In addition the advantages and limitations of the available methods for assessment of BSEP inhibition, and evaluation of its potential adverse functional consequences, will be discussed. Most commonly, BSEP inhibition by drugs is evaluated *in vitro* using inverted membrane vesicles from insect cells transiently transfected with plasmid vectors that encode the protein. When interpreting the data provided by these and other *in vitro* methods, it is important to assess potency by determination of an IC50. When considering potential DILI risk, the *in vitro* BSEP IC50 value should be compared to the maximum total plasma drug concentration at steady state. Various follow up studies can further aid the interpretation of *in vitro* BSEP inhibition data and these should be undertaken on a case by case basis. For example, since some drugs have been shown to exhibit non-competitive BSEP inhibition, consideration of the BSEP inhibition mechanism may be required. *In vitro* studies of BSEP inhibition can also be undertaken using sandwich culture hepatocytes, which exhibit physiologically relevant expression of BSEP and other compensatory biliary transporters and /or adaptive mechanisms. *In vivo*, quantification of bile acid concentrations in serum or plasma can provide indirect insight into whether BSEP inhibition has occurred following administration of a drug that inhibits transporter function *in vitro*. Use of *in silico* tools may aid the design of drugs with reduced BSEP inhibition liabilities. The processes by which bile acid accumulation within hepatocytes following BSEP inhibition/deficiency causes hepatocyte injury are complex, as are the mechanisms by which DILI may arise in susceptible humans (which include drug-related processes such as BSEP inhibition, and also patient-related susceptibility factors). It is important to consider this complexity when extrapolating from *in vitro* BSEP inhibition data to the human DILI risk. In particular, the ITC recommends that BSEP screening should be undertaken in parallel with screening for other potentially important DILI liabilities, for example formation of chemically reactive metabolites or potent inhibition of mitochondrial function.

SC1.5 - A REGULATORY PERSPECTIVE “TRANSPORTERS IN DRUG DEVELOPMENT: SCIENTIFIC AND REGULATORY CONSIDERATIONS”**Lei K. Zhang**

Silver Spring, USA

Transporters can affect a drug's pharmacokinetics by controlling absorption, distribution, and elimination processes. They can also affect a drug's pharmacodynamics by influencing its access to the site of action. More recently, transporters have become important as drug targets, for example, urate transporter inhibitors as treatment for gout and sodium/glucose co-transporter-2 (SGLT2) inhibitors for treating type 2 diabetes. As such, it is important to consider the role of transporters during drug development. Evaluation of transporters during drug development and post-approval is an integral part of risk assessment for the optimal use of therapies in the intended patient populations. Regulatory guidance documents on drug-drug interactions (DDIs) from global regulatory agencies including the European Medicines Agency (EMA), Japan's Pharmaceuticals and Medical Devices Agency (PMDA), and the U.S. Food and Drug Administration (FDA) include recommendations on how to evaluate transporter-mediated DDIs during drug development. Decision criteria proposed by regulatory agencies help predict DDI potential and guide further DDI evaluation in drug development, which may inform the labeling and safe use of the drug. Due to the limitations of the *in vitro* assays and complexity of *in vivo* DDIs, these criteria need to be further evaluated and refined when more data are available. Biomarkers may help elucidate the *in vivo* transporter effect in early phase clinical studies. Recently, *in silico* methods (e.g., PBPK) have been used in drug development to inform study design or labeling recommendations. These methods have potential utility and require continued collaborative efforts to fill the knowledge gaps, particularly for transporter-mediated DDI or safety/efficacy prediction. This presentation will provide an overview on key elements in the regulatory and scientific considerations that can guide the evaluation of transporters during the drug development process that integrates multiple strategies including *in vitro*, *in silico* and *in vivo* evaluations.

SC2.1 - AN OVERVIEW OF NON-P450-MEDIATED METABOLISM IN DRUG DEVELOPMENT**Cyrus Khojasteh***Genentech Inc., USA*

Drug metabolism is one of the major mechanisms of drug clearance from the body. This includes a wide array of enzymes that cover oxidation, reduction, hydrolysis, and conjugation. Over the past decades, extensive research focus on cytochrome P450 enzymes has allowed for better understanding of the role these enzymes in determining ADME properties of drugs. Though P450s in most part still play a significant role of drug metabolism, understanding other drug-metabolizing enzymes are becoming important. This is especially vital to enable discovery of novel drug modalities that beyond the traditional Ro5. In this short course, we will highlight what is known about non-P450 drug metabolizing enzymes (UGT, AO and beyond) and their application in ADME and safety. We hope this workshop will enable the participants to better utilize this knowledge during drug discovery optimization.

SC2.2 - ROLE OF GLUCURONIDATION IN DRUG METABOLISM AND TOXICITY**Ryoichi Fujiwara***University of Arkansas, USA*

Conjugation of xenobiotics, such as glucuronidation and sulfation, is known as Phase II metabolism. Transfer of hydrophilic groups by phase II drug-metabolizing enzymes makes parent compounds and oxidized metabolites more water soluble, enhancing their elimination into bile and urine. UDP-Glucuronosyltransferases (UGTs) are one of the major phase II enzymes, utilizing UDP-glucuronic acid as a co-substrate and transferring glucuronic acid to an oxygen, nitrogen, or sulfur atom of their substrates. In addition to the liver, extrahepatic tissues such as intestine and kidneys also contribute to glucuronidation of drugs in the human body. To certain drugs, UGT-catalyzed glucuronidation is a rate-limiting step in the clearance of drugs. In such cases, inhibition of UGTs by co-administered drugs or genetic deficiency in the UGT gene can result in elevation of blood concentrations of the drugs. The promoter region of the UGT gene contains recognition sites for a variety of transcription factors. Induction of UGTs, therefore, is often observed, resulting in decreased blood concentrations of the drugs. Age, gender, diseases, and epigenetic status such as modulation of microRNA expression and gene methylation can also be determinants of UGT activities. UGT-overexpressing cell lines, human tissue microsomes, and primary culture cells are widely accepted tools to understand glucuronidation of drugs and drug candidates in humans. Except for primary culture cells, however, the clearance rates estimated from data obtained with *in vitro* studies are typically lower than the rates observed *in vivo*. Although animal study using mice and rats is another way to understand human drug glucuronidation, species difference in UGT function can be a major obstacle for extrapolation from animal data to humans. Major species differences associated with UGTs include the lack of UGT1A4 in mice and rats, which is one of few UGT isoforms that can glucuronidate tertiary amines. To overcome the species differences, gene-modified mouse and rat models where UGT genes are humanized have been recently developed. Studies using such humanized animals have shown better correlations between human and animal data. Importantly, conjugated metabolites are not always pharmacologically inactive. Acyl-glucuronides, which are formed by glucuronidation of the carboxylic group of substrates, can be highly reactive and toxic. This might, in part, explain severe hepatotoxicity of compounds with carboxylic group. DNA damage has also been reported by sulfated and acetylated metabolites of N-hydroxylated aniline derivatives. In this session, the basic information of phase II drug-metabolizing enzymes as well as their importance in drug clearance will be provided. Classical and recently-developed tools to estimate drug glucuronidation in humans will be discussed. Finally, cases of conjugation-induced toxicity *in vitro* and *in vivo* will be introduced.

SC2.3 - ENZYMOLOGY AND CLINICAL IMPORTANCE OF REDUCTASES AND HYDROLASES**Matthew A. Cerny***Pfizer Inc., USA*

After cytochromes P450 (P450s) and uridine 5'-diphospho-glucuronosyltransferases (UGTs), hydrolases and reductases are the next most prevalent families of drug metabolizing enzymes involved in the metabolism of exogenous substances. This presentation will be focus on the major hydrolases and reductases. Details regarding the characteristics of these enzymes (e.g. enzyme mechanism, tissue distribution, polymorphism, etc.) as well as commonly used assays systems, substrates, and inhibitors for these enzymes will be presented. Additionally, examples of the involvement and description of hydrolases and reductase in the metabolism of drug molecules will be described.

SC2.4 - TOXICOLOGICAL RELEVANCE OF NON-P450 ENZYMES: HYDROLASES AND ALDEHYDE OXIDASE**Tatsuki Fukami***Kanazawa University, Japan*

Non-P450 enzymes are receiving attention because new chemical entities that are not metabolized by P450 are recently increasing. Because non-P450 enzymes as well as P450 enzymes are often associated with drug-induced toxicity, the functional characterization of non-P450 enzymes is required to disclose the mechanisms of toxicity. Our research group has conducted studies focusing on enzymes catalyzing hydrolysis and reduction that are relevant with the interindividual variability of pharmacokinetics or drug-related toxicity.

Hydrolases catalyze the hydrolysis of compounds containing ester, amide, and thioester bonds. Carboxylesterase (CES) enzymes, CES1 and CES2, are well known to catalyze the pharmacological activation of prodrugs. In addition, arylacetamide deacetylase (AADAC) has been getting known as an enzyme hydrolyzing drugs in the liver and gastrointestinal tracts. However, these enzymes had not been investigated from the viewpoint of drug-related toxicity. Recently, we demonstrated that CES2 is involved in the quinonimine formation from flupirtine, which causes liver injury as a side effect, because the cytotoxicity by flupirtine in HepG2 cells was increased by overexpression of CES2. In addition, we demonstrated that AADAC is involved in hydrolysis of ketoconazole, which causes liver injury when it was orally administered. Cytotoxicity by ketoconazole was observed in AADAC-overexpressed HepaRG cells and human primary hepatocytes. Thus, hydrolases should receive more attention as perpetrators of drug-induced toxicity.

It has been suggested that reduction of nitroaromatic drugs appears to be involved in toxicity, because hydroxylamine, which are generally unstable, could be produced as intermediates of reduction or via hydroxylation of reduced metabolites, arylamines. Recently, we identified aldehyde oxidase 1 (AOX1), which has been generally accepted to catalyze oxidation of drugs, to be a reductase of nitrazepam and dantrolene from the human liver. Production of unstable intermediates via reduction was detected as glutathione or N-acetylcysteine conjugates. In addition to nitrazepam and dantrolene, nitroaromatic drugs that rarely cause hepatotoxicity as a side effect, such as flutamide and nilutamide, were reduced by AOX1. Thus, AOX1 would be related with drug-induced toxicity.

In this short course, newly uncovered function of hydrolases and AOX1 from the viewpoint of drug-induced toxicity and pharmacokinetics will be shared.

SC3.1 - INTRODUCTION TO QSP**Piet van der Graaf***Certara, United Kingdom*

The challenges of sustaining and improving a pharmaceutical R&D model that meets the needs of patients and society are well known and have been documented in numerous analyses. In recent years, a new era of optimism and opportunity has emerged around “personalised medicine” (backed by compelling evidence of breakthroughs in areas like immunoncology) which provides a unique opportunity to (re)position model-informed drug development (MID3) in general and quantitative systems pharmacology (QSP) specifically as essential enablers to guide the development of individualised therapy. Indeed, QSP is now increasingly being employed to translate the rapidly growing understanding and mapping of complex biology and pathophysiology into a solid foundation for the efficient and rational development of novel modalities, combination therapy and innovative treatment regimens to treat diseases in high medical need areas. However, despite this uptake and rapid scientific and technical evolution of the discipline, the impact of QSP in drug development and regulatory decision making is still limited. This workshop will discuss the challenges and opportunities for QSP and potential solutions will be proposed, illustrated by case studies. The workshop will end with a hands-on tutorial how to build a QSP model.

SC3.2 - APPLICATION OF QSP IN DRUG DISCOVERY**R. Adam Thompson***Abbvie, USA*

Drug discovery is fraught with challenges. Furthermore, a recent metanalysis has shown that oncology is the indication with the lowest probability of success for regulatory approval [1]. QSP modeling, which quantitatively describes dynamic interactions between a therapeutic and the biological system, aspires to improve success rates by facilitating informed decision-making at various stages in the drug discovery and development process. Examples of applications include improving the mechanistic understanding of a pathway, prioritizing potential targets and biomarkers, or assessing therapeutic combinations. In this presentation, we describe the development and implementation of a QSP model of apoptosis signaling and present specific use-cases in which the model has informed oncology discovery and translational decisions.

Reference:

1. Chi Heem Wong, Kien Wei Siah, Andrew W Lo; Estimation of clinical trial success rates and related parameters, *Biostatistics*, kxx069, <https://doi.org/10.1093/biostatistics/kxx069>

SC3.3 - THERAPEUTIC AREA CASE STUDY: QSP IN IMMUNO-ONCOLOGY**Shihem Bihorel**

University of Florida, USA

Immuno-oncology (I-O) is a young and growing field on the frontier of cancer therapy. Contrary to cancer therapies that directly target malignant cells, I-O therapies stimulate the body's immune system to target and attack the tumor, which is otherwise invisible to, or inhibiting the immune response. Quantitative systems pharmacology (QSP) is a burgeoning modeling field that seeks to better understand pathophysiology and pharmacological mechanisms from the levels of molecular pathways (–omics), regulatory networks, cells, tissues, organs, and ultimately the whole organism. QSP is a multi-disciplinary endeavor, borrowing from genomics, transcriptomics, proteomics, metabolomics, and signaling network modeling. As a result, computational methods for QSP analyses provide a powerful means for analyzing experimental findings, interpreting biological behaviors, testing hypotheses, and designing experiments by integrating large amounts of experimental data into mathematical models of regulatory networks and cell behavior. This presentation will explore I-O strategies to treat cancer and how QSP models are being used to gain a better understanding of cancer and I-O drugs responses.

SC3.4 - THERAPEUTIC AREA CASE STUDY: QSP IN INFLAMMATORY DISEASE**Kapil Gadkar**

Genentech Inc., USA

Asthma is an airways disorder, characterized by chronic inflammation of the airways involving numerous underlying immunological and stromal pathways. Various treatments that have been approved or are in development target activities or proteins in these pathways, and show differential impact on clinical outcomes and pathway biomarkers. Although specific molecular pathways are being characterized more thoroughly, the understanding of the link between the underlying mechanisms and the functional clinical outcomes is still very limited.

We have developed a mechanism-based mathematical model representing different cellular and soluble contributors to asthma, including: (1) innate immune, adaptive immune, and airway resident cells; (2) soluble proteins such as IL-5, IL-13, IL-4, IL-33, and IgE; and (3) clinical and biomarker endpoints such as FeNO and FEV1. The *in silico* model has been developed utilizing *in vitro* and *in vivo* data and clinical data for multiple drugs including anti-IgE, anti-IL5, anti-IL13, and anti-IL-4Ra. Virtual subjects of different patient phenotypes (e.g. Type 2 high vs Type 2 low) and with varying disease severities (e.g. mild asthmatics vs severe asthmatics) are represented in the model.

The model was used to predict responses to a neutralizing antibody to ST2, the receptor for IL-33, for which limited clinical data are available. These predictions include comparison of FEV1 and FeNO responses to anti-ST2 vs. other drugs such as anti-IL-13 and anti-IL-4Ra across virtual subjects with different phenotypes and disease severity. The effect of anti-ST2 on circulating biomarkers such as blood eosinophils & Type 2 cytokines were evaluated as surrogates of ST2 target engagement in the airway tissue and downstream effects.

In addition, the model is used to determine clinical trial doses and dose regimens to establish proof of concept for a class of NMEs. The model is used to understand the impact of the different characteristics of the NMEs on the underlying pathophysiology and potential improvement in disease prevention. These analyses supported selection of lead candidate for clinical development.

The model has proven useful in exploring and elucidating biological pathways underlying observed effects of the different interventions, and has allowed us to explore and predict the impact of novel interventional strategies for which little to no clinical data are available. It also serves as a platform for evaluating alternate hypotheses and designing experiments to experimentally test them.

SC3.5 - TUTORIAL: HOW TO BUILD A QSP MODEL IN LESS THAN ONE HOUR**Andrzej Kierzek**

Certara, United Kingdom

The purpose of this tutorial is to introduce basic concepts of Quantitative Systems Pharmacology. I will present example model, which development can be completely described within one hour presentation. I will introduce basic structure, where a Pharmacokinetic model is connected to a Systems Biology model describing disease and drug action. Subsequently, I will introduce common bi-partite graph notation, where state variables and rate laws are represented by different symbols. Further common features such as compartments, assignments, events and doses will be introduced. I will then present translation of the model to the system of Ordinary Differential Equations (ODEs) and simulation in both

Matlab and R. Subsequently, I will discuss parameterisation of the model using both literature and project data. I will review recent approaches to model calibration and validation. Finally, I will briefly describe modular graphical notation used in Certara QSP platform and show how modules can facilitate team development. I will also introduce our novel notation for indexed species and parameters, which provides intuitive way of automatically generating large collections of reactions and ODEs for repetitive parts of the model. This feature can be used to streamline development of models, where binding sites, allosteric transitions, clones or polymers lead to combinatorial explosion of possible interactions. In summary, the tutorial will use biological process maps to introduce basic structure of QSP model and will give brief introduction to QSP model calibration and validation.

SC4.1 - THE ROLE OF INFLAMMATORY MEDIATORS IN IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY

Jack Uetrecht

University of Toronto, Canada

Idiosyncratic drug-induced liver injury (IDILI) represents a significant risk factor for drug development. The unpredictable nature of IDILI makes prospective studies in humans virtually impossible, and IDILI is also idiosyncratic in animals; therefore, mechanistic studies are very difficult. However, clinical data suggest that most IDILI is immune mediated. If so, it might be possible to develop an animal model by stimulation of antigen presenting cells. However, multiple attempts using this strategy have failed to reproduce liver injury similar to IDILI in humans. This is consistent with the observation that inflammatory conditions such as ulcerative colitis are not significant risk factors for IDILI. We also tried immunization of animals with drug-modified proteins followed by treatment with the drug, but this led to upregulation of tolerogenic factors rather than liver injury. Drugs that cause serious IDILI always cause a higher incidence of mild IDILI that often resolves despite continued treatment. If IDILI is immune mediated this resolution must involve immune tolerance. In fact, the dominant immune response in the liver is immune tolerance, and blocking immune checkpoints and treatment with amodiaquine led to liver injury in mice very similar to IDILI in humans. Specifically, we used PD-1^{-/-} mice treated with anti-CTLA-4 to impair immune tolerance. This model also unmasked the ability of other drugs such as nevirapine, isoniazid, and carbamazepine to cause liver injury; however, the injury with these agents was less severe and resolved despite continued treatment. This model was also able to differentiate the IDILI risk of troglitazone from pioglitazone and tolcapone from entacapone. It appears that one aspect of the mechanism by which drugs cause IDILI is by causing hepatocyte cell stress leading to the release of drug-associated molecular pattern molecules (DAMPs). We found that the supernatant from hepatocytes incubated with amodiaquine or nevirapine led to activation of inflammasomes and release of IL-1 β from THP-1 cells, a human macrophage cell line. This is likely an early event in the induction of the immune response that in some patients can lead to IDILI. This could represent an *in vitro* method to screen drug candidates for IDILI risk. Taking the observation that drugs that cause serious IDILI always cause a higher incidence of mild liver injury one step farther, it may be that most patients and animals have a clinically silent innate immune response to drugs that cause IDILI. This is clearly true with some drugs, e.g. most patients treated with clozapine have a transient increase in serum IL-6 and IL-1 β . If this is true, it may be possible to study the innate immune response to drugs that cause IDILI without impairing immune tolerance. This would provide mechanistic clues to the early steps in the immune response to drugs even though it only rarely results in severe IDILI. It is only with a better mechanistic understanding of IDILI that we are likely to be able to significantly decrease risk.

SC4.2 - CLINICAL ASPECTS OF HEPATOCELLULAR AND CHOLESTATIC DRUG-INDUCED LIVER INJURY

Einar S. Björnsson

Landspítali - The National University Hospital of Iceland, Iceland

Idiosyncratic drug-induced liver injury (DILI) is an acute adverse hepatic reaction occurring in only a small proportion of subjects exposed to a drug, DILI can lead to jaundice, other symptoms, disability and sometimes hospitalization, including life threatening liver failure and the need for liver transplantation. DILI is a major cause of failure of drugs during development and also a major reason for withdrawal of otherwise promising drugs from the market post marketing. DILI is the most common cause of acute liver failure in western countries. There are no pathognomic clinical, biochemical or histological features that can confirm drug causality. Thus, attribution of toxicity from a particular drug is challenging, requiring temporal relationship, biochemical signature, exclusion of competing etiologies and knowledge of the hepatotoxicity potential of the implicated drug. In recent years a considerable improvement in understanding the complexity of idiosyncratic DILI has taken place, such as the importance of dose dependency, hepatic metabolism, lipophilicity of the drug and genetic susceptibility. However, reliable biomarkers that can predict the risk of DILI in individual patients are lacking and that might with high sensitivity prevent serious or even fatal DILI in clinical trials are needed. Recent findings of new phenotypes of DILI, risk of chronic liver injury following DILI and increasing proportion of developing DILI in patients treated with immunomodulatory treatment of malignancy, illustrate further the complexity and the clinical problems clinicians, the pharmaceutical industry and the regulators are facing. There is an important need for deeper understanding of this complex and clinically challenging disorder.

SC4.3 - CELLULAR AND ANIMAL MODELS OF IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY**Tsuyoshi Yokoi***Nagoya University, Japan*

Drug-induced liver injury (DILI) is one of leading causes of attrition during both early and late stages of the drug development and post-marketing. DILI is generally classified into intrinsic and idiosyncratic types. Intrinsic DILI is dose-dependent and predictable as exemplified by acetaminophen toxicity. However, occurrence of idiosyncratic DILI with very low incidence and severe liver damage is difficult to predict because of the complex nature of DILI and a poor understanding of its mechanism. Idiosyncratic DILI has been attributed to metabolic idiosyncrasy. However, recently, it comes to be recognized that most of idiosyncratic DILI-inducing drugs are associated with various types of immune- and inflammation reactions. In this lecture, current knowledge and our experimental findings on the mechanisms of DILI with focuses on reactive metabolites of drugs, and involvement of immune- and inflammation-related responses, an *in vitro* prediction system for risk assessment of DILI, and microRNA (miRNA) as a potential biomarker or early detecting of DILI are summarized.

Considering drug metabolism and toxicokinetics, we have established non-clinical animal models of DILI for 12 kinds of clinical drugs that have been known to cause idiosyncratic DILI in human. By using animal models, it has shown that the formation of reactive metabolites and both innate and adaptive immunity (mainly Th2- and Th17-related factors) are involved in the pathogenesis of hepatotoxicity of drugs. Then, based on the information of biomarkers obtained from animal models, we developed a cell-based system that predicts the potential DILI risks of drugs.

We sought to develop a novel cell-based assay for the risk assessment of DILI that considers drug metabolism as well as immune- and inflammatory-related gene expression. To accomplish this goal, human hepatoma HepaRG or HepG2 cells were treated with 96 drugs with different clinical DILI risks. The conditioned media were subsequently used to treat human pro-myelocytic leukemia HL-60 cells or human PBMC (peripheral blood mononuclear cells), and the mRNA expression levels of immune- and inflammatory-related genes were measured. An integrated score calculated from the levels of 4 or 5 genes precisely determined the DILI risks than individual gene expression did. Advanced methods of co-culture systems of PBMCs with liver cell lines demonstrated that the selected biomarkers included the 5 new biomarkers correctly predict DILI risk in the *in vitro* assay.

Recently, study on circulating miRNAs as potential biomarkers of organ injury, including DILI, has received increasing attention. It has been demonstrated that miR-122 and miR-192, which are liver enriched, could be potential biomarkers of DILI. However, these miRNAs cannot discern types of injuries. Comprehensively analyzed time-dependent plasma miRNA profiles in rats with drug-induced hepatocellular injury, cholestasis, and steatosis with high-throughput miRNA sequencing (NGS) were performed. Several miRNAs were dramatically changed earlier than traditional biomarkers such as ALT and AST, indicating the utility of miRNAs as specific biomarkers for the early detection of DILI.

These studies increased our understanding of mechanisms of DILI and may assist in selecting candidates that are more likely to be safe in humans in preclinical drug development and in clinical pharmacotherapy.

SC4.4 - PROGRESS MADE IN UNDERSTANDING GENETICALLY-INHERITED SUSCEPTIBILITY FACTORS, INCLUDING HLA GENOTYPE**Ann K. Daly***Newcastle University, United Kingdom*

Genome-wide association studies (GWAS) on DILI in the past 10 years has resulted in considerable progress in understanding genetic risk factors for this form of toxicity. Initially, a small number of HLA associations with strong effect sizes which related to common drug causes of this reaction were detected in relatively small cohorts. Associations involving 10 different HLA alleles in 3 different HLA genes have now been reported for a total of 12 drugs. These associations are generally very drug specific but a few alleles appear to be risk factors for DILI due to several structurally unrelated drugs. The underlying mechanism for these HLA associations is still understood quite poorly but there is some evidence that it involves inappropriate presentation to T cells of self-peptides with covalently attached drug or metabolite. Direct interaction of drug with HLA proteins has not been demonstrated for drugs causing DILI.

It is clear that not all forms of DILI show HLA associations; reactions due to diclofenac, statins and anti-tuberculosis drugs such as isoniazid have not shown HLA associations by GWAS approaches and genetic risk factors for DILI due to these drugs remain unclear, possibly due to limited statistical power. One recent large GWAS involving >2000 cases collected via collaboration between iDILIC, a predominantly European network, and the USA-based DILIN network detected an association with a variant in the gene PTPN22 which is known to also be a risk factor for autoimmune disease. This association extends to a number of drugs, some of which also show HLA associations. It seems likely that the PTPN22 gene product modulates inappropriate immune and inflammatory response.

Further genetic studies need to focus on collecting large numbers of cases due to individual drugs but may provide new insights into underlying mechanisms for DILI, especially where HLA genotype does not contribute to risk.

K1 - MODEL-INFORMED PRECISION DOSING AT THE BEDSIDE: CHALLENGES AND OPPORTUNITIES**Lawrence J. Lesko***Professor Emeritus, University of Florida College of Pharmacy, Florida*

Google Now is one of several amazing personal assistant applications at your fingertips. This application presents itself as “just the right information, at just the right time.” It combines incredible model-based artificial intelligence and the power of a search engine giant that stores all kinds of individual information. Can you imagine an analogous future where your physician will be able to access evidence-based precision dosing at his or her fingertips?

Why is this important to health care? Because most drugs fail to work as intended. Simply giving a fixed dose of a drug to someone, and hoping for the best, does not take into account the vast interpatient biological variation in how that dose is handled. The clinical significance of inadvertent underdosing or overdosing cannot be over-stated.

Model-informed precision dosing (MIPD) refers to a quantitative platform coupled with drug modules that provide clinical decision support for use at the point-of-care. Ideally, if the critical patient factors that define drug handling are known before selecting a drug dose, an individualized dose can be determined on the first dosing cycle. More often, fine-tuning of doses in subsequent cycles is needed and will be based on exposure-response metrics (e.g., C_{min}), pertinent clinical characteristics of a patient (e.g., renal function), dosing history including co-medications, measurable lab-based biomarkers (e.g., HbA1c) and inherited gene differences that influence drug metabolizing pathways. MIPD incorporates a model-based estimation method (e.g., Bayesian forecasting) to frame an individual's PK/PD relationship and simulate untested dosing scenarios. This determines his or her optimal dosing regimen. There are many challenges to overcome with MIPD such as accessing high quality patient data with predictive ability, integration into a physician's workflow and electronic health records, and reimbursement for all or part of the cost of MIPD by commercial or government insurance plans. But the opportunities for MIPD are astonishing such as the ability to learn from each drug:patient interaction to optimize dosing for the next patient. It remains to be seen how far MIPD will go given the complexities of biology and the numerous contexts for its use.

L1 - HOW TECHNOLOGY, BIG DATA AND SYSTEMS APPROACHES ARE TRANSFORMING 21ST CENTURY HEALTHCARE**Leroy Hood***Providence St. Joseph Health, USA*

When I started at Caltech in 1970 as an Assistant Professor—I was fascinated by the complexity of humans and how few technologies and strategies we had for assessing this complexity. Over the next 50 years of my career, I participated in seven paradigm changes that both dealt with biological complexity and framed my view of 21st century medicine. The first of these paradigm changes centered on bringing engineering to biology with the development of 6 instruments to characterize and synthesize DNA and proteins. The first four of these instruments led to my co-founding Applied Biosystems, a company that successfully commercialized each of these instruments, made them available to the scientific community and thus revolutionized the practice of biology. A second paradigm change had to do with the initiation of systems biology—the view that rather than studying biology one gene or one protein at a time, one should take a holist or global view of biological systems and operate in a cross-disciplinary and integrative (e.g. for both technologies and data) environment. I will discuss these and the remaining paradigm changes and how they also changed how biology is practiced. Most fascinatingly, they framed a strikingly new view of medicine. For example, the conviction that healthcare should be predictive, preventive, personalized and participatory (P4) which led to the conviction that healthcare really has two central domains—wellness, then largely ignored, and disease.

In an attempt to place wellness on a quantitative basis, we carried out a pilot wellness program in 2014 where 108 individuals agreed to be densely phenotyped (e.g., genome sequence, quantification of blood analytes (clinical chemistries, proteins and metabolites) every 3 months, quantification of the different species in the gut microbiome every 3 months and measurements of quantified self with a fitbit and other devices). Each individual thus had a data cloud that when analyzed led to a host of actionable possibilities that could improve individual wellness or avoid disease. Examples will be discussed. We came to call this scientific (or quantitative) wellness.

The subjects of the wellness pilot effort were so enthusiastic about continuing their scientific wellness program that in mid-2015 we started a company called Arivale that brought scientific wellness to consumers. This company now has almost 5000 clients—each with their own longitudinal data cloud. These data clouds, collectively, allowed us to view human biology and disease with unparalleled resolution which, in turn, has led to striking observations about both wellness and disease. These insights will both change the practice of healthcare and offer new approaches to the discovery of biomarkers and drug targets. I will discuss these new insights and delineate just how profoundly medicine of the 21st century will differ from medicine of the 20th century (which almost all healthcare systems are practicing).

S1.1 - WARFARIN: A PARADIGM FOR INDIVIDUALIZED DOSING**Munir Pirmohamed***University of Liverpool, United Kingdom*

Warfarin remains a very widely used oral anticoagulant despite the advent of direct oral anticoagulants (DOACs). Warfarin dose is difficult to predict, with some patients requiring 0.5mg/day, while others require 20mg/day. The current paradigm is to start on a standard dose, and check INR, a biomarker for the degree of anticoagulation, to reach a therapeutic dose which maintains the INR between 2-3. However, this trial and error approach can lead to over- or under-anticoagulation with the likelihood of complications. At least 60% of the variability in individual daily dose requirements for warfarin can be predicted based on genetic and clinical factors. The genetic factors include polymorphisms in CYP2C9 and VKORC1. Many different algorithms, incorporating both genetic and clinical determinants of dose, have been developed to predict warfarin dosing. In the EU-PACT randomised controlled trial, we used a loading dose algorithm to initiate warfarin. Genotyping was performed using a point-of-care genotyping platform which provided results on 3 alleles in 2 hours. The result of the trial showed that genotype guided dosing was superior to standard dosing: this has been replicated in several other trials more recently. We have gone onto implement this into clinical practice – the genotyping platform was refined to produce results in 45 min, and nurses undertook the genotyping and used a web-based algorithm to initiate dosing. The results of this implementation study were identical to the randomised trial. Genotype-guided dosing of warfarin was also shown to be cost-effective. The limitation of our dosing algorithms is that they only predict dosing for individuals of Northern European Ancestry. We are therefore now undertaking patient recruitment in Uganda and South Africa to identify clinical and genetic factors which are important for determining dose variation in African populations, where the use of warfarin is the mainstay for anticoagulation given the cost of DOACs.

S1.2- RELEVANCE OF TRANSPORTER AND METABOLIC SNPS TO PEDIATRIC TREATMENT**Tsuyoshi Fukuda** and Chie Emoto*Cincinnati Children's Hospital Medical Center, USA*

It is not easy to discuss the relevance of pharmacogenetics in pediatrics, due to the limited number of patients enrolled and sampling points taken in studies and developmental changes over the pediatric period as compared to adults. Progress over decades in two major areas, Pharmacogenetics and Pediatric Clinical Pharmacology, has contributed to pharmacological management in pediatric patients. In order to predict drug behavior in pediatric patients, pharmacogenetic data need to be integrated with age-dependent anatomical and physiological information. Recently, a pharmacometric approach has increasingly been recognized as an integrating methodology to fill in the information gap, which can incorporate the concept of growth and development for children with pharmacogenetic effects on the functions of drug metabolizing enzymes (DMEs) and/or transporters.

Simplified uniform dosing is ideal, but realistic dosing should be considered with multiple static and dynamic changes, which influence drug disposition. Traditionally, pediatric doses have been scaled down from adult doses using body-size mass (e.g. weight, BSA). However, due to the nonlinear nature of dynamic changes in various physiological parameters, the simple scaling methods, based on body mass, often fail to predict drug exposure, especially in neonates and infants. As static parameters, single nucleotide polymorphisms (SNPs) in DMEs and/or transporters are well-known determinant factors for drug disposition. For pediatric patients, the genotype-phenotype relationship may be influenced by the developmental changes that occur between birth and adolescence. Thus, it would be attractive to have a simulation platform which can integrate different aspects of knowledge, such as pharmacogenetic effects and age-dependent changes including the ontogeny of various biological components.

This presentation will discuss the pharmacogenetic effects of SNPs in DMEs and/or transporters for pediatric drug dosing and potential approaches to tackle the clinical relevance of PGx in pediatric patients. In addition to the effects, the impact of growth and development on pediatric PKs will be highlighted, including practical examples, using a pediatric physiologically-based PK (PBPK) model as a knowledge-integrating platform.

S1.3 - CPIC GUIDELINES AND IMPLEMENTATION TO DATE**Kelly Caudle***St. Jude Children's Research Hospital, USA*

The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international consortium of over 300 members of clinical pharmacists, physicians, nurses, genetic counselors and scientists who are interested in facilitating use of pharmacogenetic tests for patient care. CPIC provides freely available, peer-reviewed, updatable clinical practice guidelines that enable the translation of genetic laboratory test results into actionable prescribing decisions for specific drugs. CPIC also coordinates projects that facilitate the adoption of pharmacogenetics into clinical practice and the electronic health record (<https://cpicpgx.org/informatics/>). Prior to the creation of CPIC, one barrier to implementation of clinical pharmacogenetics was the lack of detailed gene/drug clinical practice guidelines. CPIC guidelines are developed using established and rigorous methods and include a standard system for grading levels of evidence and strength to

each prescribing recommendation. CPIC has published 23 guidelines, providing genotype-based therapeutic recommendations for 17 genes covering over 40 drugs (<https://cpicpgx.org/guidelines/>). CPIC guidelines are used by many programs and laboratories to facilitate the implementation of pharmacogenetics. For a list of pharmacogenetic implementers using CPIC guidelines see <https://cpicpgx.org/resources/>. CPIC provides additional resources to facilitate the adoption of CPIC guidelines into a variety of clinical practice settings and the electronic health record by providing tables that translate genotypes into phenotypes, example consult and clinical decision support language and workflows to implementing genetic test results into an electronic health record. CPIC guidelines and resources provide information and education for successful integration of pharmacogenetics into clinical care.

S1.4 - PERSONALISED TREATMENT OF CANCER

Howard McLeod

Moffitt Cancer Center, USA

Precision medicine has become a focus of research and policy and has the opportunity to also change medical practice. The use of genomics in this context may impact many areas of medicine, but is becoming a real part of oncology, with implications for therapy selection, treatment avoidance, dosing, and risk prediction. The presence of clinically predictive germline variants has also opened the hope that objective predictors of patient toxicity will be in the future. This has mainly focused on cytochrome P450 enzymes (CYPs), such as CYP2C19/voriconazole or CYP3A5/tacrolimus. More recently there has emerged robust data for membrane transporters having an important association with ototoxicity, neurotoxicity, and cardiotoxicity. Now there is data showing that moderate penetrance variants in known biological pathways have important clinical impact. For example, genes involved in the Charcot-Marie-Tooth peripheral neuropathy syndromes and risk of chemotherapy-induced peripheral neuropathy. This constellation of data from pharmacology and biology now will allow for the development of robust risk/benefit models, whereby decisions between apparently equal treatment options can be made for an individual patient. These probabilistic strategies are important ways to make pharmacogenomic findings of relevance to modern cancer care. There is a need for personalized medicine approaches to also go beyond DNA, to include biomarkers that reflect the patients current situation. While this can include immunoproteomic or metabolomics strategies, blood level guided therapy remains an underexplored clinical tool. It is also clear that there are many barriers to clinical application. There are also critical non-science issues, such as integration of new tests into health systems, changing old habits to allow application of new data, and the reality that the cost of both testing and the therapeutic options are a key driver in health care. As the scientific evidence matures, we must think beyond our favorite aspect of translational science if we are to overcome the many obstacles to delivering more careful selection of cancer therapy.

S2.1 - "TISSUES ON CHIPS"- A NOVEL TOOL FOR TOXICITY AND EFFICACY TESTING ON HUMAN TISSUE

Bo Yeon Lee

National Institute of Health, NCATS, USA

More than 30% of promising medications have failed in human clinical trials because they are determined to be toxic, and another 60% fail due to lack of efficacy despite promising pre-clinical studies in 2-D cell culture and animal models. The challenge of accurately predicting drug toxicities and efficacies is in part due to inherent species differences in drug metabolizing enzyme activities in *in vivo* studies, and cell-type specific sensitivities to toxicants. To address this challenge in drug development, the NCATS (National Center for Advancing Translational Sciences) of NIH (National Institute of Health) has been supporting the development of Microphysiological Systems (MPS; also known as "organs-on-chips" or "tissue chips") through the Tissue Chips program.

Tissue Chips are *in vitro*, three-dimensional organ systems from human cells on bioengineered platforms. These mimic *in vivo* tissue architecture and physiological conditions for safety and efficacy studies, as well as disease modeling. The Tissue Chip platform will provide a tool for predictive toxicology and efficacy assessments of candidate therapeutics. This technology carries significant potential to be used to inform clinical trial design by; further elucidating the disease pathophysiology, assisting with the selection of the best drug candidates for clinical trials, improving the selection of patient populations, and identifying the reliable clinical trial endpoints. Tissue chips can also contribute to studies in precision medicine, environment exposures, reproduction and development, infectious diseases, microbiome, and countermeasures agents.

S2.2 - KIDNEY ORGANIDS FOR DISEASE MODELING AND HIGH THROUGHPUT SCREENING

Benjamin Freedman

University of Washington, USA

Kidneys cannot naturally regenerate lost tissue, and few preventive medications exist, limiting treatment options to temporary salves of dialysis or transplant with substantial side effects. We have developed a simple, commercially

available method to differentiate human pluripotent stem cells into intricately patterned, multi-segment organoids that resemble kidney tissues. While beautiful, how to translate organoids into innovative therapies for organs as complex as human kidneys remains a critical question. To address this challenge, we have applied CRISPR gene editing and high throughput automation to reveal disease mechanisms in organoids and test therapeutic interventions. Mutations associated with polycystic kidney disease or cilia cause organoid tubules to swell thousands of times in size, producing large, fluid-filled cysts of centimeter diameters. In contrast, mutations associated with podocytes, the filtering cells of the kidney, do not affect tubules but cause junctional deformities that explain urinary defects *in vivo*. Harnessing the power of automation, scRNA-seq analysis of organoids reveals sixteen different cell types, and identifies a novel three-gene signature of glomerular disease that appears in human patients. To improve organoid function and seek therapies, thousands of organoids can be manufactured simultaneously in high throughput screening formats, and analyzed for multi-dimensional phenotypes of differentiation, toxicity, and disease. Screening reveals treatments that dramatically increase the vascular endothelium, and identifies factors capable of reversibly modifying kidney cystogenesis. Organoids with live fluorescence reporters and in microfluidic kidney-on-a-chip formats provide next-generation platforms for phenotypic screening, toxicity testing, and illumination of intracellular mechanisms at the tissue scale. Collectively, these findings delineate key strategies and focus areas for advancement of kidney therapeutics using human organoids as surrogates for drug discovery, gene therapy, and regeneration.

S2.3 - REAL-TIME TOXICITY ASSESSMENT ON PERFUSED 3D EPITHELIAL TUBES

Bas Trietsch

Mimetas, Netherlands

In vitro models that better reflect *in vivo* epithelial barrier physiology are urgently needed to predict adverse drug effects. We used a standardized microfluidic 3D tissue culture platform, called the OrganoPlate® to culture up to 96 perfused ECM supported epithelial tubules without using artificial membranes. This organ-on-a-chip platform is implemented to culture tubules representing various tissues including human kidney proximal tubules, colon, liver and blood vessels. Models representing both healthy and diseased states as well as the effect of pharmacological intervention can be interrogated using standard assays and equipment including (time-lapse) high-content imaging, automated liquid handling, high resolution confocal imaging, and fluorescent, luminescent or colorimetric biochemical assays. Providing both apical and basolateral access and avoiding artificial membranes provides optimal flexibility in studying barrier function, transport and migration processes as well as cell-cell interaction. In addition to using fluorescent probes to monitor barrier function we have recently developed the OrganoTEER, an instrument that enables the real-time and parallel measurement of trans-epithelial electrical resistance (TEER) on all tubes of an OrganoPlate. The (co-)culture of best in class human tissue models on a platform compatible with industry standard equipment offers the combination of unsurpassed physiological relevance, throughput and assay flexibility.

S2.4 - BIOFABRICATION OF LIVER CONSTRUCTS FOR DRUG TOXICITY STUDIES

Kerstin Schneeberger¹, Manon Bouwmeester¹, Riccardo Levato², Nynke Kramer¹, Jos Malda², Bart Spee¹

¹*Utrecht University, Netherlands*, ²*University Medical Center Utrecht, Netherlands*

Current *in vitro* systems do not accurately predict drug-induced liver injury (DILI). DILI still occurs in clinical phases of drug development or even post-marketing, which makes it a serious health concern. New *in vitro* models that can reliably predict DILI are thus necessary. Human adult stem cell-derived liver organoids are long-lived and can differentiate into hepatocyte-like cells. As such, they represent an exciting new *in vitro* model.

In this study, we combined human liver organoids with a photocrosslinkable gelatin methacryloyl (GelMA) hydrogel to create 3D liver tissue constructs. The simultaneous deposition of poloxamer 488 as a sacrificial support material allowed for the formation of a perfusable porous construct. Human liver organoids efficiently differentiated towards hepatocytes in GelMA and remained viable in the 3D printed hepatocyte constructs. We also confirmed that the constructs are suitable for acute toxicity studies, as dose-dependent cell damage could be induced by treatment with prototypical hepatotoxicants. Furthermore, we developed a custom-designed flow bioreactor to perfuse the construct. Our results indicate that perfused 3D liver constructs in a bioreactor system have the potential to predict DILI in a reliable and patient-specific manner.

S3.1 - DEVELOPMENT AND APPLICATION OF A PBPK MODEL FOR LARGE MOLECULES IN PFIZER

Hannah Jones

Pfizer Inc., USA

'Best practice' approaches for the prediction of traditional mAb PK in humans have largely been empirical relying on allometric scaling and don't allow for differences in FcRn affinity or clearance/distribution mechanism which effect the PK of large molecules. PBPK modelling offers promise in this area and is well established for small molecule PK prediction,

but the development of PBPK models for large molecules has been limited. We have successfully developed a large molecule PBPK model that describes the intracellular trafficking and FcRn recycling of mAbs in the TG32 mouse and in human. This model utilizes available mAb specific *in vitro* data on FcRn affinity and species specific FcRn expression tissue data, tissue volume and blood flow physiology to predict a priori the *in vivo* PK of mAbs. A novel feature of this model is its ability to describe the binding of the mAb to FcRn at pH 6, the trafficking through the endosome and release at pH 7.4. The model predicts accurately the PK of ~25 mAbs (covering a 10-fold T1/2 range 3-30 days) in the majority of cases. In addition the mechanistic nature of this model allows us to accurately predict the PK and target binding of mAbs engineered to have pH dependent binding to its target or FcRn. Use of this model in early discovery could revolutionize our approaches to compound selection.

S3.2 - PBPK AND PKPD MODELING TO INVESTIGATE ENGINEERED ANTIBODIES

Tatsuhiko Tachibana

Chugai Pharmaceutical Co., Ltd., Japan

There has been an increase in the number of monoclonal antibodies (mAbs) approved for therapeutic use. However, there are a limited number of promising antigens which can be targeted by conventional mAbs. To expand the target antigen space and/or to deliver substantial benefits to patients over conventional mAbs, a series of innovative antibody engineering technologies have been developed. These include our Recycling Antibody and Sweeping Antibody technologies. The recycling antibody can pH-dependently bind to an antigen, enabling the antibody to escape from lysosomal degradation after target-mediated internalization. The sweeping antibody has both pH-dependent antigen-binding and increased FcR-binding at neutral pH, properties which accelerate the degradation of soluble target antigen. Both recycling and sweeping antibodies are useful for blocking targets too abundant for conventional antibodies. Pharmacokinetic/pharmacodynamic (PK/PD) and physiologically-based pharmacokinetic (PBPK) modeling play a crucial role in our understanding of the quantitative impact of new technologies, enabling us to find possible therapeutic applications. My talk will describe how we used these models and simulations to investigate engineered antibodies for drug discovery and development.

S3.3 - PBPK MODELS FOR PREDICTING HUMAN PK FOR MONOCLONAL ANTIBODIES AND OTHER LARGE MOLECULE MODALITIES

Iain Gardner

Certara UK Limited (Simcyp Division), United Kingdom

Physiologically based pharmacokinetic (PBPK) models combine information on the biological system with xenobiotic specific information to allow simulation of the xenobiotic concentrations in blood and tissues following dosing to an animal or human subject. Over the last 20 years there has been a large increase in the use of PBPK models to simulate the disposition of small molecule drugs and regulatory guidance has been issued outlining the steps needed to qualify the performance of a PBPK model for a small molecule drug (https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-qualification-reporting-physiologically-based-pharmacokinetic-pbpbk-modelling_en.pdf). To develop PBPK models that describe the disposition of therapeutic proteins although some of the same physiological information (e.g. tissue volumes and blood flows) used to construct small molecule PBPK models can be used, a number of additional considerations need to be taken into account. Generally therapeutic proteins have poor membrane permeability and move from the tissue vascular space to the interstitial space via convection and diffusion through pores between the endothelial cells. These processes are slow relative to the delivery of proteins to the tissue by blood and necessitate the use of permeability-limited models for each organ. In addition, therapeutic proteins can enter the endothelial cells by fluid phase endocytosis and then undergo catabolism in the cell lysosomes. For some classes of therapeutic proteins (eg IgG and albumin), FcRn acts as a salvage pathway protecting the proteins from lysosomal degradation. Once in the interstitial space therapeutic proteins generally have limited cellular uptake, unless a specific uptake mechanism exists, and return to the blood via the lymphatic system. The clearance of therapeutic proteins from the body is dependent upon processes including lysosomal and enzymatic degradation, renal filtration and target mediated disposition (TMD), depending on the particular protein in question. TMD occurs when a protein binds with high affinity to its biological target forming a complex that dissociates slowly, with the resultant complex being subject to internalization/degradation providing an additional clearance pathway. Despite the inherent challenges in constructing PBPK models for therapeutic proteins, much progress has been made over the last 30 years. Approaches to link distribution of proteins from the vascular to interstitial space of different tissues dependent on protein size have been described (Gill et al, 2016, AAPS J, 18, 156). PBPK models have been developed for both small and large proteins, monoclonal antibodies (e.g. Li et al., AAPS J, 2014, 16, 1097; Glassman and Balthasar, 2016, J PKPD, 43, 427) and antibody drug conjugates (e.g. Khot et al., 2017, AAPS J, 19, 1715). PBPK models for proteins have also been linked to TMD models adapted to account for the binding of bispecific antibodies to two independent targets or to account for target shedding (Li et al., CPT: PSP, 2014, 3, e96). Lastly, due to the separation of physiological system information from the inherent compound properties, PBPK models have the ability to account for

physiology changes occurring with age and disease, allowing protein disposition to be simulated in children and renally impaired subjects for example. Examples of these different applications will be highlighted in the presentation.

S3.4 - SYSTEMS PK/PD MODEL INFORMED DISCOVERY AND DEVELOPMENT OF CANCER IMMUNOTHERAPY

Dhaval Shah

University of Buffalo, USA

Cancer immunotherapy involves the use of biological agents to help body's immune system fight cancer. These agents typically involve monoclonal antibody-based therapeutics (e.g. immune-checkpoint modulator, T-cell engaging bi-specific molecules, antibody-drug conjugates etc.), cell-based therapeutics (e.g. CAR-T cells, TCR-T cells etc.), and other therapeutics like viruses, vaccine, and cytokines. This presentation will provide select examples that demonstrate how systems-based pharmacokinetics-pharmacodynamics modeling and simulation can be used to facilitate the discovery, development, and preclinical-to-clinical translation of cancer immunotherapy.

S4.1 - IMPACT OF INTESTINAL FLORA ON HOST METABOLISM OF DRUG, SUGAR AND LIPID

Sumio Ohtsuki¹ and Kuno Takuya²

¹Kumamoto University, Japan, ²Otsuka Pharmaceutical, Japan

Intestinal bacteria have attracted recent attention since it was discovered that they can influence various physiological functions and diseases in humans. It has been reported that the profile of intestinal bacteria in obese and non-obese people tend to have different. Intestinal bacteria may affect energy consumption and fat accumulation of host body. In addition, it is known that these bacteria are also associated with lifestyle diseases such as type 2 diabetes, nervous diseases such as autism, and intestinal diseases such as colon cancer. One factor that alters intestinal bacteria is the administration of antimicrobials, which used to treat and prevention of bacterial infections. It can be expected that these drugs cause dysbiosis in the qualitative and quantitative balance of bacterial populations in the intestine and have various effects on host physiology. It can be also considered that intestinal dysbiosis changes the pharmacokinetics by altering drug metabolism. Since antibiotics are often administered together with other drugs, it is important to investigate whether, and to what extent, intestinal dysbiosis induced by antibiotics impacts on drug-drug interactions and the efficacy of co-administered drugs. To clarify the effect of dysbiosis by antibiotics administration, we have conducted quantitative and focused proteomic analysis of liver and other tissues in the mouse treated with non-absorbable antibiotics active against Gram-positive bacteria and Gram-negative bacteria. In this presentation, we will introduce our recent study about changes of hepatic proteome in dysbiosis mouse relating to drug, sugar and lipid metabolism, and mechanism involved in this proteome changes.

S4.2 - ACTIVITY-BASED PROFILING OF THE MICROBIOME RESPONSE TO CHEMICAL EXPOSURES

Aaron Wright

Pacific Northwest National Laboratory, USA

We are developing chemical biology approaches to determine and quantify xenometabolic activities at the host-gut microbiome interface and to delineate how exposures create metabolic susceptibilities and impact host physiology throughout human development. Using activity-based protein profiling we can quantify phase I and II enzyme activities in the liver, lung, and intestine, and determine how environmental and chemical exposures impact these activities. Most recently we are measuring the contribution of the gut microbiome in xenometabolism. The human gut microbiota can have a major impact on the metabolism of chemical agents, either by modulating host metabolism or by transforming the agents or their metabolites directly. To accurately predict how an individual will respond to a xenobiotic exposure or drug treatment regimen it is necessary to identify which microorganisms present in the gut interact with that agent and related metabolites. However, the inter-individual diversity of gut microorganisms has complicated the development of such screening tools. We are developing approaches to measure key metabolic activities in the gut, which are primarily hydrolysis and reduction reactions. We have revealed that taxonomically diverse populations can account for the same enzyme activity upon chemical exposures to the gut microbiota, illuminating the need for functional analyses within the gut. By combining ABPs to probe directly for function in situ, we isolated and identified functionally active microbial subpopulations from the gut microbiome and provide an estimation of functional redundancy. Using our approach, we can directly characterize and quantify the functional response of individual microbes and specific enzymes in the gut microbiome and host organs to xenobiotic exposures. We are now expanding our approach for numerous other enzyme targets in the gut microbiome and host tissues.

S4.3 - IMPACT OF THE HUMAN GUT MICROBIOME ON DRUG DISPOSITION**Peter Turnbaugh***NCATS, University of California San Francisco, USA*

Although the importance of human genetic polymorphisms in therapeutic outcomes is well-established, the role of specific genotypic or copy number variants in our “second genome” (the microbiome) has been largely overlooked. Our research group takes a microbiome-centric approach to pharmacology, working to elucidate the direct and indirect mechanisms through which the human microbiome shapes the efficacy and toxicity of small molecule and biologic therapies. I will discuss our ongoing studies focused on the impact of human gut bacteria on the efficacy of anti-inflammatory drugs used for rheumatoid arthritis. These results emphasize the broad impact of gut bacteria metabolism on drug pharmacokinetics and pharmacodynamics, and a starting point for more systematic studies of the complex interactions between pharmaceuticals and human-associated microbes.

S4.4 - DEVELOPMENTAL REPROGRAMMING OF THE GUT MICROBIOTA BY ENVIRONMENTAL TOXICANTS**Julia Yue Cui***University of Washington, USA*

During postnatal development, the maturation of the gut-liver axis is important for xenobiotic biotransformation and detoxification in newborns and children. Growing evidence in the literature suggests that early life exposure to environmental chemicals may lead to delayed onset of diseases later in life. Most studies on developmental origins of health and disease (DOHaD) have focused on host signaling pathways, whereas relatively less is known regarding to what extent gut microbiome contributes to this process. We tested the hypothesis that early life exposure to human health relevant environmental toxicants causes persistent dysbiosis, leading to epigenetic reprogramming of hepatic xenobiotic biotransformation genes by microbial metabolites. Using multi-omics approach, we have systemically characterized the basal ontogeny of the gut microbiome, microbial metabolites such as bile acids and short chain fatty acids (SCFAs), as well as the necessity of gut microbiome in modulating the ontogeny of host hepatic drug metabolizing enzymes. Maternal exposure to the formerly used flame retardant polybrominated diphenyl ether (PBDE) congener BDE-47, current-use PBDE alternative TBBPA, or the bisphenol A (BPA) alternative BPS, altered the composition and functions of gut microbiome in adult pups. Specifically, principle coordinate analysis showed a distinct separation among the three chemical exposure groups, and especially between BPS and vehicle exposure groups. A total of 73 taxa were persistently altered by at least 1 chemical exposure, among which 12 taxa were commonly regulated by all 3 chemicals. Most notably, there was a persistent decrease in many taxa of the Clostridia class in the Firmicutes phylum by early life exposure to all 3 chemicals, whereas many taxa in the Bacteroidia class of the Bacteroidetes phylum were persistent up-regulated. The most representative microbial biomarkers for each exposure condition were Clostridiales for vehicle, S24-7 for BDE-47, Rikenellaceae for TBBPA, and Lactobacillus for BPS. BPS down-regulated fecal acetic acid, whereas TBBPA up-regulated propionic acid and succinate in these pups. In addition to maternal chemical exposure, neonatal oral exposure to various environmental contaminants, and especially BDE-99 (another diet- and breast milk-enriched PBDE congener), produced persistent gut dysbiosis in adult age, including a persistent decrease in the richness of the bacterial community (alpha diversity), as well as a profound increase in the SCFA-generating *Akkermansia muciniphila* in both male and female adult pups in duodenum, jejunum, large intestine, and feces. Corresponding to the increase in *A. muciniphila*, in BDE-99 neonatal exposed mice, there was a persistent increase in the relative abundance of the hepatic SCFAs succinate and lactate, as well as an increasing trend in the known HDAC inhibitor acetate in adult age. In addition, adult male mice that were neonatally exposed to BDE-99 had 12,606 more peaks for the permissive gene transcription mark histone 3 lysine 27 acetylation (H3K27ac; $p < 0.01$; MACS2) associated with 4,495 protein-coding genes (threshold: ± 10 kb of the gene loci; PAVIS). Examples of these genes include cytochrome P450s as well as genes involved in circadian entrainment. Taken together, early life environmental chemical exposure persistently altered the gut-liver axis in adult age, and this may contribute to the pathogenesis of DOHaD.

L2 - EVALUATION OF DISEASE PROGRESSION / DRUG EFFECTS BY EXPANDING THE PK CONCEPT**Hiroshi Suzuki***The University of Tokyo Hospital, Japan*

Disease progression / drug effects may be described in a semi-quantitative manner by expanding the concept of PKs. By expanding the concept of physiologically based PK to the systems-biological analysis, we may be able to understand the mechanism of disease progression / drug effects. In the first part, we will discuss on our PK/PD and systems-biological method to understand the mechanism of toxicity of molecular target drugs. As an example, we have focused on the toxicity of sunitinib. We focused on the fact that the toxicity of sorafenib is lower than that of sunitinib at the pharmacological doses, and PK/PD analysis revealed that phosphorylase kinase gamma-subunit (PHKG) 1/2 are inhibited by sunitinib but not by sorafenib. Based on the systems-biological pathway map analysis, it was suggested that the inhibition of PHKG 1/2 by sunitinib results in the exposure of cells to the oxidative stress, which was confirmed by *in vivo*

experiments in mice. Moreover, it was revealed that the administration of anti-oxidants results in the recovery in the reduced cellular glutathione levels, and indeed, the simultaneous administration of alpha-tocopherol resulted in the reduced toxicities. This is an example that the systems-biological analysis is useful in understanding the mechanism of development of disease / drug effects. In the second part, we would like to discuss on our medical record analysis method by expanding the PPK concept. PPK is usually used to reconstruct the plasma drug concentration profiles from one-point data taken from numerous patients. We wanted to reconstruct the time-profiles of biomarkers changes of chronic diseases from the fragmented patients' data. We developed a novel method (SReFT (Statistical Restoration of Fragmented Time-course) analysis) to reconstitute the long-term disease progression from temporally fragmented data by extending the nonlinear mixed-effects model to incorporate the estimation of the time after the onset of the disease of each subject. Application of this method to Alzheimer's disease successfully depicted disease progression over 20 years. This is an example that SReFT analysis is useful in understanding the disease progression / drug effects from patients' outcome. Integrative studies considering both of the mechanistic and outcome analyses will enable us to (1) identify the novel biomarkers to estimate the disease state / the prediction of disease progression, (2) identify patients who really need the drug administration, (3) identify the suitable time point to start the medication, and (4) identify the novel target for the disease treatment.

S5.1 - PROTEOMICS BASED STUDIES OF DISEASE EFFECTS ON THE BRAIN BARRIER TRANSPORTERS

Tetsuya Terasaki

Graduate School of Pharmaceutical Sciences, Tohoku University, Japan

For the development of effective neuroprotective drugs, it is an important subject to understand regulatory mechanism of the drug transporter at the blood-brain barrier (BBB) in the diseased condition. P-glycoprotein (P-gp/MDR1) is the most important drug efflux transporter at the BBB, while its activity is altered in several CNS diseases. Inflammation and oxidative stress are associated with a variety of CNS disease. Phosphorylation signaling could be one of the most important MDR1-regulatory mechanisms, as protein kinases are key proteins for modulation of MDR1 function. In this work, phosphoproteomics technology has been used to clarify regulatory mechanism in inflammatory or oxidative stress-induced decrease in apparent MDR1 efflux function in human brain capillary endothelial (hCMEC/D3) cells. TNF-alpha (10ng/mL, 1hr) treatment and hydrogen peroxide (0.05-5 mM, 20min) treatment were used for inflammation model and oxidative stress model, respectively. TNF-alpha and oxidative stress treatment reduced the apparent efflux rate of MDR1 significantly in the D3, while MDR1 protein expression amount was not changed. Actin filament-associated protein-1 (AFAP-1), AP-1 and MAPK1 were significantly phosphorylated after TNF-alpha treatment. Knockdown of AFAP-1 protein expression inhibited the reduction in MDR1 efflux rate by TNF-alpha treatment, while knockdown of AP-1 expression or inhibition of MAPK function did not. These results demonstrate that AFAP-1 is a key molecule in the TNF-alpha mediated inflammatory signaling pathway inducing rapid reduction of intrinsic MDR1 efflux rate in the BBB. The reducing effect of oxidative stress to the MDR1 efflux rate in the D3 was attenuated in the presence of dynasore, an inhibitor of dynamin-dependent endocytosis. Abl and Src kinases were activated by oxidative stress, reducing the plasma membrane localization of MDR1 in the D3. These results suggest that the apparent reducing effect of MDR1 efflux rate by oxidative stress is caused by the signaling of Abl and Src kinase, facilitating the internalization of MDR1 protein in the BBB. Glucocorticoids are stress-responsive hormones and their increase is associated with neurotoxicity and decreased proliferation of nerve and hippocampus cells. A brain perfusion study was performed to confirm reducing effect of oxidative stress to BBB P-gp efflux activity of cortisol *in vivo* in rats. H₂O₂ administration significantly decreased cortisol efflux at the BBB. Interestingly, inhibitors of Abl kinase and Src kinase suppressed decrease of cortisol efflux *in vivo*. These findings suggest that AFAP-1, Abl kinase and Src kinase inhibitors could be effective for maintaining MDR1 mediated efflux of glucocorticoids at the BBB, causing neuroprotective effect at inflammatory and/or oxidative stress condition in humans.

S5.2 - SLC16A11: FROM HUMAN GENETICS TO NOVEL MECHANISMS UNDERLYING TYPE 2 DIABETES

Eitan Hoch

Broad Institute of MIT and Harvard, USA

Genetic variants in the orphan transporter MCT11 (SLC16A11) were recently identified as one of the largest genetic risk signals for type 2 diabetes (T2D).

Our work shows that the T2D-associated variants at this locus have two distinct effects: (1) decreasing SLC16A11 expression in liver, and (2) disrupting a key interaction with basigin, thereby reducing cell-surface localization of MCT11. Both independent mechanisms have the same direction-of-effect in disrupting MCT11 function, and thus support the causality of SLC16A11 in increasing T2D risk.

To illustrate how disruption of MCT11 function leads to increased risk of T2D, we demonstrate that MCT11 functions as a proton-coupled monocarboxylate transporter, and that genetic perturbation of SLC16A11 induces changes in fatty acid and lipid metabolism. These alterations in cellular metabolism are consistent with those observed in insulin resistance and T2D. This work, therefore positions SLC16A11 as a promising therapeutic target for T2D.

S5.3 - TRANSPORTERS IN POLYCYSTIC KIDNEY DISEASE**Kim Brouwer***University of North Carolina Eshelman School of Pharmacy, USA*

Autosomal dominant polycystic kidney disease (ADPKD), the most common hereditary form of kidney disease in humans, is characterized by the development of fluid-filled cysts in the kidney and a decline in renal function, with frequent development of hepatic cysts. The polycystic kidney (PCK) rat, a rodent model of ADPKD, is used to study disease progression and evaluate therapeutic interventions because the renal and hepatic histopathological features in PCK rats resemble human ADPKD. Previous data have shown that bile acids are elevated in the PCK rat, indicating that polycystic kidney disease could influence the disposition of endogenous or exogenous compounds, which could lead to altered efficacy or toxicity of medications.¹ For example, a higher incidence of elevated liver enzymes was first reported with tolvaptan (TVP) in patients with ADPKD.² To investigate potential disease-mediated alterations, hepatic and renal transport protein levels were measured in PCK and wild-type (WT) Sprague Dawley rats. In addition, coproporphyrin (CP)-I and CP-III, transporter probes of organic anion transporting polypeptides (Oatps) and multidrug resistance-associated protein 2 (Mrp2), were analyzed in serum, urine, and bile of PCK and WT rats. The disposition of TVP and metabolites was assessed in PCK and WT rat isolated perfused livers (IPLs), and transporter function was evaluated with 5(6)-carboxy-2',7'-dichlorofluorescein (CDF), a substrate of Oatp1a1, Mrp2, and Mrp3. Western blot analysis revealed downregulation of Mrp2 (3.1-fold) and Oatp1a4 (2.9-fold) in PCK rat liver samples. In the kidney, Mrp2, Mrp4, and organic solute transporter beta (Ost β) protein levels in PCK rats were increased by 3.3-, 2.6-, and 1.8-fold, respectively. Serum and urine CP-I and CP-III concentrations were elevated in PCK rats. Although biliary CP-I concentrations did not change significantly, biliary CP-III concentrations were decreased in PCK compared to WT rats, consistent with altered hepatic and renal transporter expression in PCK rats. The biliary excretion of CDF was decreased 28-fold in PCK compared to WT rat IPLs. TVP and metabolite disposition also was altered in PCK rat IPLs.³ Quantitative systems pharmacology modeling and simulation demonstrated that *in vivo* hepatic exposure to TVP and a TVP metabolite (DM-4103), coupled with altered bile acid disposition and inhibition of mitochondrial respiration, could account for the initiation of TVP-mediated liver injury.⁴ Differences in transporter expression and function may contribute to altered drug disposition and toxicity in polycystic kidney disease.

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S5.4 - LIVER TRANSPORTER ACTIVITY IN PATIENTS WITH RENAL DISEASE**Aubrey Stoch***Merck & Co. Inc., USA*

Renal impairment has a well-recognized influence on drug clearance. Both chronic renal failure (CRF) and ESRD have been shown to not only reduce the systemic clearance of drugs but also impact protein and tissue binding. While this is to be anticipated with drugs typically eliminated renally, it is also appreciated that CRF can modify non-renal disposition of drugs, including those typically cleared hepatically. Data continues to emerge suggesting the influence of renal failure on transporter function, including hepatic and renal uptake transporters, as well as the cytochrome P450 family. These alterations may have a meaningful impact on drug disposition and impact clinical decision-making. The underlying mechanisms responsible for alterations in drug disposition (transporter and enzyme activity) in the setting of renal failure remain poorly characterized.

We have recently completed a clinical study to investigate the influence of renal impairment on the pharmacokinetics and drug-drug interactions of hepatically eliminated drugs. This study leveraged a Merck-characterized microdose cocktail of substrates to efficiently probe three pathways of disposition: CYP3A using midazolam, P-glycoprotein (P-gp) using dabigatran, OATP1B1/1B3 using rosuvastatin, pitavastatin and atorvastatin, and breast cancer resistance protein (BCRP) using rosuvastatin¹. The relative contribution of renal impairment to substrate disposition was examined across the spectrum of renal impairment (mild, moderate, severe, and ESRD). Furthermore, the impact of a single dose of rifampin, an index inhibitor of OATP1B1/1B3 and BCRP and P-gp, on the cocktail components was also examined across the spectrum of renal function.

In these subjects who received the microdose cocktail, we also evaluated the effect of renal disease with and without co-

administration of rifampin on endogenous substrates of OATP1B, such as bilirubin, coproporphyrins I and III, and sulfated bile salts. These are being explored to assess DDI risk in early clinical development, but the effect of CRF on their levels is not known. In this presentation, I will share initial pharmacokinetic and endogenous biomarker results from this clinical study, and how these data are informing how we can use this data to better manage drug interaction risk in this vulnerable population in drug development.

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S6.1 - PREDICTING ROUTES, SITES, AND PRODUCTS OF METABOLISM

Matthew Segall

Optibrium Limited, United Kingdom

Predicting the metabolism of potential drug candidates can help to guide the design of compounds with improved metabolic stability, highlight potential drug-drug interactions and suggest the most likely metabolites to guide experimental metabolite identification. Potentially active, reactive or toxic metabolites can also be flagged for further investigation. The most widely studied enzymes, due to their major contribution to phase I metabolism, are the Cytochrome P450s (P450s). We will describe a method for predicting P450 metabolism, based on a mechanistic understanding of the reactions leading to oxidative metabolism. This combines quantum mechanical (QM) simulations, to estimate the reactivity of potential sites of metabolism on a compound, with a ligand-based approach to account for the effects of orientation and steric constraints due to the binding pockets of different P450 isoforms [1]. The metabolites resulting from oxidation at each potential site of metabolism can also be predicted; however, as the regioselectivity of metabolism may be different for each isoform, it is also necessary to identify which isoforms will be responsible for the metabolism of a compound in order to predict the profile of metabolites that are likely to be formed. We will describe an accurate 'WhichP450' model [2] that can be coupled with isoform-specific regioselectivity models to generate a ranked list of expected metabolites. These principles and the resulting models of P450 metabolism are now well established and we will describe our latest research that applies the same principles to other drug-metabolising enzymes, including UDP-glucuronosyltransferase (UGT) and Flavin-containing monooxygenase (FMO). Detailed QM simulations have improved our understanding of the reaction mechanisms of these enzymes and we will illustrate how this enables us to build similar, accurate models for these enzymes. Modelling of additional phase I and phase II enzymes will improve our ability to predict the metabolic fate of compounds and more effectively guide the design of efficacious and safe drug candidates.

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S6.2 - SUBSTRATE RECOGNITION BY CYTOCHROME P450s

Emily E. Scott¹ and Aaron G. Bart²

Departments of Medicinal Chemistry¹ and Pharmacology¹ and Biophysics Program² University of Michigan, USA

Predicting drug metabolism by cytochrome P450 enzymes is highly valuable, but typically very difficult given the flexibility and promiscuity of most of these enzymes. Although structures are now available for many of the human xenobiotic-metabolizing enzymes, a single structure isn't always very informative. For example, CYP1A1 is well-known for oxidizing planar polyaromatic hydrocarbons and, until recently, the only structure available was with such a compound: alpha-naphthoflavone. However it was immediately clear from this initial structure with a planar enclosed active site that conformational changes would be required to accommodate validated larger and/or nonplanar substrates. To probe such conformational changes, a series of structures were determined with a structurally diverse set of validated substrates. Substrates somewhat larger than alpha-naphthoflavone with both planar and non-planar components included the grapefruit juice furanocoumarin bergamottin and the lung cancer drug and tyrosine kinase EGFR inhibitor erlotinib. While the planar portions of these substrates generally occupied a similar region of the active site, the nonplanar portion of bergamottin extended toward the heme consistent with the formation of inactive metabolites, while the nonplanar portions of erlotinib extended away from the heme, consistent with the formation of reactive metabolites that can result in toxicity. Both perturbed residues composing the active site roof to somewhat expand the CYP1A1 active site volume, creating a channel to the protein surface in the case of erlotinib. A larger and even more structurally diverse pan-Pim kinase inhibitor candidate also bound in an orientation consistent with its observed metabolism, with the CYP1A1 active site varying even more significantly. As with erlotinib, a channel is also observed from the active site proper to the protein surface, but the active site is no longer planar due to substantial conformational changes in the F helix forming the active site roof. These three new structures of CYP1A1 significantly extend the known conformational landscape of this P450,

demonstrating that the active site topology is not exclusively planar or compact. Current efforts are assessing whether these four CYP1A1 structures are sufficient to understand the binding of other tyrosine-kinase inhibitors activated by CYP1A1 into reactive metabolites and potentially linked to clinical toxicities. Structural similarities with human CYP1A2 and CYP1B1 suggest they may undergo similar conformational changes. This new structural information on a flexible P450 enzyme may help anticipate the metabolic liabilities of new pharmaceutical agents.

S6.3 - APPLICATION OF *IN SILICO* DMPK IN DRUG DISCOVERY AND DEVELOPMENT

Fabio Broccatelli

Genentech Inc., USA

Drug discovery requires multi-parameter optimization to achieve ideal drug efficacy, dose and safety profiles. Identifying a clinical candidate typically involves the synthesis and testing of 1000's of molecules, contributing significantly to R&D cost. In Silico ADME modeling has already proven an effective tool in this cycle; upcoming technologies at the interface of Artificial Intelligence, Physiologically-Based PK and PKPD modeling promise to further transform the field. This presentation will describe real life *in silico* ADME modeling applications and project impact.

S6.4 - BIOSYNTHESIS OF DRUG CANDIDATES AND METABOLITES

W. Griffith (Griff) Humphreys

Aranmore Pharma Consultants, USA

While modern synthetic chemistry provides the ability to synthesize an incredible variety of new structures, the natural world provides unmatched chemical diversity. This diversity has provided numerous new drugs over the years. Natural product isolation continues to provide novel structure that may be drug candidates, however, are more often the starting point for chemistry efforts. Another way that natural enzymes have been used in candidate discovery is through use of enzymes to generate chemical diversity in conjunction with synthetic efforts. The most common use of biosynthesis is for targeted metabolite synthesis. In this setting the most typical enzyme sources are either native or expressed enzymes. Other sources include artificial and mutant enzymes as well as microbial enzymes. This presentation will provide an overview of how synthetic efforts can be augmented through use of enzyme systems to provide either drug candidates or key metabolites.

S7.1 - PREDICTION OF DIFFERENCES IN PHARMACOKINETICS BETWEEN CHINESE, JAPANESE, AND CAUCASIAN POPULATIONS - AN UPDATE

Geoffrey Tucker

University of Sheffield, United Kingdom

A previous analysis of predicted exposure in Chinese vs Caucasian populations of model CYP substrates (desipramine - 2D6; tolbutamide - 2C9; omeprazole - 2C19; phenacetin - 1A2; bupropion - 2B6; midazolam - 3A4/5) (Barter et al, 2013) based on PBPK modelling will be summarised and updated with more recent information. On average across the CYPs oral clearance in Han Chinese was predicted to be 65% (48-93%) of that in Caucasians (observed - 62% (33-107%)). Similarly, the results of recent exercises to predict exposure in healthy Japanese subjects will be reviewed. An evaluation of virtual compounds metabolised mainly by CYPs 2C19 indicated oral clearance ratios of 0.3 - 0.5 in Japanese vs Caucasians (Patel et al, 2017), and Matsumoto et al (2018) predicted/observed AUC ratios of 9 investigational compounds in Japanese of about 0.9 (0.8 - 2.7). The differences between the Asian and Caucasian populations largely reflect the combined impact of liver weight, enzyme abundance and the frequency of genetic polymorphisms. At least with regard to metabolism by CYPs the expectations of Chinese vs Japanese vs Caucasian differences in drug exposure are reasonably clear, and should inform clinical and regulatory decisions on the need or otherwise for dosage adjustment. More data are needed with regard to such differences due to other drug metabolising enzymes, transporters, the impact of national and regional differences in diet and the incidence of achlorhydria as it affects drug absorption. Furthermore, although Chinese and Japanese populations are relatively homogenous, there are small sub-groups (e.g. Hmong, Ryukyuan, Aynu) that might benefit from further study. Importantly, pharmacokinetic differences do not necessarily translate into significant pharmacodynamic differences, but inter-racial differences in intrinsic activity require further investigation. In this context, no difference was detected by PKPD modelling in the response to a monoclonal antibody in Chinese and Caucasian subjects (Wang et al, 2019).

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S7.2 - TRANSPORTER POLYMORPHISMS DRIVE INTER-ETHNIC DIFFERENCES IN DRUG RESPONSE**Kathy Giacomini***University of California San Francisco, USA*

Transporters in two major superfamilies, the ATP Binding Cassette (ABC) Superfamily and the Solute Carrier Superfamily (SLC), play major roles in drug absorption, disposition and response. A wealth of new information on genetic polymorphisms available in large sequencing databases such as gnomAD (<https://gnomad.broadinstitute.org/>) indicates that there are large inter-ethnic differences in the allele frequencies of many transporter polymorphisms. Such differences may underlie inter-individual and indeed, inter-ethnic variation in the pharmacokinetics and pharmacodynamics of many drugs. After a brief overview of important drug transporters, I will describe inter-ethnic differences in allele frequencies of common polymorphisms in drug transporters, along with the underlying evolutionary pressures that may have contributed to allele frequency differences. The role of transporter polymorphisms in determining inter-ethnic differences in the disposition and response to drugs used in the treatment of diabetes and cardiovascular disease will be described. In particular, I will focus on large inter-ethnic differences in response to the anti-diabetic drug, metformin and therapeutic and adverse response to rosuvastatin. Time-permitting, I will describe the relationship between transporter polymorphisms and endogenous metabolite levels, and how transporter polymorphisms may contribute to inter-ethnic differences in metabolomic biomarker levels. Collectively, large sequencing and pharmacogenomic studies have ushered in a new understanding of the role of transporter polymorphisms in inter-ethnic differences in drug response.

S7.3 - ENABLING ASIA-INCLUSIVE GLOBAL DRUG DEVELOPMENT THROUGH QUANTITATIVE AND TRANSLATIONAL SCIENCE**Karthik Venkatakrishnan***Takeda Pharmaceuticals International Co., USA*

Delay in approval (“drug lag”) of innovative therapies in Asia has led to increased interest in increasing the efficiency of drug development for Asian patient populations. Approaches to simultaneous global clinical development inclusive of Asia are on the rise [1]. Recent progressive changes in the global regulatory landscape coupled with advances in clinical, translational and quantitative sciences inspire commitment to a patient-centric “Totality of Evidence” approach to Asia-inclusive drug development [2]. The ICH E17 guideline for multi-regional clinical trials (MRCTs) has formalized definition of a pooled East Asian region when supported by scientific considerations of relevant drug and disease-related intrinsic and extrinsic factors [3]. ICH E5 principles have been central to regulatory review of ethnic sensitivity as exemplified by the Bridging Study Evaluation review process adopted in Taiwan. ADME and PK/PD sciences are key foundational disciplines that are integral to objective assessment of ethnic sensitivity of drug response across global patient populations. This presentation will illustrate application of concepts of translational science and quantitative clinical pharmacology to influence development of pan-Asian development strategies designed to define the right dose and increase efficiency in global trial designs. Opportunities to apply population pharmacology models (PBPK, population PK and exposure-response) and the importance of understanding regional variations in disease progression, epidemiology and pathophysiology to streamline drug development for the East Asian region informed by ICH E5 and ICH E17 principles will be discussed using illustrative examples in the oncology therapeutic area. A roadmap for prospective integration of PK/PD considerations and dose decisions in globalization of drug development inclusive of Asia will be presented, including discussion of an exposure-matched global clinical trial strategy to support Asia-inclusive drug development in the setting of PK-related dose differences between populations [4-6].

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S7.4 - IMPACT OF RACIAL/ETHNIC DIFFERENCES IN DRUG RESPONSE: REGULATORY PERSPECTIVES**Anuradha Ramamoorthy***U.S. Food and Drug Administration, USA*

Race/ethnicity is an important intrinsic factor that should be considered during drug development. Similarly, genetics is another important intrinsic factor that can have profound impact on both the exposure and response to a drug. About one in five new drugs approved by the FDA between 2008 and 2013 reported some difference in exposure and/or response across racial/ethnic groups in PK, safety, efficacy, dose change, and pharmacogenetics (PMID 25669658). In some instances, this also translated into population-specific prescribing recommendations as well as needing postmarketing studies.

Differences in frequencies of functional variants are common for some important pharmacogenes that are involved in drug metabolism and transport (e.g., CYP2D6, CYP2C9, and CYP2C19), drug target or pathway (e.g., EGFR), and immunological reactions (e.g., HLA-B) across different racial/ethnic populations. Additionally, racial/ethnic differences in frequencies of certain genetic variants can also affect clinical trial enrollment patterns (e.g., EGFR mutations in non-small cell lung cancer) of multiregional clinical trials. This talk will highlight the impact of inter-relationships between race/ethnicity and genetics on inter-individual variation in drug exposure and response and consequently, the benefit-risk assessment. This talk will also more broadly reflect upon the impact of these intrinsic factors in global drug development and the implications for regulatory decision-making.

S8.1 - BIOMARKERS FOR *IN VIVO* ASSESSMENT OF TRANSPORTER FUNCTION**Martin F. Fromm***Friedrich-Alexander-University, Germany*

Transporter-mediated drug-drug interactions are a concern during pharmacotherapy and drug development. There is great interest in reducing the number of studies in healthy volunteers aiming at the investigation of transporter-mediated drug-drug interactions during drug development. One approach could be the use of endogenous compounds, which are substrates of certain transporters. A suitable endogenous biomarker for function of e.g. hepatic or renal transporters could be measured in plasma and / or urine during administration without and with simultaneous administration of a new molecular entity. Alterations of e.g. AUC or renal clearance of the potential biomarker could indicate the possibility of transporter-mediated drug-drug interactions due to the new molecular entity. It will be discussed, which requirements should be fulfilled for a biomarker of transporter function. Moreover, an overview will be given for currently investigated biomarkers of transporter function including the respective limitations. Taken together, endogenous biomarkers could be suitable for assessing transporter function and transporter mediated drug-drug interaction, but considerably more work needs to be done to clearly establish suitable biomarkers.

S8.2 - MECHANISTIC MODELS FOR COPROPORPHYRIN I AND CREATININE AS ENDOGENOUS BIOMARKERS FOR TRANSPORTER DRUG-DRUG INTERACTIONS**Aleksandra Galetin***University of Manchester, United Kingdom*

Endogenous biomarkers are increasingly considered as clinically relevant tools for the assessment of transporter function *in vivo* and corresponding drug-drug interactions (DDIs). The presentation illustrates application of mechanistic modelling to support qualification of endogenous biomarkers, focusing on coproporphyrin I (CPI) and creatinine as examples. Minimal physiologically-based pharmacokinetic (PBPK) model was developed for CPI to investigate the impact of OATP1B1 c.521T>C genotype on CPI plasma exposure and inter-individual variability in its baseline concentration. Power calculations to support optimal clinical DDI study design with CPI as endogenous OATP1B biomarker are illustrated. Physiologically-based model of creatinine renal disposition accounted for glomerular filtration, passive permeability by para- and transcellular routes, and creatinine active transport via different renal transporters. Assumption that OCT2 acts either as an uptake transporter or a bidirectional transporter driven by the electrochemical gradient was investigated. Evaluation of this mechanistic model for quantitative prediction of creatinine-drug transporter-mediated interactions with a range of inhibitors is illustrated. Biomarker models developed represent useful translational tools to facilitate the design of clinical studies and evaluation of transporter-mediated DDI risk in early drug development.

S8.3 - KNOCKOUT MICE, TRANSCRIPTOMICS AND METABOLOMICS APPROACH FOR DISCOVERY OF ENDOGENOUS BIOMARKERS FOR RENAL DRUG TRANSPORTERS**Sanjay Nigam***University of California San Diego, USA*

Metabolomics analyses of knockout animals, as well as other types of *in vivo* studies, have now defined numerous likely endogenous substrates of drug transporters. These studies should lead to identification of new biomarkers of transporter-associated function. We will discuss in detail the knockouts of the organic anion transporters Oat1 (a gene originally identified by us as NKT) and Oat3. These are the main transporters of many drugs, toxins and natural products. Systems biology studies of the knockout mice support a central role for these and other "drug" transporters in inter-organ and inter-organismal (eg. gut microbiome-host) communication via metabolites and signaling molecules--and other essential aspects of endogenous physiology. This has led to a new conceptual framework known as the Remote Sensing and Signaling Hypothesis (Nigam SK, Nature Drug Discovery 2015), which has broad implications for tissue targeting of drugs and for understanding drug-metabolite interactions in health and disease (eg. kidney failure).

S8.4 - INTEGRATED QUANTITATIVE PROTEOMICS AND METABOLOMICS APPROACH FOR DISCOVERY AND VALIDATION OF UGT2B17 BIOMARKER TO PREDICT DRUG METABOLISM**Bhagwat Prasad***University of Washington, USA*

UGT2B17, an important androgen- and drug-metabolizing enzyme, demonstrates dramatic inter-individual variability in humans, which is associated with variable efficacy and pharmacokinetics (PK) of several medications. UGT2B17 is also implicated in multiple diseases such as various cancers, osteoporosis, obesity, insulin sensitivity, as well as inconsistent testosterone doping test results. However, these clinical observations are often controversial likely due to high non-genetic variability in UGT2B17 activity. We identified using a series of omics experiments that genetics, age and sex can partially explain UGT2B17 variability in humans, however, the cause of the majority of this variability (~74%) is unknown. Particularly, UGT2B17 protein abundance showed >150-fold variation in adult male human liver donors that are harboring 1 or 2 gene copy number. Human intestinal microsomes isolated from small intestine also showed high variability with significantly higher abundance compared to liver microsomes. The intestinal UGT2B17 abundance increases along the distal intestinal segments, potentially leading to intra-individual variability. As part of an effort towards predicting non-genetic variability, overarching objective of our research is to validate a phenotypic biomarker for the prospective prediction of *in vivo* UGT2B17 activity. Our compelling data suggest that a combination of androgen glucuronides in serum and urinary can reasonably predict UGT2B17 activity, which was supported by using an exogenous substrate (dihydroexemestane). The proposed UGT2B17 biomarker after further clinical validation will allow i) precise and prospective dose-selection of UGT2B17 substrate drugs including exogenous testosterone and ii) identification of individuals with greater risk to UGT2B17-associated pathophysiological conditions.

L3 - DRUG METABOLISM, PHARMACOGENOMICS AND THE QUEST TO PERSONALIZE HIV TREATMENT AND PREVENTION**Namandjé Bumpus***Johns Hopkins University School of Medicine, USA*

HIV pre-exposure prophylaxis, the administration of anti-HIV drugs to HIV naïve individuals in order to prevent HIV infection, is a promising approach to decreasing the incidence of HIV infection. Likewise, the use of antiretroviral therapy in HIV infected individuals suppresses viral load and decreases the risk of HIV transmission. However, gaps in knowledge remain regarding the role of drug metabolism and the impact of genetic variation in metabolism on governing the responses to antiretrovirals of various classes. For instance, nucleotide reverse transcriptase inhibitors must be phosphorylated to triphosphate analogs in order to become pharmacologically active, however, insight into the enzymes responsible for this activation has been limited. We have systematically tested and identified kinases that phosphorylate and thereby activate the nucleotide reverse transcriptase inhibitors, tenofovir and emtricitabine. In the case of tenofovir, we find that adenylate kinases play a key role in tenofovir activation within cells and tissues that are susceptible to HIV infection. In addition, through sequencing of genomic DNA isolated from HIV prevention clinical trial participants we have discovered dozens of genetic variants that may contribute to interindividual variability in tenofovir and emtricitabine outcomes. Taking a different approach, we have also investigated the impact of antiretroviral drug metabolites on cells. Using efavirenz, a non-nucleoside reverse transcriptase inhibitor, as a model compound we have demonstrated that efavirenz and the primary cytochrome P450-dependent metabolite of efavirenz differentially activated cellular stress-induced signaling. Specifically, we have found that efavirenz stimulates the activation in primary hepatocytes of key cell stress regulators, inositol-requiring 1 α and X-box binding protein 1. In contrast, these signaling molecules were not readily activated by efavirenz metabolites, although an intersecting signaling pathway, the c-Jun N-terminal kinase pathway was preferentially activated by an efavirenz metabolite. Finally, MALDI-mass spectrometry has been employed to probe the

distribution of antiretroviral drugs including tenofovir, emtricitabine and efavirenz and interestingly, the patterns of distribution in tissue correlate with known clinical safety profiles for the drug informing outcomes further. Upon integration, these findings provide mechanistic insight into the relationship between drug metabolism, pharmacogenomics and the pharmacology of HIV treatment and prevention.

S9.1 - ALBUMIN-MEDIATED HEPATIC UPTAKE OF ORGANIC ANION TRANSPORT POLYPEPTIDE SUBSTRATES

Yuichi Sugiyama

Sugiyama Laboratory, RIKEN Baton Zone Program, RIKEN, Japan

The hepatic uptake process is most important for the overall drug dispositions by the liver. In conventional view, it has been assumed that an unbound ligand governs the hepatic uptake rate which is called "free drug" hypothesis. In the liver, the hepatic uptake of organic anions is very efficient although these organic anions are avidly bound to serum albumin, and consequently, it has been argued that the hepatic uptake of organic anions is more efficient than can be accounted for by its unbound concentration. This phenomenon is called "albumin-mediated" hepatic uptake (1-5). In the kinetic model which the authors proposed previously (3), the ligand-albumin complex binds to the cell (hepatocyte) surface, and then the ligand dissociation rate from albumin is accelerated by the interaction of the complex to the cell surface. If the ligand is a good substrate for transporters such as OATPs, the locally dissociated ligand interacts with transporters followed by the translocation to inside the cell.

Our recent studies indicated that *in vitro-in vivo* extrapolation (IVIVE) of hepatic uptake clearance was not so good by using pooled hepatocytes (considered to represent the average values) and by measuring the hepatic initial uptake of many organic anions, substrates of OATPs in the absence of albumin. In this extrapolation, the IVIVE was performed based on "free-hypothesis"(4,5). Such experimental results suggest that the hepatic uptake clearance of drugs contributed not only from unbound form but also from bound form, and the albumin-mediated effect on uptake clearance should be considered.

In conclusion, the albumin-mediated hepatic uptake was well and quantitatively accounted for by a model considering the binding of albumin and albumin-ligand complex to cell surface (Estimated K_d value for the binding of albumin to the cell surface was ca. 50 μM). IVIVE was improved much better by predicting the hepatic uptake clearance in the presence of physiological albumin concentration (5 % HSA based on this model).

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S9.2 - EFFECTS OF ALBUMIN ON PREDICTION OF LIVER K_{PUU} AND HUMAN HEPATIC CLEARANCE FOR ENZYME- AND TRANSPORTER-MEDIATED MECHANISMS

Li Di

Pfizer Inc., USA

Accurate prediction of human hepatic clearance and liver unbound partition coefficient (K_{puu}) is challenging for compounds that are eliminated by both enzyme- and transporter-mediated mechanisms. Typically, empirical scaling factors are needed to recover the *in vivo* pharmacokinetic (PK) profiles and they tend to be compound- and assay-dependent, which makes it difficult to apply these scaling factors to novel drug discovery compounds. The empirical scaling factors can sometimes be very large and, therefore, particularly sensitive to small signals of the active processes. This requires the *in vitro* assays to have the sensitivity to detect weak signals and most of the current assays are not sensitive enough, especially for highly permeable compounds. In addition, liver K_{puu} tend to be over-predicted using these approaches, as significant compound concentrations are assigned to the liver in order to recover the human PK. In this presentation, we will discuss a novel method that uses hepatocytes supplemented with 4% BSA (bovine serum

albumin) to accurately predict hepatic clearance and liver K_{puu} with a completely bottom-up approach without any empirical scaling factors. The addition of albumin to the assay is of critical importance as clearance and K_{puu} are significantly underpredicted without albumin. For the 32 structurally diverse compounds with many of them OATP substrates, the human clearance prediction for most of the compounds are within 3-fold of observed values. For liver K_{puu} prediction in rats and humans, most of the compounds are within 2-fold of the observed values. The potential underlying mechanisms of the albumin effect will also be discussed.

S9.3 - IMPACT OF ALBUMIN AND PLASMA ON THE RENAL UPTAKE OF OAT1 SUBSTRATES

Rosalinde (Roos) Masereeuw

Utrecht Institute for Pharmaceutical Sciences, Netherlands

In the kidney, multiple active and facilitative transport systems regulate the excretion of xenobiotics, like endogenous wastes and drugs from the blood in addition to glomerular filtration of the free fraction. These transport systems are predominantly located in the proximal tubule and are notable for their high transport capacity and wide variety of substrates accepted. In patients with chronic kidney disease (CKD) adequate renal clearance is compromised, resulting in the accumulation of xenobiotics. The endogenous waste solutes retained in uremic conditions, also named uremic toxins, are a heterogeneous group of organic compounds with intrinsic biological activities, of which many are too large to be filtered and/or are protein-bound. The renal excretion of protein-bound toxins depends largely on active tubular secretion, shifting the binding and allowing for active secretion of the free fraction. In CKD, protein binding is compromised due to posttranslational modifications resulting in increased unbound fractions of that uremic toxins that are related to the many complications of CKD. Whether altered protein binding also affects their renal clearance is unknown. To address this, we used a recently established microfluidics renal tubule system and indoxyl sulfate as prototypical and highly plasma albumin-bound uremic solute. Indoxyl sulfate is cleared via active tubular secretion, with predominant involvement of organic anion transporter 1 (OAT1). Bioengineered renal proximal tubules were created by seeding conditionally immortalized proximal tubule epithelial cells transduced with OAT1 (ciPTEC-OAT1) on hollow fiber membranes and perfused with indoxyl sulfate in absence or presence of healthy human serum albumin (healthy-HSA), or *in vitro* guanidylated HSA (CKD-HSA) to mimic uremic conditions. Our results demonstrate that indoxyl sulfate showed the highest transport rate and the highest affinity for OAT1 in presence of healthy-HSA. Furthermore, we demonstrated that the renal tubules-on-a-chip were capable of actively secreting the uremic toxin from healthy spiked and uremic plasma, again with a higher rate in healthy conditions. In conclusion, active, OAT1-mediated, secretion of indoxyl sulfate is compromised in uremic conditions due to changes in plasma albumin binding as well as altered OAT1 kinetics. Moreover, our study emphasizes the importance of studying xenobiotic kinetics in a physiologically or pathologically relevant environment.

S10.1 - ONTOGENY OF HUMAN MEMBRANE TRANSPORTERS: FROM DATA TO APPLICATION

Saskia de Wildt

Radboud University Medical Center, Netherlands

Membrane transporters play an important role in the disposition of endogenous compounds and drugs. Hence, variation in transporter activity may importantly impact a drug's efficacy and safety. Age-related variation in drug transporter expression has been extensively studied in animals, but human data are only recently emerging. These human data support age-related variation, which appears both tissue and transporter-specific. In this presentation, the challenges and opportunities to extrapolate the existing human data to real-world clinical application is discussed. This will include the pros and cons of the different *ex vivo* and *in vivo* methods used to generate these data, limits to available human tissue and study patients, remaining gaps in current biological data, challenges to scale to pharmacokinetic data and simulation of dosing guidelines.

S10.2 - PEDIATRIC PBPK: DEALING WITH UNCERTAIN PARAMETERS AND DECIDING ON SUCCESS CRITERIA FOR PREDICTABILITY

Trevor Johnson

Certara UK Limited (Simcyp Division), United Kingdom

PBPK models are increasingly used in the pharmaceutical industry from early discovery through to regulatory submissions. Recent data (2018) from the FDA indicates that although 60% of applications using this approach were for drug-drug interaction prediction an increasing number are for other applications and 15% were for pediatric indications. For pediatrics, a predict, learn, confirm and apply paradigm is encouraged with PK data being used to optimize and verify the PBPK model firstly in adults before moving on to pediatric verification and application. Many submissions involving pediatric PBPK (p-PBPK) as part of a pediatric study or investigation plan have been for dose extrapolation or optimal trial design, prior to undertaking clinical studies, a moderate regulatory impact as defined by EMA. However, as p-PBPK

models 'mature' there are cases where they have been used to avoid studies altogether, a high regulatory impact (EMA) where robust model verification is needed [1].

P-PBPK models, including those for premature neonates, incorporate additional algorithms describing the age related changes in physiology and biochemistry. Whilst many of the 'systems' parameters are now more robust, knowledge gaps still exist giving rise to various levels of model uncertainty. Research into the ontogeny of enzymes and transporters is still ongoing e.g. CYP3A4, CYP2D6, Pgp, OATP1A1 but is being facilitated by advances in proteomics and application of *in vivo* ontogeny data. The lack of good quality pediatric drug studies performed across the age range and lack of PK data are a limitation to the performance verification of p-PBPK models. The first part of the presentation will cover these issues. The quality of data and context of use of PBPK models both influence the acceptance criteria. Traditionally, the 2-fold level of prediction was viewed as acceptable for clearance and drug-drug interaction predictions, especially in early drug development. However, depending on the PBPK application, as more data are gathered and clinical results used to optimize parameters, then a higher level of accuracy would seem more reasonable such as 0.8 to 1.25-fold. A recent publication has set the acceptance criteria in the context of the variability seen in the observed data. Predictions within the 99.998% CI for the geometric mean are seen as a reasonable predictions and those within the 95% CI as a better prediction [2].

More recently, p-PBPK models have seen applications in the clinical setting to guide dose changes in neonates, infants and children and there is a suggestion to apply such models in the precision medicine arena. These novel applications require detailed understanding of the drug and system parameters at the patient level and require new acceptance metrics. The second half of the presentation will discuss predictability and acceptance issues.

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S10.3 - USE OF 'VIRTUAL CHILDREN' TO INFORM EXPOSURE-CONTROLLED DOSING OF DRUGS IN CHILDREN

Jean C. Dinh

Children's Mercy Hospital, USA

Anticipating dose-exposure relationships in children is difficult due to the inherent heterogeneity in the pediatric population. The National Institute of Child Health and Human Development defines seven subpopulations within the ages of 0 – 21 years: neonatal, infancy, toddler, early childhood, middle childhood, early adolescence, and late adolescence. Conducting clinical trials to inform dosing guidelines for children is therefore not always feasible, given the large number of participants needed to characterize drug pharmacokinetics for each subpopulation. A common strategy to address this difficulty is to use adult data to inform pediatric dosing. However, this approach may not be optimal when a drug is developed for a largely pediatric indication. Construction of guidelines is further complicated if a polymorphic pathway is involved in drug clearance. We hypothesized that using physiologically-based pharmacokinetic (PBPK) models may be a useful for: 1) understanding and characterizing the impact of maturation and genetic variability in variable drug disposition, and 2) developing a mechanism to understand dose-exposure relationships and individualize therapy. This investigation used atomoxetine as the pilot substrate given that this drug was developed for patients with attention deficit/hyperactivity disorder (ADHD), most of whom are diagnosed in early to middle childhood. This drug is also a sensitive CYP2D6 substrate; such that the Food and Drug Administration lists atomoxetine as an index substrate for CYP2D6.

Physiologically-based pharmacokinetic (PBPK) models are computational tools that can be used to predict drug exposure relationships for select populations of interest. However, bottom-up PBPK models for CYP2D6 substrates often under-predict CYP2D6 contribution to drug clearance. These models typically rely on an intrinsic clearance (Cl_{int}) generated from adult tissues. Scalars are then applied to predict drug exposure. We hypothesized that more accurate PBPK models for children could be built using Cl_{int} values derived from individual pediatric livers with enriched data, such as P450 protein content, genotype information, gender, age and sex. A screen of ~150 human liver microsome (HLM) preparations of varying age and of known CYP2D6, CYP2C19, and CYP2B6 genotype at three therapeutically relevant concentrations of ATX was conducted to further understand and describe variability in atomoxetine metabolism as a function of both genotype and ontogeny. These data were used as the basis of the "virtual children" project, which are essentially individual PBPK models which have been scaled up to anticipate atomoxetine exposure for each HLM donor. This methodology may be used to anticipate the impact of CYP2D6 genetic variability for a given drug in a population, particularly if there are multiple polymorphic pathways involved. Additionally, these models may potentially be used as a dosing tool to anticipate drug exposure in an individual patient, through selection and aggregation of virtual children that most resemble the characteristics of the individual patient.

S10.4 - BUILDING, VERIFYING AND VALIDATING PEDIATRIC PBPK MODELS: VULNERABILITIES AND KNOWLEDGE DEFICITS**Ping Zhao***Bill & Melinda Gates Foundation, USA*

PBPK analyses are routinely used by drug developers and regulators to evaluate and inform drug-drug interaction (DDI) liabilities for new molecular entities (NMEs). In 2017, the US FDA published draft clinical and *in vitro* DDI guidances, which encourage the use of PBPK as a type of DDI study that “can be used in lieu of some prospective DDI studies” with detailed recommendations. The lecture will review findings from regulatory science researches that support PBPK recommendations in the new draft DDI guidances. Predictive performance of PBPK to assess CYP induction for an NME as a victim and a perpetrator will be discussed.

S11.1 - NAVIGATING THROUGH DISCOVERY AND DEVELOPMENT PAINLESSLY: SNAPSHOTS WITHIN THE NAV1.7 PROGRAM**Jae Chang***Genentech Inc., USA*

Approximately 20% of adults in the United States report of chronic pain. Unfortunately, the current standard of care for chronic pain falls short in addressing this prevalent medical need due to suboptimal efficacy, as well as for poor safety and tolerability, especially around addiction. Nav1.7 is a voltage-gated sodium channel encoded by the SCN9A gene. It is expressed extensively in peripheral nociceptive sensory neurons such as the dorsal root ganglion and trigeminal ganglia. Strong genetic evidence in human implicates Nav1.7 in pain processing, as gain-of-function mutations are associated with increased sensitivity to pain, whereas loss-of-function mutations are associated with chronic insensitivity to pain. As opposed to an opioid receptor, it is hypothesized that a selective Nav1.7 inhibitor would provide pain relief without safety liabilities plaguing the current standard of care including the potential for abuse. GDC-0276 is a potent, selective Nav1.7 inhibitor. This presentation will tell the story of GDC-0276, focusing on the challenges in early development to identify GDC-0276 and to predict its human PK, as well as outlining hurdles encountered in clinical development including MIST, hepatotoxicity and how to best assess preclinical abuse liability.

S11.2 - ELAGOLIX: A MECHANISTIC UNDERSTANDING OF DRUG-DRUG INTERACTIONS**David Stresser***AbbVie, USA*

Elagolix is an orally administered gonadotropin-releasing hormone antagonist indicated for the management of moderate to severe pain associated with endometriosis. During development, Elagolix was characterized to address potential interactions with clinically relevant drug metabolizing enzymes and drug transporters. This presentation will give an overview of some of the various approaches used to develop an understanding of Elagolix disposition and interrogate drug-drug interaction potential. Additionally, this presentation will provide some retrospective evaluation of our prediction performance with respect to clinical observations.

S11.3 - ZEPATIER: THE TALE OF TWO NOVELS**Christine Fandozzi***Merck & Co. Inc., USA*

ZEPATIER®, consisting of two novel drugs, grazoprevir (GZR) and elbasavir (EBR), gained FDA approval for the treatment for Hepatitis C Virus (HCV) in January 2016. The combination of GZR, which potently inhibits HCV Protease, and EBR, which inhibits the NS5A protein, allowed for HCV treatment without ribavirin, but required the development of two novel drugs as a combination therapy. Grazoprevir is a substrate for CYP3A, P-gp, and OATP1B1/3, and elbasavir is a substrate for CYP3A and P-gp. Based on *in vitro* data, GZR has a potential to inhibit CYP3A4 (weak), CYP2C8, OATP1B1/3, BCRP, BSEP, MRP2-4, and EBR has a potential to inhibit BCRP. Important concomitant medications in the HCV treatment population include ritonavir-boosted HIV protease inhibitors, as well as statins. This talk will take you through the discovery of the two novel agents, how the approaches influenced the drug-drug interaction (DDI) profiles, and the clinical DDI studies supportive of registration. The discussion will also comment on the likely underlying mechanisms of the clinically observed DDIs, shared learnings, and suggestions for best practices moving forward.

S11.4 - MANAGEMENT OF MIST IN A CASE OF MULTIPLE METABOLIC SPECIES DIFFERENCES**Lars Weidolf***AstraZeneca R & D, Sweden*

I will present the *in vivo* metabolism across species of a small molecule drug candidate that is currently in early clinical development. Structurally, the compound may look innocuous, but metabolite profiling revealed interesting pathways for each species investigated. For instance:

- N-acetylation with possible influence of NAT polymorphism in man.
- Chemical and/or enzymatic internal ring-closure.
- Deamination forming a carbonyl metabolite in animals, but with hardly a trace in man, while the corresponding alcohol was abundant in man, with hardly a trace in animals.
- A very dominant carbamoyl glucuronide in human plasma, with surprisingly high plasma protein binding in man compared to the animal species.

Relating to the somewhat fuzzy classification of Phase 2-metabolites as being generally without toxicological concern as outlined in regulatory guidance (FDA Nov 2016 and ICH M3(R2) 2009) we set out to assess plasma exposure ratios in animals compared to man. The plasma exposure ratios across species for this metabolite benefitted from relatively high NOAEL doses to the animals as compared to low therapeutic doses to man. Despite this metabolite constituting about 80% of the total drug related exposure and having almost 100-fold lower free fraction in man, the exposure ratio in one animal species was > 1 and should conclude the exposure as being adequate as per MIST guidance.

As a bonus, I will also mention how metabolite knowledge was used to qualify a product of chemical degradation in the drug substance/product by utilizing that it was also formed as a metabolite as loosely guided by ICH Q3A(R2) and ICH Q3B(R2).

PRE-DOCTORAL/GRADUATE POSTER FINALISTS (A1 – A6)**A1 - IS URINARY TESTOSTERONE GLUCURONIDE A POTENTIAL BIOMARKER FOR UGT2B17 ONTOGENY IN AGES 7 TO 18 YEARS?***Haeyoung Zhang, University of Washington, Seattle, WA, USA***A2 - DIFFERENTIAL REGULATION OF ALDEHYDE OXIDASE BY PREGNANE X RECEPTOR AND RETINOID X RECEPTOR AGONISTS IN HUMAN CELL MODELS***Shiyang Chen, National University of Singapore, Singapore, Singapore***A3 - A HIGH-THROUGHPUT DOUBLE COCKTAIL APPROACH FOR EVALUATING TIME-DEPENDENT INHIBITION OF NINE MAJOR HUMAN CYTOCHROME P450 ENZYMES***Helinä Kahma, University of Helsinki, Helsinki, Finland***A4 - UNDERSTANDING THE PHYSIOLOGICAL FUNCTIONS OF THE XENOBIOTIC-SENSING NUCLEAR RECEPTORS ON GUT MICROBIOME USING GENETICALLY-MODIFIED MICE***Mallory Little, University of Washington, Seattle, WA, USA***A5 - THE EFFECT OF CHANGES IN PHARMACOKINETICS OF TRANSPORTER SUBSTRATES ON PBPK MODELING APPROACHES***Jasleen Sodhi, University of California San Francisco, San Francisco, CA, USA***A6 - INVESTIGATING THE ROLE OF DRUG TRANSPORTERS IN FUROSEMIDE ABSORPTION, FOOD-EFFECT AND ELIMINATION USING A PROTEOMICS INFORMED-MECHANISTIC PBPK MODELING APPROACH***Revathi Chapa, University of Washington, Bellevue, WA, USA***POSTDOCTORAL POSTER FINALISTS (A7 – A12)****A7 - INTERACTION WITH YAP IS A POSSIBLE UNDERLYING MECHANISM FOR CAR-DEPENDENT HEPATOCARCINOGENESIS***Ryota Shizu, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Shizuoka, Japan***A8 - APICAL AND BASOLATERAL FLOW RATE INFLUENCE INTESTINAL ABSORPTION IN FRESH INTESTINAL TISSUE INSIDE A NOVEL INTESTINE ON A CHIP MODEL***Hossein Eslami Amirabadi, TNO & Utrecht University, Zeist, Netherlands***A9 - ALDEHYDE OXIDASE CONTRIBUTES TO ALL-TRANS-RETINOIC ACID BIOSYNTHESIS IN HUMAN LIVER***Guo Zhong, University of Washington, Seattle, WA, USA***A10 - CHARACTERIZATION OF DIFFERENTIAL TISSUE ABUNDANCE OF MAJOR NON-CYP ENZYMES IN HUMAN***Naveen Neradugomma, University of Washington, Seattle, WA, USA***A11 - OPTIMIZED RENAL TRANSPORTER QUANTIFICATION BY USING AQUAPORIN 1 AND AQUAPORIN 2 AS ANATOMICAL MARKERS: APPLICATION IN CHARACTERIZING THE ONTOGENY OF RENAL TRANSPORTERS AND ITS CORRELATION WITH HEPATIC TRANSPORTERS IN PAIRED SAMPLES***Cindy Yanfei Li, University of Washington, Seattle, WA, USA***A12 - MECHANISTIC KIDNEY MODELS FOR CREATININE: RELEVANCE OF OCT2 TRANSPORT DRIVING FORCE TO PREDICTION OF CREATININE-DRUG INTERACTIONS***Daniel Scotcher, University of Manchester, Manchester, United Kingdom***ASSAY DEVELOPMENT FOR BIOLOGICS (P1)****P1 - DEVELOPMENT OF A HIGHLY SENSITIVE LC/MS/MS ASSAY FOR PEGYLATED FGF21 PROTEIN MEASUREMENT FROM BIOLOGICAL MATRICES***Xiaomei Gu, Bristol-Myers Squibb, Princeton, NJ, USA***BIOANALYSIS (P2)****P2 - QUANTITATIVE ANALYSIS OF A THYROID HORMONE BIOMARKER EXTRACTED FROM WHOLE BLOOD USING A MITRA MICROSAMPLING DEVICE***Jason Watts, Alturas Analytics, Moscow, ID, USA***BIOANALYTICAL METHOD DEVELOPMENT (P3)****P3 - OPTIMIZATION OF THE SAMPLE COLLECTION PROCEDURES FOR SELECTIVE BCRP INHIBITOR, KO143, IN RODENTS***Alicia Pietrasiewicz, Biogen, Cambridge, MA, USA***BIOAVAILABILITY (P4)****P4 - ELUCIDATION OF DOSE PROPORTIONALITY PHARMACOKINETICS AND SPECIES DIFFERENCES IN THE CYP-MEDIATED METABOLISM OF CLADRIN, A POTENT ANTI-OSTEOPOROTIC ISOFLAVONE***Mamunur Rashid, CSIR-Central Drug Research Institute, Lucknow, Uttarpradesh, India***CLEARANCE PREDICTION (P5 - P8)****P5 - USE OF THE H_μREL[®] HUMAN HEPATOCYTE CO-CULTURE MODEL TO DETERMINE INTRINSIC CLEARANCE AND ELUCIDATE METABOLIC PATHWAYS OF STABLE COMPOUNDS***Philip Butler, Cyprotex, Macclesfield, United Kingdom***P6 - COMPARISON OF TECHNIQUES TO ASSESS NON-SPECIFIC MICROSOMAL BINDING***Tom Chan, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA***P7 - UTILIZATION OF A MICROPHYSIOLOGICAL SYSTEM TO MODEL RENAL SECRETION OF OPIOIDS IN HUMANS***Tomoki Imaoka, University of Washington, Seattle, WA, USA***P8 - INTERSPECIES DIFFERENCES IN RENAL CLEARANCE BASED ON PHYSICOCHEMICAL DRUG PROPERTIES***Katja Jansen, Utrecht University, Utrecht, Netherlands***CONJUGATION REACTIONS AND ENZYMES (P9 - P12)****P9 - EFFECTS OF HISTONE DEACETYLASE INHIBITOR ON UGT1A1 AND UGT1A6 EXPRESSION IN CACO-2 CELLS***Miki Katoh, Meijo University, Nagoya, Japan***P10 - USE OF MICROBIAL BIOTRANSFORMATION TO PRODUCE SCALABLE QUANTITIES OF A GLUCURONIDATED METABOLITE OF THE CLINICAL-STAGE SOLUBLE GUANYLATE CYCLASE INHIBITOR PRALICIGUAT***Julia Shanu-Wilson, Hypha Discovery Limited, Slough, England, United Kingdom*

P11 - EXPANDING THE PHENOTYPING TOOL KIT FOR GLUTATHIONE S-TRANSFERASES*Timothy Strelevitz, Pfizer, Gales Ferry, CT, USA***P12 - ABSTRACT WITHDRAWN****CYTOCHROME P450 (P13 - P29)****P13 - ASSESSMENT OF DRUG METABOLIZING ENZYME EXPRESSION AFTER HIGH-FAT FEEDINGS, AND AFTER A SWITCH BACK TO A NORMAL DIET IN RATS***Hamdah Mohammed S Al Nebaihi, University of Alberta, Edmonton, AB, Canada***P14 - EFFECT OF THE CYP2C8*3 VARIANT ON ASTHMA SYMPTOM CONTROL AND MONTELUKAST EFFICACY***Marysol Almestica, University of Utah, Salt Lake City, UT, USA***P15 - VITAMIN D DEFICIENCY ALTERS CYP3A GENE EXPRESSION AND PHARMACOKINETICS OF DEXAMETHASONE***Kavya Annu, St Jude Children's Research Hospital/University of Tennessee Health Science Center, Memphis, TN, USA***P16 - DRUG-INDUCED LIVER INJURY ALTERS EXPRESSION AND ACTIVITIES OF DRUG-METABOLIZING CYTOCHROME P450 ENZYMES IN MOUSE LIVER AT DIFFERENT AGES***Yifan Bao, University of Connecticut, Storrs, CT, USA***P17 - IMPACT OF AGE ON CYP450 ACTIVITIES IN BOVINE LIVER, LUNG AND KIDNEY***Steven Hu, Zoetis, Kalamazoo, MI, USA***P18 - BIOTRANSFORMATION OF METHOXYLATED BROMODIPHENYL ETHER NATURAL PRODUCTS***Margaret James, University of Florida, Gainesville, FL, USA***P19 - DESIGN AND CHARACTERIZATION OF SELECTIVE MECHANISM-BASED INHIBITORS OF CYP4Z1***John Kowalski, University of Washington, Seattle, WA, USA***P20 - DUAL EFFECTS OF METHYLATED INDOLES ON CYP1A1 IN HUMAN HEPATOCYTES***Kristyna Krasulova, Palacky University Olomouc, Olomouc, Czech Republic***P21 - DRUG LIBRARY SCREENING FOR HUMAN CYP1A1 SUBSTRATES***Dieter Lang, BayerAG, Wuppertal, Germany***P22 - ENDOBIOTICS FOR PHENOTYPING OF HUMAN CYTOCHROME P450 ENZYMES: USE OF METABOLOMICS FOR THE IDENTIFICATION OF NEW CYP2D6 ENDOGENOUS BIOMARKERS ON HEALTHY VOLUNTEERS***Jules Desmeules, UNIGE, Genève, Switzerland***P23 - EFFECT OF DEXAMETHASONE TREATMENT ON CYTOCHROME P450 3A-UDP-GLUCURONOSYLTRANSFERASE 1A INTERACTIONS IN RAT LIVER***Yuu Miyauchi, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan***P24 - ADVERSE EFFECTS OF SYNTHETIC CANNABINOIDS RELATIVE TO Δ9THC ARE ASSOCIATED WITH ATYPICAL METABOLIC PROFILES AND PROPERTIES AT CANNABINOID RECEPTORS CB1 AND CB2***Anna Radomska-Pandya, University of Arkansas for Medical Science, Little Rock, AR, USA***P25 - ACCESSING MAMMALIAN DRUG METABOLITES USING POLYCYPS® ENZYMES***Julia Shanu-Wilson, Hypha Discovery Limited, Slough, England, United Kingdom***P26 - A RAPID FLUORESCENCE BASED SCREEN FOR CYP51 INHIBITION***Michael Voice, Cypex Ltd, Dundee, United Kingdom***P27 - ASSOCIATION OF CYTOCHROME P450 REACTIVITY AND REPEATED-DOSE TOXICITY OF CHEMICAL COMPOUNDS***Kouichi Yoshinari, University of Shizuoka, Shizuoka, Japan***P28 - STEREOSELECTIVE OXIDATION KINETICS OF DEOXYCHOLATE IN RECOMBINANT AND MICROSOMAL CYP3A ENZYMES: DEOXYCHOLATE 19-HYDROXYLATION IS AN *IN VITRO* MARKER OF CYP3A7 ACTIVITY***Jian Zhang, Sichuan University, Chengdu, Sichuan, China***P29 - TERTIARY OXIDATION OF SECONDARY BILE ACIDS, DEOXYCHOLATE, IS CONSERVATIVE IN HUMAN, MONKEY, DOG, RAT AND MOUSE***Pingping Zhu, Sichuan University, Chengdu, Sichuan, China***DIFFERENCES IN METABOLISM (SPECIES, GENDER, AGE, DISEASES) (P30 - P40)****P30 - AN EXAMINATION OF THE REVERSIBILITY IN THE EFFECTS OF HIGH FAT DIET ON LIDOCAINE MICROSOMAL METABOLISM IN RATS***Hamdah Mohammed S Al Nebaihi, University of Alberta, Edmonton, AB, Canada***P31 - RATES AND ROUTES OF EXCRETION OF 14C-AQX-1125 FOLLOWING ORAL ADMINISTRATION TO RATS, DOGS AND HUMANS***Philip Bond, Pharmaron UK, Rushden, Northamptonshire, United Kingdom***P32 - COMPARATIVE METABOLISM OF 14C-AQX-1125 FOLLOWING ORAL ADMINISTRATION TO RATS, DOGS AND HUMANS***Eleanor Barton, Pharmaron UK, Rushden, Northamptonshire, United Kingdom***P33 - SPECIES DIFFERENCES IN THE MASS BALANCE, EXCRETION AND METABOLISM OF [14C] LY3202626 IN HUMAN, DOG AND RAT***Ken Cassidy, Eli Lilly and Company, Indianapolis, IN, USA***P34 - RATES AND ROUTES OF EXCRETION OF 14C-ERAVACYCLINE FOLLOWING ADMINISTRATION TO RATS AND HUMANS***Philip Bond, Pharmaron UK, Rushden, Northamptonshire, United Kingdom***P35 - THE USE OF PRIMARY CRYOPRESERVED HUMAN HEPATOCYTES AS A MODEL FOR NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) RELATED CHANGES IN PHASE I AND PHASE II ENZYME ACTIVITIES***Karissa Cottier, BioIVT, Baltimore, MD, USA***P36 - PERINATAL BDE-47 EXPOSURE ALTERED THE DEVELOPMENTAL TRAJECTORY OF GUT MICROBIOTA IN RATS***Joanna Gillette, University of Washington, Seattle, WA, USA*

P37 - SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS EVALUATION OF CHIGLITAZAR, A NOVEL IDENTIFIED PPAR PAN-AGONIST FOR THE TREATMENT OF TYPE 2 DIABETES IN DIFFERENT AGE GROUPS

Xiaojiao Li, Phase I Clinical Trial Unit, First Hospital, Jilin University, Changchun, China

P38 - EXPLORING SPECIES DIFFERENCES IN ALDEHYDE OXIDASE ACTIVITY

Pranav Shah, National Center for Advancing Translational Sciences, Rockville, MD, USA

P39 - METABOLISM OF DESLORATADINE IN TK-NOG CHIMERIC MICE WITH HUMANIZED LIVERS

Shotaro Uehara, Central Institute for Experimental Animals, Kawasaki, Japan

P40 - DIET-INDUCED OBESE ALTERS THE EXPRESSION AND FUNCTION OF HEPATIC DRUG-METABOLIZING ENZYMES AND TRANSPORTERS IN RATS

Feng Xu, Southern Medical University, Shanghai, Fengxian, China

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Weiqi Chen, BMS, Princeton, NJ, USA

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Lindsay Czuba, University of Washington, Seattle, WA, USA

P43 - HUMAN AND ANIMAL ADME USING WS-CAVITY RING-DOWN SPECTROSCOPY.

Stephen Dueker, BioCore, KIRAMS, Seoul, Korean, South Korea

P44 - TISSUE DISTRIBUTION OF EDP-305, A HIGHLY SELECTIVE AND POTENT FARNESOID X RECEPTOR (FXR) AGONIST, IN PRECLINICAL SPECIES

Meng Huang, Enanta Pharmaceuticals, Inc., Watertown, MA, USA

P45 - DETERMINATION AND COMPARISON OF SKIN AND PLASMA CONCENTRATIONS OF MULTIKINASE INHIBITORS AND THEIR ACTIVE METABOLITES IN MICE

Ayaka Kojima, Meijo University, Nagoya, Japan

P46 - UNDERSTANDING THE EXCRETION PROFILE AND THE METABOLISM OF PITOLISANT IN CYP450 2D6 POOR AND EXTENSIVE METABOLISERS AT STEADY STATE

Iain Shaw, Quotient Sciences Ltd, Nottingham, Nottinghamshire, United Kingdom

P47 - AN INTEGRATED RADIOLABELLED STUDY TO DETERMINE THE ABSORPTION, METABOLISM, EXCRETION AND ABSOLUTE BIOAVAILABILITY OF PEMA FIBRATE IN HEALTHY MALE SUBJECTS

Iain Shaw, Quotient Sciences Ltd, Nottingham, Nottinghamshire, United Kingdom

P48 - REGIONAL INTESTINAL DISPOSITION OF DESAMINOTYROSINE, AN IMMUNOMODULATORY MICROBIAL METABOLITE OF FLAVONOIDS

Rashim Singh, University of Houston, Houston, TX, USA

P49 - UNCOVERING NOVEL LYSOSOMAL PH CHANGES CAUSED BY DMDNA31, A NOVEL RIFALOG PAYLOAD OF AN ANTIBODY-ANTIBIOTIC CONJUGATE (AAC) IN DEVELOPMENT TO TREAT STAPHYLOCOCCUS AUREUS INFECTIONS

Jennifer Vogt, Genentech, South San Francisco, CA, USA

P50 - QUANTITATIVE WHOLE BODY AUTORADIOGRAPHY OF 14C-ENV-101 IN CYNOMOLGUS MONKEY

Guy Webber, ENVIGO, Huntingdon, United Kingdom

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P51 - ROUTINE METABOLITE IDENTIFICATION FOR COMPLEX CYCLIC PEPTIDES BASED ON IMS ENABLED QTOF DIA DATA ACQUISITION AND MASS-METASITE DATA PROCESSING

Nathan Anderson, Waters Corporation, Milford, MA, USA

P52 - DETERMINATION OF AMINOGLYCOSIDE TOXICITY IN HUMAN RENAL PROXIMAL TUBULE MONOLAYERS USING CLINICAL RELEVANT BIOMARKERS OF NEPHROTOXICITY

Colin Brown, Newcells Biotech, Newcastle upon Tyne, United Kingdom

P53 - OPIUM ALKALOID - NOSCAPINE: A POTENTIAL CHEMOTHERAPEUTIC AGENT

Ramesh Chandra, University of Delhi, Delhi, Delhi, India

P54 - ENDOPLASMIC RETICULUM STRESS SUPPRESS MOTILITY AND METABOLISM OF PROSTATE CANCER CELLS: UNCOVERING ENTEROLACTONE'S MECHANISMS OF ACTION

Franklyn De Silva, University of Saskatchewan, Saskatoon, SK, Canada

P55 - CHARACTERIZATION OF *IN VIVO* DISPOSITION OF CTI-1601: A MITOCHONDRIA TARGETED THERAPY FOR FRIEDREICH'S ATAXIA

Eric Gonzalez, NIH-NCATS, Rockville, MD, USA

P56 - QUANTITATION OF A NOVEL ENOLASE-2 SELECTIVE INHIBITOR PRODRUG BIS-POM-HEX, ITS INTERMEDIATE HEMI-POM-HEX, AND PARENT DRUG HEX IN MOUSE PLASMA AND BRAIN TISSUES USING LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

Yongying Jiang, University of Texas MD Anderson Cancer Center, Houston, TX, USA

P57 - APPLICATION OF AN ABBREVIATED RADIOLABELLED ADME STUDY UTILIZING 3H-LABELED TEST ARTICLE IN THE TRANSITION BETWEEN DISCOVERY AND DEVELOPMENT

Heasook Kim-Kang, Q² Solutions, Indianapolis, IN, USA

P58 - UTILISING A DUAL HUMAN TRANSPORTER MDCK_MDR1_BCRP CELL LINE TO ASSESS EFFLUX AT THE BLOOD BRAIN BARRIER

Dermot McGinnity, AstraZeneca, Cambridge, United Kingdom

P59 - DEVELOPMENT OF A NOVEL PROFILE DATA ANALYSIS METHOD TO AID DEEP UNDERSTANDING OF PHARMACODYNAMICS BY DECOMPOSING THE COMPLEX EFFECTS OF A DRUG

Tadahaya Mizuno, The University of Tokyo, Bunkyo, Tokyo, Japan

P60 - COMPARISON OF TOXICITY AND PERMEABILITY AMONG ANGIOTENSIN RECEPTOR BLOCKERS TO THE FETUS*Tomohiro Nishimura, Keio University, Tokyo, Japan***P61 - INVESTIGATIONS INTO DEFINITIVE *IN VITRO* DDI ASSAYS TO SATISFY FDA GUIDELINES***Chuong Pham, Alliance Pharma, Inc., Malvern, PA, USA***P62 - DEVELOPMENT OF LC-MS/MS METHODS FOR THE QUANTITATION OF COMMONLY CO-ADMINISTERED DRUGS AND AN ASSESSMENT OF THE IMPACT OF THEIR PRESENCE ON THE ANALYSIS AND STABILITY OF NOVEL DRUG CANDIDATES IN SUPPORT OF A CLINICAL STUDY***Ryan Schmich, Alturas Analytics, Moscow, ID, USA***P63 - FAST-ACTING SMALL MOLECULES INHIBITING MALARIA INFECTION AT MULTIPLE LIFE STAGES***Snigdha Singh, Delhi University, Delhi, India***P64 - STRATEGIES AND CONSIDERATIONS FOR HUMAN PK OPTIMISATION AND PREDICTION IN DRUG DISCOVERY: CLEARANCE***Beth Williamson, AstraZeneca, Cambridge, United Kingdom***P65 - STRATEGIES AND CONSIDERATIONS FOR HUMAN PK OPTIMISATION AND PREDICTION IN DRUG DISCOVERY: VOLUME OF DISTRIBUTION AND ORAL ABSORPTION***Beth Williamson, AstraZeneca, Cambridge, United Kingdom***P66 - EVALUATION OF SPECIES DIFFERENCES IN MAJOR CLEARANCE PATHWAYS BETWEEN HUMANS AND ANIMALS.***Saki Yamauchi, Japan Tobacco Inc., Takatsuki, Osaka, Japan***P67 - A RANDOMIZED, DOSE-ESCALATION, DOUBLE-BLIND AND PLACEBO-CONTROL STUDY TO ASSESS THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF JAKTINIB IN HEALTHY CHINESE VOLUNTEERS***Hong Zhang, The First Hospital of Jilin University, Changchun, Jilin, China***P68 - A SYSTEMIC REVIEW OF DRUG DISPOSITION AND DRUG INTERACTION DATA FOR 16 NEW SMALL-MOLECULE DRUGS DEVELOPED AND APPROVED IN CHINA BETWEEN 2007 AND 2018***Mingshe Zhu, MassDefect Technologies, Princeton, NJ, USA***DRUG INTERACTION (P69 - P87)****P69 - PHARMACOKINETIC INTERACTION STUDY OF NOVEL COMBINATION OF HMG CO-A REDUCTASE INHIBITOR WITH GREEN COFFEE BEAN FORMULATION FOR OBESITY ASSOCIATED COMPLICATIONS IN SD RATS USING UHPLC/QTOF/MS***Lavanya Bolla, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, Telangana, India***P70 - STEREOSELECTIVE INHIBITORY POTENTIALS OF GINSENOSE-RH2 EPIMERS TOWARD ORGANIC ANION TRANSPORTING POLYPEPTIDE (OATP) 1B1/3-MEDIATED UPTAKE***Soon Uk Chae, The Catholic University of Korea, Bucheon-si, South Korea***P71 - PREDICTION OF P-GP MEDIATED DRUG-DRUG INTERACTION AT THE BLOOD-BRAIN BARRIER***Hélène Chapy, UCB Biopharma, Braine l'Alleud, Belgium***P72 - QUANTITATIVE PREDICTION OF CYP3A4 AND CYP3A5-MEDIATED DRUG DRUG INTERACTIONS***Yingying Guo, Eli Lilly and Company, Indianapolis, IN, USA***P73 - EVALUATION FOR INHIBITORY POTENTIAL OF GINSENOSE-RH2 EPIMERS ON BREAST CANCER RESISTANCE PROTEIN (BCRP)-MEDIATED EFFLUX***Seongjun Jo, The Catholic University of Korea, Bucheon, South Korea***P74 - EVALUATION OF ENDOGENOUS BIOMARKERS OF OATP1B ACTIVITY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER WHO RECEIVE PACLITAXEL***Hiroyuki Kusuhara, The University of Tokyo, Bunkyo-ku, Tokyo, Japan***P75 - *IN VITRO* INHIBITORY EFFECTS OF ZIZIPH SPINOSI SEMEN EXTRACT ON CYTOCHROME P450 AND UDP-GLUCURONOSYLTRANSFERASE ENZYMES ACTIVITIES IN HUMAN LIVER MICROSOMES***Chae Bin Lee, Catholic university of Korea, Bucheon, South Korea***P76 - *IN VITRO* INHIBITORY EFFECTS OF APINACA, A SYNTHETIC CANNABINOID, ON HUMAN CYTOCHROME P450, UDP-GLUCURONOSYLTRANSFERASE ENZYMES, AND DRUG TRANSPORTERS***Hye Suk Lee, The Catholic University of Korea, Bucheon, South Korea***P77 - *IN VITRO* METABOLISM OF TICAGRELOR AND ITS METABOLIC INTERACTIONS WITH STATINS***Shuaibing Liu, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China***P78 - *IN VITRO* METABOLIC, CYP AND TRANSPORTER CHARACTERIZATION OF PTI-125, A NOVEL SMALL MOLECULE DRUG CANDIDATE FOR ALZHEIMER'S DISEASE***Gang Luo, Covance Inc., Madison, WI, USA***P79 - EFFECTS OF THE PREGNANE X RECEPTOR (PXR) ACTIVATOR RIFAMPIN ON TRANSPORTER GENE EXPRESSIONS: STUDIES IN HEPATOCYTES *IN VITRO* AND IN MONKEYS *IN VIVO****Congrong Niu, Gilead Sciences, Foster City, CA, USA***P80 - IMPACT OF PIPERINE ON CARBAMAZEPINE: MODELING-BASED PHARMACOKINETICS ANALYSIS AND PHARMACODYNAMICS EVALUATION IN ANIMAL MODELS***Tianjing Ren, The Chinese University of Hong Kong, Hong Kong, Hong Kong***P81 - THE IMPACT OF METABOLITE DETECTION ON ADHERENCE AND THE PREVALENCE OF DRUG-DRUG INTERACTIONS IN THE BEHAVIORAL HEALTH POPULATION THROUGH URINE TOXICOLOGY TESTING***Leigh Ann Saucier, Aegis Sciences Corporation, Nashville, TN, USA***P82 - PHARMACOKINETIC APPLICATION OF NORMOTHERMIC PERFUSED *EX VIVO* PORCINE AND DISEASED HUMAN LIVERS***Lianne Stevens, LUMC & TNO, Zeist, Netherlands***P83 - THE NATURAL PRODUCT KRATOM IS A POTENTIAL PRECIPITANT OF PHARMACOKINETIC INTERACTIONS WITH COMMON DRUGS OF ABUSE***Rakshit Tanna, Washington State University, Spokane, WA, USA***P84 - A PLATFORM FOR EVALUATING *IN VIVO* OATP1B INHIBITION RISK IN CYNOMOLGUS MONKEYS AND TRANSLATION TO HUMAN***Daniel Tatosian, Merck & Co, Inc., Kenilworth, NJ, USA*

P85 - *IN VITRO* INHIBITORY EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON HUMAN LIVER MICROSOMAL MORPHINE GLUCURONIDATION

Verawan Uchaipichat, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

P86 - INVESTIGATION OF THE INTERACTION MECHANISM BETWEEN METRONIDAZOLE AND 5-FLUOROURACIL

Yoshiaki Yamagishi, Musashino University, Nishitokyo-shi, Tokyo, Japan

P87 - MAIN MECHANISMS OF PHARMACOKINETIC DRUG-DRUG INTERACTIONS TRIGGERING LABEL RECOMMENDATIONS FOR DRUGS APPROVED BY THE FOOD AND DRUG ADMINISTRATION IN 2018

Jingjing Yu, University of Washington, Seattle, WA, USA

DRUG-INDUCED LUNG TOXICITY (P88)**P88 - ROLE OF MICRORNAS IN DRUG-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN HUMAN LUNG-DERIVED ALVEOLAR TYPE II EPITHELIAL CELL MODEL A549/ABCA3**

Ryoko Yumoto, Hiroshima University, Graduate School of Biomedical & Health Sciences, Hiroshima, Japan

ENZYME INDUCTION (P89 - P96, P251 AND P252)**P89 - SIMCYP PBPK MODELING AND SIMULATION OF PHARMACOKINETIC DRUG INTERACTION BETWEEN LORLATINIB AND ITRACONAZOLE: A CASE STUDY USING INDUCTION SLOPE**

Jian Lin, Pfizer Inc, Groton, CT, USA

P90 - APPLICATION OF PRIMARY HUMAN HEPATOCYTES ENRICHED WITH HUMAN PLASMA IN CYP INDUCTION STUDIES

Chuang Lu, Sanofi, Waltham, MA, USA

P91 - DETERMINATION OF ADDITIVE, SYNERGISTIC AND ANTAGONISTIC EFFECTS BY THE ANALYSES OF PROTEIN MARKERS IN PESTICIDE MIXTURE-TREATED HEPAR-G-CELLS

Oliver Poetz, SIGNATOPE, Reutlingen, Germany

P92 - CORRELATION BETWEEN CYTOCHROME P450 INDUCERS AND NUCLEAR RECEPTOR ACTIVATION- A SCREENING APPROACH

Shantanu Roychowdhury, Eurofins Panlabs, Saint Charles, Missouri, USA

P93 - TISSUE STEM CELL DERIVED HUMAN INTESTINAL ORGANIDS: A NOVEL AND PHYSIOLOGICALLY RELEVANT MODEL TO EVALUATE CYTOCHROME P450 INDUCTION IN GUT

David Stresser, AbbVie, North Chicago, IL, USA

P94 - HIGH THROUGHPUT LCMS QUANTIFICATION OF CYTOCHROME P450 PROTEINS IN PLATED HUMAN HEPATOCYTES DIRECT FROM 96-WELL PLATES

David Stresser, AbbVie, North Chicago, IL, USA

P95 - A-TO-I RNA EDITING MODULATES THE EXPRESSION OF HUMAN PREGNANE X RECEPTOR

Seiya Takemoto, Kanazawa University, Kanazawa, Japan

P96 - EVALUATION OF METHOTREXATE AND IDELALISIB AS INDUCERS OF HUMAN ALDEHYDE OXIDASE IN CELLULAR MODELS

Szu Ling Yeap, National University of Singapore, Singapore, Singapore

P251 - AHR-MEDIATED EFFECT OF BENZO(A)PYRENE AND SMOKING ON MICRORNA EXPRESSION IN LUNG CANCER

Lyudmila Gulyaeva, Federal Research Center "Basic and Translational Medicine", Novosibirsk, Russia

P252 - EFFECTS OF O,P'-DDT, P,P'-DDT AND ENDOSULFAN ON THE EXPRESSION OF HORMONE-SENSITIVE MICRORNA IN MCF-7 CELLS

Lyudmila Gulyaeva, Federal Research Center "Basic and Translational Medicine", Novosibirsk, Russia

ENZYME INHIBITION/INACTIVATION (P97 - P104)**P97 - CLINICAL PREDICTION OF DRUG-DRUG INTERACTION RISK DUE TO TIME-DEPENDENT INHIBITION OF CYTOCHROME P450 3A4 - A SIDE-BY-SIDE COMPARISON OF HUMAN LIVER MICROSOME AND HEPATOCYTE**

Elaine Tseng, Pfizer Inc., Groton, CT, USA

P98 - DELINEATION OF *IN VITRO* CUT-OFF VALUES FOR CYP3A4/5 TIME DEPENDENT INHIBITION USEFUL IN EARLY DRUG DESIGN - A SIDE-BY-SIDE COMPARISON IN HUMAN LIVER MICROSOMES AND HEPATOCYTES

Heather Eng, Pfizer, Groton, CT, USA

P99 - LC-MS/MS BASED BIOANALYSIS METHOD FOR BILIRUBIN GLUCURONIDES: APPLICATION IN UGT1A1 INHIBITION EVALUATION AND COMPARISON WITH DATA OBTAINED USING ESTRADIOL

Chunyan Han, Pharmaron, Beijing, China

P100 - *IN VITRO* EVALUATION OF UDP-GLUCURONOSYLTRANSFERASE (UGT) 1A3 AND UGT2B4 INHIBITION

Kimberly Lapham, Pfizer Inc, Groton, CT, USA

P101 - THE ROLE OF L- AND D-MENTHOL IN THE GLUCURONIDATION AND DETOXIFICATION OF THE MAJOR LUNG CARCINOGEN, NNK

Philip Lazarus, Washington State University College of Pharmacy and Pharmaceutical Sciences, Spokane, WA, USA

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Jacqueline Wen Hui Leow, National University of Singapore, Singapore, Singapore

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Lian Zhou, Eli Lilly Company, Indianapolis, IN, USA

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Yuanyuan Shi, University of Washington, Seattle, WA, USA

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Simone Stahl, AstraZeneca, Cambridge, Cambridgeshire, United Kingdom

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Toni Steiner, MilliporeSigma, St Louis, MO, USA

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Thom van der Made, University of Manchester, Manchester, United Kingdom

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David Wagner, AstraZeneca, Waltham, MA, USA

UGT1A1 INHIBITION (P249)**P249 - LC-MS/MS BASED BIOANALYSIS METHOD FOR BILIRUBIN GLUCURONIDES: APPLICATION IN UGT1A1 INHIBITION EVALUATION AND COMPARISON WITH DATA OBTAINED USING ESTRADIOL**

Chunyan Han, Pharmaron, Beijing, China

A1 - IS URINARY TESTOSTERONE GLUCURONIDE A POTENTIAL BIOMARKER FOR UGT2B17 ONTOGENY IN AGES 7 TO 18 YEARS?**Haeyoung Zhang**¹, Abdul Basit¹, J. Steven Leeder², and Bhagwat Prasad¹¹University of Washington, USA, ²Children's Mercy Hospital, USA

UGT2B17 is a highly variable androgen- and drug-metabolizing enzyme, with over a 500-fold variability in its protein abundance (1). Multiple factors contribute to UGT2B17 variability, including gene deletion, single nucleotide polymorphisms, age, and sex; however, these combined explain only one third of its variability (2). In particular, UGT2B17 exhibits unique ontogeny, where males show scarce expression below 10 years with a rapid increase in adolescence (Age₅₀ 13.6 years), while females show an earlier and attenuated increase (Age₅₀ 10.8 years) (2). This becomes concerning when UGT2B17 substrates are administered as pediatric therapeutics. Known xenobiotic substrates include vorinostat and tobacco carcinogen NNAL, whereas testosterone is a well-established endogenous substrate. Considering the high and multifaceted variability in UGT2B17 and its dynamic ontogeny, there is a need for a phenotypic biomarker to predict UGT2B17 activity. In this exploratory analysis, we propose that TG normalized by another androgen metabolite such as androsterone glucuronide (AG) or dehydroepiandrosterone sulfate (DHEAS), will reflect the ontogeny of UGT2B17, with potential use for prospective stratification in children. We analyzed archived longitudinal urine samples in 63 pediatric subjects between ages 7 to 18 years, who were followed in 7 visits over 3 years. Urine aliquots were processed with solid phase extraction (SPE) followed by targeted metabolomics analysis using a validated LC-MS/MS method. Ages were grouped in 3 categories: under 9 years (group 1), 9 to 13 years (group 2), and 13 to 18 years (group 3). Kruskal-Wallis test followed by Dunn's multiple comparison test was used to compare metabolite levels in these groups. Inter-day, intra-day, and inter-analyst variability and benchtop, multiple freeze-thaw, and auto-sampler stability were within 20%. Lower limit of quantification for TG, AG, testosterone sulfate (TS), and DHEAS were 0.5, 2, 2.5 and 3 ng/mL, respectively. Our data show that Age₅₀ for TG/AG is around 12.6 years for males and 11.1 years for females, and for TG/DHEAS were 13 and 9.1 years for males and females, respectively. Only males showed statistically significant differences in normalized TG ratios between age groups (group 1 versus 3 and group 2 versus 3). Fold differences in variability for normalized TG were more than 8 times higher for groups 2 and 3 compared to group 1, suggesting increased variability in TG formation with age. TG/TS increased by 4-fold in males and 2-fold in females from group 1 to group 3, indicating increased fraction of TG formation compared to TS. Results support the potential for normalized TG ratio as a promising phenotypic biomarker to predict age- and sex-dependent drug metabolism by UGT2B17. Further statistical analysis and quantitation of other metabolites are ongoing. UGT2B17 genotyping analysis is needed to further validate this biomarker. These results may aid in development of anti-doping criteria for young athletes during developmental stages. Future clinical studies are needed to validate and confirm whether TG ratios correlate with other reported UGT2B17 associations.

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A2 - DIFFERENTIAL REGULATION OF ALDEHYDE OXIDASE BY PREGNANE X RECEPTOR AND RETINOID X RECEPTOR AGONISTS IN HUMAN CELL MODELS**Shiyan Chen** and Aik Jiang Lau*National University of Singapore, Singapore*

Aldehyde oxidase 1 (AOX1) is a drug-metabolizing enzyme that catalyzes the biotransformation of various drugs and chemicals, but very little is known about the transcriptional regulation of AOX1 expression. Pregnane X receptor (PXR) is a nuclear receptor that regulates numerous genes involved in the metabolism and transport of xenobiotics. Upon ligand binding, PXR heterodimerizes with another nuclear receptor, retinoid X receptor (RXR), and binds to the DNA response elements of target genes, resulting in increased transcription of target genes. The aim of the present study was to determine whether PXR and RXR regulate human AOX1 expression, as determined in LS180 human colon adenocarcinoma cells and MCF-7 human breast adenocarcinoma cells. Initial experiments indicated that AOX1 mRNA level was greater in MCF-7 than LS180 cells. In LS180 cells, rifampicin (PXR agonist; 50 μ M) increased AOX1 mRNA level by 3-fold, whereas 9-cis-retinoic acid (RXR agonist; 10 μ M) had no effect. In contrast, in MCF-7 cells, 9-cis-retinoic acid increased AOX1 mRNA by 4-fold, whereas rifampicin had minimal or no effect. The induction of AOX1 by rifampicin in LS180 cells and 9-cis-retinoic acid in MCF-7 cells reached a plateau between 72 and 120 h. Given that PXR is expressed in LS180 cells but not MCF-7 cells, we hypothesized that PXR regulates the induction of AOX1 by rifampicin in LS180 cells. To test this hypothesis, both chemical and genetic knock-down approaches were used. SPA70 (a potent and PXR-selective antagonist, 10 μ M) attenuated rifampicin-mediated increase in AOX1 mRNA level by 50% and attenuated the increase in CYP3A4 mRNA level (a prototypical target gene of PXR) by 67%. As compared to control siRNA, transfection of LS180 cells with PXR siRNA decreased PXR level by 30%, and the PXR siRNA attenuated both rifampicin-mediated AOX1 and CYP3A4 induction. Given that RXR is essential for PXR function, we determined whether a RXR antagonist (HX531) would decrease the effect of rifampicin on AOX1 expression. Indeed, HX531 (1 μ M) attenuated the

induction of AOX-1 by rifampicin (30 μ M) by 43%, whereas it attenuated CYP3A4 induction by rifampicin by 95%. These decreases were not a result of cytotoxicity as determined by a lactate dehydrogenase assay. Moreover, pretreatment of LS180 cells with actinomycin D (a RNA synthesis inhibitor) completely abolished the induction of AOX1 and CYP3A4 by rifampicin, indicating that rifampicin increased AOX1 mRNA by a transcriptional mechanism. To determine whether the transcriptional increase in AOX1 mRNA led to increased protein level, we developed a quantitative proteomics method to quantify AOX1 protein expression in cell lysates using ultra-high performance liquid chromatography-tandem mass spectrometry. At 96 h after treatment, rifampicin increased AOX1 protein level in a concentration-dependent manner, thereby corroborating with the mRNA data. In conclusion, rifampicin and 9-cis-retinoic acid differentially regulate AOX1 expression in a cell-type-dependent manner, and PXR is the first nuclear receptor that transcriptionally regulates AOX1 expression. As PXR is activated by numerous drugs and endogenous chemicals, our novel findings suggest potential interaction between various PXR activators and AOX1 drug substrates.

A3 - A HIGH-THROUGHPUT DOUBLE COCKTAIL APPROACH FOR EVALUATING TIME-DEPENDENT INHIBITION OF NINE MAJOR HUMAN CYTOCHROME P450 ENZYMES

Helinä Kahma, Laura Aurinsalo, Mikko Neuvonen, Jenni Viinamäki, Terhi Launiainen, Anne M. Filppula, Aleksi Tornio, and Janne T. Backman

University of Helsinki and Helsinki University Hospital, Finland

Inhibition of human cytochrome P450 (CYP) enzymes is a major cause of clinically significant drug-drug interactions. A substrate cocktail method, in which multiple CYP activities are assessed in a single experiment, is an efficient and robust tool to screen the inhibitory potency of drugs. Many cocktail methods have been developed for assessing direct inhibition of CYP enzymes but only few reports have addressed the application of the method for the often more harmful time-dependent CYP inhibition. The aim of the study was to develop a sensitive high-throughput cocktail method with optimized incubation conditions for screening time-dependent inhibition of the nine most important CYP enzymes. The following probe substrates were selected for the method: tacrine (CYP1A2), coumarin (CYP2A6), bupropion (CYP2B6), amodiaquine (CYP2C8), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), astemizole (CYP2J2), midazolam (CYP3A4) and omeprazole (CYP3A4). *In vitro* incubations with pooled human liver microsomes were conducted in 96-well plates using a Tecan Freedom Evo 150 automated liquid handler. Based on preliminary interaction experiments, the substrates were divided into two cocktails to minimize interaction between the substrates: 5 μ M tacrine, 50 μ M bupropion, 2 μ M amodiaquine, 100 μ M tolbutamide and 2 μ M midazolam in cocktail A, and 1 μ M coumarin, 0.3 μ M astemizole, 20 μ M omeprazole and 5 μ M dextromethorphan in cocktail B. Two separate UPLC-MS/MS methods were optimized for the quantification of the metabolite concentrations. The metabolite production of all substrates was linear with a protein concentration of 0.05 mg/ml and an incubation time up to 5 min. Substrate depletion was less than 20% for all analytes. A preincubation time of 30 minutes, an incubation time of 5 minutes and protein concentration of 0.05 mg/ml were thus selected for both cocktail assays. In further validation, the CYP activities of the probe reactions were similar (difference <30%) in the cocktail and single substrate assays. An automated protocol was developed for the determination of the maximal inactivation rate (kinact) and the concentration producing half of kinact (KI) of time-dependent inhibitors using the double cocktail method. In conclusion, we were able to optimize a rapid and reliable double cocktail system, including quantitative measurement of enzyme activities of nine major CYP enzymes in human drug metabolism. The method can be used for high-throughput screening of both time-dependent and direct CYP inhibition of CYP enzymes causing clinically significant drug-drug interactions.

A4 - UNDERSTANDING THE PHYSIOLOGICAL FUNCTIONS OF THE XENOBIOTIC-SENSING NUCLEAR RECEPTORS ON GUT MICROBIOME USING GENETICALLY-MODIFIED MICE

Mallory Little and Julia Yue Cui

University of Washington, USA

The pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are important xenobiotic-sensing nuclear receptors, and their genetic polymorphisms are known to alter the pharmacokinetics of various xenobiotics. Gut microbiome is increasingly recognized as a critical modifier of host xenobiotic biotransformation, and microbial metabolites such as lithocholic acid and indole-3-propionic acid are known endogenous PXR activators. We have previously demonstrated that the gut microbiome is necessary for maintaining the constitutive expression of certain PXR- and CAR-target genes, and pharmacological activation of PXR and CAR produces gut dysbiosis and reduces secondary bile acids. However, little is known regarding to what extent PXR and CAR under basal conditions influence the gut microbiome. To determine the role of PXR and CAR on the basal regulation of gut microbiome, 24h fecal samples were collected from male and female wild-type (WT), PXR-null, CAR-null, and PXR/CAR-null C57BL/6 mice at adolescent (30-day-old) and adult (60-day-old) ages. Fecal samples were also collected from male and female humanized PXR transgenic (hPXR-TG) and WT FVB/NJ mice at both ages to evaluate species differences between human and mouse PXR. 16S rDNA sequencing was performed by amplifying the hypervariable V4 region (n=5 per group). PCoA showed distinct separations between the gut microbiota of WT mice and mice genetically deficient in PXR, CAR, or both receptors, at both genders

and both ages, between mPXR and hPXR carriers, as well as between the two different WT mouse strains (β -diversity, QIIME). The absence of PXR or CAR increased the richness of the gut microbiota, and the absence of both receptors further augmented this increase (α -diversity, QIIME). Among the two WT strains, FVB/NJ mice had higher richness in their microbiota than C57BL/6 mice. Interestingly, *Lactobacillus* sp., noted for its probiotic potential, had higher abundance in PXR/CAR-null mice of both genders and ages. The *Prevotella* genus, which is associated with improved host glucose and insulin response, was higher in feces from hPXR-TG mice at both ages and genders relative to WT mice. Conversely, *Akkermansia muciniphila*, which has been noted for its anti-inflammatory function, was lower in feces of hPXR-TG adolescent males and adolescent and adult females. Functional predictions showed a reduction in cytochrome P450-mediated xenobiotic metabolism, intermediary metabolism pathways, as well as cell motility and secretion, in adult male hPXR-TG mice relative to adult male WT mice. The family S24-7, which is associated with negatively correlating with inflammation, was less abundant in adult FVB/NJ mice than in adult C57BL/6 mice of both ages. The importance of changes in microbial composition was explored using quantitative metabolomics for the microbial metabolite bile acids and short-chain fatty acids. This research is among the first to show that the genotype of the host xenobiotic-sensing nuclear receptors PXR and CAR affect the composition and functions of gut microbiome in an age- and gender-dependent manner. The constitutive variations in gut microbiome may in part contribute to PXR- and CAR-mediated xenobiotic biotransformation, inflammation, and metabolic syndrome.

A5 - THE EFFECT OF CHANGES IN PHARMACOKINETICS OF TRANSPORTER SUBSTRATES ON PBPK MODELING APPROACHES

Jasleen Sodhi and Leslie Z. Benet

University of California San Francisco, USA

Background: Physiologic based pharmacokinetic (PBPK) modeling is an increasingly-used tool industry-wide allowing for prediction of plasma concentration-time profiles of drugs using scaled-up *in vitro* measurements of drug-specific parameters, as well as prediction of changes in concentration-time profiles following drug-drug interactions (DDIs), for pharmacogenomics variants and/or special populations. DDIs and pharmacogenomic variance related to metabolizing enzymes have the potential to alter clearance (CL), however, changes related to transporters can potentially lead to changes in both CL and volume of distribution (Vd) when transporters more-than-minimally affect drug disposition. Currently, PBPK modeling programs, such as Symcyp, GastroPlus, and PKSIM, do not account for transporter-mediated changes in Vd. However, it is crucial for PBPK models to characterize Vd changes since the final outcome attempts to predict the time-course of systemic concentrations, not just AUC changes, and uses this fitting of time-course concentrations as a measure of the effectiveness of the model. Here we investigate the importance of characterizing the Vd change (in addition to CL changes) for transporter substrates in clinical DDI studies for accurate prediction of terminal half-life. Methods: Clinical DDI studies with concomitant IV rifampin, an OATP1B1 and BCRP transporter inhibitor, were examined for 4 orally dosed victim drugs (atorvastatin[1], glyburide[2], pitavastatin[3], and rosuvastatin[3,4]). Changes in CL, Vd, MRT, and terminal half-life between the control and IV rifampin phase were examined and ratios were expressed as control / IV rifampin phase. Results: For all drugs, the magnitude change of terminal half-life is not predicted by changes in CL alone due to significant changes in Vd. A 7.7-fold in atorvastatin CL/F was observed, however, an accompanying 17.7-fold change in Vss/F resulted in only a 2.7- and 2.3-fold change in terminal half-life and MRT, respectively. Glyburide, studied in both induced and non-induced states, showed a 2.2-fold change in CL/F in both conditions, however Vss/F changed 2.3-(induced) and 3.1-(non-induced), resulting in no change in terminal half-life for the induced condition, and only a 1.4-fold change for the non-induced condition. Two rosuvastatin studies were examined and a 3.0 to 3.6-fold change in CL/F was observed, and in combination with the 7.5 to 12.2-fold change in Vss/F, terminal half-life only changed 1.6 to 2.9-fold. Changes in pitavastatin CL/F (6.7-fold) were not reflected in change of terminal half-life (1.3-fold) due to a similar change in Vss/F (5.5-fold). Conclusions: For clinically relevant transporter substrates, it is crucial to understand that although AUC or CL may significantly change in DDI or pharmacogenomics studies, these changes may not be quantitatively reflected in MRT or terminal half-life due to an equally significant change in Vd. The reported success of PBPK modelling of such clinical studies is of concern, since at present changes in Vd are unaccounted for by programs such as Simcyp. The implications of this analysis should be carefully considered when attempting to utilize PBPK to fit clinical interaction or pharmacogenomics variance data.

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A6 - INVESTIGATING THE ROLE OF DRUG TRANSPORTERS IN FUROSEMIDE ABSORPTION, FOOD-EFFECT AND ELIMINATION USING A PROTEOMICS INFORMED-MECHANISTIC PBPK MODELING APPROACH

Revathi Chapa¹, Cindy Yanfei Li¹, Abdul Basit¹, Mayur K. Ladamor², Sheena Sharma², Aarzo Thakur², Saranjit Singh², Arzu Selen³, and Bhagwat Prasad¹

¹University of Washington, USA, ²National Institute of Pharmaceutical Education and Research (NIPER), India, ³FDA, USA

Background and objectives: Furosemide is a widely used diuretic, which exhibits variable pharmacokinetics (PK) with bioavailability ranging from 10-90%. Furosemide also shows reduced exposure with food despite of its higher solubility in medium mimicking fed state. To explain these clinical observations, we hypothesized that transporter mediated permeability plays a significant role in its variable absorption. To test this, we i) characterized the role of intestinal efflux transporters in furosemide absorption and ii) predicted the contribution of renal uptake and efflux transporters on its disposition by using a proteomics-informed mechanistic physiologically based pharmacokinetic (PBPK) modeling. Furosemide transport was initially studied using vesicles overexpressing efflux transporters, i.e., P-gp, BCRP, MRP2, MRP3 and MRP4. The transport kinetic analyses were then conducted for the transporters that exhibited activity in the initial screening, i.e., BCRP and MRP4. The kinetic parameters (V_{max} and K_m) were derived by fitting the Michaelis-Menten equation. The protein abundance of transporters was determined using quantitative proteomics in both vesicles and human tissues (intestine and kidney). The *in vitro* activities were scaled using protein abundance values and corrected for inside-out vesicles. A mechanistic whole-body PBPK model was developed to describe the intravenous and oral PK of furosemide in healthy adults by integrating physico-chemical, *in vitro* dissolution, metabolism, transport and excretion parameters using Simcyp (Certara, UK) and Gastroplus (SimulationsPlus, USA)¹. The effects of food-induced delayed gastric-emptying on BCRP mediated furosemide efflux was also investigated. The initial screening results revealed BCRP and MRP4 were the main transporters for furosemide efflux. The mean V_{max} and K_m values for BCRP were 36.9 nmol/min/mg and 21.0 μ M, respectively, while for MRP4 were 0.1 nmol/min/mg and 30.9 μ M. The protein abundances of BCRP and MRP4 were as follows: vesicles (11.4 and 0.3 pmol/mg, respectively), intestine (0.34 pmol/mg of BCRP), and kidney (0.91 pmol/mg of MRP4). The mechanistic PBPK model predicted PK parameters of furosemide were within 2-fold of the observed mean data^{2,3}. Based on the sensitivity analysis, BCRP significantly affected furosemide bioavailability, which potentially also explains the negative food effect. From this study we confirmed that BCRP is important for effluxing furosemide into the gut, which may play a critical role in its variable bioavailability. The model suggests that fasting condition leads to local saturation of BCRP because of the faster gastric emptying. On the other hand, delayed gastric emptying allows efficient BCRP mediated furosemide efflux in fed condition, hence explains the negative food effect. This may also explain the "absorption window" hypothesis studied for furosemide absorption. The study could be further extended to investigate the genetic polymorphisms of BCRP and MRP4 on the absorption, disposition, efficacy and toxicity or drug-drug interactions of furosemide. Disclaimer: The approaches and conclusions in this abstract have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any agency determination or policy.

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A7 - INTERACTION WITH YAP IS A POSSIBLE UNDERLYING MECHANISM FOR CAR-DEPENDENT HEPATOCARCINOGENESIS

Ryota Shizu, Taiki Abe, Keiichiro Sobe, Mai Ishimura, Takuomi Hosaka, Takamitsu Sasaki, and Kouichi Yoshinari
School of Pharmaceutical Sciences, University of Shizuoka, Japan

Hepatic nuclear receptor CAR is activated by various types of chemicals such as antiepileptic drug phenobarbital and regulates hepatic drug and energy metabolism. In addition to these functions, CAR is also involved in the development of liver toxicities. Namely, CAR activation induces hepatocyte proliferation and hepatocarcinogenesis in rodents. However, the mechanism of CAR-dependent hepatocyte proliferation is still unknown. Besides, it is well known that CAR-dependent hepatocyte proliferation is not observed in human liver. In this study, we have investigated the molecular mechanism by which CAR induces hepatocyte proliferation and the cause of the species difference of hepatocyte proliferation between human and rodents. Treatment of mice with CAR activators (phenobarbital or TCPOBOP) induced hepatocyte proliferation. In these livers, we found nuclear accumulation of yes-associated protein (YAP), which is related to Hippo pathway, and up-regulation of its target gene expression. YAP is known as a liver cancer inducer. Its activation is frequently observed in clinical liver cancer tissues and its overexpression in mouse induces liver cancer. In an *in vitro* evaluation system of CAR-dependent hepatocyte proliferation by using AML-12 cells derived from mouse normal hepatocytes and adenovirus vector expressing mouse CAR, chemical inhibitor or siRNA for YAP inhibited CAR-dependent cell proliferations. Moreover, treatment of mice with YAP inhibitor verteporfin attenuated TCPOBOP-induced hepatocyte proliferation. Next, we investigated the interactions of human and mouse CAR and YAP using recombinant proteins by pull-down assay. As a result, the interaction between mouse CAR and mouse YAP was observed but the

interaction between human CAR and human YAP wasn't. In addition, by using mammalian two hybrid assay in HepG2 cells, we confirmed the interaction of mouse CAR with mouse YAP and no interaction between human CAR and YAP. To determine the interaction interface, pull-down assay was conducted with several domain-deleted YAP proteins. Then, we have found that the WW domain in YAP is important for the interactions with CAR. Consistently, the mutation of the WW domain attenuated the interaction between YAP and CAR. WW domain is reported to interact with specific amino acid sequence PPXY (PY motif) and mouse and rat CAR contain the PY motif (PPAY) on their protein surface but human CAR has the mutated motif (PPAH). In pull-down assay, the substitution of PPAY to PPAH in mouse CAR abolished the interaction with mouse YAP. From these results, it is suggested that CAR interacts with YAP via the PY motif-WW domain interaction and activates YAP to induce hepatocyte proliferation and the absence of the PY motif in human CAR results in the lack of the interaction with YAP and CAR-dependent hepatocyte proliferation in human livers.

A8 - APICAL AND BASOLATERAL FLOW RATE INFLUENCE INTESTINAL ABSORPTION IN FRESH INTESTINAL TISSUE INSIDE A NOVEL INTESTINE ON A CHIP MODEL

Hossein Eslami Amirabadi^{1,2}, Joline Boogaard¹, Esmée Wierenga¹, Tim Donkers¹, Mariska Grölers-Mulderij¹, Lianne Stevens¹, Birol Usta¹, Lisanne Pieters¹, Bastiaan Ingenhut¹, Irene Nooijen¹, Rosalinde Masereeuw², and Evita van de Steeg¹

¹TNO, Netherlands, ²Utrecht University, Netherlands

Drug development is currently a long and costly process and the majority of drug candidates fail to reach clinical trials. The field still lacks proper human intestinal models that could guarantee drug safety and efficacy. Systems with ex vivo intestinal tissue add more micro-environmental context present in human intestine. However, current ex vivo models are often difficult to operate, limited to maintain the tissue viability and not suitable for small tissue samples. Moreover, conventional intestinal models do not provide enough flexibility to gain a mechanistic view behind the intestinal processes. For example, there is very limited information about the effect of flow rate, both apical and basolateral, on the intestinal integrity and permeability. At TNO, we have tackled these limitations and developed a 3D printed organ on a chip system that integrates small sizes of intestinal tissues between two microchannels (leakage free), has easier operation protocol, better suits higher throughput applications and shows low adsorption to conventional test drugs (<15%). Using this technology, we studied the effect of apical and basolateral flow rate on the integrity and absorption of fresh human intestinal tissue. We used two different flow rates, namely 1.4 ml/hr and 10 ml/hr. We observed that during 24 hours incubation of the tissue inside the chip, the leakage of FITC dextran (MW4000) from the apical to the basolateral microchannel was lower than 1% per hour which shows an excellent integrity of the tissue. Furthermore, the lactate dehydrogenase (LDH) leakage from the tissue was higher in the lower than the higher flow rate (12% vs 17% of the initial LDH content). Also, we tested the absorption of the tissue for two model drugs, i.e. atenolol (transported paracellularly) and antipyrine (transported transcellularly), and observed that the ratio of transcellular to paracellular permeability was around 4 and 2 for the low and high flow rates, respectively. These results show that the tissue remains more viable in the chips with a high flow rate but transports less test drug molecules actively to the basolateral microchannel. The developed model not only expands the window to study intestinal absorption to longer time periods, but also enables us to understand the effect of flow rate on both apical and basolateral sides. Further research will be done to determine the effect of individual flows, apical vs. basolateral, on the intestinal tissue.

A9 - ALDEHYDE OXIDASE CONTRIBUTES TO ALL-TRANS-RETINOIC ACID BIOSYNTHESIS IN HUMAN LIVER

Guo Zhong¹, Chris Seaman¹, Huaqing Xi¹, Erickson Paragas², Jeffrey Jones², and Nina Isoherranen¹

¹University of Washington, USA, ²Washington State University, USA

All-trans-retinoic acid (atRA) is a critical endogenous molecule that regulates various biological events by binding to retinoic acid receptors. It is generally believed that in the liver aldehyde dehydrogenase 1A1 (ALDH1A1) is the main enzyme responsible for atRA biosynthesis. However, in previous studies in which mice were dosed with WIN18,446, a potent ALDH1A1 inhibitor, only about 50% inhibition of atRA formation was observed in mouse liver S9 fractions, indicating that other enzymes are also involved in the formation of atRA in the liver. Because mouse and rabbit aldehyde oxidase (AOX) have been proposed to catalyze RAL oxidation to atRA, we hypothesized that aside from ALDH1A1, AOX makes a significant contribution to atRA formation in human liver. Our data demonstrate that purified human recombinant AOX takes RAL as the substrate and produces atRA ($K_m \sim 1 \mu\text{M}$; $k_{cat} \sim 3.5 \text{ min}^{-1}$) and the reaction does not require NAD⁺, an obligate cofactor for ALDH1A1 catalyzed formation of atRA. The formation of atRA catalyzed by purified human AOX was inhibited by known AOX inhibitors raloxifene (IC₅₀~200 nM) and hydralazine (IC₅₀~500 nM). In the absence of NAD⁺, atRA formation was observed in human liver S9 fractions (HLS9) prepared from four individual donors. Addition of NAD⁺ increased atRA formation by 50-60%, suggesting that ALDH1A1 and AOX both contribute to approximately 50-60% of atRA biosynthesis in human liver. Furthermore, in the presence of NAD⁺ hydralazine inhibited about 50% of atRA formation in HLS9 while combining hydralazine and WIN18,446 led to approximately 90% inhibition of hepatic atRA formation in comparison to the control. Taken together this data suggest that AOX may have an important biological role in atRA synthesis in the liver and that inhibitors of AOX may impair endogenous retinoid homeostasis.

A10 - CHARACTERIZATION OF DIFFERENTIAL TISSUE ABUNDANCE OF MAJOR NON-CYP ENZYMES IN HUMAN**Naveen Neradugomma**¹, Abdul Basit¹, Ryan Takahashi², Peter Fan³, Bill Smith⁴, Bernard Murray⁴, Scott Heyward⁵, Chris Wolford¹, Edward J. Kelly¹, and Bhagwat Prasad¹¹University of Washington, USA, ²Genentech, USA, ³Merck, USA, ⁴Gilead, USA, ⁵BioIVT, USA

Non-cytochrome-P450 (non-CYP) drug metabolizing enzymes (DMEs), such as UDP-glucuronosyl transferases (UGTs), sulfotransferases (SULTs), carboxylesterases (CESSs) and aldehyde oxidase (AO) can metabolize nearly 30% of clinically approved drugs. Current drug discovery paradigms efficiently reduce the liability of drugs to CYP-mediated biotransformation, thus potentially leading to more drug candidates being prone to non-CYP metabolism. However, very little is known about the expression levels and inter-individual variability of non-CYP DMEs. The data for extrahepatic expression patterns of these enzymes is even more limited. This leads to risks of inaccurate prediction of drug metabolism. Therefore, the main objective of this study is to quantify differential tissue abundance and inter-individual variability of major non-CYP DMEs by LC-MS/MS quantitative proteomics. Twenty-one non-CYP DMEs, namely AO, CES1 and 2; UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9 and 1A10; UGT2B4, 2B7, 2B15, 2B17 and 2B28; SULT1A1, 1A3, 1B1, 1E1, and 2A1 were analyzed in the study. These non-CYP enzymes were quantified in S9 fractions and/or microsomes from human liver (n=22), kidney (n=21), heart (n=15) (UW tissue bank), intestine (n=14) and lung (n=11) (BioIVT) by a validated LC-MS/MS assay method. We used S9 fractions for the comparison of tissue abundances of the selected non-CYP DMEs, where liver S9 fraction was used as a reference sample. Since intestinal samples were only available as microsomes (HIMs), these data were scaled to derive the corresponding S9 values prior to data analysis. We used calnexin and calreticulin marker levels to ensure quality of HIM preparation and compared the protein abundances using HLM as a reference sample. Data were analyzed by Skyline software. The inter-tissue data are presented as protein abundance per mg of total S9 protein. AO was quantifiable in liver >kidney (100:13; per mg total S9 protein) but was not quantified in intestine due to non-availability of S9 fractions. CES1 was expressed in liver>lung>heart>intestine~kidney (100:5:2:0.6:0.6), whereas CES2 was expressed in intestine>liver>heart>kidney (567:100:77:59). The inter-individual variability for AO, CES1 and CES2 expression in these tissues was in the range of 2-19-fold. Among the UGT1A proteins; 1A4 and 1A6 were only detected in liver. UGT1A1 and 1A3 were expressed in both liver>intestine (100:43 and 100:13). UGT1A9 was expressed in liver<kidney (100:120) and UGT1A10 was intestine specific. Among UGT2B proteins, 2B4 and 2B15 were only detected in liver, UGT2B7 was found in liver> kidney>intestine (100:22:18) and UGT2B17 expression was higher in intestine than liver (473:100). UGT1A7, UGT1A8 and UGT2B28 were not detectable in any sample. UGT isoforms were highly variable with UGT2B17 being the most variable DME (>300-fold). Among the cytosolic proteins, SULT1A1 was detected in liver>lung (100:33) and SULT1B1 only detected in liver; however, SULTs were not quantified in intestine due to non-availability of S9 fractions. Inter-individual variability of SULTs was within 7-fold. This is the first report to provide quantitative values for various non-CYP DME proteins in liver, intestine, kidney, heart and lung. These data are important for the development of a whole body based physiologically based pharmacokinetic (PBPK) models to predict non-CYP mediated drug disposition.

A11 - OPTIMIZED RENAL TRANSPORTER QUANTIFICATION BY USING AQUAPORIN 1 AND AQUAPORIN 2 AS ANATOMICAL MARKERS: APPLICATION IN CHARACTERIZING THE ONTOGENY OF RENAL TRANSPORTERS AND ITS CORRELATION WITH HEPATIC TRANSPORTERS IN PAIRED SAMPLES**Cindy Yanfei Li**¹, Chelsea Hosey-Cojocari², Abdul Basit¹, Jashvant D. Unadkat¹, J. Steven Leeder², and Bhagwat Prasad¹ ¹University of Washington, USA, ²Children's Mercy Hospital and Clinics, USA

Background: Renal transporters, which are primarily located in the proximal tubules, play important roles in secretion and nephrotoxicity of drugs. Although the ontogeny of hepatic transporters has been examined, no characterization of renal transporter ontogeny has been performed in human. As such information is critical in predicting age-related drug secretion and tissue accumulation, the goal of this study was to characterize the protein abundance of human renal transporters across different ages. Methods: A total of 43 human kidneys, 22 of which were paired with livers from the same donors, were obtained and classified into three age groups: children (<12 years), adolescents (12 to <18 years), and adults (>18 years). Protein abundance of kidney specific anatomical markers Aquaporin 1 and 2 (AQP1, a marker of proximal tubule; AQP2, a marker of distal tubule) and transporters was quantified by quantitative LC-MS/MS proteomics. Renal transporters quantified included organic anion transporters (OAT1, OAT3, OAT4), organic cation transporter 2 (OCT2), organic/carnitine cation transporters (OCTN1 and OCTN2), organic anion transporter polypeptide (OATP4C1), multidrug resistance proteins (MDR1 and MRP2), and multidrug and toxin extrusion protein (MATE1). Quantitative proteomics was also applied to paired livers to quantify protein abundance of organic anion transporting polypeptides (OATP1B1, OATP1B3, and OATP2B1), OAT2, OCT1, bile salt export pump (BSEP), MDR1, MRP2, MRP3, and MATE1. The Grubbs' test was used to identify outliers (p<0.05) based on AQP1 and AQP2 data. Protein abundance across different age groups was compared by Kruskal-Wallis followed by Dunn's multiple comparisons test. The protein-protein correlation was analyzed using Spearman correlation test. Results: Six out of 43 kidney samples were identified as outliers (Grubbs' test) that significantly differed from the others with relatively higher expression of AQP2 over AQP1. The renal transporters were consistently lower in these six samples, which confirmed that these cortex samples likely were

contaminated by medulla. No significant age-related changes were observed for renal transporter abundance, albeit only OCT2 abundance was modestly lower (<1.5-fold) in adults than that in adolescents. The liver MRP3 abundance was higher (~2-fold) in adults than in children. The latter along with our functional data (unpublished) on efflux of steroid glucuronides suggest that MRP3 could be regulated by sex hormones. Higher protein-protein correlation was observed in kidney as compared to those in paired livers. Specifically, the protein expression of OAT1 and OAT3 in kidney was significantly correlated ($r > 0.8$), which was also observed for OATP4C1 and MRP2, MDR1 and MRP2 in kidney. The abundance of transporters expressed in both liver and kidney (i.e., MDR1, MRP2 and MATE1) was not correlated in paired samples. Conclusions: The use of AQP1 and AQP2 provides a reliable method for identifying kidney tissues with significant contamination of the distal tubules. Although kidney transporter abundance was not different across ages (>2 years), the abundance and protein-protein correlation data can be used in physiologically based pharmacokinetic (PBPK) modeling. Further investigations are required to characterize ontogeny of renal transporters in children < 2 years. (Supported in part by UWRAPT which is funded by Gilead, Genentech, Biogen, Takeda and Merck)

A12 - MECHANISTIC KIDNEY MODELS FOR CREATININE: RELEVANCE OF OCT2 TRANSPORT DRIVING FORCE TO PREDICTION OF CREATININE-DRUG INTERACTIONS

Daniel Scotcher¹, Vikram Arya², Xinning Yang², Ping Zhao³, Lei K. Zhang², Shiew-Mei Huang², Amin Rostami-Hodjegan¹, and Aleksandra Galetin¹

¹University of Manchester, United Kingdom, ²US Food and Drug Administration, USA, ³Bill & Melinda Gates Foundation, USA

Creatinine is widely used as a clinical biomarker of glomerular filtration rate and renal function. However, active renal secretion contributes on average 9% to its renal clearance. Drug-mediated inhibition of renal transporters has been associated with transient elevations in serum creatinine. Distinguishing creatinine elevations caused by transporter inhibition from those resulting from renal toxicity is important within drug development and clinical practice. The current study developed a physiologically-based creatinine model that accounts for roles of organic cation transporter 2 (OCT2), organic anion transporter 2 (OAT2) and multidrug and toxin extrusion (MATE)1 and MATE2-K transporters expressed in proximal tubule cells. The models described OCT2 kinetics using either conventional assumption (i.e., uptake only OCT2) or by accounting for electrochemical gradient driven transport (i.e., bidirectional OCT2). The models were developed in a step-wise manner, using combination of forward and reverse quantitative translation approaches. Subsequently, the mechanistic creatinine models were evaluated by assessing their ability to predict creatinine-drug interactions for a set of 10 perpetrators. The overall performance of creatinine models was comparable regardless of OCT2 directionality assumptions; 59% and 51% of n=117 measurements of changes in serum creatinine from 22 clinical studies were within prediction limits for the uptake-OCT2 and bidirectional-OCT2 models, respectively. However, only the bidirectional-OCT2 model successfully predicted that ranitidine has minimal interaction with creatinine. Current study developed the most mechanistic models of creatinine renal disposition to-date providing a framework for evaluating mechanistic hypotheses concerning the various processes in creatinine renal disposition, and their interplay.

P1 - DEVELOPMENT OF A HIGHLY SENSITIVE LC/MS/MS ASSAY FOR PEGYLATED FGF21 PROTEIN MEASUREMENT FROM BIOLOGICAL MATRICES

Xiaomei Gu, Renu Singh Dhanikula, and Yue-Zhong Shu
Bristol-Myers Squibb, USA

A PEGylated FGF21 protein consisting of human fibroblast growth factor 21 (FGF21) with polyethylene glycol (PEG) attachment was in clinical development as a treatment for Non-Alcoholic Steatohepatitis (NASH). The C-terminus region of FGF21 is responsible for interaction with β -Klotho, which stimulates glucose uptake and reduces transcription of CYP7A, and it is proteolytically processed *in vivo*, resulting in the loss of activity. The *in vitro* and *in vivo* the PEGylated FGF21 data showed truncation at C-terminus in all species. Therefore, it is of great interest to measure the C-terminus region of the intact protein and demonstrate PK and PD relations.

We developed a bioanalytical assay that utilized LC/MS/MS to measure a surrogate target peptide, formed from the C-terminus via acid hydrolysis. Using peptide mapping approach, we found out that low concentration of hydrochloric acid in 7 M guanidine chloride was much more selective for acid-labile aspartyl-prolyl amide bond cleavage. By further optimizing acid concentration and digestion temperature, we were able to achieve detection as low as 5 ng/mL of the intact PEGylated FGF21 in biological matrices. Protein precipitation with acidified N-propanol was proven to be effective in removing interference from endogenous proteins. The assay with a dynamic range established at 5–50000 ng/ml is being used for *in vivo* and *in vitro* investigative studies assessing proteolytic cleavage of the PEGylated FGF21 protein.

P2 - QUANTITATIVE ANALYSIS OF A THYROID HORMONE BIOMARKER EXTRACTED FROM WHOLE BLOOD USING A MITRA MICROSAMPLING DEVICE

Jason Watts, Ryan Schmich, Katherine Yahvah, and Chad Christianson
Alturas Analytics, Inc., USA

Thyroid hormones are critical regulators of metabolism, growth, and development. Thyroxine (T4) and 3,3',5-triiodothyronine (T3) are produced in the thyroid gland and released into circulation where the more biologically active form, T3 exerts effects on peripheral tissues. The serum level of T4 is a useful biomarker of overall thyroid function. Low or high levels of circulating thyroid hormones are indicative of thyroid or pituitary gland dysfunction resulting from disease or malnutrition. Hyperthyroidism or hypothyroidism can result from autoimmune disorders, such as Graves' disease, certain medications, thyroid cancer, and can often occur during pregnancy. Individuals suffering from thyroid dysfunction suffer from a broad range of symptoms including weakness and fatigue. The Mitra Microsampling Device (MMD) enables convenient and accurate at-home sample self-collection from a simple finger prick. Here, T4 was extracted from whole blood collected using an MMD and analyzed by MFLC-MS/MS. Thyroxine (T4) was spiked into whole blood and 10 microliters absorbed onto a Mitra Microsampling Device. The MMD was dried at ambient temperature and then placed into 500 microliters of methanol containing internal standard. After incubation for 30 minutes on a shaker, 400 microliters of the extract was transferred to a 96 well plate, evaporated to dryness, and reconstituted with 100 microliters of 80:20 Methanol:H₂O with 0.1% NH₄OH. The extract was analyzed on an API-6500 mass spectrometer operating in positive ESI mode equipped with and Optiflow source. The MFLC system was a Waters M Class operating with binary gradient method and a flowrate of 50 microliters/minute. Separation was achieved using a Phenomenex Kinetex Biphenyl column (5 cm x 1.0 mm, 3 μ m). The method is linear from 0.50 to 500 ng/ml with an r-value of 0.9973. Measurement of multiple lots of human whole blood using this method revealed endogenous T4 levels of 18.9 ng/ml. This method provides sensitive and accurate quantification of thyroid biomarkers utilizing a simple, at-home sample collection requiring only 10 microliters of sample.

P3 - OPTIMIZATION OF THE SAMPLE COLLECTION PROCEDURES FOR SELECTIVE BCRP INHIBITOR, KO143, IN RODENTS

Alicia Pietrasiewicz, Christopher Rowbottom, Elvana Veizaj, and Doug Burdette
Biogen, USA

Ko143 is known as a potent and selective inhibitor of the Breast Cancer Resistance Protein (BCRP) and can be used in the chemical inhibition towards a systemic knock down of the BCRP efflux transporter in preclinical species. In the presence of plasma, Ko143 is readily hydrolyzed and converted to its acid form which creates pharmacokinetic and bioanalytical challenges both *in-vivo* and *ex-vivo*. It has been previously reported that Ko143 is unstable *ex-vivo* in mouse plasma, and only detectable in rat up to 120 minutes *in-vivo* when dosed via IP(1). In-house development of an efficient bioanalytical method for the quantitation of Ko143 in rat plasma was needed to inform the design of future studies that involved the chemical inhibition of BCRP. The primary objective was to optimize Ko143 sample collection and handling procedures to prevent any additional degradation from occurring *ex-vivo*. The secondary objective was to develop a bioanalytical method for the acid form of Ko143 and determine the conversion rate of the ester to acid in both whole blood (WB) and plasma.

Stability of Ko143 was 90% lower in untreated plasma compared to WB when left on ice for 1 hour, while room temperature (RT) decreased stability in WB by an additional 20-fold. Comparison of that WB versus plasma derived by centrifugation at T0 displayed no difference in Ko143 concentration, but acid levels increased 8-fold. Addition of sodium fluoride (NaF) at 10 mg/mL to WB or plasma stabilized Ko143 levels and kept acid levels low up to 24 hours at 5°C. Interestingly, levels of acid also increased in treated samples where plasma was derived from WB as compared to levels in WB directly. NaF improved stability of Ko143 at both RT and 5°C, while acid levels increased only at RT. Therefore, increased acid in plasma derived from WB most likely occurred during centrifugation at RT. Other findings noted during method optimization included the impact of a freeze-thaw cycle, which decreased stability of Ko143 by 20%, and the percent of LC-MS/MS signal contribution due to the analytical interference between Ko143 and its acid form. When the concentration of Ko143 acid was at or above 4000 ng/mL, this correlated to a $\geq 90\%$ decrease of Ko143 which was seen in untreated plasma samples. If WB is spun down to plasma, it is critical that conditions are kept cold throughout all steps including centrifugation to prevent rapid acid conversion and Ko143 degradation. These data indicated that the ideal collection method for Ko143 is to collect samples into chilled tubes containing NaF at 10 mg/mL and conduct an immediate protein precipitation extraction of WB using acetonitrile on ice.

Reference:

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P4 - ELUCIDATION OF DOSE PROPORTIONALITY PHARMACOKINETICS AND SPECIES DIFFERENCES IN THE CYP-MEDIATED METABOLISM OF CLADRIN, A POTENT ANTI-OSTEOPOROTIC ISOFLAVONE

Mamunur Rashid, Sandeep Kumar Singh, Swati Chaturvedi, and Muhamad Wahajuddin
CSIR-Central Drug Research Institute, India

Flavonoids are phytochemicals which possess phenolic structures and have numerous pharmacological properties. A plethora of therapeutically active isoflavones such as formononetin, isofornonetin, cladrin, pruretin and cajanin have been isolated from *Butea monosperma*, abundantly found in Asia region. Structurally cladrin (3', 4' dimethoxy daidzein) related to formononetin and shows osteogenic activity. Cladrin (CD) improves microarchitecture of trabecular bones, cortical thickness, bone formation and induces expression of osteogenic genes in a dose-dependent manner. Thus cladrin could be a good candidate for osteoporosis and bone-related diseases. However, there is no complete preclinical pharmacokinetics of CD available in the literature. Therefore, we aimed to investigate dose proportionality pharmacokinetics, plasma protein binding blood partitioning of CD in SD rats. Dose proportionality study of CD was carried out at 5, 10 and 20 mg/kg and the maximum attainable plasma concentration (C_{max}) after oral administration was found to be 68.61 ± 4.64 , 184.00 ± 21.21 and 142.00 ± 31.11 ng/mL, respectively. The AUC of CD from zero time to last time point of analysis (AUC_{0-t}) at different doses was found to be 368.24 ± 10.53 , 919.70 ± 6.29 and 656.95 ± 179.70 h* μ g/L respectively. The absolute bioavailability of CD was found to be 16.58, 19.04 and 6.76% respectively. Cladrin was rapidly absorbed with a high apparent volume of distribution (15.03 ± 1.79 L/kg), low clearance (2.27 ± 0.30 L/h/kg) and high plasma protein binding, indicating that CD is a high extraction compound. CD was found to be absorbed in a reasonably linear manner up to 10mg/kg and afterward showed a decrease in the absorption. The decrease in AUC and C_{max} and higher CL of CD at 20mg/kg dose can be ascribed to a couple of reasons responsible for decreasing its bioavailability and saturable absorption. There are probably several reasons that it does not follow dose proportionality due to solubility constrain, saturation of drug in the gastric region and may be due to efflux/influx transporter which causes a barrier for drug absorption. Thus CD is a potent compound that can be used for osteoporosis and bone-related disease irrespective of its low bioavailability. Metabolic stability of CD was performed in rat dog, rabbit, mice, and human microsomes to illustrate its efficacy and safety. The observed Clint and hepatic Clint in the rat, dog, rabbit, mice, and human liver microsomes were found to be 0.008, 0.016, 0.049, 0.017, 0.010 and 19.20 ± 0.34 , 38.85 ± 3.85 , 121.27 ± 3.50 , 41.30 ± 0.35 and 24.0 ± 1.75 μ L/min/mg respectively. These observed data suggest that CD has low clearance in the presence of NADPH in liver microsomes for of all species. The metabolite identification was performed on UPLC for the separation and LC-MS/MS using Light Sight VR software for the identification. Two major phase I metabolites M1 and M2 were identified in all species in common. The metabolites are identified as Oxidation (M1) demethylation (M2) with hydroxylation. Further confirmation of the exact position of the oxidation and hydroxylation using 2D-NMR and exploration of phase II metabolism are in progress.

P5 - USE OF THE H_μREL® HUMAN HEPATOCYTE CO-CULTURE MODEL TO DETERMINE INTRINSIC CLEARANCE AND ELUCIDATE METABOLIC PATHWAYS OF STABLE COMPOUNDS

Philip Butler, Jo Wilcock, Lara Ward, Katie Paine, Marks Vahmjanins, and Anna Kerins
Cyprotex, United Kingdom

Hepatocyte suspensions are commonly used to predict clearance of new chemical entities. However, incubation times are relatively short, in order to maintain cell viability and enzymatic activity, which may limit the accuracy of prediction of clearance for stable compounds. The aim of this work was to determine the clearance of a range of compounds in three

systems; H μ RELhumanPool™ co-culture (72 h), 2D monoculture (24 h) and suspension (2 h), utilising the same batch of cryopreserved human hepatocytes (5-donor pool). Data generated from these assays were then scaled to predict *in vivo* human clearance to assess the suitability of each system in accurately determining clearance of stable compounds. Both plated systems predicted *in vivo* clearance well, following regression correction, however the H μ REL® human hepatocyte co-culture model demonstrated superior assay sensitivity, enabled by a longer incubation time in comparison to the 2D monoculture. Following regression correction, human *in vivo* clearance was predicted within 2-fold for 12 out of 13 compounds assessed in the H μ REL® co-culture model.

Furthermore, the utility of the H μ REL® co-culture model as an *in vitro* model for the generation of metabolites for stable compounds was demonstrated. Eight compounds were incubated in the three *in vitro* systems and metabolic profiling was performed using high resolution accurate mass spectrometry. The benefit of the longer incubation time of the H μ REL® co-culture system on the sensitivity of detection of metabolites, which were not observed in the other *in vitro* systems, was demonstrated. For example, diazepam, disopyramide, tolbutamide and warfarin were all shown to form metabolites in the H μ REL® co-culture system that were either present in lower amounts or entirely absent in 2D monoculture or suspensions of human hepatocytes.

These results demonstrate the application of the H μ REL® co-culture model, both in determining the clearance of stable compounds and in establishing the metabolic profile of a compound, realised by the longer incubation period.

P6 - Comparison of techniques to assess non-specific microsomal binding

Tom Chan, Andrea Whitcher-Johnstone, Young-Sun Scaringella, and Mitchell Taub
Boehringer Ingelheim Pharmaceuticals, USA

Determining non-specific binding of drug candidates to human liver microsomes (HLM) is important for estimating accurate enzyme kinetic parameters such as K_i , K_I and K_m , parameters that are used to predict *in vivo* drug-drug interactions and clearance. Equilibrium dialysis (ED) is considered the gold standard method to determine non-specific microsomal binding; however, this is a highly laborious technique. Several alternative methods have been established to estimate non-specific microsomal binding: *in silico* approaches relying on Log P or Log D values, filtration, ultracentrifugation, artificial HLM systems, and extrapolation from Clint and high-throughput equilibrium dialysis (RED). In this study, the above-mentioned approaches to estimate non-specific microsomal binding were compared to ED. The results indicate that for acidic compounds, low non-specific binding to microsomes contributed to overall high consistency of the results across methods. In contrast to acidic compounds, for neutral and basic compounds, a trend towards higher microsomal binding was associated greater variability of data across methods, particularly as HLM concentration was increased. The RED system generally produced the most similar results to ED; however, close estimations (within 0.8-1.2 x the value obtained using ED) could be achieved with the other evaluated methods depending on the compound and HLM concentrations used. Interestingly, no alternative method including RED was capable of producing similar results to ED for a highly bound compound, e.g. BIRT2584. For particularly challenging compounds such as BIRT2584, it may be necessary to rely on ED to achieve the most accurate prediction of non-specific binding to microsomes.

P7 - UTILIZATION OF A MICROPHYSIOLOGICAL SYSTEM TO MODEL RENAL SECRETION OF OPIOIDS IN HUMANS

Tomoki Imaoka, Sara Shum, Shih-Yu Chang, Alenka Chapron, Catherine K. Yeung, Jonathan Himmelfarb, Nina Isoherranen, and Edward J. Kelly
University of Washington, USA

Opioids are among the most commonly prescribed and administered drugs in the US for clinical pain management. With the increasing use of opioids in clinical situations, opioid overdose, dependence and addiction have become significant public health concerns. It is well recognized that special populations, such as the elderly and patients with chronic kidney disease (CKD) are at higher risk of opioid overdose. Understanding the specific elimination pathways is essential for accurate prediction of opioid pharmacokinetics in these special populations as a strategy for mitigating risk of opioid overdose. With respect to morphine and oxycodone, detailed pharmacokinetic parameters are not entirely understood although UGT2B7 and CYP3A4/2D6 in the liver are the primary routes for clearance of morphine and oxycodone, respectively. This study aimed to define characteristics of renal secretion of morphine, oxycodone and their metabolites using a vascularized human proximal tubule microphysiological system (VPT-MPS) we have recently developed. The VPT-MPS is composed of human proximal tubule epithelial cells and human umbilical vein endothelial cells in side-by-side channels. Morphine, oxycodone and their metabolites were administered to vascular channel of VPT-MPS and effluents from proximal tubule channel were sequentially collected and analyzed by LC/MS/MS. Efflux of morphine, oxycodone and their metabolites were observed in a time-dependent manner. Interestingly, efflux of morphine and morphine 6-glucuronide (M6G) was inhibited by coadministration of tetraethylammonium (organic cation transporter inhibitor) and/or probenecid (organic anion transporter inhibitor), while inhibitory effects were minimal for oxycodone and oxymorphone, suggesting carrier-mediated active secretion of morphine and M6G. In addition, *in vitro-in vivo* extrapolation analyses were carried out to evaluate predictability performance of VPT-MPS, which revealed that VPT-

MPS gave good prediction of observed *in vivo* clearance with ratio of predicted/observed < 3. Ongoing studies include using a linked liver>kidney MPS to replicate sequential hepatic metabolism and renal clearance *ex vivo* to generate a quantitative systems pharmacology model of opioid disposition.

P8 - INTERSPECIES DIFFERENCES IN RENAL CLEARANCE BASED ON PHYSICOCHEMICAL DRUG PROPERTIES

Katja Jansen¹, Carla Pou Casellas², Kim Wever³, Lucianne Groenink¹, and Rosalinde Masereeuw¹

¹Utrecht University, Netherlands, ²UMC Utrecht, Netherlands, ³Radboud UMC, Netherlands

Before new drugs are approved for human use, various animal models are applied to study drug efficacy and safety. Renal clearance (CL_r) is a standard safety measure since a significant part of drugs is cleared by the kidneys. It is assumed that glomerular filtration rate and metabolic waste removal are determined by metabolic rate, which in turn scales linearly to body weight. Thus, human CL_r should be predictable based on body weight. However, there are biological differences between species which could be reflected in pharmacokinetics. Our aim was to identify species-specific limitations in human CL_r extrapolation.

Using PubMed and EMBASE, we systematically reviewed human and animal studies that have reported CL_r or related outcome measures for 20 renally excreted drugs with different physicochemical properties. Based on the human data and simple allometry, we assessed how much animal models deviate from the expected value, and correlated these differences to the properties of the drugs.

In total, we included 264 studies with human or animal CL_r data for the selected drugs. For each animal species, we calculated average fold errors per drug, as well as the mean differences with 95% confidence intervals compared to humans. These mean differences varied between -0.56 and 1.74 for mice, -0.24 and 2.86 for rats, -0.77 and 0.57 for rabbits, -0.78 and 2.33 for dogs, and -0.55 and 0.47 for monkeys. Subgroup analyses based on physicochemical drug properties revealed significant alterations between average mean differences, hinting towards critical physiological interspecies differences at play.

This systematic meta-analysis presents a novel approach to quantify interspecies differences. We showed that rats tend to overestimate human CL_r. In general, however, animals are good predictive models for renal drug excretion.

P9 - EFFECTS OF HISTONE DEACETYLASE INHIBITOR ON UGT1A1 AND UGT1A6 EXPRESSION IN CACO-2 CELLS

Miki Katoh, Yuki Asai, Shoko Goto, and Masayuki Nadai

Meijo University, Japan

Histone deacetylase (HDAC) inhibitors are used to treat various cancers such as lymphoma and myeloma. In addition, HDAC inhibitors have been recently suggested to elicit neuroprotective activities via enhancing synaptic plasticity and memory, and thus could be potential therapeutic agents for several neurological disorders including Alzheimer's and Parkinson's disease. Valproic acid (VPA), a drug used to treat epilepsy, is a well-known powerful histone deacetylase inhibitor. UDP-glycosyltransferase catalyzes the covalent addition of sugars to a given drug and affects the pharmacokinetic properties of the drug. However, there are few reports on the effects of HDAC inhibitors on drug conjugation. In the present study, we aimed to clarify the effects of HDAC inhibitors on UGT1A1 and UGT1A6 expression in Caco-2 cells. The HDAC inhibitors used were apicidin, MS-275, SAHA, sodium butyrate, trichostatin A, and VPA. Caco-2 cells were treated with these inhibitors for 72 h, and total RNA was isolated from the cells and UGT expression was estimated by real-time PCR. Although UGT1A1 mRNA expression was not altered by the HDAC inhibitors except by VPA (0.52-fold), UGT1A6 mRNA expression was increased by all the HDAC inhibitors (apicidin, 3.0-fold; MS-275, 2.7-fold; SAHA, 2.8-fold; sodium butyrate, 3.3-fold; trichostatin A, 2.2-fold; and VPA, 2.3-fold). This indicated that HDAC inhibitors affect the expression of UGT1A6, but not of UGT1A1. Serotonin glucuronidation, which is catalyzed by UGT1A6, was increased by apicidin, MS-275, sodium butyrate, and VPA, but not by SAHA and trichostatin A. The induction ratio of serotonin glucuronidation by treatment with VPA was high, and therefore, the mechanism of induction was also investigated. It has been reported that VPA increases the phosphorylation of extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK). Hence, the p38 mitogen-activated protein kinase inhibitor SB203580, JNK inhibitor SP600125, or ERK inhibitor U0126 was co-administered with VPA to determine the effects of these inhibitors on VPA-induced UGT1A6 mRNA expression. However, these inhibitors did not affect UGT1A6 induction by VPA, suggesting that the pathway involving mitogen-activated protein kinase was not responsible for this induction. UGT1A6 is one of the erythroid 2-related factor 2 (Nrf2) target genes. VPA treatment increased Nrf-2 and heme oxygenase-1 mRNA expression by 2.3- and 2.0-folds in Caco-2 cells. Therefore, the induction of UGT1A6 may be caused by the activation of Nrf2.

P10 - USE OF MICROBIAL BIOTRANSFORMATION TO PRODUCE SCALABLE QUANTITIES OF A GLUCURONIDATED METABOLITE OF THE CLINICAL-STAGE SOLUBLE GUANYLATE CYCLASE INHIBITOR PRALICIGUAT

Julia Shanu-Wilson¹, Jonathan Steele¹, Richard Phipps¹, Ravi Manohar¹, Deepa Karunakaran¹, Alicja Borowik¹, Frank Scheffler¹, Liam Evans¹, Andrew Carvalho², and Ali Banijamali³

¹*Hypha Discovery Limited, United Kingdom*, ²*Cyclerion Therapeutics, USA*, ³*Akebia Therapeutics, USA*

In recent years, FDA guidance has advised initiating human metabolite profiling earlier in drug development, emphasizing the importance of metabolite identification and quantification to evaluate a drug metabolite's safety and pharmacological activity.

Praliguat (IW-1973) is a soluble guanylate cyclase stimulator in Phase 2 clinical trials for diabetic nephropathy and heart failure with preserved ejection fraction (HFpEF). During studies on metabolism of praliguat in preclinical species and in human hepatocytes, a prominent direct O-glucuronide metabolite was detected. Initial attempts by Cyclerion to isolate the metabolite in rat bile, via administration of praliguat to bile duct-cannulated rats, lacked scalability and resulted in low yields. Access to larger quantities of this glucuronide metabolite for structure elucidation and pharmacological activity was achieved through microbial biotransformation of the parent compound.

Initial screening of praliguat against a glucuronidating panel of filamentous microbes identified four strains capable of producing the desired metabolite whilst two other strains produced oxidized metabolites. One actinomycete strain produced the O-glucuronide in excellent yield without further chemical modifications. Following confirmation of the correct metabolite, the producing organism was scaled up to produce 66 mg of the O-glucuronide from a 4L fermentation. A further 6L scale-up was subsequently performed to provide an additional 118 mg of the O-glucuronide at > 95% purity, as determined by ELSD and 1H NMR spectroscopy. The purified glucuronide metabolite produced by the actinomycete strain was used as a quantitative analytical standard for clinical sample analysis and for other pharmacological and DMPK studies.

P11 - EXPANDING THE PHENOTYPING TOOL KIT FOR GLUTATHIONE S-TRANSFERASES

Timothy Strelevitz, Matthew A. Cerny, and Martin Dowty
Pfizer Inc, USA

In recent years targeted covalent inhibitors (TCIs) have been developed to selectively inactivate cysteine-containing drug targets. Due to the nature of the chemical warheads utilized, TCIs often undergo direct conjugation with glutathione (GSH) or are converted to GSH conjugates via glutathione S-transferase (GST). As GST-mediated metabolism may represent a major clearance pathway for these compounds and a number of GSTs are polymorphic, we sought to develop a GST phenotyping strategy using a panel of commercially available recombinant human GSTs (rhGST). To date, published methods to assess activity of rhGSTs have employed insensitive UV spectrophotometric assays using the pan-GST substrate, 1,2-chlorodinitro benzene (CDNB). Additionally, cumene hydroperoxide (CuOOH) is a commonly used substrate for GST T1 and T2 activities. To enable higher throughput and greater sensitivity, LC-MS/MS-based assays have been developed using both CDNB and CuOOH. Using these assays, we have characterized the GST-mediated metabolism of CDNB, CuOOH, and other GST substrates across the panel of rhGST. The utility of the CDNB and CuOOH assays will be discussed in the context of understanding relevant GST phenotypes as well as to study the potential for drug-drug interactions for GST mediated clearance compounds.

P12 - ABSTRACT WITHDRAWN**P13 - ASSESSMENT OF DRUG METABOLIZING ENZYME EXPRESSION AFTER HIGH-FAT FEEDINGS, AND AFTER A SWITCH BACK TO A NORMAL DIET IN RATS**

Hamdah Mohammed S Al Nebaihi, Zaid H. Maayah, Ayman O.S. El-Kadi, and Dion R. Brocks
University of Alberta, Canada

Purpose: To investigate the changes in mRNA and protein expression of drug metabolizing enzymes provoked by a high-fat (HF) diet, and whether diet normalization can overturn these changes in male and female rats. We hypothesized reductions in hepatic enzyme expression after a HF diet, with rebound increases after an additional 4 weeks of standard diet. Methods: Male and female Sprague Dawley rats (n=8 total per each) were started on a 14 week course of 45% HF rat chow with water starting at 1 month of age. At the end of the 14 week period, half of the animals were euthanized under isoflurane. In the remaining rats, the diet was switched to normal rat chow (fat content 13.4%). Four weeks after, those rats were euthanized. For each of the 14 and 18 week groups, control rats (n=4) were included where the diet was 13.4% fat content. Tissues were collected and frozen at -80°C. Liver microsomes were harvested and protein concentrations were determined using Lowry assay. Western blot was used to determine protein expression of hepatic enzymes. Those proteins measured were those involved in the metabolism of many drugs (CYP2E1, CYP3A1, CYP1A2,

CYP2C11, CYP2C12 and CYP2D1). Liver mRNA for selected cytochrome P450 (CYP3A2, CYP1A2, CYP2C11, CYP2C12) were determined via real-time PCR. Results: The HF diet led to significant increases in the body weights of male and female rats (+28% male, +20% female). After 14 weeks on HF diet, the protein expressions of CYP3A1, CYP1A2, CYP2C11 and CYP2C12 were significantly ($p < 0.05$) diminished in both male and female rats ($\geq 40\%$ reduction compared to control group using unpaired student t-test). Expression of CYP2D1 was significantly reduced in male but not in female rats. The level of mRNA of CYP3A2 and CYP1A2 exhibited a significant reduction in both male and female rats while CYP2C12 was more than three times higher than control female rats. Switching the diet to normal chow led to no further increase in body weight in male rats, and a significant decrease in female rats. Interestingly, the mRNA and protein expression levels reverted back to normal levels when the standard diet was implemented from weeks 14 to 18. Conclusions: Diet-induced obesity was associated with a reduction in some metabolizing enzyme expressions which may give rise to a reduction in the efficiency of drug metabolism. Adopting a leaner diet can cause normalization of enzymes involved in drug metabolism even if body mass is elevated as was the case for the male rats.

P14 - EFFECT OF THE CYP2C8*3 VARIANT ON ASTHMA SYMPTOM CONTROL AND MONTELUKAST EFFICACY

Marysol Almestica, Cassandra Deering-Rice, and Christopher Reilly

University of Utah, USA

Asthma causes chronic inflammation of the airways and bronchial hyper-reactivity. Despite treatment with appropriate therapeutics, many asthmatics experience suboptimal control of their symptoms due in part to variation in genes that dictate drug disposition and/or response. We previously found an association between the CYP3A4*22 and CYP3A5*3 variants with improved asthma control among individuals being treated with fluticasone propionate (FP) and beclomethasone dipropionate (BDP), respectively. We hypothesized that additional genotype-phenotype correlations may exist for asthma drug metabolism pathways. Using TaqMan Open Array technology we assayed 170 different single nucleotide polymorphisms in approximately 1500 genomic DNA samples collected from children undergoing treatment for asthma. Multiple SNPs in the CYP3A gene family, as well as other cytochrome P450 enzymes, P450 reductase, the glucocorticoid receptor, and other asthma-related genes were assayed. We found a new association between variation in CYP2C8 and asthma control scores. For the CYP2C8*3 variant, which is defined by amino acid substitutions at Arg139(Lys) and Lys399(Arg), the mean asthma control scores were lower (i.e., better asthma symptom control) in patients expressing ≥ 1 copy of CYP2C8*3 allele, when compared to patients with the wild-type CYP2C8*1/*1 genotype (4.28 [n=845] vs. 3.32 [n=172]). Furthermore, when results were stratified by treatment with montelukast, patients with ≥ 1 copy of CYP2C8*3 exhibited lower mean asthma control scores (3.56 [n=55] vs. 5.44 [n=214] ($p=0.0017$)), an effect that was not observed for several other asthma controller medications. We found that cytochrome P450 (CYP) 2C8, the principle enzyme involved in the metabolism of montelukast, is expressed in small airway epithelial cells (SAEC) and its expression is inducible. Efforts are currently underway to study the role of CYP2C8 variation in montelukast clearance and efficacy in human lung cells. These, and future findings should further our long-term goal of improving treatment of asthma through better understanding of the mechanisms associated with sub-optimal clinical responses to current therapies.

P15 - VITAMIN D DEFICIENCY ALTERS CYP3A GENE EXPRESSION AND PHARMACOKINETICS OF DEXAMETHASONE

Kavya Annu^{1,2}, Jean Cai¹, Burgess Freeman¹, and Erin Schuetz¹

¹St. Jude Children's Research Hospital, USA, ²University of Tennessee Health Science Center, USA

Vitamin D (VD3) is known to cause drug interaction with CYP3A4 substrates, as it can induce intestinal CYP3A4. Additionally, VD3 supplementation increased CYP3A4 substrate clearance in VD3 deficient patients (Schwartz, 2009). VD3 deficiency is common worldwide in the pediatric population, including those with leukemias. Furthermore, chemotherapies used to treat leukemias (e.g glucocorticoids) cause a further erosion of VD3 through induction of CYP3A4, that metabolizes active forms of VD3. Although VD3 deficiency is found in 60-70% of cancer patients, and VD3 supplementation is suggested to treat deficiency, VD3's effect on the pharmacokinetics (PK) of CYP3A4-metabolized anti-leukemic therapies has not been evaluated. Herein we hypothesize that VD3 levels (deficiency vs. sufficiency) alter intestinal expression of VDR target genes, such as Cyp3a, thereby influencing the PK of orally administered CYP3A4-metabolized leukemia therapies. Chemotherapeutics such as dexamethasone (dex) and dasatinib were chosen for this study, as they are both orally administered and Cyp3a-metabolized. To evaluate changes in Cyp3a expression, we generated C57BL/6 VD3 sufficient (Suf) and VD3 deficient (Def) mice via diet manipulation and examined intestinal expression by quantitative real-time PCR. There was 47% lower duodenal Cyp3a11 expression in 10-week-old Def mice compared to the Suf mice. To study changes in dex PK, a discontinuous dosing (8mg/L) regimen (3.5 days on/3.5 days off), in drinking water similar to the clinical setting, was used. Plasma dex concentrations were determined by LC-MS/MS at days 0 (baseline), 4, 11 and 18 (after each dex dosing cycle). Overall plasma concentrations ranged from 2.6–201.5nM (outliers removed), similar to concentrations achieved in pediatric patients on a discontinuous dex regimen. Male Def mice had higher plasma dex levels ($p < 0.05$) compared to Suf mice with a mean of 31.7nM (N=8) and 12.43nM (N=11),

respectively, on day 4, while there was no difference in dex levels between groups at day 18. A similar trend was observed in females with the plasma mean dex concentration of 63nM (N=4) and 58nM (N=5) in Def vs. 52nM (N=4) and 38nM (N=5) in Suf mice on days 11 and 18, respectively. The plasma PK of dasatinib was evaluated after two separate single oral gavage doses of 10 mg/kg to determine the effects of VD3 status, sex and age (8wk vs 10 wk) using a nonlinear mixed effects PK modeling approach. Blood samples were collected at up to 24 hr. post-dose with N=3 mice/group/time point and assayed using LC-MS/MS. There was no influence of VD3 status or sex or age on oral dasatinib PK ($p < 0.05$ threshold level). We conclude that, in mice, VD3 levels alter intestinal Cyp3a expression, and can affect plasma exposure of oral dexamethasone but not dasatinib.

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P16 - DRUG-INDUCED LIVER INJURY ALTERS EXPRESSION AND ACTIVITIES OF DRUG-METABOLIZING CYTOCHROME P450 ENZYMES IN MOUSE LIVER AT DIFFERENT AGES

Yifan Bao¹, Xueyan Shao¹, Pei Wang², Junjie Zhu³, Jingcheng Xiao⁴, Jian Shi⁴, Lirong Zhang², Haojie Zhu⁴, Xiaochao Ma³, Jose Manautou¹, and Xiaobo Zhong¹

¹University of Connecticut, USA, ²Zhengzhou University, China, ³University of Pittsburgh, USA, ⁴University of Michigan, USA

Drug-induced liver injury (DILI) is a serious liver disease globally. The potential risk of DILI may be related to the expression and activities of drug metabolizing enzymes, especially cytochrome P450s (CYPs), which are responsible for metabolizing 50-60% of prescription drugs. However, the impact of DILI on the expression and activities of CYPs was not determined yet. The aim of this study is to fill in the knowledge gap. We used acetaminophen (APAP) as a model drug to induce DILI in male and female C57BL6/J mice at ages of day 10 (infant), 22 (child), and 60 (adult). DILI was detected by levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma with further confirmation by H & E staining in liver tissue sections. The expression of CYPs was measured by RT-PCR (mRNAs) and LC-MS/MS (proteins). The activities of CYPs were determined by the formation rates of metabolites from probe drugs of each CYP using LC-MS/MS. DILI was induced at mild to severe levels on a dose-dependent manner in 200, 300, and 400 mg/kg APAP treated groups at child and adult ages, but not at infant age. The age-related sensitivity to DILI is related to the expression and activity of CYP2E1, 3A11, and 1A2 to convert APAP to its toxic metabolite NAPQI in liver. At child and adult ages, significant decreases at levels of mRNA, protein, or activities of CYP2E1, 3A11, 1A2, 2C29, and 2B10 were found, which were correlated to severe levels of DILI. These results indicate that altered levels of CYPs in severe injured liver caused by drugs may result in alterations of therapeutic efficacy of drugs mediated by CYPs metabolism. Further studies are needed to confirm the findings in human patient populations with DILI and to understand the underlying molecular mechanisms of down-regulation of CYPs in liver by DILI.

P17 - IMPACT OF AGE ON CYP450 ACTIVITIES IN BOVINE LIVER, LUNG AND KIDNEY

Steven Hu

Zoetis, USA

Age has significant impact on the activity of metabolizing enzymes in animals. Cattle is an important livestock species that we are targeting in both growth performance and disease treatment. Cattle grow almost linearly from the new born to its slaughter age (~18 months). In this work, impact of age on the major metabolizing enzymes, cytochrome P450 (CYP450), in liver, lung and kidney of beef cattle has been evaluated. Livers, lungs and kidneys were collected and processed to microsomes from the ages of new born, 3-, 7-, 10- and 14-months (n=3/age group) of Angus beef cattle. The microsomal CYP450 contents were assessed using a photospectrometric method. Their CYP450 activities were evaluated using metabolism of specific substrates of CYP450 isoforms with LC/MS analysis. CYP450 contents and activities in bovine livers were much higher than those in their kidneys and lungs at different ages. CYP450 contents and activities were low in liver, lung and kidney of new born calves. Those contents and activities increased with age and varied by different developmental patterns with different CYP450 isoforms. The cattle had the maximum metabolizing capability and activity in all three organs at the age of around 7 to 10 months. These findings will be helpful in in-vivo study design and data evaluation in bovine drug research and development.

P18 - BIOTRANSFORMATION OF METHOXYLATED BROMODIPHENYL ETHER NATURAL PRODUCTS

Margaret James¹, Katherine Cisneros¹, and Vinayak Agarwal²

¹University of Florida, USA, ²Georgia Institute of Technology, USA

Bromodiphenyl ethers (BDEs) and their metabolites are found in the environment, arising from natural and anthropogenic sources. BDEs have been used as flame retardants, while hydroxylated bromodiphenyl ethers (OH-BDEs) and

methoxylated bromodiphenyl ethers (MeO-BDEs) are biosynthesized by marine organisms. The toxicity of BDEs and their metabolites have been investigated and there is evidence that OH-BDEs affect the thyroid hormone system. Our hypothesis was that MeO-BDEs would be O-demethylated by cytochrome P450, forming the OH-BDEs. The objective of this study was to examine the demethylation of 6-MeO-BDE47, 4-MeO-BDE68 and 2-MeO-BDE68 in human liver microsomes. Individual human liver microsomes from de-identified male donors aged 21 to 49 were used. Microsomes, 0.1 mg, were incubated with varying concentrations (10 to 500 μM) of the MeO-BDE and NADPH, 2 mM, in 0.1M HEPES buffer pH 7.4 at 37°C for 30 min. Reactions were stopped by addition of methanol and an internal standard, triclosan, final concentration 2.5 μM . Precipitated protein was removed by centrifugation. The filtered supernatant was analyzed by reverse phase LC/MS/MS with electrospray ionization and detection in the negative ion mode with multiple reaction monitoring. Rates of demethylation varied between individual human liver microsomal samples. In any one sample, the 6-MeO-BDE47 was most readily demethylated, followed by 4-MeO-BDE68 and 2-MeO-BDE68. Maximum rates of demethylation (V_{max}) varied from 5 to 100 pmol/min/mg protein and K_m values varied from 20 to 79 μM . Thus, people exposed to MeO-BDEs are likely to form the potentially toxic OH-BDEs. However, rates of glucuronidation and sulfonation of the OH-BDE products in human liver microsomes and cytosol are rapid (Cisneros et al 2019), suggesting exposure to these compounds will be brief, and they will not bioaccumulate. This research was supported in part by the United States Public Health service, grants R00-ES026620 (V.A.), UL1 TR001427 and TL1 TR001428, and by an Alfred P. Sloan Foundation research fellowship to V.A.

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P19 - DESIGN AND CHARACTERIZATION OF SELECTIVE MECHANISM-BASED INHIBITORS OF CYP4Z1

John Kowalski¹, Matthew McDonald¹, Helmut Hanenberg², Constanze Wiek², and Allan Rettie¹

¹University of Washington, USA, ²Heinrich Heine University, Germany

The cytochrome P450 4 (CYP4) family constitutes thirteen enzymes in humans that are typically involved in fatty acid and eicosanoid oxidation. CYP4Z1 is selectively expressed in human mammary tissue and is the most highly upregulated CYP4 gene in breast cancer. Our recent studies demonstrated that CYP4Z1-dependent metabolism of arachidonic acid generates 14,15-epoxyeicosatrienoic acid (14,15-EET), a metabolite shown to enhance tumor growth and metastasis (reference 1). Therefore, chemical inhibitors of CYP4Z1 would be expected to be of utility in deciphering the role of the enzyme in breast cancer. Our primary goal is the development of new CYP4Z1-specific inhibitors that will be useful for studying the (patho)physiological role(s) of this relatively unknown P450. We have synthesized a series of N-1-aminobenzotriazole (ABT) analogs, which behave as mechanism-based inhibitors of CYP4Z1. Inhibitors were screened for time-dependent inhibition using luciferin benzyl ether (2) to monitor CYP4Z1 activity and against other commercially available CYP4 enzymes to evaluate isoform selectivity utilizing pro-luciferins. Inhibition of hepatic CYPs was investigated via a cocktail approach in human liver microsomes. An aliphatic chain length of six to eight carbons is ideal for potency, while the addition of a carboxylic acid moiety to inhibitors confers substantial selectivity towards CYP4Z1. Our top hits have shifted IC_{50} values for CYP4Z1 of 200 nM and show dramatic time-dependent effects. The most potent, and selective, analog displays a $\text{KI} = 2.2 \mu\text{M}$, $\text{Kinact} = 0.15 \text{ min}^{-1}$, and partition ratio of ~ 25 for CYP4Z1. Addition of multiple nucleophilic trapping agents and dialysis failed to significantly abrogate CYP4Z1 inhibition by this analog. The currently known inhibitors of CYP4Z1 are promiscuous towards many CYP isozymes (1,2). The ABT-analog design we have employed places the location of bioactivation at an internal molecular site to direct oxidative attack by CYP4Z1. To mimic the identified CYP4Z1 substrates, a carboxylic acid moiety at the alkyl terminus was added. This enables selective recognition of these inhibitors by non-covalently heme bound CYP4 enzymes, particularly CYP4Z1. Ongoing studies are assessing inhibitory activity in recombinantly expressed CYP4Z1 purified to homogeneity and probing the specific mechanism of CYP4Z1 inactivation by these compounds from investigation of heme adduct formation/destruction. We have designed, synthesized, and characterized multiple time-dependent inhibitors that are highly selective for CYP4Z1. These will be useful tools to enable biochemical characterization of CYP4Z1 and help unravel the role that bioactive lipid metabolites play in breast cancer progression.

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P20 - DUAL EFFECTS OF METHYLATED INDOLES ON CYP1A1 IN HUMAN HEPATOCYTES

Barbora Vyhldalova, **Kristyna Krasulova**, Karolina Poulikova, Iveta Bartonkova, and Zdenek Dvorak
Palacky University, Faculty of Science, Czech Republic

The aryl hydrocarbon receptor (AhR) is a central regulator of many physiological functions, such as cell cycle, apoptosis, proliferation, differentiation, cell adhesion, immune response, circadian rhythm and DNA-repair. It is also involved in chemical and microbial defense of organism and in molecular pathogenesis of inflammatory bowel disease, cancer, diabetes, neurological diseases etc. We have recently reported the entire series of methylated and methoxylated indoles, comprising both xenobiotics and intestinal microbiota products, as agonists and/or antagonists of human AhR [1]. The aim of the current study was to examine the effects of mono-methylindoles (MMI) on AhR-CYP1A1 pathway in primary human hepatocytes. Indoles methylated at positions 4, 5, 6 and 7 were strong inducers of CYP1A1 mRNA and protein. Medium induction was achieved by 1-methylindole and 3-methylindole, while 2-methylindole had no effect. Catalytic activity of CYP1A1 in human hepatocytes incubated with MMI was dose-dependently increased by 1-methylindole and 7-methylindole, whereas inverse U-shaped, concentration-dependent profiles of CYP1A1 activity were observed for 4-methylindole, 5-methylindole and 6-methylindole. In human hepatocytes pre-incubated for 24 h with TCDD, all tested MMI dose-dependently inhibited the enzyme activity of CYP1A1, with IC50 values ranging from 1.2 μ M to 23.8 μ M. With exception of 1-methylindole, all MMI caused also dose-dependent inhibition of CYP1A1 activity in human liver microsomes. Single point inactivation assay revealed time-dependent inhibition of CYP1A1 by 4-methylindole and 5-methylindole. Collectively, we show that mono-methylated indoles display dual effects on CYP1A1 in primary human hepatocytes. On the one hand, they are AhR-dependent inducers of CYP1A1 gene, thereby, increasing the levels of CYP1A1 enzyme and its activity. On the other hand, MMI are inhibitors of CYP1A1 catalytic activity. We acknowledge financial support from Czech Science Foundation [19-00236S] and Palacky University in Olomouc [PrF-2019-003].

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P21 - DRUG LIBRARY SCREENING FOR HUMAN CYP1A1 SUBSTRATES

Dieter Lang and Natalia Jungmann
BayerAG, Germany

Cytochrome P450s (CYPs), a superfamily of enzymes, are involved in the biotransformation of endogenous and xenobiotic chemicals and responsible for the metabolic clearance of widely prescribed drugs. CYP1A1, one of the 3 isoforms of the CYP1 family, is widely believed to play an important role in the metabolism and activation of a whole variety of procarcinogens, e.g. polyaromatic hydrocarbons (PAHs) or aromatic amines. It is also known that CYP1A1 is highly inducible by endogenous and exogenous factors e.g. PAHs. However, CYP1A1 was so far not considered to play a significant role in the metabolic clearance of drugs. We recently demonstrated a highly variable expression of CYP1A1 protein in human liver microsomal preparations, and showed that CYP1A1 can play an important role in the metabolic clearance of selected drugs in humans, resulting in a high clinical pharmacokinetic variability. Therefore, we screened a library of about 450 marketed or previously marketed drugs using recombinant human CYP1A1 to investigate its substrate specificity. Surprisingly, about 50% of the drugs were substrates for CYP1A1 and about 20% were considered as good substrates. Good substrates were classified based on intrinsic clearance determination with partly very high efficiency in metabolism and in comparison to *in vivo* relevant CYP1A1 substrates showing a high pharmacokinetic variability. Human CYP1A1 seems to have a very broad substrate acceptance almost comparable to human CYP3A4. Therefore, CYP1A1 should be considered as an important enzyme in drug discovery and development.

P22 - ENDOBIOTICS FOR PHENOTYPING OF HUMAN CYTOCHROME P450 ENZYMES: USE OF METABOLOMICS FOR THE IDENTIFICATION OF NEW CYP2D6 ENDOGENOUS BIOMARKERS ON HEALTHY VOLUNTEERS

Jules Desmeules¹, Gaëlle Magliocco², Nasim Bararpour³, Flavia Storelli², Youssef Daali², and Aurélien Thomas⁴
¹UNIGE, Switzerland, ²Geneva University Hospitals, Switzerland, ³University Center of Legal Medicine, Switzerland, ⁴University Center of Legal Medicine, Switzerland

CYP2D6 metabolizes ~25% of all marketed drugs. There is an important variability in the activity of this enzyme among individuals. The cause of this variability might be environmental, genetic, ethnical or even related to a disease. The administration of a CYP2D6 probe drug (e.g. dextromethorphan) is a good way to characterize CYP2D6 phenotype. Nonetheless, it is relatively invasive and the vulnerable population (e.g. pregnant women) cannot be phenotyped in this manner. Therefore, finding an endogenous substance which is metabolized by CYP2D6 could replace usual phenotyping procedure using a probe drug. This study uses global metabolomics to find an endogenous metabolite of CYP2D6.

Plasma collected from 32 healthy volunteers before and after administration of paroxetine, a strong inhibitor of CYP2D6, are analysed by metabolomics using a LC-Q-Exactive HRMS. After data filtration and normalization, a volcano plot analysis was performed to select potential endogenous metabolites which are metabolized by CYP2D6.

3 hits were significantly down-regulated after paroxetine intake compared to baseline samples (all $P < 0.05$ FDR-adjusted). It appears that these features are either fatty acid, or bile acid. Seemingly, they all contain a hydroxyl group that may potentially have been catalysed by CYP2D6. However, it has not yet been possible to fully validate the chemical structure of these features (e.g. position of the hydroxyl group). Significant Pearson correlations between the urinary metabolic ratio DEM/DOR and the three different hits were also established (all $P < 0.05$).

Complementary strategies will be used to fully characterize the features identified in this study (e.g. NMR). Confirmation with in-vitro tests (e.g. supersomes) are currently in process in order to clarify the metabolic pathway.

P23 - EFFECT OF DEXAMETHASONE TREATMENT ON CYTOCHROME P450 3A-UDP-GLUCURONOSYLTRANSFERASE 1A INTERACTIONS IN RAT LIVER

Yuu Miyauchi¹, Yoshitaka Tanaka¹, Kiyoshi Nagata², Yasushi Yamazoe³, Peter Mackenzie⁴, and Yuji Ishii¹

¹Graduate School of Pharmaceutical Sciences, Kyushu University, Japan, ²Tohoku Medical and Pharmaceut. University, Japan, ³Graduate School of Pharmaceutical Science, Tohoku University, Japan, ⁴Coll. Med. & Pub. Health Flinders Univ., Australia

Cytochrome P450 (P450, CYP) and UDP-glucuronosyltransferase (UGT) play crucial roles in drug metabolism, contributing to the metabolism of 70% of therapeutic drugs. There are large inter-individual differences in their *in vivo* functions, which are difficult to explain by differences in hepatic mRNA levels alone. To address this issue, we have been focusing on P450-UGT interactions as a novel post-translational regulation which can affect both P450 and UGT functions. Our last study revealed that UGT2B7 suppresses CYP3A4 activity by inhibiting substrate-binding to the P450 [1]. However, it remains unclear whether other UGT isoforms also suppress CYP3A4, and whether such a P450-UGT interaction can be observed *in vivo*. In this study, we have co-expressed CYP3A4, NADPH-cytochrome P450 reductase, and UGT1A9, another important human UGT, in a baculovirus-insect cell system. We prepared homogenates and microsomes from virus-infected cells, and compared CYP3A4 activity in the presence and absence of UGT1A9 to determine the role of UGT1A9 on activity of the P450. As with UGT2B7, UGT1A9 significantly suppressed CYP3A4. However, the effects of truncation of the C-terminal transmembrane and cytoplasmic regions were different between UGT1A9 and UGT2B7. The truncation mutant of UGT1A9 retained significant suppressive effects like the wild-type UGT, whereas this effect was attenuated with UGT2B7 truncation, suggesting that the mechanism underlying UGT-mediated suppression of CYP3A4 is UGT-specific. Next, we analyzed hepatic P450-UGT interactions in Wistar rats. Rats were treated with dexamethasone 21-phosphate (80 mg/kg, i.p.) for four days. Control rats were treated with vehicle, saline. One day after the last treatment, livers were removed and microsomes were prepared by differential centrifugation. Immunoblotting indicated that dexamethasone-treatment resulted in a 10-fold induction of hepatic CYP3A, while UGT1A was induced at most 4-fold. We carried out kinetic analyses and compared CYP3A turnover, activity per P450 unit, between the groups. CYP3A4 turnover was about 2-fold higher in the dexamethasone-treated group than control, implying that hepatic CYP3A-UGT1A interaction was partially canceled by the treatment, and that UGT-free and unsuppressed CYP3A may show high turnover. In conclusion, we have shown that UGTs suppressed CYP3A4 in an isoform-dependent manner, and that some xenobiotics could affect hepatic P450-UGT interaction. It is expected that studies of P450-UGT interactions may improve our understanding of the mechanisms leading to large inter-individual differences in enzyme activities in the future.

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P24 - ADVERSE EFFECTS OF SYNTHETIC CANNABINOIDS RELATIVE TO Δ^9 THC ARE ASSOCIATED WITH ATYPICAL METABOLIC PROFILES AND PROPERTIES AT CANNABINOID RECEPTORS CB1 and CB2

Anna Radomska-Pandya and Paul Prather
University of Arkansas for Medical Science, USA

There has been emerging interest in the application of cannabinoids in medicine, and several have been tested as drugs for a variety of disease processes. Classical cannabinoid use is associated with few adverse effects as compared to synthetic cannabinoids (SCBs), which are rapidly emerging drugs of abuse, that can result in extreme agitation, hallucinations, tachycardia, syncope, and seizures. To potentially explain the higher toxicity associated with SCBs as compared to Δ^9 THC, we tested the hypothesis that SCBs exhibit distinct metabolic profiles and atypical pharmacodynamic properties at CB1 and CB2 receptors. The metabolism of SCBs and the biological activity of their metabolites, produced by cytochrome P450s (P450s) and UDP-glucuronosyltransferases (UGTs), has not been thoroughly investigated. Cannabinoids underwent extensive metabolism by P450s and UGTs resulting in the biosynthesis of hydroxylated and carboxylated metabolites that were subsequently excreted in human urine, primarily as glucuronides.

In the present study, SCBs were incubated with human liver microsomes (HLMs) as well as recombinant P450s in the presence of the NADPH regenerating system. Mono- and dihydroxylated metabolites were the major oxidative metabolites of SCBs in HLMs. Subsequent studies with recombinant P450s identified CYP3A4/5 and CYP2J2 as the major isoforms involved in the hydroxylation reactions. Steady-state kinetic analyses were performed, and rigorous metabolite identification was carried out using LC-MS/MS and HPLC-UV/Vis. Subsequent mechanistic studies involving binding and activation of cannabinoid receptors (CBRs) CB1 and CB2 showed that SCBs caused psychoactive effects similar to those of Δ^9 -THC. Moreover, CBRs were able to bind several hydroxylated and glucuronidated SCB metabolites with an affinity similar to that of the parent compound. Finally, our *in vivo* data demonstrated that SCB metabolites retained biological activity in mice. In conclusion, our study has shown that the atypical pharmacodynamic properties of SCBs at CB1 and CB2 relative to Δ^9 THC (higher potency/efficacy and greater production of desensitization) coupled with an unusual metabolic profile (production of metabolically stable, active Phase II metabolites) may contribute to the pronounced adverse effects observed with the abuse of SCBs when compared to marijuana. (NIH/NIDA DA039143 ARP & PLP).

P25 - ACCESSING MAMMALIAN DRUG METABOLITES USING POLYCYPS® ENZYMES

Julia Shanu-Wilson, William Hodds, Sandie Lai, Richard Phipps, Antonio de Riso, Vincent Poon, Kinga Nytko, Aksana Khan, Liam Evans, Frank Scheffler, and Jonathan Steele
Hypha Discovery Limited, United Kingdom

Access to major and/or disproportionate metabolites as part of drug development programs is critical to ensure adherence to regulatory guidelines issued by the FDA and EMA, as well as to comply with internal safety standards within pharmaceutical companies themselves. Identification and characterization of metabolites ensures not only rigorous safety evaluation of significant metabolites, but can also expand patent coverage and reveal superior potency, reduced side effects or improved ADME and physical properties compared to the parent drug.

Strategies to produce metabolites often begin with chemical synthesis, either by direct modification of the drug candidate or via modified intermediates in the synthetic scheme, however alternative methods are required if either poor reaction yields, severe reaction conditions or unwanted by-products are encountered. Equally, constraints on medicinal chemistry resource can mean that investing significant time in chemical synthesis of metabolites may not be the best route, particularly in cases where the structure of the metabolite is not known, and where multiple possibilities need to be made. This poster illustrates the application of a new biocatalysis kit, PolyCYPs®, to enable scalable synthesis of CYP-derived metabolites of drugs. The PolyCYPs platform is comprised of a set of cloned cytochrome P450 enzymes and redox partners derived from some of the talented bacteria in Hypha's biotransformation panel. Enzymes in the kit catalyze oxidation reactions of a wide variety of substrate types to generate multiple human and other CYP metabolites. The poster features application of selected PolyCYPs isoforms to produce hydroxylated human metabolites of drugs, as illustrated below. Further, the utility of PolyCYPs enzymes for introducing oxygen into a drug candidate as part of a late stage functionalization program will be demonstrated, in which derivatives can be generated in parallel which may possess superior properties such as improved metabolic stability and LLE, and for exploration of structure-activity relationships.

P26 - A RAPID FLUORESCENCE BASED SCREEN FOR CYP51 INHIBITION

Michael Voice, Gillian Macintyre, and Michael Pritchard
Cypex Ltd, United Kingdom

Drug treatments such as fungicides and anti-parasitics often target the pathogen CYP51 (e.g. Lamb et al (1999) and Riley J. et al (2015)) and there is a risk with new drugs using the same strategy that they will exhibit crossover inhibition of human CYP51 leading to unwanted side effects. Here we have developed a quick, easy to run, inhibition screen for human CYP51 inhibition using the fluorogenic substrate BOMCC. Recombinant human CYP51, co-expressed in 10 litre fermenters with human CYP reductase and supplemented with human cytochrome b5, was incubated in 96 well plates with BOMCC. Following a preincubation at 37°C, NADPH generating system was added to the wells and the increase in fluorescence was measured over time. The K_m for BOMCC metabolism to CHC with human CYP51 was determined, 14.7 $\mu\text{M} \pm 3.8$ ($n = 3$ separate batches of CYP51) and six compounds were tested for their ability to inhibit CYP51 with a BOMCC concentration in the assay of 10 μM . 7 concentrations of inhibitor were used across an 833 fold concentration range and the data were analysed and fitted to IC_{50} curves in Excel using XLfit. The IC_{50} s were; azalanstat 0.021 \pm 0.006 μM , fluconazole > 100 μM , itraconazole 0.32 \pm 0.09 μM , ketoconazole 0.056 \pm 0.018 μM , miconazole 0.032 \pm 0.005 μM and testosterone 18 \pm 8 μM ($n = 3$ separate batches of CYP51). Azalanstat was subsequently used as a positive control in a number of related experiments using two lots of recombinant CYP51 ($n=22$ each lot). Lot 1 IC_{50} 0.020 \pm 0.006 μM , Lot 2 IC_{50} 0.024 \pm 0.005 μM .

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Development of a Fluorescence-based Trypanosoma cruzi CYP51 Inhibition Assay for Effective Compound Triaging in Drug Discovery Programmes for Chagas Disease

P27 - ASSOCIATION OF CYTOCHROME P450 REACTIVITY AND REPEATED-DOSE TOXICITY OF CHEMICAL COMPOUNDS

Michiko Watanabe¹, Takamitsu Sasaki¹, Jun-ichi Takeshita², Ryota Shizu¹, Takuomi Hosaka¹, and **Kouichi Yoshinari**¹
¹University of Shizuoka, Japan, ²National Institute of Advanced Industrial Science and Technology, Japan

[Introduction] To assess the safety of chemical compounds, several types of animal tests are carried out. Among them, repeated-dose toxicity (RDT) tests are often important to determine the no observed adverse effect level (NOAEL) of the test compounds. Since these tests need large cost and time, and require a huge number of animals, the development of alternative methods is strongly demanded. One of such approaches is *in silico* or computer-based approach using quantitative structure-activity relationship (QSAR) and/or grouping/read-across methods, which predict the toxicity of untested chemical compounds based on their chemical structures and/or other properties available from existing data. However, it remains very difficult to establish a method for RDT prediction with such *in silico* approaches and further parameters to characterize chemical compounds, especially those support the pharmacokinetic and metabolic profiles, are needed. We are thus focusing on the reactivity to drug-metabolizing enzymes such as cytochrome P450s (P450s). P450 is a major family of drug-metabolizing enzymes and comprises multiple forms with different substrate specificities. They are involved not only in the detoxification of the xenobiotics in the body but also in their metabolic activation to reactive metabolites, which is a key event for various types of toxicities. P450 reactivity of chemical compounds thus may help to characterize both structural and toxicological properties of compounds. [Methods] As test compounds, we selected ~150 compounds, whose rat RDT data is publicly available, from Hazard Evaluation Support System Integrated Platform (National Institute of Technology and Evaluation, Japan). Using P450-Glo systems (Promega) and commercially available recombinant rat P450 enzymes, namely CYP1A1, CYP1A2, CYP2B1, CYP2C6, CYP2D1, CYP2E1 and CYP3A2, inhibitory effects of test compounds at three concentrations against these P450 forms were determined. The % inhibition values (vs. vehicle control) were calculated for each compound per each form and more than 20% inhibition was judged as "positive". Molecular descriptors were calculated with Dragon 7 (Talete). Statistical analyses were performed with JMP Pro 13 (SAS institute). [Results and Discussion] Among the P450 forms tested, CYP1A1 was most reactive to test compounds (28% positive) and then CYP2C6 and CYP2B1. CYP2D1 and CYP2E1 were less reactive (less than 10% compounds inhibited these forms). Principal component analyses using inhibition results and molecular descriptors demonstrated that in the loading plots the P450 inhibition parameters were plotted differently from most of molecular descriptors. We then investigated the association between P450 inhibition data and the endpoints observed in RDT tests and found the statistical associations between inhibition of some P450 forms and liver function-related endpoints (e.g. liver weight increase, centrilobular hepatocyte hypertrophy, or γ -GTP increase) or anemia-related endpoints. Our present results suggest that P450 inhibition data (i.e. P450 reactivity) can be novel parameters to characterize chemical compounds and useful for the prediction of some endpoints of RDT tests.

P28 - STEREOSELECTIVE OXIDATION KINETICS OF DEOXYCHOLATE IN RECOMBINANT AND MICROSOMAL CYP3A ENZYMES: DEOXYCHOLATE 19-HYDROXYLATION IS AN *IN VITRO* MARKER OF CYP3A7 ACTIVITY

Jian Zhang¹, Yujie Chen¹, Pingping Zhu¹, Jianguang Huang², Changxiao Liu³, Liang Xu¹, Wei Jia⁴, Irina F. Sevrioukova⁵, and Ke Lan¹

¹Sichuan University, China, ²Huazhong University of Science and Technology, China, ³Tianjin Institute of Pharmaceutical Research, China, ⁴University of Hawaii Cancer Center, USA, ⁵University of California, Irvine, USA

The primary bile acids (BAs) synthesized from cholesterol in the liver are converted to secondary BAs by gut microbiota. It was recently disclosed that the major secondary BAs, deoxycholate (DCA), glycodeoxycholate (GDCA) and taurodeoxycholate (TDCA), are stereoselectively oxidized to tertiary BAs exclusively by the hepatic CYP3A enzymes [1]. Following the newly defined host liver (primary BAs) - gut microbiota (secondary BAs) - host liver (tertiary BAs) axis of BAs host-gut microbial co-metabolism, we investigated the stereoselective oxidation of DCA at C-1 β , -3 β , -4 β , -5 β , -6 α , -6 β and -19 in the recombinant CYP3A4, 3A5 and 3A7 enzymes in Bactosomes and the naive enzymes human liver microsomes (HLM). The stereoselective oxidations of DCA fit well with Hill kinetics at 1-300 μ M in both recombinant CYP3A enzymes and pooled HLMs. With none or trace contributions from CYP3A5, CYP3A7 favors the oxidations at C-19, C-4 β , C-6 α , C-3 β and C-1 β , whereas CYP3A4 favors the oxidations at C-5 β and C-6 β compared to each other. Correlation between DCA oxidations and testosterone 6 β -hydroxylation in 14 adult single-donor HLMs provided a proof-of-concept evidence that DCA 19-hydroxylation is an *in vitro* marker reaction for CYP3A7 activity, whereas oxidations at other sites are mixed indicators for CYP3A4 and CYP3A7 activities. Deactivation caused by DCA induced P450-P420 conversion, as shown by the spectral titrations of isolated CYP3A proteins, was observed when DCA levels were near or higher than its critical micelle concentration (about 1500 μ M). Unlike CYP3A4, CYP3A7 showed abnormally elevated

activities at 500 μ M and 750 μ M, which might be associated with an altered affinity for DCA multimers. The disclosed kinetic and functional roles of CYP3A isoforms in disposing the gut bacteria-derived DCA may help understand the structural and functional mechanisms of CYP3A.

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P29 - TERTIARY OXIDATION OF SECONDARY BILE ACIDS, DEOXYCHOLATE, IS CONSERVATIVE IN HUMAN, MONKEY, DOG, RAT AND MOUSE

Ke Lan¹, Wei Jia², Changxiao Liu³, Jiangeng Huang⁴, Yujie Chen¹, Pingping Zhu¹, Yanwen Tan¹, Jian Zhang¹, Mingming Su², and Liang Xu¹

¹Sichuan University, China ²University of Hawaii Cancer Center, USA, ³Tianjin Institute of Pharmaceutical Research, China, ⁴Huazhong University of Science and Technology, China

It was recently disclosed that the CYP3A4/3A7 catalyzed stereoselective oxidations of deoxycholate (DCA), glycodeoxycholate (GDCA) and taurodeoxycholate (TDCA) are continuum of the host-gut microbial co-metabolism of bile acids (BAs) in human adults[1]. This work aims to investigate whether the tertiary metabolism of DCA is a conserved pathway in main experimental animals. The total unconjugated BAs profiles were quantitatively determined by enzyme-digestion techniques in urine and feces from human (n=6), beagle dogs (n=6), Sprague Dawley rats (n=6) and C57BL/6 mice (n=8). It was determined that cholate (CA) and chenodeoxycholate (CDCA) are primary bile acids (BAs) of all animals, α -muricholate (α MCA) and β -muricholate (β MCA) are additional primary BAs of rodents, and lithocholate (LCA) and DCA are secondary BAs of all animals. The tertiary oxidized metabolites of DCA were found in all tested animals with some species differences. 3-dehydroDCA, DCA-4 β -ol, DCA-5 β -ol, DCA-6 β -ol and DCA-6 α -ol were prevalently found in human, dogs, rats and mice. DCA-1 β -ol, the major metabolite in human, was also detected in dogs, but was detected only with a trace level in rodents. DCA and its oxidized metabolites disappeared in C57BL/6 germ-free mice (n=3) compared with conventional raised ones, providing an *in vivo* evidence that the tertiary BAs are metabolites from DCA oxidized in host liver. *In vitro* metabolism of DCA at 1-300 μ M were subsequently performed in liver microsomes (LMs) of the corresponding species and cynomolgus monkeys in comparison to human. The human CYP3A7 specific metabolites, DCA-4 β -ol and DCA-19-ol, were detected only with a trace or much lower level than human in the tested animals. Oxidation at the other sites, C-3 β , C-1 β , C-5 β , C-6 α and C-5 β , were observed in all tested animals with monkeys demonstrating the most similar stereoselective kinetics as that of human. The most significant species difference was identified that rats preferred oxidation of DCA at C-7 β forming ursocholic acid (UCA), a BA commonly considered as an isomerized metabolite of CA by gut bacteria. Some *in vivo* - *in vitro* irrelevance might be associated with the host CYP3A catalyzed oxidations of GDCA and TDCA that require further investigations. In conclusion, this work demonstrated that the tertiary oxidation of DCA species is a conserved pathway in human, monkeys, dogs, rats and mice.

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P30 - AN EXAMINATION OF THE REVERSIBILITY IN THE EFFECTS OF HIGH FAT DIET ON LIDOCAINE MICROSOMAL METABOLISM IN RATS

Hamdah Mohammed S Al Nebaihi and Dion R. Brocks
University of Alberta, Canada

We have observed changes in drug metabolizing enzyme expression in dietary-induced obesity at both the protein and functional levels. A major treatment of obesity in humans is a change in diet to one of reduced calorie content. Using lidocaine as a test substrate, we investigated its metabolism to its active dealkylated metabolite, monoethylglycinexylidide (MEGX), in rats given standard diet (control), or high-fat-diet for 14 weeks with or without an extra four weeks of lean diet. The goal was to examine the differences that high fat caused in the metabolism of lidocaine in male and female rats, and also if any changes could be reversible. Methods: Male and female Sprague Dawley rats (n=8 total per each) were started on a 14 week course of 45% high fat rat (HF) chow with water starting at 1 month of age. At the end of the 14 week period, half of the animals were euthanized under isoflurane. In the remaining rats, the diet was switched to normal rat chow (fat content 13.4%). Four weeks after, those rats were euthanized. For each of the 14 and 18 week groups, control rats (n=4) were included where the diet was 13.4% fat content. Tissues were collected and frozen at -80°C. Liver microsomes were harvested and lidocaine was incubated with the microsomal protein (1 mg/ml) with cofactor (NADPH) at 37°C with pH 7.4 for 10 min. Enzyme reactions were terminated by adding 1M NaOH. High-performance liquid

chromatography was used to assay the formation rate of lidocaine metabolite, MEGX. Results: After 14 weeks on the high-fat diet, MEGX maximal formation rate (V_{max}) was reduced significantly ($p < 0.05$, Student unpaired t-test) in both male and female rats (>30% and >20% reduction in male and female rats respectively). Of note, MEGX formation rate was higher in male than female rats. Interestingly, the MEGX formation rates increased back to normal levels when the standard diet was implemented from weeks 14 to 18. No significant changes in the enzyme affinity (K_m) and intrinsic clearance (Cl_{int}) were seen compared to control diet. Conclusions: Diet-induced obesity was associated with a reduction in the maximum hepatic microsomal rate of N-dealkylation leading to a reduction of MEGX formation. Those changes were reversible by a switch to normal diet and matched the changes that we observed in expression levels of enzymes known to be involved in lidocaine metabolism.

P31 - RATES AND ROUTES OF EXCRETION OF 14C-AQX-1125 FOLLOWING ORAL ADMINISTRATION TO RATS, DOGS AND HUMANS

Ray Cooke¹, **Philip Bond**¹, Eleanor Barton¹, Iain Shaw², Patrick Tam³, Judy Toews³, Curtis Harwig³, Jeremy Pettigrew³, Dorothea Scholl³, Sapna Padania³, and Lloyd Mackenzie³

¹Pharmaron UK, United Kingdom, ²Quotient Sciences, United Kingdom, ³Aquinox Pharmaceuticals, Canada

AQX-1125 ((1S,3S,4R)-4-((3aS,4R,5S,7aS)-4-(aminomethyl)-7a-methyl-1-methyleneoctahydro-1H-inden-5-yl)-3-(hydroxymethyl)-4-methylcyclohexanol) is a small molecule SHIP1 activator, a phosphatase which regulates the PI3 Kinase pathway. AQX-1125 was under development by Aquinox Pharmaceuticals as a potential anti inflammatory. A series of studies were performed at Pharmaron UK in collaboration with Aquinox, to determine rates and routes of excretion of 14C-AQX-1125 following oral administration to rats, dogs and humans in both males and females. Following a single oral administration of 14C-AQX-1125 to male and female albino rats, (target dose, 30 mg/kg, radioactive dose 100 μ Ci/kg), excretion was similarly rapid and predominantly via the faecal route with means of 58.2% and 62.0% of dose recovered for males and females respectively during 0 – 168 hour collection. Urine accounted for a slightly smaller proportion of radioactivity (35.8% and 30.0% in male and female rats respectively) over the same period. Following a single oral administration of 14C-AQX-1125 to male and female dogs, (target dose, 10 mg/kg radioactive dose 20 μ Ci/kg), excretion was relatively rapid with nearly 50% of the radioactive dose recovered in the initial 0 – 24 hour post dose collection (mean 47.7% male and 47.6% for female dogs respectively). A relatively equal proportion of the dose was detected in urine (43.3 – 45.4%) and faeces (42.8 – 46.2%) with no notable gender differences in the dog. A radiolabelled clinical metabolism study was performed with 14C-AQX-1125 (target radioactive dose 50 μ Ci, 200 mg) administered orally to both male and female healthy human volunteers (n = 4 volunteers per gender). The total radioactivity recovered over the 0 - 192 hour collection period showed no gender difference between males (92.6%) and females (92.7%). The major route of elimination in humans was urinary with a mean recovery of dose related material of 74.1 and 76.5% for males and females respectively. A comparison between the 3 species would suggest no gender difference in the rates and routes of excretion occurred following oral administration. All mass balance studies showed a near complete mass balance of the radioactive dose. There would appear to be a change in the route of excretion from the rat, through the dog to ultimately the human mass balance. Following oral administration, the rat shows the major route of excretion to be faecal, the dog shows that excretion is equivalent by both urinary and faecal routes, whereas in the clinical mass balance study, the predominant route is urinary clearance.

P32 - COMPARATIVE METABOLISM OF 14C-AQX-1125 FOLLOWING ORAL ADMINISTRATION TO RATS, DOGS AND HUMANS

Eleanor Barton¹, Ray Cooke¹, Dylan Williams¹, Patrick Tam², Judy Toews², Curtis Harwig², Jeremy Pettigrew², Dorothea Scholl², Sapna Padania², and Lloyd Mackenzie²

¹Pharmaron UK, United Kingdom, ²Aquinox Pharmaceuticals, Canada

AQX-1125 ((1S,3S,4R)-4-((3aS,4R,5S,7aS)-4-(aminomethyl)-7a-methyl-1-methyleneoctahydro-1H-inden-5-yl)-3-(hydroxymethyl)-4-methylcyclohexanol) is a small molecule SHIP1 activator, a phosphatase which regulates the PI3 Kinase pathway. AQX-1125 was under development by Aquinox Pharmaceuticals as a potential anti inflammatory. A series of studies were performed at Pharmaron UK in collaboration with Aquinox, to determine the metabolic pathway of 14C-AQX-1125 following oral administration to rats, dogs and humans in both males and females. Plasma samples from these studies were analysed by LC-MS/MS with in-line fraction collection and off-line radioactivity counting to generate the metabolite profiles. High resolution, accurate mass, full scan and product ion mass spectrometry techniques were utilised in order to structurally identify the major radioactive components. The main routes of metabolism for AQX-1125 were determined to be via glucuronidation, hydroxylation and hydrolysis. Three notable circulating metabolites were observed in the human plasma AUC(0-24 hr) pools. Unchanged AQX-1125 accounted for 38.4% of the extracted plasma sample radioactivity in the overall population (males and females) and a glucuronide conjugate of AQX-1125 was identified as the major metabolite, accounting for 34.2% of the circulating radioactivity. Two further metabolites were identified; a second glucuronide conjugate of AQX-1125 (7.6%) and M355 (metabolite with molecular weight of 355 Da) (8.3%). No significant differences (>5%) were observed in the metabolite profile between males and females. Further investigation was required

to definitively identify the exact isomeric structures of these three notable circulating metabolites. Additional characterisation of the metabolites was carried out through both chemical synthesis of reference standards and isolation from biological matrices coupled with NMR analysis. The major glucuronide metabolite was identified as the 6-O- β -glucuronide of AQX-1125, the minor glucuronide as 3-O- β glucuronide and M355 as the N-hydroxy hydrolysed AQX-1125. A comparison of the metabolism of AQX-1125 across humans and the toxicity species did show some differences. All three human metabolites were observed in dog, whereas in rats only the N-hydroxy hydrolysed AQX-1125 metabolite was present. The major metabolites in humans (as defined by the FDA Metabolites in Safety Testing (MIST) guidelines) were shown to be present in one or both animal toxicity species.

P33 - SPECIES DIFFERENCES IN THE MASS BALANCE, EXCRETION AND METABOLISM OF [14C] LY3202626 IN HUMAN, DOG AND RAT

Ken Cassidy¹, Scott Monk¹, Ping Yi², and Kishore Katyayan¹

¹Eli Lilly and Company, USA, ²Covance, USA

LY3202626 is a potent beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) inhibitor that has been investigated for treating Alzheimer's disease. As part of the development program the mass balance, excretion, and metabolism of LY3202626 was determined in male human subjects, Sprague Dawley rats, and Beagle dogs. Human subjects were orally administered 10 mg (100 uCi), intact and bile duct cannulated (BDC) rats were orally administered 30 mg/kg (50 uCi/kg), while dogs were administered 10 mg/kg (50 uCi/kg) orally or 1 mg/kg (15 uCi/kg) intravenously. The excretion profiles and mass balance results were substantially different between species. Excretion of radioactivity in rat and dog was fairly rapid, with >85% of the dose recovered by 48 or 96 hours, respectively. Over a 168 hr collection period, 97% of the dose (27% urine, 70% feces) was recovered from intact rats and 94% of the dose (14% bile, 35% urine and 45% feces) was recovered from BDC rats. In the dog, over a 192 hour collection period, 87% of the dose (17% urine, 70% feces) was recovered following oral administration and 89% of the dose (22% urine, 67% feces) was recovered following IV administration. Based on these results, urinary excretion of radioactivity is predominant in the rat while biliary excretion is the predominant route of excretion in the dog. The excretion profile and mass balance results in human were unexpectedly different from those in the animals. In humans, radioactivity was eliminated slowly, with an average of only 75% of the dose recovered before releasing the subjects from the clinic following a stay of up to 21 days. In the rat and dog, LY3202626 was moderately absorbed and was subject to extensive metabolism, with < 5% of the dose excreted as unchanged parent from the BDC rat and from IV dosed dogs. The major metabolic clearance pathway in the rat was amide hydrolysis. In the BDC rat, approximately 44% of the dose was recovered as amide hydrolysis metabolites in urine and bile combined, and presumed unabsorbed parent compound represented 35% in the feces. Conversely, the major metabolic clearance pathway in the dog was O-demethylation (M2), which represented 54% of the dose in urine and feces combined after IV administration. LY3202626 was well absorbed and extensively metabolized in human with only 4% of dose excreted as unchanged parent drug. Metabolism of LY3202626 in human occurred primarily via O-demethylation (M2, M16; a combined 27% of the dose) and amide hydrolysis (M1, M4 and M5; a combined 24% of the dose). *In vitro* experiments suggest that the O-desmethyl metabolite M2 is reduced to M16 by gut microflora in the intestine. Subsequent re-absorption of M16 and oxidation in the liver by aldehyde oxidase back to M2 may result in enterohepatic cycling, which could explain the observed slow excretion of radioactivity in human.

P34 - RATES AND ROUTES OF EXCRETION OF 14C-ERAVACYCLINE FOLLOWING ADMINISTRATION TO RATS AND HUMANS

Ray Cooke¹, **Philip Bond**¹, Amy Bombardier², Nicholas Dunwoody², and Larry Tsai²

¹Pharmaron UK, United Kingdom, ²Tetraphase, USA

Eravacycline (7-fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline) is a novel, fully synthetic broad spectrum fluorocycline. As Tetraphase's lead product, marketed as XERAVA™ it is approved for the treatment of complicated intra-abdominal infections. A series of studies were performed at Pharmaron UK, in collaboration with Tetraphase, to determine the rates and route of elimination of 14C-Eravacycline following administration to rats and humans.

Following a single intravenous administration to male rats (target dose, 30 mg free base/kg, 100 μ Ci/kg), the recovery of radioactivity was measured in excreta for a collection period of 168 hours. At the end of the collection period, the recovery of radioactivity was essentially complete (93.5% of administered dose) with less than 1.5% of drug related material retained in the carcass at 168 hours. The major route of elimination was via the faeces with a mean of 66.1% of the administered radioactivity recovered by 168 hours post dosing. Urinary excretion accounted for 25.05% of the administered dose. The majority of the administered radioactivity was excreted in urine and faeces within the first 48 hours following dosing. When 14C-Eravacycline was administered (iv) to bile duct of cannulated rats and mass balance determined over a shorter time period of 48 hours, there was a greater amount of drug related material retained in the carcass at 48 hours (approximately 6.6% of dose). The largest proportion of dose was measured in the urine, followed by the bile and then the faeces, at 34.1%, 23.0% and 16.9% of dose respectively. The overall recovery of radioactivity was determined in bile duct cannulated rats to be 83.4%.

The rates and routes of elimination of drug related material were determined following administration of ^{14}C -Eravacycline to 2 groups ($n = 4$ and 5 per group) of healthy volunteers either as a single oral dose (100 mg, 130 μCi) or a single 60 minute intravenous infusion (60 mg, 105 μCi). At the end of the collection period (288 hours), the mean recovery for each dose route was remarkably similar at 82.5% and 82.8% for oral and intravenous dosing respectively. Following oral administration, the great majority of the radioactivity was associated with the faeces (mean value, 75.3% of dose) with a much smaller proportion found in the urine (mean 7.2%). The majority of the radioactivity was excreted in the initial 120 hours post dose. Intravenous dosing of ^{14}C -Eravacycline to healthy volunteers resulted in a slightly different elimination profile compared to the oral administration. Following intravenous administration, approximately 35% of the dose was excreted in the urine with 47.8% in the faeces. Similarly with the intravenous dose, the majority of the radioactivity was eliminated in the initial 120 hours post dose.

P35 - THE USE OF PRIMARY CRYOPRESERVED HUMAN HEPATOCYTES AS A MODEL FOR NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) RELATED CHANGES IN PHASE I AND PHASE II ENZYME ACTIVITIES

Karissa Cottier, Xuan Le, and Scott Heyward
BioIVT, USA

Primary human hepatocytes are the current gold standard for *in vitro* predictions of *in vivo* drug metabolism. Inter-individual variability in activity of drug metabolizing enzymes based on age, BMI, and gender have been assessed, however, it's not known if activity changes in complex liver disorders such as non-alcoholic fatty liver disease (NAFLD) can be accurately modeled using primary hepatocytes. NAFLD is a liver disease characterized by hepatic steatosis, which can progress to non-alcoholic steatohepatitis (NASH). To investigate changes in enzyme activities associated with this disease state we utilized cryopreserved isolated hepatocytes from donor livers. In parallel, formalin fixed paraffin embedded (FFPE) tissue samples from the same donor livers were blindly examined by a board-certified pathologist and given an NAFLD Activity Score (NAS) comprised of graded assessment of hepatic steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2), with $\text{NAS} \geq 5$ commonly used as a histologic diagnosis of NASH. Additionally, a graded assessment of fibrosis (0-4) was performed. Metabolic activity for both cytochrome p450(CYP) (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4) and conjugating enzymes (UGT and SULT) was generated by assessing metabolite formation with prototypic substrates in suspension incubations. Donors with $\text{NAS} \geq 5$ had significantly lower activity of CYP1A2 and CYP2C19 and trending decreased activity of CYP3A4 and the multiple enzyme substrate 7-ethoxycoumarin (ECOD) compared to those with a NAS of 0. To determine if individual pathological features contribute to metabolic changes, we next evaluated enzyme activity in donor hepatocytes separated only by their steatosis, lobular inflammation, ballooning, and fibrosis scores. We found that separating by steatosis scores alone uncovered a host of changes in metabolic enzyme activities, including some which were not seen when total NAS was evaluated. Compared to donors with steatosis scores of 0, hepatocytes from donors with a score of 3 had significantly reduced activity of CYP1A2, CYP2C8, CYP2C19, CYP3A4 and a trending reduction in CYP2C9 ($P=0.0509$) and UGT1A1 activity ($P=0.10$). No changes in enzymatic activity were seen based on inflammation score, while minimal changes were seen based on ballooning score (reduced CYP2E1 activity). Examination of metabolism based on fibrosis score showed significant reductions in activity of CYP1A2, CYP2C19, and trending reductions in ECOD ($P=0.055$) and CYP3A4 ($P=0.97$). These results suggest that, although inflammation and hepatocyte ballooning contribute to the complex pathological manifestations of NASH, steatosis and fibrosis may be the most prominent factors leading the changes in drug metabolizing enzymes. Therefore, when assessing potential drug-drug interactions in a NASH population, utilization of hepatocytes from livers with high levels of steatosis and fibrosis may give a more accurate prediction for severe cases.

P36 - PERINATAL BDE-47 EXPOSURE ALTERED THE DEVELOPMENTAL TRAJECTORY OF GUT MICROBIOTA IN RATS

Joanna Gillette
University of Washington, USA

Maternal exposure to persistent environmental toxicants polybrominated diphenyl ethers (PBDEs) is associated with many human diseases, notably hepatotoxicity, thyroid disorders, and reproductive toxicity. PBDEs, previously seeded into manufactured products as flame retardants in the United States, were phased out during the 2000s. A 2017 study of women in California showed modest average annual percent increases in serum concentrations of PBDEs. Accordingly, the gut microbiome is increasingly recognized as the "second genome", impacting crucial health-related factors like host xenobiotic biotransformation and energy homeostasis. Persistent dysbiosis may contribute to altered susceptibility of disease in adulthood. The goal of this study was to test the hypothesis that perinatal exposure to BDE-47, a common PBDE congener that can cross the placenta and enrich in breast milk, modifies the developmental trajectory of gut microbiota. Pregnant Wistar rats were orally exposed to BDE-47 (0.2mg/kg) from gestational day 8 to postnatal day (PND) 21. Fecal samples were collected from mothers at PND21 and male pups at PND65 and 120. Microbial DNA was isolated using 16S rDNA sequencing ($n=5-9/\text{group}$) and analyzed using QIIME. Perinatal BDE-47 exposure had minimal effect on the richness of gut microbiota in PND65 pups and mothers, but profoundly increased richness of gut microbiota in

PND120 pups. In PND65 pups, only 4 taxa were persistently regulated by perinatal BDE-47 exposure. Interestingly, the effect of perinatal BDE-47 exposure was evident in PND120 pups, with an increase in 3 taxa in the Lactobacillales order and 11 taxa in the Clostridiales order. Targeted metabolomics has confirmed alterations of distinct microbial metabolites (short-chain fatty acids and secondary bile acids) corresponding to BDE-47-induced dysbiosis. In summary, the present study showed that perinatal BDE-47 exposure modified the developmental trajectory of the offspring's microbiota, possibly producing delayed onset of diseases in adulthood.

P37 - Safety, pharmacokinetics and pharmacodynamics evaluation of chiglitazar, a novel identified PPAR pan-agonist for the treatment of type 2 diabetes in different age groups

Xiaojiao Li¹, Qianqian Li¹, Min Wu¹, Hong Zhang¹, Jingrui Liu¹, Jinwen Zhang¹, Jia Yu², and Yanhua Ding¹

¹Phase I Clinical Trial Unit, First Hospital, Jilin University, China, ²Chipscreen Biosciences Co., Ltd., China

Chiglitazar, a peroxisome proliferator-activated receptor (PPAR) pan agonist, is a potential new therapeutic strategy for treating type 2 diabetes (T2D). This study was designed to determine the pharmacokinetics, short-term pharmacodynamics, and tolerability of multiple doses of chiglitazar in different age group Chinese T2D patients, particularly the elderly. The study was designed as an open-label and multiple-dose (7 days) study. It was planned to enroll 2 cohorts (35~65 and 65~85 age groups) of 10 patients each. Chiglitazar 48 mg, the dose used in two pivotal phase 3 clinical studies, was administered once-daily for 7 days. Intensive PK blood samples were collected after the first and last dose. Fingertip fasting blood glucose was measured pre-dose on day 1 to day 7, day 8 and day 9. Changes in FPG, TC, TG, HDL and LDL from baseline to day 9 were also evaluated. The plasma concentrations of the chiglitazar were determined using a validated LC-MS/MS method after sample preparation. A total of 20 T2D patients were selected and divided into two age categories: under 65 years (group 1, n=10), and over 65 years (group 2, n=10). Chiglitazar 48 mg was well tolerated after single and multiple administration for 7 days in different age groups. No statistically significant differences were observed in C_{max} or AUC for chiglitazar between the different age groups, with geometric mean ratio of AUC₀₋₂₄ (group 2/group 1) was 96.83%, 90%CI was 76.92%-121.90% for single dose. After reaching the steady state (day 7), the AUC₀₋₄₈ of chiglitazar was slightly higher (10%~20%) in group 2, with geometric mean ratio (group 2/group 1) was 113.32%, 90%CI was 94.30%-136.18%. However, there is no significant differences of the exposure between two groups (P>0.05). After 5 days of daily administration, chiglitazar reached its steady states, and there was no significant accumulation with multiple doses (accumulation factor of ~1) for each age group. Due to the short treatment period (7 days), the fingertip blood glucose profiles and FPG were not improved significantly from day 1 to day 9. But the decrease changes in fasting plasma lipid parameters (TC, TG, LDL-C) was found after 7 days treatment. In conclusion, chiglitazar pharmacokinetics and the short-term pharmacodynamics were similar in patients with different age groups. This suggested that no dose adjustment of chiglitazar is necessary in elder patients with T2D.

P38 - EXPLORING SPECIES DIFFERENCES IN ALDEHYDE OXIDASE ACTIVITY

Pranav Shah¹, Vishal Siramshetty², Gregory Tawa², Dac-Trung Nguyen², Alexey Zakharov², Noel Southall², Emre M. Isin³, R. Scott Obach⁴, Xin Xu², and Nenad Manevski³

¹National Center for Advancing Translational Sciences, USA, ²NIH Bridgms and TRND - Rockville, MD, USA, ³UCB Biopharma, United Kingdom, ⁴Pfizer, USA

Over the last few decades, chemists have become increasingly proficient at bypassing cytochrome P 450 (CYP) - mediated clearance in an effort to optimize the clearance of drug candidates as well as minimizing drug-drug interactions. This has led to the emergence of other drug metabolizing enzymes such as aldehyde oxidase (AO) as noteworthy contributors to drug clearance. Typical early-stage screening assays are performed in liver microsomal fractions and it is possible to miss the contribution of non-microsomal clearance until much later in drug development. Several well-known structure modification strategies exist to guide the medicinal chemist through CYP-mediated metabolism; however, strategies to attenuate non-CYP-mediated liabilities are scarce. Several projects at NCATS were recently discovered to have species dependent AO-mediated liabilities. Our goal is to (i) explore these differences in four species using a large, chemically diverse, dataset and (ii) provide chemists with guidelines that can be used to rationally design compounds to attenuate AO-mediated metabolism. To begin, we identified 365 compounds most likely to be susceptible towards AO-mediated liabilities from our NCATS_ADME 5K library. We performed cytosolic stability assays in human, cyno monkey, CD-1 mouse and SD rat cytosol fractions using our high-throughput automated system. Briefly, each reaction mixture (110 µL) consisted of a test compound (1 µM), allopurinol (50 µM) and cytosol fractions (2 mg/mL) in phosphate buffer (100 mM) at pH 7.4. Samples were incubated in 384-well plates at 37°C for 0, 5, 10, 15, 30 and 60 minutes. Since gender differences in AO-mediated metabolism have been reported, we performed these experiments in male cytosol fractions. The highest number of compounds were found to be metabolized by AO (AO substrates defined as t_{1/2}<30 min) in mouse (70) followed by human (64), monkey (52) and rat (49) respectively. The maximum substrate overlap was found between mouse and human (76.2%). We plan to explore these datasets further by performing matched molecular pair analysis to derive a set of species-dependent molecular transformations that have a high probability of attenuating AO-mediated metabolism. We also plan on building QSAR models to help us predict AO activity of new molecules found by screening

or designed by application of said transformations. To understand the physical basis of AO metabolism and, more specifically, how the species-dependent transformations work we will develop 3D models where AO metabolism is viewed as a two-step process, (i) recognition of the compound by AO, (ii) modification made by AO which depend on the electronics of the compound. The knowledge gained from this dataset, and the various models derived from it, will facilitate lead optimization by enabling structure-based modifications. The code and the data will be incorporated in the publicly available NCATS web services (<https://predictor.ncats.io>).

P39 - METABOLISM OF DESLORATADINE IN TK-NOG CHIMERIC MICE WITH HUMANIZED LIVERS

Shotaro Uehara¹, Yuichiro Higuchi¹, Nao Yoneda¹, Hiroshi Yamazaki², and Hiroshi Suemizu¹

¹Central Institute for Experimental Animals, Japan, ²Showa Pharmaceutical University, Japan

Desloratadine is a long-acting tricyclic H₁-receptor histamine antagonist used for treating seasonal allergies and hives. It is primarily converted to 3-hydroxydesloratadine followed by O-glucuronidation to 3-hydroxydesloratadine O-glucuronide in humans; however, these metabolites are detectable in trace amounts in rodents. In contrast, 5-hydroxydesloratadine and 6-hydroxydesloratadine are the majorly excreted metabolites of desloratadine metabolism in rodents. Against this background, present study aimed to investigate the metabolism of desloratadine in chimeric TK-NOG mice with humanized livers (Hu-Liver mice) *in vivo* and *in vitro*. Desloratadine was orally administered at a single dose of 10 mg/kg to Hu-Liver mice and control non-humanized TK-NOG mice, and plasma and urine samples were obtained. For *in vitro* metabolic studies, hepatocytes obtained from Hu-Liver mice were treated with desloratadine, and the formation of hydrolyzed metabolites and their glucuronide conjugates was analyzed. Concentrations of desloratadine and its metabolites in these samples were measured by liquid chromatography-tandem mass spectrometry. After oral administration of desloratadine, Hu-Liver mice had higher concentrations of 3-hydroxydesloratadine and its glucuronide in plasma than did the control mice. Concentrations of 3-hydroxydesloratadine and its glucuronide in 0–72 h accumulated urine samples from Hu-Liver mice were markedly higher than those from control mice, whereas 5-hydroxydesloratadine were significantly in urine of control mice. Consistent with the *in vivo* data, the *in vitro* 3-hydroxydesloratadine formation was detected in hepatocytes from humans and Hu-Liver mice, but not in those of control mice that mainly converted desloratadine to 5-hydroxydesloratadine. These results suggest that desloratadine metabolism in Hu-Liver mice is closer to that in humans. Thus, Hu-Liver mice are potentially useful as an *in vivo* model for drug metabolism studies.

P40 - Diet-induced Obese Alters the Expression and Function of Hepatic Drug-metabolizing Enzymes and Transporters in Rats

Feng Xu

Southern Medical University, China

Obesity increases the incidences of metabolic syndrome, including type 2 diabetes, fatty liver, dyslipidemia, hyperglycemia, heart disease, hypertension and cancer. These diseases usually require medication, but pharmacokinetics and pharmacodynamics of many drugs have changed in obese patients. In particular, little is known about the hepatic drug-metabolizing enzymes and transporters that are influenced by diet-induced obese.

Methods: We established obesity and fatty liver models in rats by high-fat diet. The expression profiles of drug-metabolizing enzymes and transporters were studied by quantitative real-time PCR and Western blotting analysis. The function of these enzymes and transporters were assessed by their substrates and cocktail methods.

Results: The expression and activity of phase I enzymes (CYP1A2, CYP2B1, CYP2C11, CYP3A1, CYP4A1 and FMO1) and phase II enzymes (UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B7, NAT1 and GSTT1) were decreased in the liver of obese rats. In addition, the mRNA levels of hepatic transporter Slco1a2, Slco1b2, Slc22a5, Abcc2, Abcc3, Abcb1a and Abcg2 decreased significantly in obese animals, while Abcb1b increased significantly. Furthermore, the decreased expression of hepatic phase I and II enzymes and transporter may be due to changes of Hnf4 α , LXR α and FXR.

Conclusion: A number of drug metabolic enzymes and transporters were significantly altered in the diet-induced obese rats. The data based on this study implies that similar alterations of hepatic drug metabolizing enzymes and transporters may occur in patients with obesity and associated fatty liver, thereby impacting drug metabolism and pharmacokinetics.

P41 - QUANTITATIVE TISSUE DISTRIBUTION OF CONJUGATED AND FREE PAYLOAD IN TUMOR BEARING MICE AFTER INTRAVENOUS DOSING OF GLYPICAN 3-TARGETING ADNECTIN DRUG CONJUGATE

Weiqi Chen, Iyer Ramaswamy, Wenying Li, and Jinping Gan

BMS, USA

Tumor specific delivery of cytotoxic agents offers great potential in maximizing the therapeutic window in cancer chemotherapy. Human glypican-3 is a cell membrane protein that is over-expressed in hepatocellular carcinoma, offering an attractive target protein for cytotoxic drug conjugates. Here we describe a tissue distribution study to test the hypothesis that a glypican-3-binding adnectin drug conjugate (A1H-tub, A1) can achieve tumor specific targeted delivery

of cytotoxic payload in glypican-3 expressing tumor-bearing mice, in comparison with a conjugate with non-binding adnectin (A-RGE-H-tub, RGE). Both adnectins were conjugated to a tubulysin payload via a linker that consisted of a maleimide group and a cathepsin-B-cleavable dipeptide linker (Figure 1). After intravenous dosing of A1 and RGE respectively, multiple tissue samples were collected at various timepoints up to 168 hours and homogenized with saline. Plasma and tissue homogenate samples were subjected to immunocapture followed by on-bead cathepsin-B digestion to release conjugated payload or direct protein precipitation to obtain the free payload. A polyclonal antibody against an adnectin epitope that is not involved in glypican-3 binding was selected for the immunocapture. The bioanalysis was carried out on a Shimadzu Nexera UHPLC coupled with a Sciex 6500 mass spectrometer through selective reaction monitoring in positive ionization mode. The recovery of conjugated payload by immunocapture was assessed by spiking of standards in various tissue homogenates, and it was found to be greater than 60% across tissue types for both drug conjugates. The LC-MS/MS assay was sensitive, selective, and linear across the concentration range for the payload. The concentrations of the free payload in the kidney were the highest, consistent with kidney being the major catabolism and elimination organ for adnectins. While no difference in free payload was observed between RGE and A1 in plasma, liver, and kidney, more than 10 fold higher free payload was observed in tumor dosed with A1 than that with RGE, supporting the tumor targeting effect. Similar results were observed for conjugated payloads, with tumor being the only tissue that demonstrated differential distribution favoring A1 at early timepoints up to 5 hours. While the conjugated payloads rapidly declined in the A1 arm after 5 hours, the free payload stayed high in tumor up to 168 hours, consistent with initial binding event of A1 with glypican-3 on the tumor surface, followed by internalization and quasi-irreversible binding to tubulins in cancer cells. In summary, we have achieved proof of concept *in vivo* by the demonstration of glypican-3 specific tumor uptake and retention of tubulysin payload of a glypican-3 targeting adnectin drug conjugate.

P42 - MATERNAL-FETAL PHARMACOKINETICS OF BUPROPION AND ITS METABOLITES

Lindsay Czuba, Emily Fay, Jennifer Sagar, Alyssa Stephenson-Famy, Sara Shum, and Nina Isoherranen
University of Washington, USA

Bupropion is a norephenephrine-dopamine reuptake inhibitor commonly used in pregnancy to treat depression and aid in smoking cessation. Racemic bupropion is metabolized to several active metabolites including threohydrobupropion (threo), erythrohydrobupropion (erythro), and S,S-hydroxybupropion (S,S-OH-bup), and to inactive R,R-hydroxybupropion (R,R-OH-bup). S,S- and R,R-OH-bup are believed to be responsible for the major adverse effects associated with bupropion. OH-bup is predominantly formed by CYP2B6, while threo and erythro are formed by 11 β -HSD1 and several aldo-keto reductases. In addition, CYP2C19 hydroxylates bupropion, threo and erythro to 4'OH-metabolites. A clinical study suggested that the exposure of racemic bupropion during pregnancy is unchanged. That study did not evaluate the CLf of OH-bup (CYP2B6 activity), the formation of the 4'OH-metabolites (CYP2C19 activity), or the exposure to the individual stereoisomers and metabolites. In human hepatocytes, CYP2B6 is induced by estradiol, thus we predicted that OH-bup formation clearance (CLf) will increase during pregnancy as a result of increased estradiol concentrations. As CYP2C19 activity is decreased during pregnancy, we also predicted that the CLf of the 4'OH-metabolites will decrease during pregnancy leading to complex changes in Bup and its metabolites PK-PD during pregnancy. We hypothesized that the CLf of OH-bup by CYP2B6 will increase, and the CLf of 4'OH-threo or 4'OH-erythro by CYP2C19 will decrease during pregnancy when compared to postpartum, resulting in an increase in the steady-state concentrations of OH-bup stereoisomers and threo and erythro. To test this hypothesis, we recruited eight pregnant women who were on a chronic bupropion regimen at enrollment. All subjects were genotyped for CYP2B6 and CYP2C19. Plasma and 24-hour urine samples were collected in the 2nd (T2) and/or 3rd (T3) trimesters, and at 6-12 weeks postpartum (PP). In three subjects, maternal and umbilical cord plasma were collected at the time of delivery to determine the maternal to fetal plasma concentration ratio of bupropion and its metabolites. Plasma and urine samples were analyzed via LC-MS/MS to quantify (R)- and (S)-Bupropion, (R,R)- and (S,S)-OH-bup, erythro, threo, and the 4'OH- and conjugated metabolites. Steady-state plasma concentrations of bupropion and its metabolites were variable between subjects and unchanged when compared to matched PP concentrations. Threo was the main metabolite quantified in urine and only negligible amounts of unchanged Bup were excreted in urine. Both the S,S- and R,R-OH-bup to Bup metabolite to parent ratio increased in three subjects, decreased in one subject, and remained unchanged in one subject in T3 in comparison to postpartum. There was no significant difference in the threo and erythro metabolite-parent ratios or CLf, when compared to the matched postpartum values. Additionally, there was no significant difference in the 4'OH-threo or 4'OH-erythro CLf, suggesting CYP2C19 activity was unchanged during pregnancy. The maternal-to-fetal concentration ratios were >1 for all analytes, with greatest ratio observed with R,R-OH-bup, suggesting that the placenta plays a role in decreasing bupropion and its metabolite exposure to the fetus. Together, the data suggests that there are no clinically significant changes to the exposure to bupropion and its metabolites during pregnancy.

P43 - Human and Animal ADME using WS-Cavity Ring-Down Spectroscopy.**Stephen Dueker**¹, Hye Jin Yoon¹, Feng Dong², Anhye Kim³, Soo Hyeon Bae⁴, and Howard Lee⁵¹BioCore, South Korea, ²Picarro, South Korea, ³CHA Bundang Medical Center, CHA University, South Korea, ⁴Korean Institute of Radiological and Medical Sciences, South Korea, ⁵Seoul National University Hospital, South Korea

Radiocarbon (¹⁴C) is widely used for drug metabolism and distribution studies. This isotope brings high specificity (low natural background), stable molecular inclusion, and an inherent means of quantitation by decay counting (LSC/decay counting). Decay counting for ¹⁴C however is limited by low efficiency; accordingly, relatively large quantities of radioactive “doses” must be administered for *in vivo* ADME studies. Accelerator Mass Spectrometry brought high sensitivity (attomole) and speed by directly measuring atoms using high energy mass spectrometry. However, this instrument was engineered for the task of carbon dating and its complexity has slowed its adoption in biomedicine. Laser-based optical methods are poised to fill the measurement gap between AMS and LSC. One platform, Wavelength-Scanned Cavity Ring-Down Spectroscopy (WS-CRDS) (developed at Picarro in collaboration with Lawrence Livermore National Lab) builds upon years of work with high sensitivity IR lasers for quantification of isotopic signatures in small molecules. The application to ¹⁴C has been propelled by improvements in laser power, cell optics, and spectral models. A first commercial prototype instrument was installed at BioCore’s ADME lab in Korea in 2017 for a performance evaluation of CRDS against AMS for measurement of trace levels of ¹⁴C in biological samples. Test samples were generated from two studies: 1) a Human microtracer ADME study (1 uCi oral dose; plasma and urine collected at Seoul National University Hospital) and 2) Mouse tissues samples from a large molecule biodistribution study (2.1 nCi infusion, all major tissues and organs collected at Hoffmann-La Roche, Switzerland). Sample isotope ratios of ¹⁴C/C were tested over a large dynamic range from the natural ¹⁴C background (1 Modern is 98 amol ¹⁴C/mg C) to ~16,000 Modern. CRDS samples were analyzed directly after dry-down, whereas samples above 300 Modern required a carbon dilution step prior to AMS analysis. The comparison results showed CRDS accuracy of >94% compared to AMS for well-mixed samples (plasma), with RSDs <15% for replicates at the LLOQ and much lower at enriched ¹⁴C levels. Carryover was estimated to <0.15% between injections. PK modeling of both studies showed no significance difference between CRDS and AMS datasets. Conclusion: We showed this early CRDS commercial prototype can support ¹⁴C clinical and animal studies that previously were only possible using AMS sensitivity. AMS and CRDS have greater importance than just being “better scintillation counters”, and we expect CRDS to create greater demand for radiolabeled tool molecules in many areas of investigation.

P44 - TISSUE DISTRIBUTION OF EDP-305, A HIGHLY SELECTIVE AND POTENT FARNESOID X RECEPTOR (FXR) AGONIST, IN PRECLINICAL SPECIES**Meng Huang**, Li-Juan Jiang, Peng Dai, Matthew Ronsheim, and Yat Sun Or*Enanta Pharmaceuticals, Inc., USA*

Background and Aims: EDP-305, a selective and potent small molecule FXR agonist, is currently being developed for the treatments of Non-alcoholic steatohepatitis (NASH) and primary biliary cholangitis (PBC). Herein, we report the tissue distribution of EDP-305 in preclinical species.

Methods: Male C57BL/6 mice and male and female CD-1 mice were administered a single oral dose of radioactive-labeled [¹⁴C]EDP-305 at 10 mg/kg (100 µCi/kg) formulated in 0.5% methylcellulose/water. The [¹⁴C]EDP-305 concentrations in fifty-seven (57) tissues were determined using validated quantitative whole-body autoradiography (QWBA). Pharmacokinetic analysis in plasma and tissues was calculated using Phoenix® WinNonlin®.

Results: [¹⁴C]EDP-305 was well absorbed in male C57BL/6 mice and male and female CD-1 mice, regardless of strain and sex. Tissues with the highest [¹⁴C]EDP-305 exposure were small intestine, liver and gall bladder, with tissue-to-plasma exposure ratios of 69.9, 10.7 and 10.5, respectively. All the other fifty-four (54) tissues tested had less exposure than plasma. Very little radioactivity was found in melanin-containing tissues (e.g., skin and ocular system) and central nervous system. High exposure in gall bladder suggested that biliary excretion was a major elimination pathway for EDP-305. The concentrations in all of the tissues were similar between male and female mice.

Areas under the curve (AUC) in plasma, small intestine and liver were 50, 536 and 3,513 µg equivalent-hr/mL, respectively, with long half-lives of 6, 27 and 45 hours. Plasma, small intestine and liver had [¹⁴C]EDP-305 concentrations of 0.24, 1.34 and 35.7 µg equivalent/mL, respectively, at 24 hours post dose, which was consistent with the observed long half-lives in these tissues.

Conclusions: EDP-305 preferentially penetrated two NASH target organs, the liver and small intestine, with sustained pharmacokinetic exposure. Current data demonstrates that EDP-305 is attractive for further investigation in NASH and PBC.

P45 - DETERMINATION AND COMPARISON OF SKIN AND PLASMA CONCENTRATIONS OF MULTIKINASE INHIBITORS AND THEIR ACTIVE METABOLITES IN MICE

Ayaka Kojima, Miki Katoh, Yuki Asai, and Masayuki Nadai
Meijo University/ Faculty of Pharmacy, Japan

Multikinase inhibitors (MKIs), including sorafenib and sunitinib, have the potential to cause particular side effects such as hand-foot skin reactions (HFSR), which is characterized by erythematous skin lesions on the hands and feet accompanied by keratinization. HFSR is a dose-limiting toxicity of MKIs and may necessitate dose reductions and lead to decreased quality of life. HFSR has been shown to be caused by sorafenib and sunitinib with a higher frequency, compared to the other MKIs, nintedanib and vandetanib. The HFSR mechanism has considered to be direct stimulation of skin cells, but the details remain unclear. In the present study, we aimed to determine and compare the distribution profiles of four MKIs (sorafenib, sunitinib, nintedanib and vandetanib) in the mouse skin and plasma. Each MKI was administered orally at 40 mg/kg (single dose) or 30 mg/kg (14-day) to 9-week male C57/BL6 mice. Abdominal skin and blood were collected at 6 hours after the final administration. The MKIs in the skin and plasma was extracted with organic solvents and then quantified by LC-MS/MS. After a single administration, all MKIs used in the present study could be detected in the skin. On the other hand, after the 14-day administration, the skin and plasma concentration of four MKIs were higher than those after the single administration. The S/P ratios of all the MKIs increased, except that of sunitinib, were 0.48, 2.85, 10.50, and 29.50, respectively. Taking these results into considerations, the HFSR frequencies may not be related to the S/P ratios. The active metabolites of sorafenib and sunitinib was detected in the skin after 14-day administration. The S/P ratios of these active metabolites were 1.20 and 5.90, respectively, which is higher than those of the parent drugs, but the production rate of the active metabolite from sorafenib was extremely low. Therefore, it is suggested that the active metabolites of sunitinib may tend to accumulate in the skin. There have been reports of breast cancer resistance protein and P-glycoprotein being expressed in the skin and this may be responsible for the skin distribution profile of some drugs¹). These MKIs have been reported to be substrates of those transporters; therefore, it is necessary to consider the impact of the transporter expression in the skin.

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P46 - UNDERSTANDING THE EXCRETION PROFILE AND THE METABOLISM OF PITOLISANT IN CYP450 2D6 POOR AND EXTENSIVE METABOLISERS AT STEADY STATE

Iain Shaw¹, Philippe Robert², Stephane Piris², Thierry Duvauchelle², Stuart Mair¹, Helen Walker³, and Stuart Wood⁴
¹Quotient Sciences, United Kingdom, ²Bioprojet Pharma, France, ³Consultant, United Kingdom, ⁴Pharmaron-UK, United Kingdom

Introduction: Pitolisant (BF2.649) is a “first in class” novel, highly potent, selective, orally active histamine H3 receptor antagonist/inverse agonist with a ligand inhibition constant (Ki) between 0.3 nM and 2.4 nM at the human receptor, marketed in Europe as a treatment for narcolepsy with or without cataplexy.

A previous single dose human ADME study conducted in healthy male subjects demonstrated that pitolisant was well tolerated and provided a mean recovery of 88% of administered radioactivity, 25% of which was recovered as CO₂ in expired air. This study was designed to better define the absorption and elimination pathways, and circulating metabolites of pitolisant at steady state. Additionally, the molecule was labelled in a position where recovery of radioactivity through CO₂ expiration which had been a feature of the previous ADME study was not likely.

Pitolisant is partly metabolised by CYP2D6, which is known to have genetic polymorphisms resulting in some individuals having reduced or increased activity of the enzyme. Therefore, subjects were genotyped to ensure data in at least 3 subjects who had a predicted phenotype of poor metabolisers (PM). This would provide understanding of the absorption and elimination pathways and circulating metabolites of pitolisant at steady state in various CYP2D6 phenotypes, and allow characterization of the pitolisant elimination phase.

Materials and Methods: The subjects received an oral administration of pitolisant (17.8mg tablet) once daily for 7 days (steady state). They then received a single oral dose of [14C]-pitolisant (17.8mg capsule) containing not more than 148uCi 14C, on Day 8.

Radiolabelled drug product manufacturing was performed in a GMP manufacturing suite that is integrated with the clinical pharmacology unit. Blood, serum, urine and feces samples were collected and assayed for total radioactivity. Serum samples were assayed via LC-MS for parent drug and three main metabolites quantitation. Serum, urine and feces samples were analysed for metabolite profiling and identification via radioactivity analysis and high resolution mass spectrometry.

Results: Pitolisant was well tolerated when administered over a period of 8 days at a dose of 17.8mg per day. The cumulative mass balance recovery of [14C]-pitolisant was 92% with approximately 55% excreted within 24 hours post-dose. The majority of radioactivity (89%) was recovered in urine and a small part in feces (3%). There was no radioactivity recovered in expired air.

There was no significant difference in the overall radioactivity recovered from CYP450 2D6 poor and extensive

metabolisers however the rate of excretion appeared slower in poor metabolisers. The exposure (C_{max} and AUC) of pitolisant was approximately 3-fold greater in the CYP2D6 PMs.

Formation of the major metabolites of pitolisant was rapid and the combined exposure for parent and the three main metabolites quantified represented 49% and 33% of circulating radioactivity in poor and extensive metabolisers respectively. Other main metabolites were identified in this study leading to the characterisation of the complete metabolite profile.

Conclusion: This study and associated sample analysis provided a thorough understanding of the mass balance and metabolism of pitolisant as required by the regulatory agency for the additional post marketing investigation.

P47 - AN INTEGRATED RADIOLABELLED STUDY TO DETERMINE THE ABSORPTION, METABOLISM, EXCRETION AND ABSOLUTE BIOAVAILABILITY OF PEMAFIBRATE IN HEALTHY MALE SUBJECTS

Iain Shaw¹, Neil Hounslow², Stuart Mair¹, Helen Walker³, and Stuart Wood⁴

¹Quotient Sciences, United Kingdom, ²Kowa Research Europe Ltd, United Kingdom, ³Consultant, United Kingdom,

⁴Pharmaron-UK, United Kingdom

Introduction: Pemafibrate is a highly selective peroxisome proliferator-activated receptor alpha modulator, developed and marketed by Kowa Pharmaceuticals as a treatment for dyslipidemia. As part of the development programme for pemafibrate, an open labelled, single sequenced, two part radiolabelled crossover study was performed in male subjects. The study assessed the absorption and disposition of pemafibrate after intravenous (IV) and oral administrations and enabled determination of mass balance, absolute bioavailability, routes of excretion and metabolism(1).

Materials and Methods: In Part 1, subjects received a single oral dose of 0.2 mg pemafibrate followed by a 15 minute IV infusion of 0.2µg [¹⁴C]-pemafibrate (containing not more than [NMT] 270 nCi ¹⁴C) ending at approximately T_{max} for the oral dose.

There was a minimum three day washout between Part 1 and Part 2.

In Part 2, subjects received a single oral dose of 0.8 mg [¹⁴C]-pemafibrate (containing 121 µCi ¹⁴C).

Radiolabelled drug product manufacturing was performed in a GMP manufacturing suite that is integrated with the clinical pharmacology unit. Blood, urine and feces samples were collected and assayed for total radioactivity, parent drug and ¹⁴C parent drug.

Results: The IV and oral pharmacokinetics of pemafibrate were characterised. Mass balance data showed that an average of 87% of the administered radioactivity was recovered after oral dosing with the majority (73%) recovered in the feces. Pemafibrate showed good oral bioavailability (61.5%) and was well tolerated in normal healthy male volunteers. Extensive metabolism was observed however most parent drug and metabolites were excreted via the liver. In plasma, only two main metabolites were identified at levels >10% of total radioactivity.

Conclusion: This study and the associated sample analysis provided a thorough understanding of the absolute oral bioavailability, mass balance and metabolism of pemafibrate in a very efficient clinical trial.

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1. Pemafibrate Has High Bioavailability and is Principally Excreted via the Liver. Neil Hounslow,
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P48 - REGIONAL INTESTINAL DISPOSITION OF DESAMINOTYROSINE, AN IMMUNOMODULATORY MICROBIAL METABOLITE OF FLAVONOIDS

Rashim Singh¹, Jin Ke², Taijun Yin¹, Song Gao³, and Ming Hu¹

¹University of Houston, USA, ²Yunnan University of Chinese Medicine, China, ³Texas Southern University, USA

Purpose: The purpose of this study was to determine the intestinal disposition of desaminotyrosine (DAT), [chemical name: 3-(4-Hydroxyphenyl)propionic acid], an immunomodulatory microbial metabolite of flavonoids with protective effects against influenza, using *in vitro* Caco-2 cell TC7 monolayers and *in situ* four-site single-pass rat intestinal perfusion model with bile-duct cannulation. Hypothesis: We hypothesize that flavonoids are metabolized into DAT by gut microbiota in colon, which then enters enterohepatic recirculation via biliary excretion of its sulfate and glucuronide and subsequent hydrolysis by microbial enzymes. Method: Male Fisher (F344) rats (70-110 days old) (n=3) weighing between 260 and 350 g, were perfused with DAT (at 25 µM concentration) at a flow rate of 0.191 ml/min for 2.5 hours. Intestinal perfusates and bile samples were collected for each 30-min interval. Blood samples were collected once every 30 min and urine was collected from urinary bladder at the end of the experiment. Apical (AP) to basolateral (BL) and BL to AP transport of 50 µM DAT was measured in 21 days old Caco-2 TC7 cells in HBSS buffer (pH 7.4) at 37°C. Experiments were run in triplicate and samples were collected from AP and BL side at 30, 60, 90, 120, 180 min. Results: The effective intestinal permeability (P^{eff}) of DAT (at 25 µM concentration) in rat intestine at four sites (duodenum, jejunum, ileum and colon) of F344 rats showed significant regional preference. DAT showed highest absorption in duodenal region followed by colon, jejunum and ileum. Duodenum (P^{eff} = 1.61 ± 0.22 x 10⁻⁶ cm/sec) showed about 8-folds higher absorption than ileum (P^{eff} = 0.23 ± 0.10 x 10⁻⁶ cm/sec) and about 2- and 1.7-folds higher absorption than jejunum (P^{eff} = 0.84 ± 0.21 x 10⁻⁶

cm/sec) and colon, ($P^{\text{eff}} = 0.93 \pm 0.41 \times 10^{-6}$ cm/sec) respectively. The results indicated the involvement of an active uptake transporter, most probably monocarboxylic acid transporter (MCT), in the transport of DAT (a hydroxy monocarboxylic acid) across intestine. DAT (at 50 μM concentration) was moderately transported from both AP to BL ($P_{\text{app},A \rightarrow B} = 1.43 \pm 0.29 \times 10^{-6}$ cm/sec) and BL to AP ($P_{\text{app},B \rightarrow A} = 1.21 \pm 0.39 \times 10^{-6}$ cm/sec) membrane in Caco-2 cells. However, we saw no significant difference between AP to BL and BL to AP transport in Caco-2 cells, possibly because of saturation of uptake transporter at this concentration. Additionally, no significant amount of DAT metabolites were detected in Caco-2 cells or rat intestinal perfusates. However, both DAT-sulfate and DAT-glucuronides were detected in significant quantities in the bile and urine samples but not in the blood samples. Concentrations of DAT sulfate in bile and urine were significantly higher than its glucuronide. Conclusion: In conclusion, DAT was moderately absorbed in Caco-2 cell and throughout the length of rat intestine except ileum. DAT was metabolized *in vivo* into mainly sulfate and some glucuronide, which were excreted via both urinary and biliary routes. Role of uptake and/or efflux transporters in DAT intestinal absorption, biliary disposition and subsequent enterohepatic recycling needs further investigation.

P49 - UNCOVERING NOVEL LYSOSOMAL PH CHANGES CAUSED BY DMDNA31, A NOVEL RIFALOG PAYLOAD OF AN ANTIBODY-ANTIBIOTIC CONJUGATE (AAC) IN DEVELOPMENT TO TREAT STAPHYLOCOCCUS AUREUS INFECTIONS

Jennifer Vogt, Wouter Hazenbos, Thomas Pillow, and William Proctor
Genentech, USA

Staphylococcus aureus (*S. aureus*) represents a worldwide public health issue as it is the leading causative agent of bacterial infections in humans, in both hospital and community settings. Disseminated infections causing endocarditis, osteomyelitis, and necrotizing pneumonia are increasingly difficult to treat due to their invasive nature. Standard of care drugs (e.g. vancomycin and daptomycin) are inefficient at eliminating intracellular populations of *S. aureus*. We are developing a novel THIOMAB™ antibody antibiotic conjugate (AAC) therapeutic aimed at circumventing these issues by specifically targeting intracellular bacteria. The AAC is comprised of an antibody targeting the wall-teichoic acid of *S. aureus* conjugated to a rifamycin analogue (rifalog) payload, dimethyl-DNA31 (dmDNA31), through a cathepsin-cleavable ValCit linker. This AAC is designed to kill intracellular bacteria following opsonization and phagocytosis via dmDNA31 cleavage and release inside the phagolysosome (1, 2). The goal of this study was to assess lysosomotropic properties of dmDNA31 as a potential contribution to the mechanism of the AAC activity. We employed a high content imaging assay using LysoTracker DND26® (a fluorescent marker which accumulates in acidic compartments) in primary plated human hepatocytes to evaluate the lysosomotropism of dmDNA31, other rifalogs and antibiotics, and known lysosomotropic compounds (ie chloroquine, amiodarone, and propranolol). Unlike prototypical lysosomotropic compounds, dmDNA31 did not induce lysosomal swelling. Instead, dmDNA31 caused a dose-dependent decrease in LysoTracker signal in primary hepatocytes, suggesting potential alkalization of lysosomes. Washout experiments demonstrated this effect on LysoTracker signal was durable for up to 24h after the removal of compound, suggesting sustained intra-lysosomal retention of dmDNA31. To test if our observations were resulting from pH perturbation of the acidic lysosomal lumen, we implemented an additional assay using LysoSensor Yellow-Blue®. This dye exhibits pH-dependent dual emission spectra, fluorescing yellow in acidic compartments, and blue in more neutral environments. We found that dmDNA31 behaves similarly to bafilomycin A (an inhibitor of vacuolar-ATPase proton pumps), where we observed a near complete shift from yellow to blue fluorescence supporting alkalization of lysosomes. Lastly, we assessed the effects that dmDNA31 had on lysosomal pH by observing changes in the emission ratio 490/440λ of dextran-conjugated fluorescein to measure intracellular/intralysosomal pH. Murine macrophages were then treated with sub-cytotoxic concentrations of dmDNA31, rifampicin, chloroquine, and bafilomycin A1 for 24 hours. Similar to bafilomycin A1, dmDNA31 significantly alkalized the lysosomes (lysosomal pH shift from 5.1 to 6.6) while rifampicin and chloroquine have lesser effects on lysosomal pH (5.1 to 5.9 and 5.1 to 5.8, respectively). Taken together, we demonstrated that dmDNA31 is lysosomotropic and caused alkalization of the phagolysosome. While additional studies are warranted, these preliminary findings suggest this phenomenon may play a role in the mechanism of action of the AAC, considering clinically relevant strains of *S. aureus* have been reported to require an acidic environment for expression of virulence genes, and subsequent bacterial persistence.

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P50 - QUANTITATIVE WHOLE BODY AUTORADIOGRAPHY OF 14C-ENV-101 IN CYNOMOLGUS MONKEY

Guy Webber and Sarah Townley

ENVIGO, United Kingdom

Quantitative Whole-Body Autoradiography (QWBA) is an increasingly employed technique used to assess the distribution of radiolabelled drug within the whole animal. It provides a quantitative and visual record of drug distribution and concentrations in tissues and organs including brain and eye. The technique is often limited to small animals but we have developed our procedures to accommodate for larger animals. In this study we assessed quantitative drug distribution of a development compound, ENV-101, a novel centrally active dopamine D2 antagonist evaluated for the treatment of schizophrenia and bipolar disorder, in male cynomolgus monkey (*macaca fascicularis*) of 2.4 kg bodyweight and a single oral radioactive dose of 40 micro-curies/kg (0.3 mk/kg). Information concerning the tissue distribution of the compound in cynomolgus monkeys was required to support pharmacology and toxicology studies. After 1 and 8 hours post-dose, animals were frozen in hexane/carbon dioxide, sectioned using a Leica microtome and subject to phosphor imaging using radioactive standards prepared in whole blood. Distribution and tissue/organ concentrations for 14C-ENV-101 are presented including visual autoradiographs displaying drug penetration and distribution.

Following administration of single oral doses of 14C-ENV-101 to male cynomolgus monkeys, radioactivity (representing ENV-101 and/or its metabolites) was well absorbed and widely distributed into all tissues (including the target organ, the brain) throughout the animal body, with concentrations in the majority of tissues being generally higher than those in blood and plasma throughout. Highest observed concentrations occurred in the vast majority of tissues at 1 hour post-dose (the first sampling time) and were mainly associated with brown fat, visceral tissues (liver, kidney cortex and medulla), secretory tissues (pancreas, pituitary, salivary gland and mandibular lymph node), and adrenal cortex, where they were approximately 6-30 fold higher than that observed in blood or plasma. Low concentrations were generally observed in plasma, vena cava, choroid plexus, meninges, mammary gland, testis, fur and intervertebral disc, throughout. Concentrations of radioactivity had generally declined, but were still quantifiable in all of the tissues sectioned at 8 hours post-dose. At this time, radioactivity in visceral tissues were approximately 20-30% of the highest observed concentrations, whereas in glandular or secretory tissues, these were approximately 30-50% of the highest observed concentrations. The presence of high concentrations of radioactivity in the uveal tract at both time points and in the skin at 8 hours may suggest some association of drug-related material with melanin, although this was not reflected in the radioactivity concentrations in meninges. Although radioactivity concentrations in the brain were generally greater than in plasma and whole-blood, there was no specific localisation or affinity of radioactivity with individual regions of the brain. Concentrations in the choroid plexus and meninges were notably lower than in other regions of the brain and more similar to these in whole-blood and plasma.

P51 - ROUTINE METABOLITE IDENTIFICATION FOR COMPLEX CYCLIC PEPTIDES BASED ON IMS ENABLED QTOF DIA DATA ACQUISITION AND MASS-METASITE DATA PROCESSINGYun Alelyunas, Mark Wrona, and Nathan Anderson*Waters Corporation, USA*

The metabolic fates of peptide drug candidates need to be thoroughly and rapidly investigated in order to assess their potential to advance in discovery and development. Inclusion of unnatural amino acids, backbone modifications, conjugations, and cyclizations are common strategies to both improve efficacy and ADME profiles. To identify catabolites of these peptide types, data rich acquisition modes coupled with tailored software tools capable of effectively dealing with the complicated permutations of peptide LC-MS data are needed. In this study, catabolites of several structurally complex cyclic peptides were investigated using ion-mobility enabled HDMSE acquired data and processed using the Mass-MetaSite and WebMetabase macromolecule software packages (Molecular Discovery Ltd). Cyclic peptides, including daptomycin, dalbavancin, oritavancin, anidulafungin, and lanreotide, were incubated at 10 μ M in simulated intestinal fluid (SIF) in the presence of 500 μ g/mL chymotrypsin, respectively. Each incubate was sampled at 0 min, 5, 15, 45, and 120 min and quenched by adding 2 volumes of acetonitrile containing 1% formic acid. The LC system used was an ACQUITY H-Class UPLC equipped with an ACQUITY UPLC Peptide BEH C18 Column, 300 \AA , 1.7 μ m, 2.1 mm X 100 mm. A linear gradient from 5 to 40-70 B% in 8 minutes was used (mobile phase A was water + 0.1% formic acid, and mobile phase B was acetonitrile + 0.1% formic acid (v/v)). Flow rate was 0.4 ml/min and column temperature was 60°C. Data were acquired in UNIFI using HDMSE (ion mobility enabled MSE) on a Vion IMS QToF with intelligent data capture enabled for file size compression and processed using Mass-MetaSite and WebMetabase for metabolite identification. Key metabolites for five FDA approved cyclic peptide drugs of varying non-natural structural complexity were identified and elucidated. Ion mobility afforded additional resolution that was shown to help further discriminate, characterize and resolve metabolites from matrix ions having similar m/z, but significantly different ion mobility. Intelligent data capture, now directly implemented in raw data acquisition, afforded >70% reduction of file sizes. The macromolecule processing settings for Mass-MetaSite and WebMetabase made possible access to and analysis of IMS-enabled DIA (HDMSE) data directly from the UNIFI software platform. Key preliminary results for daptomycin incubations in SIF included the observation of a major ring opened metabolite from ester hydrolysis. The metabolite was confirmed through common fragment identification. The

software also identified minor metabolites from amide hydrolysis at the terminal region of the parent and ring opened metabolites. 2 Ion mobility enabled non-targeted HDMSE acquisition coupled with Mass-MetaSite and WebMetabase provides seamless data acquisition and processing for routine metabolite determination of complex cyclic peptides.

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P52 - DETERMINATION OF AMINOGLYCOSIDE TOXICITY IN HUMAN RENAL PROXIMAL TUBULE MONOLAYERS USING CLINICAL RELEVANT BIOMARKERS OF NEPHROTOXICITY

Colin Brown, Lyle Armstrong, Mike Nicholds, Git Nicholds, and Keith Pye
Newcells Biotech, United Kingdom

Around 50 % of preclinical *in vivo* toxicity screens fail to predict subsequent human toxicity, leading to significant attrition of drug molecules during drug development. Renal toxicity accounts for about 20 % of the unexpected toxicity at first in man. Understanding nephrotoxicity has been hampered by the lack of a good renal model. Recently we reported the utility of aProximate™ human proximal tubule cell (hPTC) monolayers as a powerful *in vitro* tool to investigate nephrotoxicity by screening a toolkit of 36 diverse compounds (19 nephrotoxic and 17 non nephrotoxic). ROC analysis gave a specificity of 88.2%, a sensitivity of 68.4% and an overall accuracy of 77.8% suggesting that aProximate™ human proximal tubule cell monolayers are highly predictive in detecting nephrotoxicity. Here we report the effects of a panel of aminoglycosides upon the release of the clinically relevant biomarkers NGAL, KIM-1 and clusterin by the cells in response to aminoglycoside challenge. hPTCs were isolated from fresh kidney cortex using a collagenase digest and Percoll density gradient protocol. hPTCs grown on Transwells formed confluent monolayers and produced average TEER value of more than 90 Ω.cm² after day 7 in culture. To test the utility of hPTC monolayers as predictive models of aminoglycoside nephrotoxicity, cells were challenged with a range of aminoglycoside analogues (tobramycin, gentamicin, neomycin, amikacin and streptomycin over a concentration range 0-300µM for 72 hours. At 72 hours the apical supernatant was collected and analysed for the biomarkers; KIM-1, NGAL and clusterin using a Mesoscale Discovery (MSD) platform. In addition, transepithelial resistance (TEER) was recorded as a measure of monolayer integrity, cell viability was determined by intracellular ATP levels and cell death was assessed using LDH release as a measure of cell death. In response to neomycin, tobramycin or gentamicin challenge, there was a significant concentration dependent rise in KIM-1, NGAL and clusterin release (e.g. clusterin production was 5.3-fold, 5.1-fold, 4.9- fold above control levels). And a significant fall in cell ATP-content, LDH release and TEER. In contrast, exposure of the cells to amikacin resulted in a more modest 1.5-fold increase in biomarker and cell viability endpoints. 72-hour exposure to streptomycin had no significant impact on any of the nephrotoxicity endpoints measure. These results report the successful application of primary human proximal tubule model as a predictive, *in vitro*, model of aminoglycoside nephrotoxicity.

P53 - Opium Alkaloid - Noscapine: A Potential Chemotherapeutic Agent

Ramesh Chandra and Snidgha Singh
University of Delhi, India

At cellular level, cancer is a disease of the cell that develops because of failures in the mechanisms that regulate cell growth. An individual cell multiplies without restraint until it and its progeny eventually overwhelm tissues and organs. Cancer development is a multistep process in which the summation of events is required to produce malignant tumor. Although there is tremendous progress in understanding the molecular events that lead to malignancy, progress in the development of clinically innovative drugs that can cure cancer is not moving at that pace.

A recent discovery that might challenge the existing knowledge of cancer and methods to treat it, researchers found that “cancer cells spread to organs much earlier than was thought.” In fact, cancer cells can spread even before a tumour develops, remain quiet for long periods of time and then awaken to form aggressive and fatal metastasis. The findings also offer an explanation for why some 5% of cancer patients have metastases but no original tumour. Cancer cells are able to spread from a nascent tumor much earlier than scientists long thought and are more adept than later emigrants at forming potentially lethal metastases at distant sites such as the brain and bones.

Chemotherapy with cytotoxic anticancer agents is still the main way of therapy targeted at specific cellular mechanisms in the malignant tissues. Some chemotherapeutic agents disrupt the crucial parts of the cell that are critical for cell division such as mitotic spindle. Most common cancers require combination of radiation and chemotherapy for the treatment. Noscapine is a very safe cough suppressant (antitussive) which has been in use for many decades. In recent years, noscapine's anti-cancer effect has been demonstrated when taken at doses higher than those used for cough suppression. It is currently in off-label use by a number of physicians in treatment of cancers of the breast, lung, prostate,

ovaries and brain, and lymphomas, to name but a few. It is being clinically studied in non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL). Noscapine, a non-addictive derivative of opium, has also demonstrated outstanding clinical effectiveness in reducing death rates from strokes. Its long safety record, widespread availability and ease of administration make it an ideal candidate for fighting several life-threatening conditions.

Our laboratory established that noscapine inhibits the progression of cancer cells by interfering with microtubular functions at the cellular level and induces apoptosis as like taxanes and the vinca alkaloids. Noscapine binds to tubulin and alters its conformation, resulting in a disruption of the dynamics of microtubule assembly (by increasing the time that microtubules spend idle in a paused state) unlike other tubulin inhibitors such as taxanes and vinca alkaloids which affect microtubule polymerization. Perhaps more importantly, noscapine was able to inhibit cancer at doses (300mg/kg body weight) which produced little or no toxicity, including no adverse effects on the primary immune response. In addition, noscapine also demonstrated potential anti-angiogenic activity as an alternate anti-cancer mechanism.

P54 - ENDOPLASMIC RETICULUM STRESS SUPPRESS MOTILITY AND METABOLISM OF PROSTATE CANCER CELLS: UNCOVERING ENTEROLACTONE'S MECHANISMS OF ACTION

Franklyn De Silva

University of Saskatchewan, Canada

Background: When a cell is under stress, it attempts to restore ER homeostasis and cellular functions, by activating several signaling pathways. However, if the stress unresolved, pro-survival signaling is diverted to pro-apoptotic signaling. Evidence of similarities and interactions among cardiovascular disease, cancer, obesity, and inflammation, indicates an overlapping disease-biology linking cancer prevention, treatment and survival. Naturally occurring plant lignans abundantly present in flaxseed (FLN) are capable of modulating serum and hepatic cholesterol levels. Diverse plant lignanoid constituents can be precursors of enterolactone (ENL) a mammalian lignan. Despite the information gathered throughout the years, the effects and mechanisms of action of bioactive mammalian lignans and their phase II metabolites are not completely understood. As drug discovery efforts continue to move towards multi-targeted effects, ENL's drug-like characteristics warrants further attention to fully grasp FLN enriched products' as well as ENL analogs' potential use in the clinic. A clinical trial done by our lab with a lignan enriched oral product indicates that it can provide a clinically relevant dose without significant toxicities. A link between FLNs anti-cancer effects might exist through the ability of FLNs to modulate ER stress and metabolism in dysregulated cells, while TGF β signaling is reported to be involved in the epithelial mesenchymal transition of prostate cancer (PC) cells especially with metastases and recurrence. Interestingly, metformin, and statins, are reported to interfere with GTPase activity, cytoskeleton, and motility.

Hypothesis: Anti-cancer effects of ENL in PC cells are a result of increased ER stress and decreased TGF β .

Methods: Key targets in metabolism, ER stress, survival, and trafficking were evaluated using cancerous and non-cancerous cell lines (LNCap, PC3, C4-2, RWPE-1, and 3T3-L1).

Results: ENL alone did not cause cytotoxicity (sulforhodamine B) to non-cancerous cells at 1000 μ M, but to cancerous cells at 50 - 200 μ M. Binding assay (PolarScreen™ PPAR γ -competitor), transactivation assay (Signal reporter), and uptake assay (labeled glucose) revealed ENL as a PPAR γ partial agonist compared with controls Rosiglitazone (full agonist) and FMOG (partial agonist). ENL modulated metabolism markers (FASN, SREBPs, LDLR, PPAR γ , GLUT1 and PKM2), reduced EMT markers (TGF β) and increased ER stress markers (ATF4, CHOP, GADD34 and GRP58). ENL reduced mitochondrial redox function (Alamar blue) and caused mitochondrial toxicity (Cell-Glo ATP) in glycolytic and non-glycolytic phenotype representing cells using glucose and galactose media. ENL sensitized select clinically relevant anticancer drugs; microtubule inhibitors (Cabazitaxel and Docetaxel), and androgen-receptor / synthesis inhibitors (Enzalutamide and abiraterone) to decrease cell viability (Calcein AM, colony forming assay, trypan blue exclusion) and cell motility (migration, adherent, wound healing assay) and increased apoptosis (Caspase 3/7 Assay). Microscopy using F-actin stain (Phalloidin conjugate) revealed changes in cytoskeleton.

Conclusions: There might be a novel connection in the ER stress regulated metabolism and motility, by ENL connecting PPAR γ and TGF β . ENL reduce metabolism, modulate ATP generation, increased oxidative stress (reactive oxygen species), activate pro-apoptotic factors leading to mitochondrial toxicity related cell death, and reduced migration and invasion.

All these suggest that the combination of enterolactone (lignans) with clinically relevant drugs could be an efficacious therapeutic strategy for the treatment of PC.

P55 - CHARACTERIZATION OF *IN VIVO* DISPOSITION OF CTI-1601: A MITOCHONDRIA TARGETED THERAPY FOR FRIEDREICH'S ATAXIA

Eric Gonzalez¹, Erik Wagner¹, Nicholas Mess², Amy Wang¹, Mark Payne³, David Bettoun⁴, Elizabeth Ottinger¹, Bonnie Rup⁵, and Xin Xu¹

¹NIH/NCATS, USA, ²Altasciences, Canada, ³Indiana University School of Medicine, USA, ⁴Chondrial Therapeutics, USA, ⁵Bonnie Rup Consulting, USA

Friedreich's Ataxia (FA) is a rare disease that is caused by a defect in the gene which codes for human Frataxin (FXN), a mitochondrial matrix protein which is integral in the assembly of iron-sulfur protein clusters. As a treatment for FA, Chondrial Therapeutics is developing a protein replacement therapy, CTI-1601, which is posited to deliver functional FXN to mitochondria. The efficacy of CTI-1601 relies on the cell-penetrating ability of the trans-activator of transcription (TAT) peptide, which has been added to the amino terminus of the full-length human FXN protein, and the subsequent endogenous processing into mature FXN, and other possible active degradant(s), after translocation into the mitochondria. In order to understand the *in vivo* disposition of CTI-1601, pharmacokinetic studies were conducted in healthy C57BL6 male mice following administration of a single 5 mg/kg intravenous (IV), or 10 mg/kg subcutaneous (SC) dose. An exploratory hybrid LBA-LC-MS/MS method was developed to quantify the molecule, in which samples were prepared using an anti-human FXN immunocapture step prior to proteolysis and LC-MS/MS analysis. Three tryptic peptides of CTI-1601 were monitored, which correspond to (1) the TAT moiety, (2) the mitochondrial signal peptide (MSP) of full-length FXN, and (3) the mature human FXN protein. Interestingly, the half-life of the full-length CTI-1601 was very short in plasma, in that the TAT moiety is cleaved from the molecule, as shown by the loss of the TAT peptide as early as 5 min post dose (the first sampling time point). The plasma half-lives of the remaining fragments ranged from 1.5 – 2.1 hours, with the C_{max} value for mature FXN reaching ~2800 ng/mL following the IV dose (LLOQ=25 ng/mL). The observation of rapid elimination of the TAT moiety warranted proof-of-concept that CTI-1601-derived mature FXN does in fact enter the mitochondria in tissue cells *in vivo*. For this purpose, the tissue samples collected were split, and one half was used to prepare mitochondrial isolates, while the other was used for the standard tissue homogenate preparations. Following sensitivity improvements to the exploratory hybrid LBA-LC-MS/MS assay (~20-fold), a substantial amount of CTI-1601-derived mature human FXN was observed in the liver mitochondria of mice dosed SC at 10 mg/kg. The pharmacokinetic analysis of CTI-1601 continued with the SC administration of 10 and 50 mg/kg CTI-1601 in FXN knockout (KO) mice, which have diminished expression of FXN in muscle tissue. Using two electrochemiluminescence (ECL) immunoassays developed for the quantification of either CTI-1601 or mature human FXN, preliminary analyses of the plasma samples show that the AUC of both molecules increased with the higher dose, while the T_{max} for the mature human FXN was extended from 30 min to 1 hour. The remaining tissue and mitochondria sample analyses are underway. In conclusion, these studies will not only provide the disposition kinetics of CTI-1601 in various tissues, they also determine the amount of the drug-derived mature FXN delivered to the target organelle, the mitochondria.

P56 - QUANTITATION OF A NOVEL ENOLASE-2 SELECTIVE INHIBITOR PRODRUG BIS-POM-HEX, ITS INTERMEDIATE HEMI-POM-HEX, AND PARENT DRUG HEX IN MOUSE PLASMA AND BRAIN TISSUES USING LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

Yongying Jiang

University of Texas MD Anderson Cancer Center, USA

Enolase (ENO) is an essential glycolysis enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Our previous studies has revealed a specific dependency on ENO2 in ENO1-/- glioma cells and thus targeting ENO2 in ENO1 homozygously deleted tumors represents an attractive therapeutic strategy. Recently, we have discovered HEX as an inhibitor with selectivity toward ENO2, whose pro-drug Bis-POM-HEX shows selective toxicity against ENO1-/- glioma cells in the low nM range and efficacy in eradicating intracranial ENO1 deleted tumors in orthotopic xenograft models. The Bis-POM-HEX prodrug is converted to Hemi-POM-HEX and subsequently HEX in cells by carboxy esterases. In order to explore Bis-POM-HEX and its derivatives for further development as anticancer drugs, we have developed and validated for the first time an analytical method to determine the concentration of these three analytes in the same plasma or brain samples. Quantitation of Bis-POM-HEX, Hemi-POM-HEX, and HEX in a same sample is highly challenging due to the intrinsic instability of Bis-POM-HEX and hemi-POM-HEX, and the polarity difference between the hydrophobic Bis-POM-HEX and hydrophilic Hemi-POM-HEX and HEX as phosphoric acid derivatives. The analytical method we developed employs protein precipitation treatment for quantitation of the non-polar Bis-POM-HEX and solid phase extraction (SPE) coupled with in-cartridge derivatization for quantitation of polar Hemi-POM-HEX and HEX. To quantitate Bis-POM-HEX, an aliquot of the sample was mixed with ice-cold acetonitrile and the extract was analyzed directly by LC-MS/MS. To quantitate Hemi-POM-HEX and HEX, an aliquot of the same sample was treated with ammonium bicarbonate (10 mM) containing Bn-HEX as an internal standard and then extracted by SPE. After in cartridge derivatization by trimethylsilyldiazomethane (TMS-DAM), the collected sample was then analyzed by LC-MS/MS. This method was validated by a linear calibration range of 5 - 1000 ng/mL for Bis-POM-HEX, 0.1 – 5 μ M for both Hemi-POM-HEX and HEX. This sensitive method has been successfully applied to determine PK profiles of Bis-POM-

HEX, Hemi-POM-HEX, and HEX in CD-1 mice and various tumor mouse models for PK, PD, and toxicity studies. The preliminary *in vivo* and ex-vivo results suggest that Bis-POM-HEX was quickly converted into HEMI-POM-HEX, followed by a more gradual conversion to HEX, the ultimate species responsible for the observed *in vivo* activities.

P57 - APPLICATION OF AN ABBREVIATED RADIOLABELED ADME STUDY UTILIZING 3H-LABELED TEST ARTICLE IN THE TRANSITION BETWEEN DISCOVERY AND DEVELOPMENT

Heasook Kim-Kang¹, Lian Zhou², Yu-Hua Hui², Kenneth Cassidy², and Richard Smith¹

¹Q² Solutions, USA, ²Eli Lilly, USA

When a compound enters Development phase, absorption, distribution, metabolism and excretion (ADME) studies traditionally utilize 14C- labelled-test article (TA) versus 3H-labelled-TA due to the following critical potential reasons: 1) biological instability of 3H label, 2) metabolic pathway switching due to an isotope effect, 3) unreliable quantitative data for some of the metabolites. Nevertheless, 14C-TA synthesis may be challenging, costly and time consuming, thus posing a risk for obtaining ADME information early. With these potential drawbacks in mind, if carefully used, 3H-TA can provide answers to key questions to support successful execution of future 14C-ADME studies. This study, conducted to facilitate quick and reliable metabolism data generation, describes the successful use of 3H-TA (bearing a tricyclic structure) in an *in vivo* ADME study during the transition between Discovery and Development interface. The study was abbreviated since only one BDC dog with a single IV dose was used. If the 3H-label is positioned at a stable site, it can successfully be used for a full ADME study as in case of a semi-synthetically prepared macrolide, [3H]-emamectin benzoate¹. The advantages of using 3H-TA include its easier/faster synthesis, higher specific activity (maximum of 28.8 Ci/mmol), and lower cost. 3H-TA used for the current study was available within 1 month and provided a quick understanding of in-vivo metabolic stability of the parent moiety toward amide-cleavage, a radioPK profile, excretion routes and metabolite profiles. Collected biological samples were subjected to total radioactivity determination before and after drying to determine the degree of tritium exchange. Overall, 3H-TA was considered sufficiently stable for the study's main goal since the loss was observed only in later time-interval/point samples. Selected samples were also subjected to radioprofiling using in-line radiodetection, yielding no unretained radiopeaks, supporting the presence of no/negligible amount of tritiated water (resulting from tritium exchange) in the samples. During the study, a major metabolite (challenging to identify due to coeluting matrices), was identified utilizing an online pre-concentration setup (described by Koppen²), followed by secondary chromatography. This analysis yielded a single radioactive peak with informative mass spectral data, showing no ring-cleavage and oxidative in nature, owing to the separation of coeluting matrix in the region. Identification of this metabolite detected in all three matrices was critical to assist in the decision for the optimal 14C-labeling position. The structures of major metabolites revealed all three rings remained intact except for a few minor metabolites. Overall, although the study was abbreviated, based on the results, dual-labeling of the compound was deemed unnecessary for future 14C-study and the desirable 14C-labeling position could be determined.

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P58 - UTILISING A DUAL HUMAN TRANSPORTER MDCK_MDR1_BCRP CELL LINE TO ASSESS EFFLUX AT THE BLOOD BRAIN BARRIER

Dermot McGinnity¹, Nicola Colclough¹, Ravindra Alluri¹, Danxi Li², Kan Chen³, and Yumei Yan³

¹AstraZeneca, United Kingdom, ²Pharmaron, China, ³AstraZeneca, China

Patients with brain metastases or primary brain tumours are difficult to treat, have a poor prognosis and represent a high unmet need. In part this is believed to be due to the blood brain barrier (BBB) limiting drug exposure in the brain. To facilitate the design of brain penetrant compounds at AstraZeneca we have established a comprehensive suite of BBB assays as part of our CNS strategy. A new double human efflux transporter transfected MDCK_MDRP_BCRP cell line has been established as our first line in-vitro BBB screen. This cell line has been characterised using MDR1 and BCRP transporter inhibition studies using a range of single and dual transporter substrates. This reveals good activity of both transporters in the cell line and enables the relative contributions to overall efflux ratios (ER) of dual substrates such as imatinib to be determined (ER without inhibition = 35, with MDR1 inhibition = 20, with BCRP inhibition = 14 and with both transporters inhibited ER = 0.5). A strong relationship between the human ER and rodent free brain/free plasma ratio K_{puu} gives confidence in the use of the MDCK_MDR1_BCRP assay to filter out candidate drugs unlikely to be sufficiently brain penetrant. Highly brain penetrant compounds with K_{puu} > 0.3 show ER < 2 in the MDCK_MDR1_BCRP assay whilst compounds predominantly excluded from the brain, K_{puu} < 0.05, have ER > 20. An assessment of 53 structurally diverse compounds demonstrated that all 7 (100%) compounds with a measured K_{puu} ≥ 0.3 also had an ER ≤ 2. Likewise all 8 compounds with a measured ER ≥ 20 had a K_{puu} ≤ 0.05. However, 8/21 (38%) compounds with a measured K_{puu} ≤ 0.05 had an ER ≥ 20, and 7/20 (35%) compounds with a ER ≤ 2 had a K_{puu} ≥ 0.3). These data helped established guidance target criteria for the MDCK_MDR1_BCRP assay in drug discovery projects. An

ER of 0.3 and an ER>20 is very likely to lead to low K_{puu} <0.05. The MDCK_MDR1_BCRP efflux assay, with its enhanced throughput and rapid turnaround features, is a key assay in the AstraZeneca oncology project test cascade with particular importance in the compound design phase allowing scientists to design out efflux substrate liability and so maximise likelihood of brain exposure.

P59 - DEVELOPMENT OF A NOVEL PROFILE DATA ANALYSIS METHOD TO AID DEEP UNDERSTANDING OF PHARMACODYNAMICS BY DECOMPOSING THE COMPLEX EFFECTS OF A DRUG

Tadahaya Mizuno, Shotaro Maedera, and Hiroyuki Kusuhara

The University of Tokyo, Japan

Not all the effects of a drug are fully discovered by researchers or developers. Therefore, to separate the complex effects of a drug into basic components is a prerequisite for a deep understanding of the pharmacological properties of drugs, which contributes to drug discovery processes such as drug screening, drug repositioning, and prediction of toxicity. To achieve separation, we focused on profile data analysis. The important characteristic of omics is the conversion of the biological information of a sample into numeric values by its comprehensiveness, which enables mathematical approaches to the analysis of biological samples. We have extended factor analysis and developed a novel profile data analysis method, orthogonal linear separation analysis (OLSA), which analyzes any types of profile data matrix as an input (Mizuno T, Sci Rep, 2019). In this study, we tested its performance.

We investigated transcriptome data of MCF7 cells treated with 318 compounds in Connectivity Map. OLSA contracted 11,911 genes to 118 factors. Ontology of the main genes constituting the factors detected significant enrichment of the ontology in 65 of 118 factors and similar results were obtained in two other data sets. Interestingly, structurally similar compounds showed high scores of several factors which did not exhibit significant enrichment of ontology, implying that OLSA detects biological responses not characterized yet. In further analysis of the Connectivity Map data set, one factor discriminated the properties of two Hsp90 inhibitors, geldanamycin and radicicol, while clustering analysis could not. Doxorubicin and other topoisomerase inhibitors were estimated to inhibit Na⁺/K⁺ ATPase, one of the suggested mechanisms of doxorubicin-induced cardiotoxicity. Moreover, we have established a mathematical method for transcription factor detection from the main genes constituting OLSA-derived factors and connected the factors with transcription factors. Luciferase assays revealed the novel aspects of several drugs and low molecular weight compounds such as the induction of p53 and SREBF1 with ciclopirox and tetrandrine, respectively. Finally, based on the factor including PI3K/AKT/mTORC1 inhibition activity, 5 compounds were predicted to be novel inducers of autophagy, and other analysis including western blotting revealed that 4 of the 5 actually induced autophagy. These findings indicate the potential of OLSA to decompose the effects of a drug and identify its basic components, which contributes to deep understanding of pharmacodynamics.

P60 - COMPARISON OF TOXICITY AND PERMEABILITY AMONG ANGIOTENSIN RECEPTOR BLOCKERS TO THE FETUS

Tomohiro Nishimura, Yu Ishikawa, Saki Noguchi, and Masatoshi Tomi

Keio University, Japan

Hypertensive disorders of pregnancy is one of common and severe diseases in pregnant women. Angiotensin II receptor blockers (ARBs) are widely used as antihypertensives but those are contraindicated in pregnant women due to the risk of oligohydramnios. However, differences of fetal transfer and risk for oligohydramnios among ARBs have not been compared and evidences for the adverse fetal effect against individual ARBs remain elucidated. FDA - AERS (Adverse Event Reporting System) is an open database and used for the analysis of clinical case reports. The aim of this study was to extract an ARB that shows relatively low risk of oligohydramnios by using FDA-AERS database and to have the experimental evidences of the differences of fetal adverse effects among ARBs. We compared the relative frequencies of representative adverse events and oligohydramnios associated with ARBs which were recorded in FDA- AERS database. Disease model rats for gestational hypertension were prepared by administrating L-NAME, non-selective NOS inhibitor, from gestational day of 12.5 (GD12.5). Olmesartan and irbesartan were treated from GD13.5 by osmotic pump. Plasma concentration of olmesartan and irbesartan was measured by LC/MS/MS. Reported frequencies of representative adverse events of ARBs such as renal failure were similar between ARBs, and the frequency of oligohydramnios associated with irbesartan was much less than that with olmesartan. L-NAME-induced gestational hypertension model rats which show hypertension and fetal growth retardation were used for the analysis of pharmacological effect and fetal transfer of olmesartan and irbesartan. The model rats were treated with olmesartan (50 mg/kg/day) and irbesartan (40 mg/kg/day) from GD13.5. The blood pressure was decreased into normal level by treatment with olmesartan or irbesartan. Therefore, olmesartan and irbesartan sufficiently exhibited their pharmacological effect in the pregnant rats. Contrary to the similarities of pharmacological effect to the dams between olmesartan and irbesartan, the weight of placenta and fetus isolated from irbesartan-treated rats were ameliorated but those from olmesartan-treated rats were little changed at GD18.5. Plasma unbound concentrations of olmesartan and irbesartan in the fetuses were equal to and much less than those in the dams, respectively, implying that fetal transfer of irbesartan across the placenta is limited compared to

olmesartan. Organic anion transporting polypeptide (OATP) 2B1 is expressed at the fetal-facing plasma membrane of syncytiotrophoblasts at least in human. Irbesartan was significantly taken up by OATP2B1 but olmesartan was not. Therefore, it is possible that extended fetal-to-placenta transfer of irbesartan results in low fetal distribution compared to olmesartan. Both olmesartan and irbesartan showed blood pressure-lowering effect to the similar extent, but only irbesartan ameliorated fetal growth. Irbesartan but not olmesartan was transported by OATP2B1 which is reportedly expressed at the fetal-facing plasma membrane of syncytiotrophoblasts in the placenta.

P61 - INVESTIGATIONS INTO DEFINITIVE *IN VITRO* DDI ASSAYS TO SATISFY FDA GUIDELINES

Taylor MacArthur¹, Lu Huo², Christopher Welsh¹, and Chuong Pham¹

¹Alliance Pharma, Inc., USA, ²Incyte, USA

Drug drug interactions (DDI) may often confound clinical predictions of drug exposure extrapolated from *in vitro* drug metabolism data. The FDA recommendations outline strategies for evaluating perpetrators and victims of cytochrome P450 (CYP) and drug transporter DDI. Clear definitive results for drug candidates reduce the risk of negative clinical outcomes. Here, we describe our efforts to generate definitive assays for evaluating drug candidates as CYP targets, inhibitors, and inducers, as well as transporter targets and inhibitors to satisfy the FDA guidelines. Using commercially available compounds, we were able to demonstrate the rigor of each assay and the corresponding DDI risk assessments in agreement with literature values.

P62 - DEVELOPMENT OF LC-MS/MS METHODS FOR THE QUANTITATION OF COMMONLY CO-ADMINISTERED DRUGS AND AN ASSESSMENT OF THE IMPACT OF THEIR PRESENCE ON THE ANALYSIS AND STABILITY OF NOVEL DRUG CANDIDATES IN SUPPORT OF A CLINICAL STUDY

Ryan Schmich, Jason Watts, Katherine Yahvah, Jennifer Zimmer, Ann Hoffman, and Shane Needham

Alturas Analytics, USA

Clinical studies involving novel drug candidates are at an all-time high as their use in immunotherapies and other targeted therapies begins to swell. The 2018 FDA Guidance for the Validation of Bioanalytical Methods requires an assessment of the impact of the presence of co-administered drugs on the analytical measurement and stability of novel drug candidates. In support of a clinical study that featured co-administration of Cimetidine, Itraconazole and Paroxetine with a proprietary drug candidate, GLP-compliant methods were developed and validated for each of these commonly co-administered drugs. In accordance with the FDA guidance, the impact of the presence of these co-administered drugs on the sensitivity and selectivity of the measurement of the proprietary drug and its metabolite was assessed. Additionally, the impact of the presence of the co-administered drugs on the freeze/thaw stability, bench top stability and long term storage stability of the proprietary drug and its metabolite were also assessed. Analytical results demonstrated that these co-administered drugs and the proprietary novel drug and metabolite could be measured with acceptable accuracy and precision when analyzed in samples that contained combinations of these analytes. Further, the data indicate that samples containing combinations of the proprietary drug and metabolite along with the co-administered drugs were stable when submitted to conditions similar to the conditions that clinical samples would be subjected to. Subsequent sample analysis in support of the clinical study generated 4031 reportable results for 2783 samples across five analytes. Four HPLC-MS/MS instruments were employed during the study and 93% of analytical runs met acceptance criteria. This work was performed with a 1 month turn-around time for QA reviewed data after the receipt of the final sample shipment.

P63 - Fast-Acting Small Molecules Inhibiting Malaria Infection at Multiple Life Stages

Snigdha Singh, Ramesh Chandra, and Aarushi Singh

Delhi University, India

The eradication of malaria remains challenging due to the complex life cycle of Plasmodium and the rapid emergence of drug-resistant forms of Plasmodium falciparum and Plasmodium vivax. New, effective, and inexpensive antimalarials against multiple life stages of the parasite are urgently needed to combat the spread of malaria. Here, we synthesized a set of novel hydroxyethylamines and investigated their activities *in vitro* and *in vivo*. All of the compounds tested had an inhibitory effect on the blood stage of P. falciparum at submicromolar concentrations, with the best showing 50% inhibitory concentrations (IC₅₀) of around 500 nM against drug-resistant P. falciparum parasites. These compounds showed inhibitory actions against plasmepsins, a family of malarial aspartyl proteases, and exhibited a marked killing effect on blood stage Plasmodium. In chloroquine-resistant Plasmodium berghei and P. berghei ANKA infected mouse models, treating mice with both compounds led to a significant decrease in blood parasite load. Importantly, two of the compounds displayed an inhibitory effect on the gametocyte stages (III-V) of P. falciparum in culture and the liver-stage infection of P. berghei both in *in vitro* and *in vivo*. Altogether, our findings suggest that fast-acting hydroxyethylamine-phthalimide analogs targeting multiple life stages of the parasite could be a valuable chemical lead for the development of novel antimalarial drugs.

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P64 - STRATEGIES AND CONSIDERATIONS FOR HUMAN PK OPTIMISATION AND PREDICTION IN DRUG DISCOVERY: CLEARANCE

Beth Williamson, Barry Jones, and Dermot McGinnity
AstraZeneca, United Kingdom

Drug metabolism and pharmacokinetic (DMPK) assays are employed early in drug discovery to reduce compound attrition in later phases and prioritise progression of compounds with sufficient quality. To achieve this, a cascade of DMPK assays have been established in AstraZeneca that can be followed sequentially for oral delivery projects. However, when challenges arise it is important to consider each compound or chemical series on a case-by-case basis. This work highlights, with examples, strategies employed for clearance (CL) optimisation and prediction.

Oral delivery projects strive for low CL to achieve optimal (or acceptable) pharmacokinetic half-life, bioavailability and human efficacious dose.

Hepatic *in vitro in vivo* extrapolation (IVIVE) of metabolic CL in animals provides an initial indication to the route of elimination. Data generated from preclinical species and human hepatic matrices, provide a degree of confidence when predicting human hepatic metabolic CL. Likewise, poor IVIVE demands further mechanistic investigation to understand the relevance to human CL estimates. Initial hepatic metabolic investigations are run in enhanced-throughput screening assays at a single substrate concentration (typically ~1 μM , on the assumption this concentration is at or below the K_m). Data confirms 1 μM is a suitable concentration for these assays. Intrinsic clearance at 0.1 and 1 μM was determined, for multiple chemistries, in human and dog hepatocytes, and 89% and 82% of compounds generated values within 2-fold for both concentrations, respectively. Exceptions are presented and do occur contributing to poor IVIVE. Hence, *in vitro* studies avoiding unnecessary *in vivo* experiments should be the starting point for investigations of poor IVIVE.

For chemical series exhibiting poor IVIVE, additional *in vivo* investigations may be warranted. These include investigating additional routes of elimination such as renal and biliary CL. Biliary and renal CL from rat and dog was determined for a diverse set of compounds and incorporated into a metabolic IVIVE analysis as additional CL pathways. This improved the IVIVE by 8 and 22% in the rat and dog, respectively. Further, comparison of CL in bile duct cannulated (BDC) rats and standard PK showed good agreement with 96% of compounds having CL within 3-fold. However, 75% lie above the unity line noting a trend towards higher CL in the standard PK compared to the BDC animals.

Additional considerations when investigating poor IVIVE include *in vivo* study design. Cassette PK studies allow efficient profiling of multiple compounds with a significant reduction of animal use compared to discrete dosing. However, PK drug-drug interactions between compounds in the cassette has the potential to introduce error in PK parameter estimation.

Data from AstraZeneca projects over the last several years shows excellent agreement for CL between discrete and cassette PK in rat and dog (86% and 91% within 2-fold, respectively) providing confidence that cassette PK is not a typical source of parameter error, but discrete PK should always be considered for key compounds.

Identifying the challenges in an efficient yet case by case approach through detailed mechanistic investigations of the CL of lead compounds is important to ensure effective identification of high-quality candidates.

P65 - STRATEGIES AND CONSIDERATIONS FOR HUMAN PK OPTIMISATION AND PREDICTION IN DRUG DISCOVERY: VOLUME OF DISTRIBUTION AND ORAL ABSORPTION

Beth Williamson, Nicola Lindsay, Barry Jones, and Dermot McGinnity
AstraZeneca, United Kingdom

Drug metabolism and pharmacokinetic (DMPK) assays are employed early in drug discovery to reduce compound attrition in later phases and to prioritise progression of compounds with sufficient quality. To achieve this, a cascade of DMPK assays have been established in AstraZeneca that can be followed sequentially for oral delivery projects. However, when challenges arise it is important to consider each compound or chemical series on a case by case approach. This work highlights, with examples, strategies to consider for prediction of volume of distribution (V_d) and oral absorption (Fabs).

Physicochemical properties e.g. ion class and lipophilicity, can be used to generate initial hypotheses regarding *in vivo* PK properties including estimation of V_d and human V_d can be predicted from values determined in animals(1). Cassette PK studies allow efficient profiling of multiple compounds with a significant reduction of animal use compared to discrete

dosing. However, PK drug-drug interactions between compounds in the cassette has the potential to introduce error in PK parameter estimation. Data from AstraZeneca projects over the last several years shows excellent agreement for Vd between discrete and cassette PK in rat and dog (81% and 82% within 2-fold, respectively) showing that cassette PK is not a common source of parameter error, but discrete PK should always be considered for key compounds.

Oral delivery projects strive for high Fabs and oral bioavailability (F) to achieve optimal (or acceptable) exposure and efficacy *in vivo*.

As F is dependent upon many biological and physicochemical properties e.g. solubility, pKa, formulation, first-pass extraction in the gut and liver, permeability and efflux transporter potential, a suite of *in vitro* and *in vivo* studies are required to understand each parameter and the individual or combined impact. Further, *in silico* models complement experimental data to influence compound design and prioritise compounds into specific assays.

Whilst CaCo2 and MDCKII transfected cells at a set concentration can confirm intrinsic permeability and efflux potential, the impact on absorption can only be fully understood with *in vivo* studies, typically in rats and dogs. *Ex vivo* Ussing chamber assays are particularly useful to understand species differences in intestinal metabolism and absorption.

Cassette PK studies in rat and dog can be used to assess F and Fabs. Data from AstraZeneca projects highlights the considerations and complexities (unlike Vd) involved when determining F. These factors are particularly evident when differences in dose and dose formulation are utilised between cassette and discrete PK studies. By excluding poorly soluble compounds (85 % for both species). Understanding these complications allows appropriate and informed comparison of F

Identifying the challenges in an efficient yet case by case approach through detailed mechanistic investigations of the absorption of lead compounds is important to ensure effective identification of high-quality candidates.

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P66 - EVALUATION OF SPECIES DIFFERENCES IN MAJOR CLEARANCE PATHWAYS BETWEEN HUMANS AND ANIMALS

Saki Yamauchi, Kota Asahina, Yusuke Aratsu, Yukihiro Nomura, and Motohiro Kogayun
Japan Tobacco Inc., Japan

In vitro-in vivo extrapolation (IVIVE) and allometric scaling are widely known prediction methods for human clearance (CL). It is reported that, for drugs cleared mainly via metabolism, IVIVE that incorporates species differences of metabolism provides higher accuracy, and for those cleared mainly via excretion, allometric scaling provides higher accuracy. These methods are used under the assumption that there are no species differences in the major CL pathways of the drugs. There are no reports as far as we know that comprehensively investigated species differences in CL pathways while several articles reported about species differences in the excretion of parent drugs to bile or urine. In this study, we evaluated species differences in major CL pathways between humans and animals (rats, dogs and monkeys) using marketed drugs. A dataset of marketed drugs whose excretion ratio data in bile and urine are available was used. We classified the marketed drugs as metabolism-type and excretion-type on the basis of their major CL pathways in humans. For drugs whose major CL pathway ratios were more than 70%, no significant species differences were observed. The major CL pathway of the metabolism-type drugs was metabolism also in animals, even if they had significant species differences in *in vitro* metabolic stability. For excretion-type drugs, no significant species differences were observed in the sum of bile and urine excretion ratios of parent drugs, while they tend to be excreted in urine in humans and bile in rats. This study suggested that there are no significant species differences in the major CL pathways of marketed drugs between humans and animals. This implies that determining the CL pathways of drug candidates in rats in the discovery stage enables to select the appropriate prediction method for human CL. For compounds mainly cleared via metabolism in rats, IVIVE incorporating species differences in *in vitro* metabolic stability is an appropriate prediction method for human CL. In addition, for compounds mainly cleared via excretion in rats, allometric scaling is considered as an appropriate prediction method for human CL, because no significant species differences were observed in the total excretion ratios of parent drugs.

P67 - A RANDOMIZED, DOSE-ESCALATION, DOUBLE-BLIND AND PLACEBO-CONTROL STUDY TO ASSESS THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF JAKTINIB IN HEALTHY CHINESE VOLUNTEERS

Hong Zhang¹, Jingrui Liu¹, Xiaoxue Zhu¹, Cuiyun Li¹, Xiaojiao Li¹, and Yanhua Ding²
¹The First Hospital of Jilin University, China, ²Phase I Clinical Research Center, China

HYPOTHESIS: About half of patients with myelofibrosis carry a gain-of-function mutation in the Janus kinase 2 gene (JAK2 V617F) that contributes to the pathophysiology of the disease. Jaktinib is a potent and selective oral Janus kinase 1 and 2 inhibitor in clinical development for the treatment of myelofibrosis, and its hydrogen is replaced by deuterium in a specific position, and showing more excellent pharmacokinetic and pharmacodynamic characteristics in preclinical

studies. **OBJECTIVE:** The purpose of this study was to determine the pharmacokinetic, food effect and tolerability profile of single and multiple doses of Jaktinib in healthy Chinese subjects. **METHODS:** This was a randomized, double-blind, placebo-controlled study with dose-escalation and food effect. Within each cohort, healthy subjects were randomized to receive single daily doses of Jaktinib of 25, 50, 100, 150, 200, 250, 300 and 400mg at single ascending dose study (SAD), and 100mg q24h, 150mg q24h, 100mg q12h, 200mg q24h and 150mg q12h for 10 days at multiple dose ascending study (MAD) in the fasted state. 6 subjects received Jaktinib and 2 subjects received matching placebo at each cohort in SAD and 8 subjects received Jaktinib and 2 subjects received matching placebo at each cohort in MAD. In food effect study, subjects (N = 12) received single dose of Jaktinib 200mg in the fasted and fed states with a 5-day washout period. Jaktinib PK parameters were estimated and summarized by dose. Liquid chromatography tandem mass spectrometry (LC-MS/MS) method was applied to determine plasma concentration of Jaktinib. Pharmacokinetic parameters were calculated using non-compartmental models. Dose proportionality of Jaktinib was examined across evaluated doses. Food effect was assessed. Safety was examined throughout the study. **RESULTS:** There were no dose restricted toxicity events and serious adverse events in this study. Decreasing absolute neutrophil counts were observed in five subjects treated q12h for 100 mg (n=1) and 150 mg (n = 4). These events were manageable and reversible upon drug discontinuation. 200 mg q24h was the Maximum tolerated dose (MTD) of this study. Jaktinib was rapidly absorbed (T_{max}:1.25-3.5h) and the half-life was approximately 2.952-9.040 h. It displayed a dose-dependent in exposure following single-dose administration, and the (90%CI) values were 1.15(0.96-1.34) and 1.00(0.86-1.15) for AUC_{inf} and C_{max} respectively. The steady state was achieved by day 4 of multiple dosing. The accumulation rate was low after multiple dosing at a 12 or 24-h dosing interval (1.24-1.38 for q12h and 0.94-1.16 for q24h administration). While in fed condition at the dosage of 200 mg, The exposure (C_{max} and AUC) of Jaktinib was increased by 1.28-1.6 times compared with fasted state. The effective dose was 25-100mg/kg at JAK2V617F bone marrow transplantation mouse model, and the human equivalent dose (HED) was 120-480 mg, which covered the MTD (200 mg q24h) of this study. **CONCLUSION:** Jaktinib was safe and well-tolerated in healthy volunteers and exhibited linear pharmacokinetics. The preclinical pharmacodynamics potency, and the safety and PK profile of Jaktinib at healthy subjects supports further evaluation for the treatment of myelofibrosis.

P68 - A SYSTEMIC REVIEW OF DRUG DISPOSITION AND DRUG INTERACTION DATA FOR 16 NEW SMALL-MOLECULE DRUGS DEVELOPED AND APPROVED IN CHINA BETWEEN 2007 AND 2018

Mingshe Zhu¹, Lingling Zhang², Tingting Cai², and Lian Guo²

¹MassDefect Technologies, USA, ²WuXi AppTec, China

Regulatory agencies in China approved 16 new small-molecule drugs between 2007 and 2018, a majority of which were discovered and all of which were developed in China. These drugs (ilaprazole, antofloxacin, imrecoxib, icotinib, iguratimod, allisartan, morinidazole, apatinib, chidamide, nemonoxacin, hemoporphin, anlotinib, albuvirtide, danoprevir, pyrotinib and fruquintinib) are only used in China except for ilaprazole and iguratimod that are also marketed in South Korea and Japan, respectively. During the period of time, China has established key DMPK capabilities, such as human radiolabeled ADME study, GLP bioanalysis, *in vitro* transporter DDI experiments. In addition, regulatory guidances for drug interaction, MIST and renal and hepatic impairment studies were issued in China, which are similar to those of ICH and USFDA. In 2018, China not only joined ICH and allowed international pharmaceutical companies to conduct parallel clinical trials in China, but also speeded up importing new drugs to the China market. The main objective of this presentation was to provide an overview of drug disposition and drug interaction data of the 16 drugs available in drug labels (in Chinese) and scientific publications, which can help the better use of drugs developed in China and oversea in the term of DDI and the improvement of DMPK strategy and approach for new drug development in China. Drug elimination routes in humans were determined for 10 drugs by analysis of radioactivity (N=2), LC/UV (N=2), NMR (N=1) and LC/MS (N=4) and prediction (N=1), while the major clearance pathways of other drugs were partially determined (N=2) or not reported (N=4). Metabolism mediated by CYP (N=4), UGT (N=1) and peptide hydrolase (N=1), non-enzymatic reaction (N=1) and renal excretion (N=1) were mainly responsible for the clearance of eight drugs, but enzymes catalyzed for the major clearance pathways of other two drugs were not determined or reported. OATP1B1/1B3 were determined to be substrates of two drugs and to play roles in their disposition. Four drugs were tested as victims of CYP inhibitors in clinical drug interaction studies and one drug was investigated in clinical renal and hepatic impairment studies. In addition, eight drugs have the data of *in vitro* CYP inhibition (N=8) and induction (N=4), and four drugs were tested as perpetrators of CYP enzymes in clinical drug interaction studies. A very limited number of *in vitro* transporter inhibition and clinical transporter DDI studies were conducted, which only focused on P-gp, BCRP and/or OATP. Furthermore, a few PBPK-based predictions and clinical pharmacogenetics study were performed to address the drug interaction issues. The drug metabolism and DDI data of the 16 new drugs demonstrates that the DMPK research in China has made huge progresses in support of the development and registration of innovative drugs in the past decade. On the other hand, the improvement of the DMPK research capability, the development of industrial expertise and the implementation of the regulatory guidances are required. The presentation will also discuss current key issues and future directions of drug metabolism research in the pharmaceutical industry in China.

P69 - PHARMACOKINETIC INTERACTION STUDY OF NOVEL COMBINATION OF HMG CO-A REDUCTASE INHIBITOR WITH GREEN COFFEE BEAN FORMULATION FOR OBESITY ASSOCIATED COMPLICATIONS IN SD RATS USING UHPLC/QTOF/MS

Lavanya Bolla, Johnsi Rani P., Anusha Kavati, and Satheeshkumar Nanjappan

National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, India

Introduction: Patients with obesity disorder will receive different combinations of drugs or herbal products simultaneously because of the metabolic complexities which are associated with obesity like cardiovascular diseases and diabetes. One of such combinations is using a herbal slimming agent along with a HMG Co-A reductase inhibitor which prevents the risk of cardiovascular diseases in obese patients. Green coffee bean formulation (GBF) is widely used as herbal slimming agent and is known for attenuation of obesity by reducing lipid accumulation. Atorvastatin (ATR) is a HMG Co-A reductase inhibitor which is used for dyslipidemia to prevent cardiovascular diseases.

Method: Herb-drug pharmacokinetic interaction study was carried out in male Sprague-dawley rats using GBF and ATR. Twelve rats were divided into two groups (n=6). First group is treated with GBF (PO; 800 mg/kg) while second group received sodium carboxymethyl cellulose (vehicle) for 7 days and on eighth day, both the groups were administered with ATR (PO; 10mg/kg) and blood samples were collected at different time points post dose and plasma was separated from centrifuged blood samples and stored at -40°C until analysis. Protein precipitation technique was followed and samples were subjected to LC-MS analysis. Detection of target ions [M+H]⁺ at m/z 559.2603 for ATR and m/z 515.2452 for telmisartan (IS) was achieved in ESI positive mode.

Results: The method was found to be sensitive (1 ng/mL) and linear over a wide concentration range of 1.0-2000.0 ng/mL. The current study demonstrates that the co-intake of ATR with GBF resulted in a slight decrease in AUC levels from 944.56h*ng/mL to 1008.27h*ng/mL for GBF treated group compared to control group and with significant decrease in C_{max} of ATR from 1962.53 ng/mL to 765.61 ng/mL.

Conclusion: From the preclinical study it was observed that GBF affects ATR oral bioavailability and it demonstrates that patients undergoing ATR therapy need to avoid co-administration of GBF containing products. Further mechanistic studies are recommended to confirm the clinical significance of these interactions.

P70 - STEREOSELECTIVE INHIBITORY POTENTIALS OF GINSENOSE-RH2 EPIMERS TOWARD ORGANIC ANION TRANSPORTING POLYPEPTIDE (OATP) 1B1/3-MEDIATED UPTAKE

Soon Uk Chae, Chae Bin Lee, Doyun Kim, Zhou Chi Huang, Seongjun Jo, Jee Sun Min, and Soo Kyung Bae

The Catholic University of Korea, South Korea

Purpose: Organic anion transporting polypeptide (OATP) 1B1/3 play essential roles in the hepatic uptake of many drugs and the assessment of OATP1B1/3-mediated drug-drug interactions are recently emphasized. The main active components of ginseng are ginsenosides, a diverse group of triterpenoid saponins that exert a variety of pharmacological activities including anti-inflammatory, anti-cancer, anti-diabetic, and cardioprotective effects. Of these, ginsenoside Rh2 is mainly recognized as an anti-cancer compound, and contains the two epimeric forms, 20(R)-Rh2 and 20(S)-Rh2. It was reported that the stereochemistry of the C-20 hydroxyl group [i.e., 20(R)-Rh2 and 20(S)-Rh2] not only plays a role in the pharmacodynamics of ginsenosides but also in their pharmacokinetic properties. The aim of this study was to evaluate the human organic anion transporting polypeptide 1B3 (OATP1B1 and 1B3)-mediated drug-drug interaction potential of ginsenoside Rh2 (Rh2) epimers, 20(R)-Rh2 and 20(S)-Rh2, using human embryonic kidney 293 (HEK293) cells overexpressing OATP1B3 (HEK293-OATP1B1 or 1B3).

Method: The inhibition of estradiol 17 β -D-glucuronide transport by ginsenoside Rh2 epimers were assessed in HEK293-OATP1B1 and OATP1B3. The concentrations of estradiol 17 β -D-glucuronide were analyzed by LC-MS/MS.

Results: The results show that ginsenoside Rh2 epimers exhibited stereoselective inhibitory effects of varying degrees on the HEK293-OATP1B1 and OATP1B3 explored. 20(S)-Rh2 had the strongest inhibitory effect on OATP1B1 with the lowest IC₅₀ value of 6.03 \pm 1.01 μ M. 20(S)-Rh2 also exerted moderate inhibition on OATP1B3, with an IC₅₀ value of 15.1 \pm 2.01 μ M. Conversely, 20(R)-Rh2 weakly inhibited OATP1B1 (IC₅₀, 37.3 \pm 5.01 μ M), and did not inhibit OATP1B3 uptake. As far as we know, in humans the prediction of *in vivo* drug-drug interactions mediated via reversible inhibition typically relies on the use of the C_{max}/K_i ratio. For competitive or uncompetitive inhibition, the IC₅₀/2, not the IC₅₀, could substitute for the K_i. In our reported previous study, after oral administration of 1 g/kg of purified ginseng dry extract to SD rats, the C_{max} of 20(S)-Rh2 was approximately 1.94 μ M. Given the IC₅₀ value of 20(S)-Rh2 for inhibition of OATP1B1, the C_{max}/K_i ratio for OATP1B1 inhibition by 20(S)-Rh2 was estimated to be 0.643.

Conclusion: There may be a potential for herb-drug interactions between 20(S)-Rh2 and OATP1B1 substrates when concomitantly administered.

P71 - PREDICTION OF P-GP MEDIATED DRUG-DRUG INTERACTION AT THE BLOOD-BRAIN BARRIER

Hélène Chapy, Johan Nicolai, Delphine Viot, Kenneth Saunders, Eric Gillent, Pierre Bonnaille, Anna-Lena Ungell, Jean-Marie Nicolas, and Hugues Chanteux
UCB Biopharma, Belgium

Aim. The aim of this work was to develop a model to predict the increase of the brain distribution of P-gp substrates in the presence of a P-gp inhibitor, based on *in vitro* data.

Background. The prediction of brain distribution fold change is of importance in the evaluation DDI potential for a drug interacting with P-gp at the blood-brain barrier level. It depends on the *in vivo* concentration ([I]) and inhibition potency (IC50) of the perpetrator and on the fe parameter of the drug victim (the clearance fraction due to the transporter, here P-gp: $fe = \frac{CL_{efflux}}{CL_{efflux} + CL_{passive}}$) (Zamek-Gliszczyński et al., 2008).

Methods. To determine these parameters, *in vitro* bidirectional transport of 3 P-gp substrates, UCB1, verapamil, and metoclopramide were evaluated in the presence and absence of cyclosporine A (CsA) in Mdr1a-transfected LLC-PK1 cells. The *in vitro* data were analyzed with SIVA® (Simcyp In Vitro data Analysis toolkit) to determine the *in vitro* efflux and passive diffusion clearances (CL_{efflux} *in vitro*, $CL_{passive}$ *in vitro*). These clearances were then extrapolated to rat *in vivo* applying an in house prediction model developed to calculate the extent of unbound brain distribution in rats (Nicolai et al. Prediction of brain disposition in rat and human from *in vitro* data correction: correction of passive permeation abstract submitted to ISSX 2019). The obtained *in vivo* clearances, $CL_{passive}$ *in vivo*, CL_{efflux} *in vivo*, were used to calculate the fe for each P-gp substrate. The *in vitro* data were also analyzed with GraphPad Prism® to obtain the inhibition parameters (IC50 and Hill coefficient). The predictions of fold increase in brain distribution were performed by applying the equation $\frac{1}{fe / (1 + ([I]/IC50)^{HillSlope} + (1 - fe))}$ (modified from Kalvass et al., 2013), for each substrate.

The predictability of the present approach was verified using *in vivo* data from Sprague Dawley (SD) rats. SD rats were exposed to pseudo-steady-state systemic CsA concentrations (Hsiao et al., 2006) and the P-gp substrates were administered per os (UCB1) or by IV route. CsA and P-gp substrate concentrations were determined in plasma and brain from terminal sampling by LC/MS/MS analytical method.

Results. The proposed working model using *in vitro* data, together with SIVA® and our in house brain distribution prediction model allowed us to determine fe (from CL_{efflux} *in vivo* and $CL_{passive}$ *in vivo*), IC50 and Hill coefficient. It resulted in an good prediction of the fold increase in brain distribution of UCB1, verapamil, and metoclopramide in the presence of increasing systemic CsA concentrations. The mean fold difference between predicted vs. observed were between 1.3 and 1.7.

Conclusion. This work shows that Mdr1a-transfected LLC-PK1 cells coupled with SIVA and our in house prediction model can be used to predict the *in vivo* brain distribution change due to P-gp inhibition.

P72 - QUANTITATIVE PREDICTION OF CYP3A4 AND CYP3A5-MEDIATED DRUG DRUG INTERACTIONS

Yingying Guo¹, Aroonrut Lucksiri², Gemma Dickinson¹, Raj Vuppalachchi³, Janna Hilligoss⁴, Shiew-Mei Huang⁵, and Stephen Hall¹

¹Eli Lilly and Company, USA, ²Department of Pharmaceutical Care, Faculty of Pharmacy, Chiang Mai University, Thailand, ³Indiana University School of Medicine, USA, ⁴Department of Medicine, Indiana University, USA, ⁵US Food and Drug Administration, USA

Cytochrome P450 3A4/5-mediated drug interactions are commonly encountered, but due to the associated complexity, prediction of the magnitude of interaction is challenging. We addressed part of this shortcoming by verifying a physiologically-based pharmacokinetic (PBPK) model that brings together the relevant drug properties, physiological components, including liver and gut CYP3A4/5, and the CYP3A5 genetic polymorphism. A midazolam-ketoconazole interaction study in 24 subjects selected by CYP3A5 genotype and LC/MS-quantification of CYP3A abundance from genotyped human liver (n=136) and small intestinal (N=12) samples were employed to build the PBPK model. A mechanistic framework in SimCYP was created that lead to a better characterization of liver and gut CYP3A content by incorporating the proteomics-informed CYP3A abundance and optimized small intestinal CYP3A4 abundance based on midazolam intestinal availability (FG) of 0.44. The framework defined the CYP3A4 abundance as 80.1pmol/mg liver microsomal protein and 130 nmol/small intestine, whereas CYP3A5 abundance for CYP3A5 expressers and non-expressers, respectively, was 23.8pmol/mg liver microsomal protein and 19.1 nmol/small intestine, and 1.44 pmol/mg liver microsomal protein and 1.13 nmol/small intestine. The correlation of hepatic CYP3A4 and CYP3A5 abundance in CYP3A5 expressers was described as (in pmol/mg) $CYP3A5 = 0.18 * CYP3A4 + 9.55$. The mechanistic framework successfully replicated the clinically observed CYP3A5 genetic effects on midazolam systemic and oral clearance. Furthermore, combined with a modified and verified ketoconazole PBPK model (Chien et al., 2006), this framework recapitulated the clinically observed geometric mean ratio of midazolam areas under the curve (AUCR) following 200mg or 400mg ketoconazole, which was, respectively, 2.7-3.4 and 3.9-4.7 fold in intravenous administration, and 11.4-13.4 and 17.0-19.7 fold in oral administration, with AUCR numerically lower ($P > 0.05$) in CYP3A5 expressers than non-expressers. PBPK modeling subsequently predicted some common scenarios where CYP3A5 expression may significantly impact pharmacokinetic and DDI studies of CYP3A substrate(s). First, fractional clearance of a substrate by CYP3A5 (fm,

CYP3A5) has to be at least 0.34 for CYP3A5 genetics to become a major contributor to PK variability. Second, CYP3A5 expression may significantly impact CYP3A-mediated DDIs depending on the relative CYP3A5 metabolic contribution and the ratio of K_i toward CYP3A5 and CYP3A4. When f_m , CYP3A5 is 0.34 or the relative catalytic efficiency of CYP3A5 is twice of CYP3A4, e.g. midazolam, the oral AUCR in CYP3A5 expressers would be comparable or lower than that of non-expressers when the K_i ratio (CYP3A5/CYP3A4) is higher than 7. However if the K_i ratio (CYP3A5/CYP3A4) is 2 or lower, the magnitude of inhibition in CYP3A5 expressers may be higher than that in non-expressers even when f_m , CYP3A5 is 0.22 or the relative catalytic efficiency of CYP3A5 is equal to CYP3A4. This may have important clinical implications in designing and conducting index (e.g. itraconazole) or concomitant (e.g. simvastatin) DDI studies for CYP3A substrates. In conclusion, the developed mechanistic framework supports dynamic prediction of CYP3A-mediated DDIs in the planning of clinical studies by bridging DDIs between CYP3A5 expressers and non-expressers.

P73 - EVALUATION FOR INHIBITORY POTENTIAL OF GINSENOSE-RH2 EPIMERS ON BREAST CANCER RESISTANCE PROTEIN (BCRP)-MEDIATED EFFLUX

Seongjun Jo, Doyun Kim, Chae Bin Lee, Soon Uk Chae, Zhou Chi Huang, Jee Sun Min, and Soo Kyung Bae
The Catholic University of Korea, South Korea

Ginsenoside, as the main active compounds of Panax ginseng, is classified steroid glycosides and triterpene saponins that play critical roles in pharmacological activity like inflammatory, anti-cancer, anti-obesity effect. One of these ginsenoside, ginsenoside Rh2, which exhibits potential therapeutic effects against various cancer; Rh2 can reduce the proliferation of a variety of cultured cancer cells and can promote apoptosis. The ginsenoside Rh2 exists as the pair of stereoisomers that differ in the spatial orientation of the hydroxyl group on C-20, 20(S)-Rh2 and 20(R)-Rh2. While most naturally occurring ginsenosides are of the (S)-configuration at this chiral carbon, processed ginseng products, such as steamed or boiled ginseng, contain the two epimeric forms, 20(R) and 20(S). The two forms show difference about not only in terms of pharmacological activities but also in terms of pharmacokinetic properties. In this study, we assessed inhibitory potential of Rh2 epimers, 20(R)-Rh2 and 20(S)-Rh2, against the Breast Cancer Resistance Protein (BCRP) *in vitro*. Prazosin and estrone-3-sulfate were used to be tested as BCRP substrates in a MDCKII cell-based assay and analyzed by LC-MS/MS. Our results showed that 20(R)-Rh2 and 20(S)-Rh2 exhibited different potentials of inhibition on the BCRP efflux. 20(S)-Rh2 exhibited strong inhibition with IC50 values of $0.933 \pm 0.121 \mu\text{M}$ (for prazosin) and $0.770 \pm 0.231 \mu\text{M}$, whereas, its (R)-epimer was observed no inhibition. In addition, a docking model suggested that 20(S)-Rh2 within the transmembrane region of a human ABCG2 transporter had a significant binding affinity for ABCG2 transporter with a Glide XP score of -9.060. As far as we know, in humans the prediction of *in vivo* drug-drug interactions mediated via reversible inhibition typically relies on the use of the C_{max}/K_i ratio. For competitive or uncompetitive inhibition, the $IC_{50}/2$, not the IC50, could substitute for the K_i . In our reported previous study, after oral dosing of 1 g/kg of purified ginseng extract to male rats, the C_{max} of 20(S)-Rh2 was approximately $1.94 \mu\text{M}$. Given the IC50 value of 20(S)-Rh2 for inhibition of BCRP, the C_{max}/K_i ratio by 20(S)-Rh2 was estimated to be 5.04. Taken together, there may be a potential for herb-drug interactions between 20(S)-Rh2 or processed ginseng products and BCRP substrates when concomitantly administered. These findings may help understand pharmacokinetic properties and herb-drug interaction potential of ginsenoside Rh2 epimers, comprehensively, and provide useful information on the clinical application of ginseng products.

P74 - EVALUATION OF ENDOGENOUS BIOMARKERS OF OATP1B ACTIVITY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER WHO RECEIVE PACLITAXEL

Hiroyuki Kusuvara¹, Daiki Mori¹, Hiroo Ishida², Tadahaya Mizuno¹, Kazuya Maeda¹, and Ken-ichi Fujita²
¹The University of Tokyo, Japan, ²Showa University School of Medicine, Japan

[Purpose] Organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 (OATP1B1/1B3) are multispecific organic anion transporters, predominantly expressed in the hepatocytes. They account for the hepatic uptake of various anionic drugs from the blood circulation. Based on *in vitro* data, paclitaxel was considered to cause OATP1B-mediated drug-drug interactions (Marada VV et al, Pharmacol Res, 2015), however its clinical relevance has not been demonstrated. This study aimed to assess *in vivo* inhibition potency of paclitaxel against OATP1B1/1B3 using endogenous biomarkers, such as glycochenodeoxycholate-3-sulfate (GCDCA-S) and coproporphyrin I (CP-I).

[Methods] The clinical study was approved by the ethical review boards of Graduate School of Pharmaceutical Sciences, the University of Tokyo and Showa University. The blood samples were collected before and after paclitaxel administration (200 mg/m², iv for 3hr) in two consecutive days in 10 patients with non-small cell lung cancer. Plasma concentrations of GCDCA-S and CP-I were quantified by LC-MS/MS. We also quantified C4, an intermediate metabolite of bile acid synthesis, and GCDCA, a precursor of GCDCA-S.

[Results and Discussion] After preincubation for 30 min, the K_i values (μM) of paclitaxel were 0.15 ± 0.03 , and 0.62 ± 0.18 for OATP1B1 and OATP1B3 using estradiol-17 β -glucuronide and pitavastatin as test probes, respectively. A paclitaxel metabolite, 6 α -hydroxypaclitaxel showed similar inhibition potency: the K_i values (μM) were 0.089 ± 0.014 and 0.87 ± 0.13 for OATP1B1 and OATP1B3, respectively. Product of the unbound fraction, and plasma total concentration of paclitaxel after the infusion was comparable to K_i for OATP1B1, but below K_i for OATP1B3. The concentrations of GCDCA-S and

CP-I increased significantly after paclitaxel administration and turned to decrease at 2 hours after the end of the administration. The AUC_{0-10hr} ratio was 3.6 ± 2.4 and 3.4 ± 1.9 for GCDCA-S and CP-I, respectively. In one patient, CP-I concentration was not altered.

[Conclusions] Therapeutic doses of paclitaxel for the treatment of non-small cell lung cancer (200 mg/m²) will cause significant OATP1B1 inhibition. It is recommended to administer OATP1B substrate drugs at least 2 hours after paclitaxel administration to avoid DDIs involving OATP1B.

P75 - IN VITRO INHIBITORY EFFECTS OF ZIZIPHI SPINOSI SEMEN EXTRACT ON CYTOCHROME P450 AND UDP-GLUCURONOSYLTRANSFERASE ENZYMES ACTIVITIES IN HUMAN LIVER MICROSOMES

Chae Bin Lee¹, Soon Uk Chae¹, Zhou Chi Huang¹, Seongjun Jo¹, Jee Sun Min¹, Yu Fen Zheng², and Soo Kyung Bae¹

¹The Catholic University of Korea, South Korea, ²China Pharmaceutical University, China

Several studies have demonstrated that Ziziphi Spinosa has hypnotic and sedative effect and also it can help memory impairment problem. Thus, the standardized 50% ethanol extracts of Ziziphi Spinosa Semen (ZSS) is developed as Alzheimer's disease treatment in Korea. In this study, we evaluated the potential of ZSS and its two main constituents, spinosin and swertisin, to inhibit human liver 9 cytochrome P450 enzymes (CYPs) and 10 UDP-glucuronosyltransferases (UGTs) to assess its safety. We demonstrated that ZSS had only inhibitory effects on CYP2B6, CYP2C8, CYP2C9 and CYP2E1 activities with IC₅₀ values of 118 ± 15.1 , 171 ± 26.3 , 175 ± 27.4 and 132 ± 18.6 µg/mL, respectively. ZSS did not inhibit the five CYPs (CYP1A2, 2A6, 2C19, 2D6, and 3A4) activities. Of the tested 10 UGT enzymes, ZSS had the strongest inhibitory effect on the UGT1A3 isoform with the lowest IC₅₀ value of 46.3 ± 9.68 µM. ZSS also showed slight inhibitory potentials for UGT1A1, UGT1A4, and UGT2B7 activities with IC₅₀ values of 110 ± 5.04 , 46.3 ± 9.68 , 113 ± 4.69 and 178 ± 22.8 µg/mL, respectively. No inhibition was observed on the UGT 1A6, 1A7, 1A8, 1A9, 1A10, and 2B15 activities. However, spinosin and swertisin showed no inhibitory effects on the tested 9 CYPs and 10 UGTs, showing different inhibitory pattern to that of ZSS. Thus, *in vitro* the four CYPs and four UGTs inhibitory effect of ZSS might be attributable to other constituents present in the ZSS except spinosin and swertisin. Our data suggest that ZSS is likely to cause clinically significant drug-drug interactions mediated via inhibition of UGT1A3 involved in drug metabolism *in vivo*. However, for better prediction of the potential *in vivo* inhibition of ZSS, identifying the constituents responsible for the UGT1A3 inhibition is needed.

P76 - IN VITRO INHIBITORY EFFECTS OF APINACA, A SYNTHETIC CANNABINOID, ON HUMAN CYTOCHROME P450, UDP-GLUCURONOSYLTRANSFERASE ENZYMES, AND DRUG TRANSPORTERS

Sunjoon Kim¹, Yong Ho Shin¹, Jeong-Han Kim², Im Sook Song³, and **Hye Suk Lee**¹

¹The Catholic University of Korea, South Korea, ²Seoul National University, South Korea, ³Kyungpook National University, South Korea

APINACA, N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide, is worldwide used as a new psychoactive substance although it has been a Schedule I controlled substance in US Drug Enforcement Administration since 2013. Drug abusers take concomitantly various drugs, and therefore, it is necessary to evaluate the potential of APINACA-induced drug interactions due to modulation of major drug-metabolizing enzymes and drug transporters. The inhibitory effects of APINACA on clinically important human major eight cytochrome P450s (CYPs) and six uridine 5'-diphospho-glucuronosyltransferases (UGTs) were investigated in human liver microsomes using LC-MS/MS assay. The inhibitory effect of APINACA on the activities of six solute carrier transporters and two efflux transporters using mammalian cells overexpressing OCT1, OCT2, OAT1, OAT3, OATP1B1, OATP1B3, P-gp, and BCRP. APINACA did not significantly inhibit CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2D6, UGT1A1, UGT1A3, UGT1A4, UGT1A6, or UGT2B7-catalyzed enzyme activities over the concentration ranges of 0.1-50 µM in human liver microsomes. It exhibited time-dependent inhibition of CYP3A4-mediated midazolam 1'-hydroxylation (K_i, 4.5 µM; k_{inact}, 0.04686 min⁻¹) and noncompetitive inhibition of UGT1A9-mediated mycophenolic acid glucuronidation with K_i value of 5.9 µM in human liver microsomes. APINACA did not inhibit significantly the transport activities of OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, P-gp, or BCRP up to 250 µM, suggesting that it may not induce transporter-mediated drug interaction *in vivo*. These data suggest that APINACA can cause *in vivo* adverse drug interactions via inhibition of CYP3A4 or UGT1A9 activities.

P77 - IN VITRO METABOLISM OF TICAGRELOR AND ITS METABOLIC INTERACTIONS WITH STATINS

Shuaibing Liu

The First Affiliated Hospital of Zhengzhou University, China

Background: Ticagrelor is a widely used antiplatelet agent in clinic, the oral bioavailability of ticagrelor averages only 35% due to the first pass metabolism[1]. However, the contribution of the intestine to ticagrelor first pass metabolism is unknown[2]. Although there's study regarding drug-drug interactions (DDIs) between statins and ticagrelor *in vivo*[3], the contribution of the metabolic enzymes and transporters to the effects remains unknown. Objective: To study the *in vitro*

metabolism of ticagrelor using human intestine microsomes (HIMs) and human liver microsomes (HLMs) as well as to investigate the drug-drug interactions between ticagrelor and some commonly used statins (simvastatin, lovastatin and atorvastatin) *in vitro*, expecting to provide an insight into the role of metabolic enzymes played in observed DDIs *in vivo*. Methods: For enzyme kinetics study, the metabolic reaction of ticagrelor was performed in HIMs and HLMs; The DDIs study between ticagrelor and statins were explored *in vitro* using pooled HLMs. Inhibition potency (K_i) values were determined using the substrate ticagrelor at concentrations of 10, 20, and 40 μM , with inhibitor concentrations ranging from 0 to 100 μM . The concentrations of its active metabolite, AR-C124910XX, in the incubation system were determined by a LC-MS/MS method. Apparent kinetic constants (K_m and V_{max}) were estimated by fitting formation rates of AR-C124910XX versus substrate concentrations to simple single-site Michaelis-Menten equation by nonlinear regression analysis using GraphPad Prism software (version 5.0). K_i values were calculated using Enzyme Kinetics Modules of Sigma Plot 14.0 based on the Dixon equations. Results: The K_m and V_{max} values for formation of AR-C124910XX in HIMs and HLMs were 9.36 μM and 256.3 pmol/min/mg, and, 30.98 μM and 1009 pmol/min/mg, respectively. Inhibition studies with selected statins indicate that simvastatin significantly inhibited the production of ticagrelor active metabolites, and its inhibition was consistent with competitive reversible inhibition with an inhibition constant K_i of 5.8 μM . Lovastatin and atorvastatin showed moderate inhibition of ticagrelor with inhibition constants K_i of 33.8 and 36.9 μM , respectively. Conclusion: Kinetic studies suggested that organ specificity exists in the intrinsic clearance of ticagrelor. Simvastatin showed a significant metabolic inhibition of ticagrelor, lovastatin and atorvastatin showed moderate metabolic inhibition of ticagrelor. The study provides an insight into elucidating the role of metabolic pathway in DDI between ticagrelor and statins. Further studies aiming at exploring the contributions of transporters to drug-drug interactions between ticagrelor and statins are on the way.

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P78 - IN VITRO METABOLIC, CYP AND TRANSPORTER CHARACTERIZATION OF PTI-125, A NOVEL SMALL MOLECULE DRUG CANDIDATE FOR ALZHEIMER'S DISEASE

Gang Luo¹, Jody Wanta¹, Kaitlin Bruden¹, Macaulay Haller¹, Courtney Lechler¹, Kari Landsverk¹, Daniel Albaugh¹, Donald L. McKenzie¹, and Lindsay Burns²

¹Covance Laboratories Inc., USA, ²Pain Therapeutics Inc., USA

PTI-125 is an oral, small molecule drug candidate in clinical development for the treatment of Alzheimer's disease (AD). PTI-125 binds and reverses an altered conformation of filamin A to suppress multiple AD pathologies. In the current *in vitro* study, the metabolic stability of PTI 125 was characterized in mouse, rat, dog, and human hepatic microsomes. Potential drug interactions were assessed by inhibition and induction of human hepatic CYP enzymes and by interactions with a panel of human drug transporters. PTI 125 (1 μM) was incubated with pooled (from both male and female) mouse, rat, dog and human hepatic microsomes (1 mg/mL) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) for 0, 15, 30 and 60 minutes or in the absence of NADPH for 0 and 60 minutes before determining remaining concentrations of PTI-125 in the incubation samples. PTI-125 showed measurable NADPH dependent metabolism by rat liver microsomes, but metabolism was not measurable by mouse, dog, or human liver microsomes. PTI-125 (up to 100 μM) did not show any reversible or time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5. Under the same experimental conditions, the known reversible and time-dependent inhibitors of CYP enzymes showed marked CYP inhibition. PTI-125 (up to 100 μM) showed no apparent cytotoxicity in a primary culture of human hepatocytes from a single donor. PTI-125 (20 and 100 μM) was stable when incubated with these single-donor human hepatocytes for 24 hours. PTI-125 (20, 60, and 100 μM) showed no induction of CYP1A2, CYP2B6, or CYP3A4 mRNA levels or activities in human hepatocytes from three individual donors. In the same hepatocytes, all known inducers demonstrated marked induction of these human CYP enzymes. In transporter studies, PTI-125 was not a substrate of key solute carrier (SLC) transporters including Organic Anion Transporter (OAT) 1, OAT3, Organic Cation Transporter (OCT) 1, OCT2, Organic Anion Transporting Polypeptide (OATP) 1B1, OATP1B3, OATP2B1, Multidrug and Toxin Extrusion (MATE) 1, MATE2-K or key ATP binding cassette transporters including P-glycoprotein (P gp) and Breast Cancer Resistance Protein (BCRP). PTI-125 showed weak inhibition of OAT1, OCT1, OCT2, MATE1 and BCRP, but no inhibition of OAT3, OATP1B1, OATP1B3, OATP2B1, MATE2 K or P-gp. Although IC₅₀ values were not accurately determined, they were estimated to be >100 μM for OAT1, OCT1, OCT2 and BCRP and >40 μM for MATE1. Overall, the general lack of drug metabolism (apparent only in rat), the lack of CYP interactions and the very weak interactions with some transporters comprise a highly favorable profile for this novel drug candidate for Alzheimer's disease.

P79 - EFFECTS OF THE PREGNANE X RECEPTOR (PXR) ACTIVATOR RIFAMPIN ON TRANSPORTER GENE EXPRESSIONS: STUDIES IN HEPATOCYTES *IN VITRO* AND IN MONKEYS *IN VIVO*

Congrong Niu¹, Yujin Wang², Sam Tep¹, Xiaofeng Zhao¹, Eisuke Murakami¹, Raju Subramanian¹, Bill Smith¹, and Yurong Lai¹

¹Gilead Sciences, USA, ²Terns Pharmaceuticals, USA

Organic anion-transporter polypeptides (OATPs) expressed on basal membrane of hepatocytes are the most important players in disposition of a wide range of drugs with active hepatic uptake as rate-determining clearance. Rifampin (RIF) is a well-investigated pregnane X receptor (PXR) activator to induce several cytochrome P450 enzymes, resulting in accelerated elimination of co-administered drugs that are metabolized by the enzymes. However, its regulatory effects on drug transporter gene expression are still in its infancy or remain controversial in literature. In the present studies, RIF-mediated induction of transporter genes was first investigated in sandwich cultured hepatocytes from three donors of human and cynomolgus monkey using qRT-PCR *in vitro*. Three-day treatment of RIF significantly induced CYP3A4 (> 60-fold change), but not CYP1A2, the metabolic enzyme genes that served as positive and negative controls respectively in the *in vitro* system. The transporter gene regulations by RIF appeared to be dose-dependent. Slight inductions of SLCO2B1, SLCO4C1, ABCB1, ABCC2, ABCC6 and SLC47A1 genes (< 2-fold change) were observed in both human and monkey hepatocytes at the highest concentration of 25 μ M except SLC51B had greater than 15-fold induction and SLCO2B1 was not expressed in monkey hepatocytes. In addition, a slight down-regulation of SLCO1B3 gene (< 2-fold change) was detected in both species. In *in vivo* studies, monkeys were dosed with RIF at 20 mg/kg/day for 7 days. Pitavastatin and antipyrine that served as the probes of OATP and CYP activities respectively were intravenously dosed before and after RIF treatment. Coproporphyrin I (CP-I) and III (CP-III), the OATP functional biomarkers, were also measured in plasma. While a significant increase of antipyrine clearance was observed after RIF treatment, the plasma exposures of pitavastatin, CP-I and CP-III remained unchanged, suggesting that OATP function was not significantly altered *in vivo*. Although a slight change of OATP2B1 and OATP1B3 gene expression was detected *in vitro*, the overall OATPs functional activities in monkey measured by a sensitive substrate and endogenous biomarkers were not affected *in vivo*. The data are consistent with regulatory guidance that the characterization of OATP1B induction during drug development is not required.

P80 - IMPACT OF PIPERINE ON CARBAMAZEPINE: MODELING-BASED PHARMACOKINETICS ANALYSIS AND PHARMACODYNAMICS EVALUATION IN ANIMAL MODELS

Tianjing Ren¹, Mengbi Yang¹, Min Xiao¹, Jijia Zhao¹, Yufeng Zhang¹, Mengyun Hu², Yan Cheng², Chunbo Zhang², Xiaoyu Yan¹, and Zhong Zuo¹

¹The Chinese University of Hong Kong, Hong Kong, ²Nanchang University, China

Carbamazepine (CBZ), the frontline anti-epileptic agent with narrow therapeutic index, is a CYP3A substrate and could be metabolized to carbamazepine-10,11-epoxide (CBZE). Its co-administration with piperine, the active component from black pepper, may lead to decreased CBZ metabolism due to CYP3A inhibition effect of piperine. Thus, the present study aimed to investigate the impact of piperine on pharmacokinetics and pharmacodynamics of CBZ and quantitatively evaluate the pharmacokinetics changes of CBZ via compartmental modeling approach. Plasma and brain pharmacokinetics of CBZ were studied in rats after single or two weeks combined oral administrations of CBZ (40 mg/kg, t.i.d.) and piperine (3.5 and 35 mg/kg, q.d.) by blood sampling and brain microdialysis. The effect of piperine on CBZ liver metabolism was further evaluated with livers obtained from rats received various treatments while its impacts on brain and plasma bindings of CBZ and CBZE were studied using rapid equilibrium dialysis. A compartmental pharmacokinetics model was built with NONMEM® to describe the plasma and brain concentrations of CBZ and CBZE and quantify the magnitude of pharmacokinetics changes in presence of piperine treatment. In addition, impact of piperine on anti-epileptic effect of CBZ was studied after their co-administrations in zebrafish and mice seizure models. The results showed no pharmacokinetics change after their single co-administration whereas significantly decreased plasma and brain concentrations of CBZ and CBZE with inhibited liver rCyp3a2 were observed after their two weeks combined oral administrations. With no impact of piperine on bindings of CBZ and CBZE in plasma and brain, compartmental pharmacokinetics modeling further estimated that long-term co-administration with high dose piperine would lead to decreases of 26%, 35%, and 38% in bioavailability, metabolism, and brain uptake of CBZ, respectively. Irrespective of pharmacokinetics changes, piperine demonstrated limited impact on the anti-epileptic effect of CBZ in studied animal seizure models. In summary, long-term combined use of CBZ with piperine could lead to decreased brain and systemic exposures of CBZ with inhibited liver metabolism, which mainly attributed to reduced absorption of CBZ resulted from GI contraction stimulation effect of piperine. Thus, long-term co-administration of CBZ with piperine in patients during epilepsy control may lead to its reduced systemic exposure, which warrants further clinical verifications.

P81 - THE IMPACT OF METABOLITE DETECTION ON ADHERENCE AND THE PREVALENCE OF DRUG-DRUG INTERACTIONS IN THE BEHAVIORAL HEALTH POPULATION THROUGH URINE TOXICOLOGY TESTING

Leigh Ann Saucier, Brian Cox, Michael Williamson, David Schwope, Hina Patel, and Rebecca Heltsley
Aegis Sciences Corporation, USA

Mental illness (MI) has been a highly discussed topic in the United States in recent years. One in five adults in the United States will experience a MI at some point during their lifetime, with one in twenty five suffering from a serious MI. Pharmacotherapy plays an important role in MI treatment, with one in six adults reporting filling a prescription for a psychiatric drug in 2013; however, adherence to medication regimens is estimated to be less than 50 percent, with some behavioral health (BH) medications thought to be even less. Indirect methods for assessing medication adherence such as pill counting, pharmacy reports, and self-report are often inaccurate, expensive, and inconvenient. The most reliable method for determining medication adherence is measuring drug levels in biological samples. In order to investigate adherence to BH medication, a study analyzing BH medications and metabolites in urine was conducted. In addition, urine drug-drug interaction (DDI) testing was also conducted on a subset of the mental health patient samples evaluated for adherence in this study to assess the potential for DDIs in this patient population. For both BH and DDI analysis, urine specimens were hydrolyzed and extracted via a solid phase extraction method. Samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) under reverse-phase chromatographic conditions using electrospray ionization with separate injections for the BH and DDI analyses. A total of 2275 unique mental health patient urine samples (1215 female, 1060 male) analyzed over a one month period were found to be positive for at least one BH medication. To mitigate the potential for false negative results, metabolites were included in the analytical method where appropriate. Of the samples analyzed, 14% were positive for metabolite(s) only, confirming the value in the use of metabolites when making adherence assessments. Of the samples analyzed for DDIs, 320 (62%) were found to have at least one DDI, with two patient samples having 15 interactions identified. Additional data analyses are ongoing. The results obtained from this study highlight the need for increased collaboration between healthcare professionals and toxicologists in order to allow physicians to make better decisions with their clients to positively impact patient outcomes.

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P82 - PHARMACOKINETIC APPLICATION OF NORMOTHERMIC PERFUSED EX VIVO PORCINE AND DISEASED HUMAN LIVERS

Lianne Stevens^{1,2}, Jeroen Dubbeld¹, Jason Doppenberg¹, Steven Erpelinck², Catherijne Knibbe³, Ian Alwayn¹, and Evita van de Steeg²

¹LUMC, Netherlands, ²TNO, Netherlands, ³LACDR, Netherlands

Drawing meaningful conclusions of *in vivo* human function from current *in vitro* biliary excretion models remains difficult due to species differences or due to differences in transporter expression. An increasing number of compounds is subjective to enterohepatic circulation (EHC), making it difficult to predict plasma profiles after oral and intravenous administration (1,2). Moreover, if a compound is subjective to EHC, it is more prone to cause a drug-drug interaction (DDI) and/or drug induced liver injury (3). We therefore aimed to create a preclinical model to investigate hepatic clearance, biliary excretion and DDI by utilizing normothermic machine perfusion (NMP) on porcine and diseased human livers. A unique source of human livers was used for the perfusion studies, namely diseased human livers which are explanted during transplantation surgery. Porcine livers were made available from the slaughterhouse. After procurement of the human and/or porcine liver, portal vein and hepatic artery were cannulated and directly flushed with Ringer's lactate solution. Thereafter, the bile duct was cannulated and subsequently the liver was connected to the LiverAssist. NMP (37°C) was performed using whole blood with the addition of epoprostenol, vitamins, antibiotics, parental nutrition, insulin and glucose for the pig liver perfusion or UW machine perfusate for the human liver. Carbogen (95% O₂/5% CO₂) was delivered to the oxygenator (2L/min). To study hepatic clearance, biliary excretion and DDI in the porcine liver, rosuvastatin was used a model compound and Rifampicin as an inhibitor. A bolus injection of 3 mg of rosuvastatin was dosed through the portal vein after a stabilized flow and pressure was obtained, and blood samples were taken at 15, 30, 45 and 60 minutes after dosing. Subsequently, 75 minutes after the first rosuvastatin dose, a second bolus injection of 3

mg rosuvastatin was applied together with a 300 mg rifampicin bolus injection and continuous infusion of 300 mg rifampicin/h. Blood samples were taken at 15, 30, 45 and 60 minutes after the second dosing of rosuvastatin and rifampicin. Bile was collected every 15 minutes.

Rosuvastatin was cleared from the circulation within 60 minutes by showing 80-90% of the dose excreted into the bile as parent drug and 10% as active metabolite N-desmethyl rosuvastatin. Upon inhibition, plasma levels of rosuvastatin were 600-fold increased and biliary excretion was 4- to 5-fold reduced. These results were comparable to prior pharmacokinetics and biliary excretion results with rosuvastatin obtained in pigs. Perfusion of the human cirrhotic livers showed a stable and constant pressure and flow in time.

We have demonstrated the feasibility of NMP of porcine livers as a valuable tool to study hepatic clearance and biliary excretion of rosuvastatin. We demonstrate for the first time that it is feasible to perfuse cirrhotic diseased (NASH/cirrhosis) human livers using the LiverAssist machine, which allows us to study hepatic disposition in diseased livers, transporter expression and disease processes in a controlled *ex vivo* setting.

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P83 - THE NATURAL PRODUCT KRATOM IS A POTENTIAL PRECIPITANT OF PHARMACOKINETIC INTERACTIONS WITH COMMON DRUGS OF ABUSE

Rakshit Tanna¹, Dan-Dan Tian¹, James Nguyen¹, Nadja Cech², Nicholas Oberlies², and Mary Paine¹

¹Washington State University, USA, ²University of North Carolina at Greensboro, USA

Background. Kratom (*Mitragyna speciosa*) is a plant native to Southeast Asia that has been reported to elicit opioid-like effects. Oral supplements made from the leaves are readily available and are commonly used to self-manage opioid withdrawal and pain. Mitragynine, a major alkaloid in the plant, may contribute to the presumed effects of kratom. Calls to poison centers in the US involving kratom exposures increased annually from 2011-2017 (13 to 682, or 52-fold), one-third of which reported use of kratom with drugs of abuse, including opioids and benzodiazepines.¹ Many of these drugs are metabolized extensively by cytochromes P450 (CYPs), particularly CYP2D6 and CYP3A4/5, raising concerns for potentially dangerous CYP inhibition-based drug interactions. The objective of this work was to assess the inhibitory effects of kratom extracts and mitragynine on CYP2D6 activity (dextromethorphan O-demethylation) and CYP3A4/5 activity (midazolam 1'-hydroxylation) in humans using an *in vitro* to *in vivo* extrapolation approach. **Methods.** Methanolic extracts prepared from three well-characterized kratom products (2, 10, 20 µg/mL), along with mitragynine (1, 10, 100 µM), were screened as inhibitors of CYP activity in human liver microsomes (HLM) and human intestinal microsomes (HIM). IC₅₀ shift experiments were conducted to assess potential time-dependent inhibition. Kinetics were determined using nonlinear least-squares regression and appropriate inhibition models. Mechanistic static models were used to predict the change in dextromethorphan and midazolam area under the plasma concentration-time curve (AUC). **Results.** All extracts and mitragynine showed concentration-dependent inhibition of CYP activity. The IC₅₀ for mitragynine towards CYP2D6 activity in HLM was 0.67±0.05 µM. Although no leftward shift was observed, mitragynine was a strong competitive inhibitor of CYP2D6 activity, with a K_i of 0.97±0.07 µM. Mitragynine caused an ~7-fold leftward shift in IC₅₀ towards CYP3A4/5 activity in both HLM (18.9±1.8 to 2.6±0.3 µM) and HIM (21.9±2.7 to 3.2±0.3 µM). The K_i and kinact for mitragynine towards CYP3A4/5 activity in HLM were 5.9±0.9 µM and 0.17±0.01 min⁻¹, respectively; corresponding parameters with HIM were 7.3±2.5 µM and 0.17±0.02 min⁻¹. Mitragynine was predicted to increase dextromethorphan and midazolam AUC by 1.1- and 12-fold, respectively. **Conclusions.** The predicted increase in midazolam AUC exceeded the recommended cut-off (1.25), necessitating further evaluation of potential kratom-drug interactions via dynamic modeling and simulation and potentially, clinical assessment. An improved understanding of these potential interactions is critical for the safe use of a readily available natural product often taken with drugs of abuse.

P84 - A PLATFORM FOR EVALUATING *IN VIVO* OATP1B INHIBITION RISK IN CYNOMOLGUS MONKEYS AND TRANSLATION TO HUMAN

Daniel Tatosian, Ying Li, Kimberly Michel, Christine Bowman, Lauren Zepp, Michael Hafey, Xiaoyan Chu, Rena Zhang, Ken Anderson, Tjerk Bueters, Maria Trujillo, and Raymond Evers
Merck & Co, Inc., USA

Background: Inhibition of drug transporters can lead to clinically meaningful drug-drug interactions, and potentially limit usage in the clinic. Direct prediction of drug-drug interaction liability for drug transporters for a new chemical entity from *in vitro* data is still a developing field. In drug discovery, use of conservative risk assessments may potentially drive decisions to discontinue otherwise acceptable compound(s), delaying opportunities to investigate novel therapeutics in the clinic. **Objective:** We assessed the utility of cynomolgus monkeys (CYNO) as an *in vivo* model for: (i) characterizing OATP1B1/B3 inhibition to inform human drug-drug interaction risk assessment for drug candidates; and (ii) evaluated the

use of coproporphyrins (COPRO) I and III as biomarkers for inhibition of OATP1B. Methods: A single oral dose of rifampin (RIF) was administered at 1.5 and 15 mg/kg and cyclosporine A (CsA) at 75 mg/kg to characterize the sensitivity of the CYNO model to detect weak and strong levels of OATP inhibition. Also, a novel discovery compound (Comp A) with an OATP1B1 IC₅₀ of 0.63 μ M and predicted human OATP1B1 R-value of 1.1 – 1.4 was administered at 5 mg/kg P.O., designed to attain a comparable C_{max} and absorbed dose in CYNO to the projected human dose for clinical development. Pitavastatin (PITA), a sensitive OATP1B substrate, and the endogenous biomarkers bilirubin and COPRO I and III were measured in plasma as *in vivo* probes for OATP inhibition. Results: Oral doses of 1.5 and 15 mg/kg RIF and 75 mg/kg CsA in 4 CYNO resulted in an 2.2, 8.6 and 13.7-fold increase in PITA AUC. RIF 15 mg/kg produced comparable exposure and a modestly higher PITA DDI relative to the 4.2 - 5.7 fold observed for 600 mg RIF in human studies. Additionally, 1.5 mg/kg and 15 mg/kg RIF, and 75 mg/kg CsA increased total bilirubin AUC_{0-t} by 1.2, 1.8 and 2.0-fold, direct bilirubin AUC by 1.4, 1.6 and 1.6-fold, COPRO I AUC by 1.1, 2.1 and 3.0 fold, and COPRO III AUC by 2.1, 3.2 and 3.4-fold, respectively. Critically, the weak DDI from 1.5 mg/kg RIF was captured, with the greatest effect on PITA, likely due to first pass inhibition. The endogenous biomarker results for RIF and CsA showed similar dose dependence for pitavastatin DDI. Administration of 5 mg/kg PO of Comp A resulted in PITA, COPRO I and III AUC ratios of 1.7, 1.0, and 0.9. The C_{max} of 1.3 μ M observed in CYNO was slightly higher than the predicted human C_{max} of 0.8 μ M. Assuming similar CYNO and human IC₅₀, the CYNO R-value was 1.26, suggesting that this dose adequately assessed *in vivo* OATP inhibition compared to human. By scaling the effect in CYNO to human accounting for PK and overall DDI magnitude differences, Comp A is predicted to cause less than a 1.2-fold increase in pitavastatin AUC in human. This weak DDI prediction is supported by the negligible effect on COPRO I and III in CYNO. These results suggest potential to use cynomolgus monkeys to further inform human OATP mediated DDI risk for novel small molecules.

P85 - *IN VITRO* INHIBITORY EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON HUMAN LIVER MICROSOMAL MORPHINE GLUCURONIDATION

Verawan Uchaipichat, Nantawat Thothumpol, and Nattaya Janpae
Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand

Tricyclic antidepressants (TCA) is commonly coadministered with morphine to cancer patients who requiring palliative care. However, knowledge about metabolic drug interaction arising from TCA inhibition on morphine glucuronidation is still limited. Using human liver microsome as the enzyme source, this study aims to (i) investigate the *in vitro* inhibitory effect of four TCA (*viz.*, amitriptyline, clomipramine, imipramine and nortriptyline) on morphine glucuronidation, and (ii) predict the magnitude of *in vivo* drug interaction arising from inhibition of various TCA on morphine glucuronidation. Morphine 3- and 6-glucuronide (M3G and M6G) formations in the incubation in the absence and presence of 2% bovine serum albumin (BSA) were quantified by using the method which is modified from previous studies (1,2). To investigate the IC₅₀ values, morphine concentration at 0.7 and 7 mM which is corresponding to S₅₀ values for morphine glucuronidation kinetic in the presence and absence of 2% BSA was employed, while all TCA concentrations were screened in the range of 10-1000 μ M. Using the incubation in the presence of 2% BSA, the inhibition mechanism and inhibition constants (K_i) were further investigated. The IC₅₀ and K_i values were estimated using Enzfitter (Biosoft, Cambridge, UK). The IC₅₀ values of amitriptyline, clomipramine, imipramine and nortriptyline for M3G formation obtained from the incubation without 2%BSA were 344, 166, 691 and 264 μ M, respectively. The respective IC₅₀ values for M6G formation were 291, 217, 398 and 471 μ M. The IC₅₀ values obtained from the incubation with 2%BSA for M3G and M6G formation were 1.3- to 6.6-fold and 3.6- to 12.8-fold lower than those obtained from the incubation without BSA. In the presence of 2% BSA, the IC₅₀ values of amitriptyline, clomipramine, imipramine and nortriptyline for M3G and M6G formation were 196&72 μ M, 25&17 μ M, 186&102 μ M, and 205&130 μ M, respectively. All four TCA exhibited competitive inhibition on morphine glucuronidation. Clomipramine showed the highest inhibitory potency for M3G and M6G formation with the K_i values of 32 and 16 μ M. The K_i values for amitriptyline, imipramine and nortriptyline for M3G and M6G formation were 245&81 μ M, 202&90 μ M, and 269&177 μ M, respectively. However, using the *in vitro* and *in vivo* extrapolation approach, the magnitude of *in vivo* drug interaction arising from TCA inhibition on morphine glucuronidation were negligible (*i.e.* the predicted morphine exposure increase when coadministered with TCA were less than 3%).

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P86 - INVESTIGATION OF THE INTERACTION MECHANISM BETWEEN METRONIDAZOLE AND 5-FLUOROURACIL

Yoshiaki Yamagishi, Erika Tomokiyo, Masato Uemura, Akihiro Ogino, Hiroko Masuo, Yohei Kusunoki, Makoto Okuyama, Haruka Maekawa, Hitomi Goda, Toshiyuki Kudo, and Kiyomi Ito
Musashino University, Japan

[Aims] Metronidazole (MTZ) is an imidazole antibiotic used worldwide for protozoal and anaerobic infections. The plasma elimination of 5-fluorouracil (5-FU) has been reported to be delayed by a concomitant use of MTZ though the mechanism of this interaction remains to be elucidated. This study aimed to explore the mechanism of this interaction focusing on the effect of MTZ on dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme in the catabolism of 5-FU. [Methods] 1) Effects on *in vitro* metabolism of 5-FU: 5-FU (20 μ M) was incubated at 37°C for 60 min with pooled human liver cytosols added with dithiothreitol and NADPH in the presence or absence of MTZ. The concentrations of remaining 5-FU were determined by LC-MS/MS. 2) Effects on the expression of DPD in HepG2 cells: HepG2 cells were plated at a density of 1.0×10^6 cells/well in 12-well plates. After 48-hours incubation, cells were incubated for 72 hours in the presence or absence of MTZ and mRNA expression of DPD was quantified by real-time RT-PCR using β -actin as a house-keeping gene. 3) Rat *in vivo* study: After treatment of 7-week-old male SD rats with oral MTZ (200 mg/kg) or vehicle once daily for 3 days, 5-FU (100 mg/kg) was orally administered and the plasma concentrations of 5-FU, uracil (endogenous substrate of DPD) and dihydrouracil were determined by LC-MS/MS. [Results and Discussion] The incubation with cytosols resulted in 54.5% decrease in 5-FU concentration in the absence of MTZ. MTZ inhibited 5-FU metabolic activity by 2.8 and 5.5% at 500 and 1,000 μ M, respectively, showing almost no effect at clinically relevant concentration. MTZ (4-2,000 μ M) also did not exhibit significant change in DPD mRNA expression in HepG2 cells, suggesting that the reported interaction was not due to direct inhibition of DPD-mediated metabolism of 5-FU by MTZ. The area under the plasma concentration-time curve of 5-FU was 0.59-times lower in MTZ-treated rats compared with the vehicle-treated rats, though the difference was not statistically significant. MTZ also did not cause a significant change in dihydrouracil/uracil concentration ratio. The possibility of indirect mechanisms and involvement of drug transporters in the interaction are under investigation.

P87 - MAIN MECHANISMS OF PHARMACOKINETIC DRUG-DRUG INTERACTIONS TRIGGERING LABEL RECOMMENDATIONS FOR DRUGS APPROVED BY THE FOOD AND DRUG ADMINISTRATION IN 2018

Jingjing Yu, Ichiko Petrie, and Isabelle Ragueneau-Majlessi
University of Washington, USA

The aim of the present work was to review pharmacokinetic drug-drug interaction (DDI) data available in New Drug Applications (NDAs) for drugs approved by the US Food and Drug Administration in 2018 and analyze the mechanisms mediating interactions that triggered label recommendations. Methods: The University of Washington Metabolism and Transport Drug Interaction Database® was used to identify clinical DDI studies available in the NDAs, and interactions resulting in label recommendations due to safety concerns (from contraindication to simple monitoring for adverse reactions) were further evaluated. Results: For the 42 new molecular entities (NMEs) approved in 2018, 23 (55%) drugs-including 10 antineoplastic agents- had label recommendations based on the results of DDI evaluations (clinical trials or physiologically-based pharmacokinetic modeling and simulations). Interestingly, 41% of interactions triggering label recommendations had changes in victim area under the curve (AUC) below 2-fold, suggesting a fairly narrow therapeutic index for the victim drugs involved. CYP3A was the predominant enzyme, involved in a large majority (72%) of all interactions. Fifteen drugs were found to be substrates of CYP3A, with AUC ratios (AUCR) ≥ 1.25 or ≤ 0.8 when co-administered with a strong CYP3A inhibitor or inducer, respectively. While none of them presented an AUCR ≥ 5 in the presence of a strong CYP3A inhibitor, five drugs, namely doravirine, duvelisib, loratrexib, lorlatinib, and netupitant [the active moiety of the prodrug fosnetupitant], exhibited high sensitivity to induction, with exposure decreases of 80-88% when co-administered with the strong inducer rifampin. When NMEs were evaluated as perpetrators of enzymes, dacomitinib was found to be a strong inhibitor of CYP2D6 (dextromethorphan AUCR 9.55), apalutamide a strong inducer of CYP3A (midazolam AUCR 0.08) and CYP2C19 (omeprazole AUCR 0.15), and ivosidenib a strong CYP3A inducer (midazolam AUCR 0.10). For transporters, P-gp was the transporter most often identified and among the 15 NMEs metabolized by CYP3A, 11 were found to also be substrates of P-gp *in vitro*. The gonadotropin-releasing hormone antagonist elagolix exhibited the highest change in exposure due to transporter inhibition, with a single oral dose of rifampin significantly increasing its exposure 5.58-fold, suggesting that elagolix is a sensitive substrate of OATP1B1. When NMEs were considered as perpetrators, all transporter-mediated interactions observed were weak inhibitions (AUCR < 2), with six drugs found to be weak inhibitors of P-gp, BCRP, OATP1B1/1B3, OCT2, and/or MATE1. Conclusion: Inhibition and induction of CYP3A were the most common mechanisms observed in clinical interactions triggering dosing recommendations. The magnitude in exposure change of most transporter-mediated interactions were limited with only one drug, elagolix, being a sensitive substrate of OATP1B1.

P88 - ROLE OF MICRORNAS IN DRUG-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN HUMAN LUNG-DERIVED ALVEOLAR TYPE II EPITHELIAL CELL MODEL A549/ABCA3

Ryoko Yumoto, Ayano Yamamoto, Takashi Konaka, Shinnosuke Takenaka, Masashi Kawami, and Mikiyoshi Takano
Hiroshima University, Graduate School of Biomedical & Health Sciences, Japan

Several drugs including bleomycin (BLM), methotrexate (MTX), and paclitaxel (PTX) sometimes induce serious interstitial lung diseases such as pulmonary fibrosis. Recent studies suggest that epithelial-mesenchymal transition (EMT) of alveolar type II epithelial cells plays a key role in the pulmonary fibrosis. We have previously shown that BLM and MTX induce EMT in a manner similar to that induced by transforming growth factor (TGF)- β 1 in cultured alveolar epithelial cells. Recently, microRNAs (miRNAs), one of non-coding RNAs, have been reported to be associated with EMT. However, the role of miRNAs in drug-induced EMT and pulmonary fibrosis remains largely unknown. In this study, we examined the role of miRNAs in drug-induced EMT using human-derived alveolar epithelial cell line A549/ABCA3 having more type II-like phenotype than wild-type A549 cells. A549/ABCA3 cells were established by introducing human ABCA3 gene into wild-type A549 cells, and were cultured in DMEM with 10% FBS. A549/ABCA3 cells were treated with BLM, MTX, and PTX, and morphology of the cells was observed by phase-contrast microscopy. Microarray analysis of miRNAs was performed using 3D-Gene system, and the expression level of each miRNA and mRNA was measured by real-time PCR. Transfection of A549/ABCA3 cells with miRNA mimic was performed using Lipofectamine2000. Treatment of A549/ABCA3 cells with BLM, MTX, and PTX induced marked changes in cell morphology, and mRNA expression of α -smooth muscle actin (α -SMA), a marker of EMT, was up-regulated by these treatments, indicating that EMT was induced by these drugs in A549/ABCA3 cells. Microarray analysis of miRNAs revealed that the expression of some miRNAs such as miR-34a was significantly increased more than 2-fold by treating the cells with BLM and MTX, while the expression of some other miRNAs such as miR-484 decreased less than half by the treatments. The increased expression of miR-34a by BLM, MTX, and PTX was also confirmed by real-time PCR. Among these miRNAs, we further examined the role of miR-34a in drug-induced EMT. Transfection of A549/ABCA3 cells with miR-34a mimic markedly upregulated α -SMA mRNA expression, indicating that miR-34a would be involved in drug-induced EMT in human alveolar type II epithelial cells. miR-34a may be a possible biomarker and/or a preventive target for drug-induced lung fibrosis. The role of miR-484 will also be discussed.

P89 - SIMCYP PBPB MODELING AND SIMULATION OF PHARMACOKINETIC DRUG INTERACTION BETWEEN LORLATINIB AND ITRACONAZOLE: A CASE STUDY USING INDUCTION SLOPE

Jian Lin, Susanna Tse, Joseph Chen, Yazdi Pithavala, Angela Wolford, Theodore Johnson, and Theunis C. Goosen
Pfizer Inc, USA

Lorlatinib is a kinase inhibitor indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC). *In vitro* studies indicated that lorlatinib is a time-dependent inhibitor as well as an inducer of CYP3A and that it activates PXR, with the net effect *in vivo* being induction. The *in vitro* dose-dependent increase in CYP3A4 mRNA observed in cultured human hepatocytes was not saturable and the estimation of typical induction parameters (Emax and EC50) could be confounded. The initial induction slope was determined by fitting the data using linear regression. Lorlatinib oral clearance increased at steady-state compared to single dose, indicating autoinduction. A physiologically based pharmacokinetic (PBPK) model was developed utilizing the initial mRNA induction slope observed in human hepatocytes and was able to recover the single- and multiple-dose plasma pharmacokinetic profile of lorlatinib. The PBPK model was verified using single- and multiple-dose lorlatinib PK data, drug-drug interaction (DDI) results between single-dose lorlatinib and itraconazole or rifampin and multiple-dose lorlatinib and midazolam. The models reasonably predicted the observed changes in lorlatinib exposure with ketoconazole (predicted AUCr 1.49 vs. observed AUCr 1.42) and rifampin (predicted AUCr 0.24 vs. observed AUCr 0.15). Extrapolation of the verified PBPK model evaluated the impact when multiple-dose lorlatinib is co-administered with CYP3A inhibitors as well as changes in fractional CYP3A4 clearance in the context of complex drug-drug interactions. The PBPK model developed using the induction slope to parameterize the lorlatinib induction effects was used to simulate different DDI scenarios and to address regulatory queries.

P90 - APPLICATION OF PRIMARY HUMAN HEPATOCYTES ENRICHED WITH HUMAN PLASMA IN CYP INDUCTION STUDIES

Chuang Lu¹, Kirsten Amaral², David Ho², and Albert P. Li²
¹Sanofi, Waltham, MA, USA, ²IVAL, USA

The 2017 US FDA drug-drug interaction guidance recommends: "to obtain inhibition parameters, the sponsor may consider primary hepatocytes enriched with human plasma as an *in vitro* system that represents physiological conditions". In this study, CYP3A4/5 induction study was performed in human hepatocytes cultured in 100% human plasma for 9 marketed compounds: carbamazepine, nifedipine, phenobarbital, pioglitazone, phenytoin, pleconaril, rifampicin, rosiglitazone, troglitazone. The hepatocytes were treated with four concentrations equivalent to and flanking the

corresponding clinical C_{max} (0.5x C_{max}, 1x C_{max}, 1.5x C_{max}, and 2x C_{max}). Freshly prepared compounds in plasma were replenished every day for 7 days to mimic clinical conditions. CYP activities were determined 24 hours after last treatment. Induction was observed for each of the 9 compounds. All compounds showed a “bell shaped” dose response with maximal induction observed around the clinical C_{max}. The fold induction ranged from 3.2-fold of pleconaril to 8.8-fold of rifampin. This approach opened an avenue for *in vitro-in vivo* extrapolation without the need to correct for plasma protein binding or hepatocyte binding.

P91 - DETERMINATION OF ADDITIVE, SYNERGISTIC AND ANTAGONISTIC EFFECTS BY THE ANALYSES OF PROTEIN MARKERS IN PESTICIDE MIXTURE-TREATED HEPARG-CELLS

Felix Schmidt¹, Hannes Planatscher², Dajana Lichtenstein³, Almut Mentz⁴, Joern Kalinowski⁴, Thomas Joos¹, Albert Braeuning³, and **Oliver Poetz**²

¹NMI, Germany, ²SIGNATOPE, Germany, ³BFR, Germany, ⁴Universität Bielefeld, Germany

Pesticide mixtures are applied in agriculture. Consequently, environment and consumers are always exposed to combinations of pesticides. The number of available and new chemical substances call for the development of novel *in vitro* methods for the detection of synergistic or cumulative effects of mixtures, since it is highly desirable to reduce animal experiments. Here, we established multiplexed mass spectrometry-based immunoassays to profile toxicologically relevant proteins. After fragmentation of cell culture samples using trypsin one peptide derived from each protein of interest is enriched by TXP antibodies which recognize a very short C-terminal epitope. The peptides can be unambiguously assigned to the proteins by tandem mass spectrometry. The target proteins are indirectly quantified by referencing the endogenous peptides to spiked synthetic isotope-coded peptide standards. HepaRG cells were chosen as a model to investigate the effects of single pesticides and combinations thereof.

The developed mass spectrometric immunoassays were used to determine the protein profiles of 26 toxicologically relevant proteins after 24 hours of treatment with 30 different pesticides. These results were used to classify the pesticides into very weak, weak, moderate and very strong correlations (Pearson) due to their similarities. Based on this grouping, four mixtures were prepared to investigate potential combinatorial effects. The following mixtures were tested: Azoxystrobin/Cyproconazole, Azoxystrobin/Difenoconazole, Azoxystrobin/Thiacloprid, and Propiconazole/Difenoconazole. We propose a procedure based on the notion of the combination index, which compares predictions based on single substance models for drug combinations to measurements from drug combinations. A synergistic effect for a drug combination would lead to a difference, namely the combined single substance models would predict a lower response. We have implemented these procedures in R. In order to test the evaluation, we have generated artificial datasets with strong, weak and no synergistic, additive or antagonistic effects of the xenobiotic mixtures. We present the general workflow and the evaluation results from the experimental study.

P92 - CORRELATION BETWEEN CYTOCHROME P450 INDUCERS AND NUCLEAR RECEPTOR ACTIVATION- A SCREENING APPROACH

Shantanu Roychowdhury and Cassidy Ward

Eurofins Panlabs, USA

Cytochrome P450 (CYP) enzymes play a major role in the metabolism of xenobiotics. According to FDA Guidance (October 2017), sponsors should initiate *in vitro* metabolic studies before first-in-human studies to influence the design of clinical PK studies assessing the potential for interactions between CYPs and investigational drugs / new molecular entities. It is recommended to evaluate the potential to induce CYP1A2, CYP2B6, and CYP3A4/5, with only further investigation in the potential to induce CYP2C8, CYP2C9 and CYP2C19 should there be induction of CYP3A4/5 as induction is mediated via activation of the pregnane X receptor (PXR). AhR (Aryl Hydrocarbon Receptor), though not a member of the Nuclear Receptor Family, shares many of the same attributes as PXR and via formation of a heterodimer with ARNT, leads to increased transcription of CYP1A1, CYP1A2, CYP2B1 and UGT1A6, and could thus new molecular entities could be investigated as possible CYP1A2 inducers via AhR activation. Constitutive Androstane Receptor (CAR) is a nuclear receptor that activates the transcription of several target genes in CYP mediated metabolism, with isoform 3 the predominantly involved in the induction of CYP2B6 and thus new molecular entities could be investigated as possible CYP2B6 inducers via CAR3 activation. Nuclear receptor assays are often done using reporter cell lines in a ready to assay format with data turnaround in 24 hours. In previous work, we evaluated a high throughput format using 24 drugs with known CYP induction properties to validate the induction of CYP1A2, CYP2B6 and CYP3A4 across different donor hepatocyte lots, however, in a screening format, this could lead to a significant amount of cost in procurement of reagents and cells as well as variability in the potential to induce enzymes. Using a the same series of reference compounds, several which are CYP2C inducers, we correlate the activation of AhR, PXR and CAR3 with the induction of CYP1A2, CYP2B6, and CYP3A4 enzymes and demonstrate the potential to use these assays as screening tools prior to CYP induction experiments.

P93 - TISSUE STEM CELL DERIVED HUMAN INTESTINAL ORGANIDS: A NOVEL AND PHYSIOLOGICALLY RELEVANT MODEL TO EVALUATE CYTOCHROME P450 INDUCTION IN GUT

David Stresser, Jun Sun, Lori Patnaude, Sonia Terrillon, and Sarah Wilson

AbbVie, USA

Induction of cytochrome P450 is a major cause of drug-drug interactions and efficacy failure and is generally considered a liability in drug development. Induction affects bioavailability when it occurs in liver and gut but because preclinical methods to evaluate induction in gut are lacking, the prediction of induction response after drug exposure in this tissue is often derived from plated human hepatocytes. Organoids are physiologically relevant, three-dimensional structures originating from stem cells. Human intestinal organoids retain several traits of normal gut physiology, mirroring the microenvironment and function of the epithelial barrier and retain cell diversity of the gastrointestinal tract. We have investigated whether organoids could be used as a surrogate to evaluate cytochrome P450 induction in gut. Human ileal and colon organoids were cultured in extracellular matrix plugs within 48-well plates for 3 days, followed by a single 48h treatment with prototypical CYP inducers rifampicin, phenobarbital, omeprazole, CITCO and phenytoin. Following treatment, mRNA was extracted and analyzed by RT-PCR. Rifampicin treatment (0.01-300 μ M) increased CYP3A4 mRNA expression maximally in a concentration dependent manner up to 8.8 ± 2.7 (n=3 experiments) and 13 ± 3.1 (n=2 experiments) fold at 100 μ M, in ileal and colon organoids, respectively. Treatment by rifampicin and the other prototypical inducers at nominal concentrations known to activate nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor and/or the aryl hydrocarbon receptor did not increase mRNA expression of CYP1A2, CYP2B6, MDR1 (PGP), BCRP and UGT1A1 in ileal organoids. Omeprazole failed to induce mRNA coding for CYP1A2, an enzyme not found in gut tissue, but did induce CYP1A1 mRNA, up to 17-fold. Using RNA-sequencing analysis of colon organoids from 3 donors, we found expression of PXR, expressed as counts per million, was about an order of magnitude higher than CAR, mirroring RNA-seq data in a public database of cadaver intestine tissue, but not cadaver liver tissue where CAR expression was similar to PXR. Taken together, these data demonstrate that this adult stem-cell derived intestinal organoid model yields robust and reproducible induction responses that are distinct from hepatocytes, indicating that this model could be more appropriate to evaluate CYP3A4 induction gut.

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P94 - HIGH THROUGHPUT LCMS QUANTIFICATION OF CYTOCHROME P450 PROTEINS IN PLATED HUMAN HEPATOCYTES DIRECT FROM 96-WELL PLATES

John Savaryn, Ning Liu, Jun Sun, Junli Ma, David Stresser, and Gary Jenkins

AbbVie, USA

ADME groups across the pharmaceutical industry need a simple, robust, cost-effective, high-throughput methodology for quantifying cytochrome P450 (CYP) proteins in plated human hepatocytes. CYPs are the major route of metabolism/clearance for xenobiotics. Thus, assessing expression changes of CYPs in the presence of drugs likely to be co-administered to patients in the clinic is a key contribution from ADME groups to predicting and/or understanding drug-drug interactions (DDI) prior to market approval. Because drug metabolism is carried out by CYP proteins, protein quantification for induction studies has been considered as the desired approach. Unfortunately, protein quantification methodology has not been well-aligned with current *in vitro* DDI plate-based assay formats and workflows. Therefore, the ADME community has had to successively settle for surrogate measures of CYP induction, namely enzyme activity and subsequently mRNA. However, both of these methods have limitations. The caveat to enzyme activity assays is that enzyme activity can be inhibited/inactivated by the inducing drug. The caveat to mRNA induction is that mRNA levels may not always correlate to protein levels. Given these caveats, current induction studies have the potential to be misleading, which can lead to disconnects between *in vitro* and clinical DDI results. Protein quantification groups have partially addressed this problem by developing quantitative LCMS methods against CYP proteins. However, these methods require microsomal or immunoprecipitation enrichment, both of which add complexity such that they have yet to be adopted widely by the ADME community. Here, we provide a simple, robust, cost-effective, high-throughput LCMS methodology aligned with current 96-well plate-based human hepatocyte induction studies. Our method bypasses both microsomal enrichment and antibody-based immunoenrichment to go directly from the plate to LCMS. We use this 'plate-to-peaks' approach for quantifying CYP3A4, CYP2B6, and CYP1A2 – the major inducible hepatic CYPs representative of PXR, CAR and AhR mediated induction, respectively. We leveraged our aligned assay format to assess induction across mRNA, protein, and enzyme activity from a number of common DDI assay compounds. As expected, results from the 3 methods using model inducers were broadly concordant but the magnitude of the induction response differed: for CYP3A4 with 10 μ M rifampicin induction was 12-fold for RNA, 8-fold for protein, and 3-fold for activity; for CYP1A2 with 50 μ M omeprazole induction was 30-fold for RNA, 13-fold for protein, and 17-fold for activity; for CYP2B6 with 50 μ M phenytoin induction was 23-fold for RNA, 2-fold for protein, and 5-fold for activity. Importantly, we anticipate the relative ease of this method will enable ADME groups to routinely adopt CYP protein quantification as part of their non-clinical evaluation of CYP induction.

Disclosure: All authors are or were employees of AbbVie at the time the work was conducted and may own AbbVie stock. NL is currently retired. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

P95 - A-TO-I RNA EDITING MODULATES THE EXPRESSION OF HUMAN PREGNANE X RECEPTOR

Seiya Takemoto, Masataka Nakano, Tatsuki Fukami, and Miki Nakajima

Kanazawa University, Japan

RNA editing is a post-transcriptional process that alters the nucleotide sequence of RNA transcripts. In mammals, adenosine-to-inosine (A-to-I) RNA editing is the most frequent type of RNA editing. Inosine forms a base pair with cytidine as if it was guanosine; therefore, the conversion of the nucleotide potentially changes the amino acid sequence, splicing, microRNA targeting, or microRNA maturation. A-to-I RNA editing is catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes. They convert adenosines in double-stranded RNA structures into inosines by hydrolytic deamination. In mammals, there are three members in the ADAR family: ADAR1, ADAR2, and ADAR3. ADAR1 and ADAR2 are ubiquitously expressed and show RNA editing activity, whereas ADAR3 does not. We previously found that there is a large interindividual variation (220-fold) in ADAR1 protein expression in a panel of 32 human liver samples and that ADARs regulate the expression of drug-metabolizing cytochrome P450 (CYP) isoforms. In the present study, we aimed to clarify the possibility that ADARs regulate the expression of pregnane X receptor (PXR), a transcription factor regulating many drug-metabolizing enzymes including CYP3A4. To investigate whether ADARs regulate human PXR expression, ADAR1 or ADAR2 in HepG2 cells was knocked down by transfection of siRNA. The knockdown of ADARs resulted in an increase in PXR mRNA (by 3-fold) and protein (by 2-fold) levels. Additionally, a luciferase assay using a reporter plasmid containing CYP3A4 promoter region revealed that the knockdown of ADAR1 significantly increased transactivity of CYP3A4 by 4-fold. Consistently, the expression of endogenous CYP3A4 mRNA was increased by knockdown of ADAR1 in HepG2 cells. Interestingly, the transactivity and expression level of CYP3A4 were not changed by knockdown of ADAR2, suggesting that only the ADAR1-dependent regulation of PXR affects the expression of its downstream genes. Next, we sought to examine the underlying mechanism of the increase in the PXR mRNA by knockdown of ADARs. Under the treatment with actinomycin D, a transcriptional inhibitor, degradation of the PXR mRNA level was observed, and the degradation was delayed by transfection of siADARs, suggesting that ADARs negatively regulate PXR expression by facilitating the degradation of PXR mRNA. Finally, a luciferase assay using reporter plasmids containing the 3'-UTR of PXR was performed, revealing that the 3'-UTR would be involved in the ADARs-dependent change in the stability of PXR mRNA. In conclusion, we uncovered that ADAR1 negatively regulates the expression of PXR, and subsequently modulates the expression of CYP3A4. As the mechanism, we found that ADAR1 facilitates the degradation of PXR mRNA. Thus, ADARs appear to indirectly regulate various drug-metabolizing enzymes via the modulation of PXR expression.

P96 - EVALUATION OF METHOTREXATE AND IDELALISIB AS INDUCERS OF HUMAN ALDEHYDE OXIDASE IN CELLULAR MODELS

Szu Ling Yeap and Aik Jiang Lau

National University of Singapore, Singapore

Aldehyde oxidase (AOX1) is a cytosolic drug-metabolizing enzyme that catalyzes the biotransformation of many N-heterocyclic chemicals, including chemotherapeutic drugs such as idelalisib and methotrexate. Idelalisib, which is a phosphatidylinositol 3-kinase inhibitor used for the treatment of leukemia and lymphoma, is primarily metabolized by AOX1 to an inactive 8-oxo-idelalisib metabolite. Methotrexate is an anti-folate used to treat solid tumors and hematological malignancies at high doses and rheumatoid arthritis at low doses. High-dose methotrexate is metabolized almost exclusively by AOX1 to 7-oxo-methotrexate, which is less efficacious than the parent drug, and its poor solubility increases the risk of nephrotoxicity. In the present study, we investigated the effect of idelalisib and methotrexate, which are substrates of AOX1, on human AOX1 expression, as assessed in cell models. Human hepatic, intestinal, and mammary cells are known to express AOX1. Therefore, HepG2 human hepatocellular carcinoma, LS180 human colon adenocarcinoma, and MCF-7 human breast adenocarcinoma cells were treated with idelalisib or methotrexate to determine whether the two drugs increase AOX1 gene expression. As analyzed by real-time polymerase chain reaction, AOX1 mRNA level was greatest in HepG2 cells, followed by MCF-7 and LS180 cells. At an equimolar concentration of 10 μM , methotrexate, but not idelalisib, increased AOX1 mRNA expression in HepG2, LS180, and MCF-7 cells. Time-course experiments indicated that methotrexate increased AOX1 mRNA expression by 2.6, 5.6, 7.1, and 5.9-fold at 48, 72, 96, and 120 h post-treatment in HepG2 cells, whereas it increased the expression by 4.8, 7.3, and 3.7-fold at 72, 96, and 120 h post-treatment in LS180 cells, respectively. A plateau was achieved at 72-96 h in both cell lines. Treatment of HepG2 and LS180 cells with varying concentrations (0.0001-10 μM for HepG2 cells; 0.001-10 μM for LS180 cells) of methotrexate once every 24 h for 72 h increased AOX1 mRNA level in a concentration-dependent manner. The minimum effective concentration was 0.06 μM , EC₅₀ was 0.025 \pm 0.002 μM , and E_{max} was 4.8-fold in HepG2 cells, whereas the minimum effective concentration was 0.1 μM , EC₅₀ was 0.11 \pm 0.02 μM , and E_{max} was 7.8-fold in LS180 cells. When

HepG2 and LS180 cells were pre-treated with actinomycin D (a RNA synthesis inhibitor) for 1 h prior to treatment with methotrexate (1 μ M) for 48 h, the increase in AOX1 mRNA expression by methotrexate was abolished completely, indicating that methotrexate induced AOX1 by a transcriptional mechanism. In conclusion, methotrexate (at clinically-relevant concentrations), but not idelalisib, induces AOX1, suggesting the possibility that methotrexate may induce its own metabolic clearance and may be a contributory factor to the development of resistance to methotrexate therapy. The findings also suggest potential interaction between methotrexate and other AOX1 substrate drugs.

P97 - CLINICAL PREDICTION OF DRUG-DRUG INTERACTION RISK DUE TO TIME-DEPENDENT INHIBITION OF CYTOCHROME P450 3A4 - A SIDE-BY-SIDE COMPARISON OF HUMAN LIVER MICROSOME AND HEPATOCYTE

Elaine Tseng, Heather Eng, Jian Lin, Matthew A. Cerny, Theunis C. Goosen, and R. Scott Obach
Pfizer Inc., USA

Successful prediction of clinical drug-drug interactions (DDI) from *in vitro* data to mitigate clinical studies remains an area of focus for pharmaceutical industry, academics, and regulatory agencies. As such, the tool box is constantly being re-evaluated to ensure that the most appropriate and relevant reagents are used. Human hepatocytes (HHEP) are generally regarded as the most complete system to study drug metabolism as it retains the full complement of hepatic phase I and phase II metabolizing enzymes. However, human liver microsomes (HLM) are most widely used as *in vitro* tool in drug discovery when assessing inhibition and inactivation of cytochrome P450 (CYP) enzymes due to simplicity, ease of manipulation, and relatively low cost. Much emphasis has been placed on the need to generate data used for DDI predictions in a physiologically relevant, robust system. In this research, comparison of DDI predictions for known time-dependent inhibitors (TDI) of CYP3A from data generated in both HHEP and HLM systems were evaluated.

In vitro TDI kinetic parameters, KI, kinact, and unbound fractions for twelve diverse compounds with clinical CYP3A DDI studies were generated in suspension HHEP and HLM. All data were incorporated into the projection of the magnitude of DDIs using mechanistic static and dynamic (Simcyp) modeling. Assessments using static models suggest that most compounds are within 2-fold of observed DDI when kinetic parameters generated from HHEP were used. Conversely, most of the compounds fall outside of the 2-fold prediction using HLM generated parameters.

The analyses presented in this study reinforce the hypothesis that HHEP is a more physiologically relevant reagent than HLM and improves clinical DDI prediction success. While the underlying mechanisms for these differences may be harder to establish, the utility of HHEP to support more accurate DDI predictions is clearly beneficial.

P98 - DELINEATION OF *IN VITRO* CUT-OFF VALUES FOR CYP3A4/5 TIME DEPENDENT INHIBITION USEFUL IN EARLY DRUG DESIGN – A SIDE-BY-SIDE COMPARISON IN HUMAN LIVER MICROSOMES AND HEPATOCYTES

Heather Eng, Elaine Tseng, Jian Lin, Theunis C, Goosen, Matthew A. Cerny, and R. Scott Obach
Pfizer, USA

We are continually developing our suite of cytochrome P450 (CYP) time-dependent inhibition (TDI) assays to improve clinical drug-drug interaction (DDI) risk assessment. Here we discuss interpretation of screening data as well as a comparison of kinetic parameter estimates generated in human liver microsomes (HLM) and suspension human hepatocytes (HHEP) as they relate to utilization in decision-making during early drug design.

In vitro test systems can reliably detect slow rates of inactivation (kobs), often flagging compounds as positive for *in vitro* TDI for which there is no clear mechanistic rationale. To establish boundary criteria for *in vitro* kobs associated with a clinically meaningful DDI (i.e. 2-fold), we generated TDI data for 50 compounds with published midazolam clinical DDI (ranging from 0.7 to 25-fold interactions). Studies were done in both HLM and HHEP, using a test concentration of 30 μ M and multiple timepoints to 40 min. The boundary for CYP3A4/5 kobs values was ≥ 0.015 min⁻¹ and ≥ 0.004 min⁻¹ for HLM and HHEP, respectively. This allows for quick judgment of the kobs magnitude in a discovery setting, agnostic of other parameters (e.g. dose, exposure, plasma protein binding) required for clinical DDI risk assessment.

It is interesting that the apparent rate of inactivation observed in HHEP relevant to a clinically meaningful midazolam DDI (AUCR >2-fold) was approximately 3.8-fold slower than HLM. The exact mechanisms for this difference are not clear and could be related to differences between *in vitro* partition ratio of inactivation. We hypothesize that using HHEP, a more physiologically relevant reagent than HLM, will improve DDI predictions. For this, we generated KI and kinact data for 12 diverse compounds in both HLM and HHEP. Results of these experiments are described in this poster. The use of these data in projections of DDI using static and dynamic models are reported in an accompanying poster. Nevertheless, the *in vitro* TDI boundary conditions established in this study should be useful for early decisions on clinical DDI risk for CYP3A4/5 substrates.

P99 - LC-MS/MS BASED BIOANALYSIS METHOD FOR BILIRUBIN GLUCURONIDES: APPLICATION IN UGT1A1 INHIBITION EVALUATION AND COMPARISON WITH DATA OBTAINED USING ESTRADIOL

Chunyan Han, Dong Yang, and Mandy Xu
Pharmaron, China

Endogenous (4Z, 15Z)-bilirubin IX_a, the end product of heme catabolism, is eliminated through UDP-glucuronosyltransferases 1A1 (UGT1A1) catalyzed glucuronidation. Inhibition of UGT1A2 might lead to hyperbilirubinemia, thus UGT 1A1 is an important content in DDI potential evaluation of new chemical entities. Estradiol is widely used as a probe for evaluation of UGT1A1 inhibition with estradiol-3-glucuronidation as the target metabolite, but it is not a selective substrate since UGT1A3 is also involved in estradiol-3-glucuronidation. Bilirubin is highly specific to UGT1A1, and is the substrate of the interest. However, current bioanalysis method for bilirubin glucuronides are all HPLC-UV based, and the elution time tend to be long, which is not compatible with instrumental set-up and experimental design of high throughput screening. Besides, bilirubin could form multiple conjugates, but most reported method use bilirubin total glucuronide (the sum of monoglucuronides and diglucuronide) as end point, enzyme kinetics with monoglucuronides and diglucuronide as target metabolites were not fully evaluated. In the present study, we developed robust LC-MS/MS method for bilirubin, bilirubin monoglucuronides and bilirubin diglucuronide. Km values for mono-, di- and total glucuronides were determined in both human liver microsomes and recombinant human UGT1A1 (rhUGT1A1) reaction systems. Inhibition potential of ten compounds (silybin, atazanavir, lopinavir, ritonavir, baicalein, 4-methylumbelliferone, riluzole, carvedilol, daidzein and ketoconazole) on the formation of mono-, di- and total glucuronides were evaluated in both human liver microsomes and recombinant human UGT1A1 reaction systems and results were compared with that obtained using estradiol.

P100 - IN VITRO EVALUATION OF UDP-GLUCURONOSYLTRANSFERASE (UGT) 1A3 AND UGT2B4 INHIBITION

Kimberly Lapham, Nicholas Ferguson, Jonathan Novak, Mark Niosi, and Theunis Goosen
Pfizer Worldwide Research and Development, USA

The scientific community has made tremendous strides developing *in vitro* assays that predict metabolic clearance and enable reaction phenotyping of UDP-glucuronosyltransferase (UGT) substrates. Our laboratory has previously characterized UGT inhibitors in HLM that can be utilized to estimate the fraction of metabolism by the major hepatic UGTs – 1A1, 1A4, 1A9, 2B7, and 2B15. In this study, we expanded our efforts to include identification of UGT1A3 and UGT2B4 inhibitors. The inhibition of UGT1A3 and UGT2B4 were studied in recombinantly expressed UGTs (rUGTs), with lithocholic acyl glucuronide and canagliflozin M5 glucuronide formation as selective probe activities, respectively. Chemical inhibitors evaluated were selected from 200 highly prescribed drugs. Initially, compounds were screened for inhibitory potential in rUGT1A3 and rUGT2B4 at 50 and 500 µM in the absence of bovine serum albumin (BSA). Comparing percent inhibition data to inhibition data previously generated for UGTs 1A1, 1A4, 1A6, 1A9, 2B7, and 2B15, approximately 50 compounds were re-evaluated for rUGT1A3 and rUGT2B4 IC₅₀s with and without 2% BSA. In addition, previously characterized UGT inhibitors against UGT1A1 (atazanavir and erlotinib, UGT1A9 (digoxin, tranilast, and magnolol), UGT1A4 (hecogenin), and UGT2B7/2B15 (16 α - and 16 β -phenyllongifolol) were evaluated for selectivity against UGT1A3 and UGT2B4. Based on rUGT IC₅₀ values in the presence of 2% BSA, indinavir with a rUGT1A3 IC₅₀ values of 5.2 µM and clotrimazole with rUGT2B4 IC₅₀ values of <0.100 µM, respectively, could be useful in characterizing fractional clearance mediated by UGT1A3 and UGT2B4.

P101 - THE ROLE OF L- AND D-MENTHOL IN THE GLUCURONIDATION AND DETOXIFICATION OF THE MAJOR LUNG CARCINOGEN, NNK

Shannon Kozlovich¹, Gang Chen¹, Christy Watson¹, William Blot², and **Philip Lazarus**¹

¹Washington State University College of Pharmacy and Pharmaceutical Sciences, USA, ²Vanderbilt University Medical Center, USA

Menthol, which creates mint flavor and scent, is often added to tobacco in both menthol and non-menthol cigarettes. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a potent tobacco carcinogen, is extensively metabolized to its equally carcinogenic metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) as (R)- or (S)-NNAL enantiomers. NNAL is detoxified by UDP-glucuronosyltransferase (UGT) enzymes with glucuronidation occurring on either NNAL's pyridine ring nitrogen (NNAL-N-Gluc) or the chiral alcohol [(R)- or (S)-NNAL-O-Gluc]. Evidence suggests that menthol may decrease NNAL detoxification, yet the underlying mechanism of menthol interaction within this pathway remains unclear. To better characterize the mechanisms underlying menthol effects on NNAL glucuronidation, *in vitro* menthol glucuronidation assays and menthol inhibition of NNAL-Gluc formation assays were performed. Additionally, NNAL and menthol glucuronides (MG) were measured in the urine of smokers (n=100) from the Southern Community Cohort Study. UGTs 1A9, 1A10, 2A1, 2A2, 2A3, 2B4, 2B7 and 2B17 all exhibited glucuronidating activity against both L- and D-menthol. In human liver microsomes, both L- and D-menthol inhibited the formation of each NNAL-Gluc, with a stereospecific difference observed between the formation of (R)-NNAL-O-Gluc and (S)-NNAL-O-Gluc in the presence of D-menthol but

not L-menthol. Urinary MG was detected in menthol and non-menthol smokers with D-MG contributing 1.3% to total urinary MG. Levels of urinary NNAL-N-Gluc were significantly ($p < 0.05$) lower among subjects with high levels of total urinary MG. These data suggest that the presence of menthol could interfere with the major detoxification pathway for NNK/NNAL, leading to longer retention of NNAL in tobacco target tissues, increasing the opportunity for NNAL to damage DNA, and leading to the development of tobacco-related cancers.

P102 - PREDICTING RISK OF CARDIAC ARRHYTHMIAS BASED ON INHIBITORY POTENCIES (K_i) AGAINST CYP2J2-MEDIATED ARACHIDONIC ACID METABOLISM

Jacqueline Wen Hui Leow¹, Amos Boon Hao Lim¹, Ravi Kumar Verma², Hao Fan², and Eric Chun Yong Chan¹

¹National University of Singapore, Singapore, ²Agency for Science, Technology and Research (A*STAR), Singapore

The epoxidation of arachidonic acid (AA) to regioisomeric epoxyeicosatrienoic acids (EETs) via cytochrome P450 2J2 (CYP2J2), the predominantly expressed CYP450 in human cardiomyocytes[1], has garnered attention due to implications of EETs in cardiac electrophysiology (e.g. ion channel inhibitory effects)[2]. However, the inhibition of CYP2J2-mediated AA-EETs metabolism by drugs and its contribution to the risk of fatal cardiac arrhythmias such as torsades de pointes (TdP) remain poorly understood.

EET regioisomers were quantified following a series of *in vitro* enzyme kinetics experiments using AA as a physiologically relevant probe substrate and other chemical probe substrates in the absence and presence of a library of CYP2J2 cum human ether-a-go-go-related gene (hERG) channel inhibitors (astemizole, cisapride, pimozone, terfenadine and tamoxifen). Molecular docking was further performed with a three-dimensional homology model of CYP2J2 using vitamin D2 bound crystal structure of CYP2R1 as a template. To investigate substrate inhibition, AA was docked into the orthosteric binding site (OBS) before the docked structure was screened for a secondary binding site (SBS) for a second AA molecule. Relevant electrophysiological endpoints derived from human-induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) assays[3] were subsequently correlated with experimentally-derived inhibitory constants associated with CYP2J2 (K_i,CYP2J2) and literature-reported hERG channel inhibition (IC₅₀,hERG).

When fitted to the empirical uncompetitive substrate inhibition model modified with Hill's coefficient (h), AA exhibited substrate inhibition of CYP2J2 beyond 40 μM and its kinetic parameters for various EET regioisomers are as follows: maximal reaction velocity (V_m) of 0.12-0.19 pmol/min/pmol CYP2J2, affinity constant (K_m) of 10.1-25.7 μM, substrate inhibitory constant (K_{si}) of 77.3-81.2 μM and h of 2.33-2.87. Consistent with substrate inhibition, AA achieved binding poses to OBS and SBS with highly favourable glide-scores of -3.1 and -3.8 respectively and the salt-bridge interaction with arginine 117 is shared between both OBS- and SBS-bound AA. K_i,CYP2J2 derived from endogenous AA versus xenobiotic probe substrates characterized the nature of substrate-dependency in K_i determination. Correlation analysis of K_i,CYP2J2 and IC₅₀,hERG of astemizole, cisapride, pimozone, terfenadine and tamoxifen against their repolarisation duration endpoints in hiPSC-CMs further shed light on the arcane role of CYP2J2 inhibition in defining risk of TdP.

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P103 - VALIDATION OF UGT INHIBITION ASSAYS FOR DDI ASSESSMENT

Guy Webber and Arabella Leggat

ENVIGO, United Kingdom

Potential drug-drug interactions (DDI) mediated by inhibition of uridine di-phospho -glucuronosyltransferase (UGT) enzymes are of increasing focus. To complement our current CYP and Transporter DDI assessment program we have undertaken a validation of UGT-inhibition assays in multi-well plate format for the major UGT isoforms using in the first instance recombinant rUGT enzymes and a universal UGT inhibitor, nicardipine (subsequent work will include human liver microsomes). The assays included using low levels of protein (0.01 – 0.25 mg/mL/5-125 pmol UGT equivalents) and a membrane activation phase using alamethacin (15 mg/mL). Following linearity experiments, results obtained were: rUGT1A1-mediated glucuronidation of estradiol vs nicardipine as inhibitor IC₅₀ = 0.23 μM, rUGT1A4-mediated glucuronidation of trifluoroperazine vs nicardipine as inhibitor IC₅₀ = 2.7 μM, rUGT1A6-mediated glucuronidation of propofol vs nicardipine as inhibitor IC₅₀ = 11.0 μM and rUGT1A9-mediated glucuronidation of propofol vs nicardipine as inhibitor IC₅₀ = 0.61 μM and UGT2B7-mediated glucuronidation of 7-Hydroxy-4-trifluoromethylcoumarin vs nicardipine as inhibitor IC₅₀ = 9.1 μM.

P104 - EVALUATION OF CYTOCHROME P450 SELECTIVITY FOR HYDRALAZINE AS AN ALDEHYDE OXIDASE INHIBITOR FOR REACTION PHENOTYPING

Xin Yang, Nathaniel Johnson, and Li Di
Pfizer Inc., USA

Hydralazine has been reported as a selective mechanism-based inactivator of aldehyde oxidase (AO) and it is widely used in the pharmaceutical industry for reaction phenotyping to estimate fraction metabolized by AO and to identify AO substrates. In this study, hydralazine selectivity against 7 cytochrome P450s (CYPs) under mechanism-based inactivation conditions was evaluated to understand the broader applications of hydralazine as a selective AO inhibitor in hepatocytes when used for reaction phenotyping. The results showed that hydralazine inhibited CYP1A2 (56%-74%), 2B6 (22%-36%), 2D6 (31%-65%) and 3A (25%-37%) at concentrations that chemically knocked out most of the AO activities ($\geq 50 \mu\text{M}$) in human hepatocytes. Furthermore, hydralazine is a time-dependent inhibitor of CYP1A2 with k_{obs} values of 0.0072 min^{-1} in hepatocytes and 0.012 min^{-1} in liver microsomes at $50 \mu\text{M}$ respectively. The results helped to define the limitations of using hydralazine for AO reaction phenotyping, F_m , AO determination, and AO substrate identification. Based on these findings, precautions need to be taken when using hydralazine as an AO inhibitor for *in vitro* studies because fraction metabolized by AO is likely to be overestimated and the likelihood of false positives in identifying AO substrates increases. This study also highlighted the importance of using relevant substrate concentration for selectivity studies. When substrate concentration is too high, inhibitors may appear to be more selective than they are.

P105 - BDE-99 REPROGRAMS THE LIVER EPIGENOME PERSISTENTLY ALTERING THE TRANSCRIPTOME

Joseph Dempsey, Joe Lim, James MacDonald, Theo Bammler, Terrance Kavanagh, and Julia Yue Cui
University of Washington, USA

Background: Growing evidence in the literature suggests that early life exposure to environmental chemicals may lead to delayed onset of diseases later in life. Polybrominated diphenyl ethers (PBDEs) are a class of recently banned flame retardants found in consumer products and in the environment. Concentrations in human specimens, such as blood, breast milk, and adipose tissue, have increased exponentially over the past 30 years. Infants and toddlers are particularly vulnerable to PBDE-induced adverse effects due to ingestion of PBDE-contaminated breast milk and household dust. During liver development, profound changes occur in the chromatin epigenetic architecture that regulates the ontogenic expression of many genes involved in xenobiotic biotransformation. The goal of this study was to test our hypothesis that neonatal exposure to PBDEs reprograms the liver epigenome and persistently alter alters transcription of xenobiotic biotransformation genes. **Methods:** Two-day-old male and female C57BL/6J mice were exposed to corn oil or 57 mg/kg BDE-99 (an enriched PBDE congener in humans), supralingually once daily for three days. At 60-days adult age, whole transcriptome sequencing was performed ($n=3$ per exposure per gender), and data were analyzed using HISAT2 and Cufflinks. In parallel, ChIP-Seq of histone 3 lysine 27 acetylation (H3K27ac), which is a permissive epigenetic mark for enhancers and promoters, was performed in these samples, and data were analyzed using bowtie 2 and MACS2. **Results:** Neonatal exposure to BDE-99 persistently altered the RNA expression of distinct xenobiotic biotransformation enzymes, and this was associated with altered H3K27ac. In adult mice, there were 10,843 H3K27ac peaks in control livers and 12,374 peaks in livers of early life BDE-99 exposed groups. These peaks were associated with 4378 and 3747 unique protein-coding genes (PCGs), respectively, and the expression of 218 of the PCGs were persistently altered by early-life exposure to BDE-99. Pathway analyses of these PCGs (STRING) identified metabolism of xenobiotics by cytochrome P450s (Cyp), drug metabolism (other enzymes), and glutathione metabolism as significantly enriched KEGG pathways for the differentially regulated PCGs that carry the H3K27ac mark. Interestingly, Cyp2c29, 2c38, and 2c50 gained an enriched acetylation peak in the BDE-99 exposed male mice but had decreased RNA expression; whereas Cyp3a16 gained an H3K27ac peak and had increased RNA expression, indicating that site specificity of H3K27ac has dual functions in gene transcription. Similarly glutathione-S-transferase alpha 3 (Gsta3), which had decreased expression following BDE-99 exposure, had no H3K27ac peaks in control conditions, but gained 3 peaks (one significantly enriched) following BDE-99 exposure. **Conclusion:** Our results demonstrate that neonatal exposure to BDE-99 reprograms the epigenetic mark H3K27ac in liver, corresponding to persistently altered expression of certain xenobiotic biotransformation genes in adult age.

P106 - APPLICATION OF CRYOPRESERVED HUMAN INTESTINAL MUCOSA (CHIM) IN THE EVALUATION OF REGIONAL DIFFERENCE IN INTESTINAL DRUG METABOLISM

Albert P. Li, Novera Alam, David Ho, Kirsten Amaral, and Walter Mitchell
In Vitro ADMET Laboratories Inc., USA

Oral ingestion represents the most desirable route of drug administration. An orally administered drug is firstly subjected to first pass metabolism by the small intestines before absorption into the portal vein followed by hepatic metabolism. Understanding potential regional differences in drug metabolism in the small intestine may aid our understanding of oral bioavailability, enteric drug toxicity, and enteric drug-drug interaction during the transit of an ingested drug in the small

intestine. This is especially important for controlled-release capsules with prolonged retention durations in the small intestine. We recently reported the establishment of a novel *in vitro* experimental model of the human small intestine, namely the cryopreserved human intestinal mucosa (CHIM) which retained high viability and drug metabolizing enzyme activities upon recovery from cryopreservation. To investigate potential regional differences in intestinal drug metabolism, CHIM were isolated from 10 12-inch segment of the small intestines. Drug metabolizing enzyme activities evaluated were: CYP1A1 (resorufin 7-deethylation), CYP1A2 (phenacetin hydroxylation), CYP2A6 (coumarin hydroxylation), CYP2B6 (bupropion hydroxylation), CYP2C8 (paclitaxel 6a-hydroxylation), CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (s-mephenytoin 4-hydroxylation), CYP2D6 (dextromethorphan hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A4 (midazolam 1-hydroxylation and testosterone 6b-hydroxylation), UGT (7-OH-coumarin glucuronidation), SULT (7-hydroxycoumarin sulfation), GST (NAPQI-GSH conjugation), FMO (benzylamine N-oxidation), MAO (kynuramine 4-hydroxylation), AO (carbazeran 4-hydroxylation), and NAT-1 (4-aminobenzoic acid N-acetylation). Regional difference in activity was observed, with the regions proximal to the stomach in general having the higher activities. The manifestation of regional difference, however, differ among individuals. Our observation of regional differences in intestinal metabolism suggest that drugs may be metabolized during transit upon ingestion. The individual differences suggest environmental factors may play a role in the regional differences in intestinal drug metabolism activity in the small intestine.

P107 - A COMPARISON OF ENTERIC AND HEPATIC METABOLISM USING *IN VITRO* ENTERIC AND HEPATIC EXPERIMENTAL SYSTEMS: CRYOPRESERVED HUMAN ENTEROCYTES, METMAX CRYOPRESERVED ENTEROCYTES, CRYOPRESERVED HUMAN INTESTINAL MUCOSA, CRYOPRESERVED HUMAN HEPATOCYTES, AND METMAX CRYOPRESERVED HUMAN HEPATOCYTES

Albert P. Li, Novera Alam, David Ho, Kirsten Amaral, and Walter Mitchell
In Vitro ADMET Laboratories Inc., USA

An ingested drug is firstly subjected to intestinal metabolism by the enterocytes in the intestinal mucosa and then hepatic metabolism upon absorption into the portal circulation. It is important to understand the difference between enteric and hepatic metabolism for an accurate prediction of the *in vivo* metabolic fates of an ingested drug. In our laboratory, we have developed *in vitro* experimental systems for the liver (cryopreserved human hepatocytes and MetMax™ (permeabilized, cofactor supplemented) cryopreserved human hepatocytes for the liver), and the small intestine (cryopreserved enterocytes, MetMax™ cryopreserved enterocytes, and cryopreserved human intestinal mucosa (CHIM)). We report here the drug metabolizing enzyme activities of these experimental systems to define the similarities and differences between hepatic and enteric drug metabolism. The drug metabolizing enzyme activities evaluated were: CYP1A1 (resorufin 7-deethylation), CYP1A2 (phenacetin hydroxylation), CYP2A6 (coumarin hydroxylation), CYP2B6 (bupropion hydroxylation), CYP2C8 (paclitaxel 6a-hydroxylation), CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (s-mephenytoin 4-hydroxylation), CYP2D6 (dextromethorphan hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A4 (midazolam 1-hydroxylation and testosterone 6b-hydroxylation), UGT (7-OH-coumarin glucuronidation), SULT (7-hydroxycoumarin sulfation), GST (NAPQI-GSH conjugation), FMO (benzylamine N-oxidation), MAO (kynuramine 4-hydroxylation), AO (carbazeran 4-hydroxylation), NAT-1 (4-aminobenzoic acid N-acetylation), and NAT-2 (sulfamethazine N-acetylation). For these experimental systems, cryopreserved enterocytes had the lowest activity and were used for the qualitative evaluation of the relative abundance of pathways, while the others were used for the quantitative comparison of hepatic and enteric drug metabolism. Drug metabolism pathways with substantially higher hepatic than enteric activities include CYP2A6, CYPB6, CYP2C8, CYP2D6, CYP2E1, CYP2J2. An interesting finding is that enteric CYP3A4 activity in some individuals were similar to that observed in hepatocytes. Other pathways with similar hepatic and enteric activities include MAO, NAT1, and NAT-2. Substantial individual differences were observed for both enteric and hepatic drug metabolizing enzymes. Our results show that both the small intestine and the liver are highly active in drug metabolism, with the liver having pathways with activities substantially higher than that in the intestine. The differences in drug metabolizing enzyme composition and activities between the intestine and the liver suggest that drugs are metabolized differently in the two organs. The *in vitro* enteric and hepatic experimental systems developed in our laboratory can be applied towards the definition of enteric and hepatic drug metabolism, providing information to further our understanding of the metabolic fates and the subsequent toxicity, and pharmacology of orally administered drugs.

P108 - EFFECTS OF ORGANIC SOLVENTS ON PRAVASTATIN UPTAKE IN HUMAN HEPATOCYTES

Albert P. Li, Kirsten Amaral, Novera Alam, David Ho, and Walter Mitchell
In Vitro ADMET Laboratories Inc., USA

Transporter-mediated drug uptake into hepatocytes is an important drug property with impact of hepatic clearance, metabolism, drug-drug interactions, toxicity, and pharmacology. *In vitro* evaluation of hepatic uptake is now a routine practice in drug development to define the role of transporter in the uptake of a drug candidate as well as whether a drug candidate may cause drug-drug interactions via inhibition of uptake transporters. We have recently developed a robust and relatively higher throughput uptake using human hepatocytes cultured as attached monolayers in a 96-well plate format. The assay involves the use of our highly plateable cryopreserved human hepatocytes (999Elite™ Cryopreserved

human hepatocytes). After thawing, the hepatocytes were cultured for 6 hrs followed by addition of uptake substrates as staggered times with termination of the assay consisting of removal of the substrate-containing culture medium, multiple washing of the hepatocytes to remove extracellular substrates, extraction of the hepatocytes with acetonitrile followed by LC/MS-MS quantification of the extracts. Using this assay, we reproducibly observed time- and concentration-dependent uptake of transporter substrates, as well as rifampin inhibition of transporter-mediated uptake. We report here results for our study on the effects of the commonly used organic solvents: DMSO, methanol, ethanol, and acetonitrile on the uptake of a model transporter substrate, pravastatin. Pravastatin uptake was evaluated at concentrations of 1.6, 3.1, 6.25, 12.5, 25, 50 and 100 μM using 0.0625%, 0.125%, 0.25%, 0.5%, 1%, and 2% (v/v) of each of the organic solvents. Dose-dependent uptake of pravastatin uptake was observed, with the expected saturation kinetics typical of transporter-mediated uptake up to 50 μM . An increased uptake above the saturation plateau was observed at 100 μM , suggesting diffusion uptake as a function of chemical concentration gradient at this high concentration. Inhibition of pravastatin uptake was observed with DMSO, ethanol and methanol but not acetonitrile. For instance, at the commonly used concentration of 1% and at the lowest concentration of pravastatin evaluated of 1.6 μM , approximately 80% inhibition by DMSO and 50% inhibition by methanol and ethanol were observed. Percent inhibition was found to decrease with higher pravastatin concentrations, with no inhibition observed at the 100 μM concentration. Our results show that the choice of organic solvents and the concentration of the solvents used needs to be carefully considered for the quantification of drug uptake in human hepatocytes. The commonly used DMSO concentration of 1%, for instance, is likely to underestimate the rate of transporter-mediated uptake.

P109 - Aconitum alkaloids from Radix Aconiti Lateralis Preparata induced liver injury in rats: mechanistic consideration on bile acid perturbations

Mengbi Yang, Xiaoyu Ji, and Zhong Zuo

The Chinese University of Hong Kong, Hong Kong

Radix Aconiti Lateralis Preparata (Fuzi) has been widely used as a potent Chinese medicine for the treatment of cardiovascular diseases, rheumatoid arthritis and other diseases. However, Fuzi's clinical use is limited by its toxicity. Six Aconitum alkaloids, namely aconitine (AC), hypaconitine (HA), mesaconitine (MA), benzoylaconine (BAC), benzoylhypaconine (BHA), benzoylmesaconine (BMA), are believed to be the principal toxins of the herb. Most of the previous toxicity studies on Fuzi focused on its acute cardiotoxicity, while its chronic hepatotoxicity remains unclear. The current study aims to evaluate the potential hepatotoxicity of Fuzi and related mechanism in rats. Commercially available concentrated granule of Fuzi was orally given to Sprague Dawley rats ($n=8$) at 6 g/kg/day for a bolus dose or for consecutive 15 days. Based on quality control results, the doses of six toxic Aconitum alkaloid were BMA (1814 $\mu\text{g}/\text{kg}$), BHA (321 $\mu\text{g}/\text{kg}$), BAC (254 $\mu\text{g}/\text{kg}$), HA (6.61 $\mu\text{g}/\text{kg}$), AC (0.39 $\mu\text{g}/\text{kg}$), and MA (0.38 $\mu\text{g}/\text{kg}$). Two hours after the last dose, rats were sacrificed followed by collection of blood, liver and heart after cardiac perfusion with 150 - 200 ml saline. Histological analyses were conducted to identify potential tissue injury, and the collected plasma and tissue samples were analyzed for the concentrations of the six Aconitum alkaloids and hepatic bile acids using a validated LC/MS/MS method. The current results revealed that after long-term exposure to the studied toxic Aconitum alkaloids at its current dose level, liver injury was more obvious than previously reported cardiac dysfunction. After 15-day exposure to the Fuzi granule, liver damage was evidenced by the histological observation of edema and vein dilation, while no cardiac damage was observed. The liver concentrations of the six toxic Aconitum alkaloids were significantly higher in rats receiving 15-day treatments of Fuzi (total Aconitum alkaloids: 175.7 ± 21.7 ng/g) compared with rats receiving single treatment (total Aconitum alkaloids: 93.5 ± 8.7 ng/g), suggesting accumulation of toxic Aconitum alkaloids in rat liver. Moreover, our mechanistic studies revealed that liver bile acid levels, including glycocholic acid (Fuzi 69.7 ± 21.1 nmol/mg vs control 4.4 ± 2.8 nmol/mg) and glycodeoxycholic acid (Fuzi 14.1 ± 3.8 nmol/mg vs control 0.9 ± 0.3 nmol/mg), were increased after 15-day treatment of Fuzi with similar trends observed in plasma. The current results for the first time indicated that long-term exposure of the six Aconitum alkaloids could induce hepatotoxicity, which could be related to the perturbations of bile acid homeostasis. (The current study is funded by Health and Medical Research Fund 15161541 by the Food and Health Bureau, Hong Kong SAR, China.)

P110 - THE PHENOTYPING OF SOLUTE CARRIER TRANSPORTERS IN HUMAN PRIMARY HEPATOCYTES: A CHEMICAL INHIBITION APPROACH

Yi-an Bi, Sumathy Mathialagan, Laurie Tylaska, Sarah Lazzaro, Chester Costales, Emi Kimoto, Anna Vildhede, Wenyi Hua, David Rodrigues, Larry Tremaine, and Manthena V. Varma
Pfizer Inc., USA

Purpose: Hepatic (sinusoidal) uptake is the first step and often considered as rate-determining in the hepatic elimination of drugs. Such uptake is mediated by various combinations of different solute carriers (SLCs); e.g., sodium-independent organic anion transporting polypeptides (OATP1B1/1B3/2B1), organic anion transporter 2 (OAT2), organic cation transporter 1 (OCT1), and sodium-dependent taurocholate co-transporting polypeptide (NTCP). Importantly, the expression and function of these transporters is modulated by genotype, disease (e.g., non-alcoholic fatty liver disease),

drug-drug interactions (DDI), and age. Moreover, liver SLCs are often paired with drug-metabolizing enzymes with regard to drug disposition, commonly referred to as “transporter-enzyme interplay. Therefore, quantitative delineation of active uptake clearance (versus passive) *in vitro*, as well as the contribution of individual SLCs to total active uptake (SLC phenotype), is critical when modeling hepatic drug clearance and drug-drug interactions (DDI). However, challenges and controversies still remain regarding the most appropriate methods and the tools to assess different SLC activities (and their contributions) in human primary hepatocytes are not well developed. In this presentation, the PHH (plateable human hepatocytes)-mediated uptake of various substrates was studied after the addition of different SLC inhibitors that presented as phenotyping tools.

Methods: PHH, HEK293-cell lines and a passive permeability assay (RRCK) were applied. The cells were preincubated for 10 min in HBSS buffer with or without a pan-SLC inhibitor (rifamycin SV) and selective inhibitors (low concentration of rifamycin SV and rifampicin, OATPs; ketoprofen, OAT2; quinidine, OCT1 and HBV seq1 peptide, NTCP) at target concentrations. The incubation conditions were optimized; uptake clearance (CL_{up}) and passive diffusion rate were determined. Samples were analyzed by LC/MS/MS and liquid scintillation counting.

Results: In this study, about 20 compounds were screened as inhibitors across 6 major uptake transporters (individually expressed in HEK293 cells) in an attempt to identify, characterize, and validate selective inhibitor conditions for “SLC phenotyping” using plated human hepatocytes (PHH). The fraction transported (ft) by individual transporters and passive diffusion were then determined via PHH incubations for a set of 20 diverse drugs/compounds, which represent various classes of the ECCS: estradiol 17 β -glucuronide (E17G), estrone 3-sulfate (E3S), cholecystokinin octapeptide (CCK8), resveratrol-3-O-glucuronide (R3G), taurocholic acid, cyclic 3',5'-guanosine monophosphate (cGMP), rosuvastatin, pitavastatin, pravastatin, fluvastatin, bromfenac, entacapone, fluorescein, tolbutamide, meloxicam, R/S-warfarin, metformin, ranitidine and thiamine. Finally, we assessed *in vitro-in vivo* extrapolation (IVIVE) of ft, OATP1B1/1B3 leveraging the clinical DDI data of statins with single dose rifampicin, a probe OATP1B1/1B3 inhibitor.

Conclusion: For a given substrate, hepatic phenotyping of OATPs, OAT2, OCT1, NTCP and passive diffusion can be achieved using a pan-SLC inhibitor and inhibitors selective for different SLCs, or combinations of SLCs, at the defined concentrations.

P111 - ASSESSMENT OF CULTURE SYSTEMS ON PERFORMANCE OF PRIMARY HUMAN HEPATOCYTE IN 3D SPHEROIDS

Katherine Dunnick¹, Stefanie Buesch², Maureen Bunger¹, Jenny Schroeder², and Magdalene Stosik²

¹Lonza, USA, ²Lonza, Germany

Limitations of animal models' translation to humans contributes to challenging predictions of drug metabolism and toxicity in the drug discovery and development process. Furthermore, the current *in vitro* models utilized for these studies, such as primary human hepatocytes (PHH) in 2-dimensional (2D) formats, often lack phenotypic and metabolic relevance. To improve predictions of *in vivo* hepatic phenotypes *in vitro*, use of 3-dimensional (3D) cell systems, such as spheroids, can be utilized. When implementing spheroid formation with PHH cells, the cells form improved cell-cell contacts and organize in a more *in vivo* like manner, and therefore mimic more closely the *in vivo* responses compared to PHH in standard 2D culture. In addition, the long-term viability and functionality of PHH spheroids is improved, thus providing a platform for extended hepatocyte exposures and repeat dosing studies necessary for drug metabolism and toxicology experiments. In this study, we analyzed the formation, culture, and performance of PHH in three different spheroid culture systems by comparing metabolic function and viability of the PHH over 28 days in culture. Results indicate that in the presence of serum, PHH formed spheroids in the majority of the tested culture systems within 5 days. After formation, serum was not required for further spheroid culture. Albumin production, viability, and bile canaliculi formation was comparable between spheroids in various ultra-low attachment plates and hanging drop culture. CYP3A4 enzyme activity was inducible by treatment with Rifampicin 5-fold in both hanging drop culture and U-bottom ULA plate conditions. Further, spheroids remained compact and stable during the full examination period independent of culture vessel. Comparing multiple donors using U-bottom ULA plates and the hanging drop system, basal CYP3A4, CYP1A2 and CYP2B6 activity was clearly detectable for up to 4 weeks of culture in spheroids generated with PHH. Overall, donor-to-donor variability in metabolic activity was more pronounced in the later stages of culture. In general, these results demonstrate the ability to culture human hepatocytes for 28-days in 3D spheroid culture with maintained metabolic function, providing a more suitable *in vivo* relevant *in vitro* model for long-term drug metabolism and toxicology studies.

P112 - PRIMARY HUMAN HEPATOCYTE 3D SPHEROIDS FOR STUDYING HEPATIC FUNCTION AND DRUG METABOLISM

Sujoy Lahiri, Julia Tritapoe, Kate Comstock, Theresa Nguyen, Deborah Tieberg, and David Kuninger

Thermo Fisher Scientific, USA

Primary Human Hepatocyte (PHH) culture provides the closest *in vitro* model to human liver that can produce a metabolic profile of a given drug very similar to that found *in vivo*. Hence, PHH culture is the gold standard for studying the *in vitro* hepatic biology, liver function, and drug-induced hepatotoxicity. The conventional 2-dimensional (2D) PHH culture is

limited by de-differentiation and rapid loss of hepatic specific functions. Therefore, there is a need for more robust *in vitro* models that reflects *in vivo* liver biology with better longevity.

Recently, 3-dimensional (3D) *in vitro* models for hepatocytes have gained a lot of attention for their ability to recapitulate the hepatic function with greater longevity. Here we describe the development of an easy to assemble 3D-culture method using PHH, which can be used for various functional assays including drug metabolism, toxicology and disease modeling. The PHH spheroids can live up to 28 days in culture and can retain hepatocyte-specific functions. To assess whether hepatocyte-specific functions were maintained in the PHH spheroids during prolonged culture, albumin secretion, CYP3A4 activity, and levels of ATP synthesized were analyzed. We also present results of metabolite identification using Orbitrap Q-Exactive Mass Spectrometry (MS) for metabolic analysis using *in vitro* PHH 3D-spheroids. The MS results indicate that the 3D-hepatic model out-performed the 2D-model using fewer cells and longer active period. Moreover, we also show that the expression of various genes, reflecting hallmark hepatic functions, in primary hepatocytes, are highly elevated in 3D-culture compared to conventional 2D-culture.

Together, our data clearly shows superior functionality of the 3D hepatic culture model. This easy to assemble 3D-model that we have developed paves way to a new direction of biomedical research using 3D-hepatic culture that can be used for disease modeling and studying drug metabolism of low turnover compounds.

P113 - APPLICATION OF METMAX HUMAN HEPATOCYTES IN THE IDENTIFICATION OF DETOXIFICATION PATHWAYS OF PROTOXICANTS

Albert P. Li and Ivy Wei

In Vitro ADMET Laboratories Inc., USA

We have recently developed a novel experimental system, cofactor supplemented permeabilized cryopreserved human hepatocytes (MetMax™ Cryopreserved Human Hepatocytes, MMHH). MMHH as an experimental system has the complete phase I oxidation and phase II conjugation drug metabolizing enzyme pathways as that for intact hepatocytes, and the convenience of liver microsomes including storage at -80 deg. and use directly upon thawing without a need for centrifugation and microscopic examination. Further, MMHH can be used at toxic drug concentrations that would diminish drug metabolizing enzyme activities in intact hepatocytes, and that one can select drug metabolizing enzyme pathways via the specification of cofactors. We report here the use of pathway-specific MMHH via supplementation with individual cofactors to identify key detoxification pathways. In this study, cytotoxicity of two protoxicants that require metabolic activation, acetaminophen and cyclophosphamide in HEK293 cells using pathway-specific MMHH as an exogenous metabolic activating system. The pathway-specific MMHH systems were: 1. Oxidation only (MMHH-O); 2. Oxidation and glucuronidation (MMHH-OG); 3. Oxidation and sulfation (MMHH-OS); 4. Oxidation and acetylation (MMHH-OA), 5. Oxidation and methylation (MMHH-OM); and 6. Oxidation and glutathione conjugation (MMHH-OT). The results showed that the cytotoxicity of both acetaminophen and cyclophosphamide was enhanced in the presence of MMHH-O, confirmation metabolic activation of these two protoxicants to cytotoxic metabolites. Cytotoxicity was diminished for acetaminophen in MMHH-OG, MMHH-OS, and MMHH-OT, suggesting that all three pathways were important its detoxification. For cyclophosphamide, diminished cytotoxicity was only observed for MMHH-OT, suggesting that GSH-conjugation is the key detoxifying pathway. Our results suggest that pathway-specific MMHH can be used as a tool to aid the identification of key drug metabolic enzyme pathways for metabolic activation and detoxification.

P114 - RECOVERY OF DRUG METABOLIZING ENZYME AND TRANSPORTER GENE EXPRESSION IN PROLONGED CULTURES OF CONFLUENT 2D-HUMAN HEPATOCYTE CULTURES

Albert P. Li and Qian Yang

In Vitro ADMET Laboratories Inc., USA

Primary cultured human hepatocytes are known to have time-dependent decrease in P450 activities, a phenomenon often attributed to dedifferentiation, presumably due to the inadequacy of the culture medium to maintain hepatocyte-specific properties. Recent findings show that culturing hepatocytes as 3D spheroids and as 2D monolayer co-culture with nonhepatocytes would lead to prolonged maintenance of drug metabolizing enzyme activities. Both hepatocyte spheroids and 2D-hepatocyte/nonhepatocyte co-cultures share one common feature, namely, the hepatocytes need to be cultured for multiple days for the establishment of the cultures. Recently, we have further optimized our procedures for isolation and cryopreservation of human hepatocytes, leading to exceptionally high quality cryopreserved human hepatocytes with high viability which form >90% confluent monolayer cultures (999Elite™ Human Hepatocytes). Furthermore, the 999Elite hepatocytes can be cultured for over 30 days while retaining the confluency. As confluent cultures of the hepatocytes have extensive cell-cell contact akin to hepatocyte spheroids, we investigated the possibility that prolonged confluent cultured hepatocytes may retain differentiation properties similarly. Multiple lots of 999Elite™ human hepatocytes were cultured for >three weeks in culture, with medium change performed on Monday, Wednesday, and Friday of the week. RNA was extracted from the hepatocytes at various culture durations. Results show that in all the lots evaluated, the expected daily decreases in CYP3A4 gene expression was observed in the first week of culture. However, gene expression of CYP3A4 was found to increase after the first week, with expression on day 14 to be similar to that in

hepatocytes before culturing, thereby presumably akin to *in vivo* levels. Similar recovery of other P450 isoforms as well as transporter gene expression were observed. Our results suggest that long-term confluent cultures of high quality cryopreserved human hepatocytes may be applied towards the evaluation of prolonged studies such as hepatic clearance of slowly metabolized chemicals, chronic drug toxicity, as well as long-term P450 induction/suppression. The relatively simple handling procedures of confluent human hepatocytes over hepatocyte spheroids is one major advantage, especially in the performance of higher throughput applications.

P115 - PROTEIN RECOVERY AND QUANTIFICATION OF DRUG METABOLIZING ENZYMES AND TRANSPORTERS IN MEMBRANE EXTRACTS PREPARED BY DIFFERENTIAL DETERGENT FRACTIONATION: COMPARISON WITH ESTABLISHED METHODS

Rani Qasem¹, John Fallon², Manisha Nautiyal², Merrie Mosedale², and Philip Smith²

¹King Saud Bin Abdulaziz University for Health Sciences, Saudi Arabia, ²The University of North Carolina at Chapel Hill, USA

Introduction: Differential detergent fractionation (DDF) of biological samples is one attractive alternative to the preparation of tissue proteins for quantification by mass spectrometry. Using the DDF protocol described by Ramsby and Makowski, 1999, in extracting membrane associated drug metabolizing enzymes (DMEs) and transporters from biological samples, it was hypothesized that DDF would be superior (e.g. higher protein yield, more convenient) in isolating membrane proteins to conventional differential centrifugation (DC) and a commonly used commercial membrane protein extraction kit.

Methods: A varying number of isolated mouse primary hepatocytes (50,000 to 5 x 10⁶ cells) and 20-40 mg of mouse liver tissue were processed by DDF. Two PIPES buffered nonionic detergents, the first containing digitonin and the second Triton-X100, were used in sequence to prepare cytosolic and membrane proteins fractions. For comparison, cytosolic and membrane proteins were also extracted in a hypotonic buffer by DC and with a common commercial membrane protein extraction kit. The protein fractions of the different extraction procedures were digested with trypsin following a standard protocol and proteotypic peptides for a range of DMEs and transporters in mouse liver were analyzed by quantitative targeted absolute proteomics (QTAP). **Results:** In processing varying numbers of isolated mouse hepatocytes by DDF, a linear increase in cytosolic and membrane protein yields occurred with increasing numbers of hepatocytes. A similar increase was also observed for the commercial extraction kit. DDF produced a higher ratio of cytosolic to membrane protein yield per million cells than the kit (1.65 ± 0.56 vs 1.03 ± 0.08) suggesting better cellular permeabilization and release of cytosolic proteins and enrichment of membrane proteins with the surfactants employed. This was also observed in extracted fractions from mouse liver tissue (1.74 ± 0.04 vs 1.11 ± 0.12). DDF compared to homogenization in a hypotonic buffer and DC produced an average membrane protein yield from mouse liver that was at least 3-fold higher. This is particularly advantageous in membrane protein extraction from limited material. DDF was compatible with QTAP and there were no signs of ion suppression by the detergents. In analyzing samples extracted by DDF, the concentrations of DMEs and transporters were highly consistent irrespective of the starting number of cells or tissue material. In contrast, the concentrations tended to fall with decreasing number of cells for the commercial kit. Finally, the processing of mouse liver with DDF yielded higher concentrations for most enzymes and transporters compared to the commercial kit suggesting enhanced enrichment of membrane proteins while the use of a hypotonic buffer and application of DC produced the lowest concentrations among the studied extraction procedures. **Conclusions:** DDF is less expensive, more efficient and consistent in the extraction and enrichment of membrane associated proteins compared to DC and a commercially available membrane protein extraction kit. DDF is particularly effective with small sample sizes and is compatible with LC-MS/MS proteomic quantification of DMEs and transporters. Ref: Ramsby, M. and Makowski, G. Differential detergent fractionation of eukaryotic cells. Analysis by two-dimensional gel electrophoresis. *Meth Mol Biol*, 1999; 112: 53-66.

P116 - TOXICITY OF HYDROPHOBIC AND HYDROPHILIC MICROCYSTIN CONGENERS ON PRIMARY HUMAN HEPATOCYTES

Vicki Richardson¹, Joseph Strasser², Elizabeth Hilborn¹

¹US EPA, USA, ²US EPA/ORISE, USA

Microcystins (MCs) are among the most common cyanotoxins found in the environment. Because they are found in many surface waters as well as in drinking and recreational waters, MCs pose a risk to humans and animals. More than 200 MC congeners have been identified with variable amino acid compositions. The amino acid composition determines the structural, physical, and chemical characteristic including hydrophilicity and is thought to determine the toxicokinetic and toxicodynamic profiles of each congener. The most widely researched congener, microcystin LR (MC-LR), is considered a potent hepatotoxin and induces apoptosis and serine/threonine protein phosphatase inhibition when administered to laboratory animals. In this study, the effects of the more hydrophobic (LA, LF, LW, LY) and the more hydrophilic (LR, RR, WR, YR) congeners on cell viability, ROS production, glutathione (GSH) depletion, and protein phosphatase 2A (PP2A) inhibition were determined using primary human hepatocytes (HH). HH were plated in 96-well microtiter plates at a density of 50,000 cells/well (viability, ROS, GSH) or in 24-well microtiter plates at 400,000 cells/well (PP2A). 24 hours

after plating, HH were exposed to LR, LW, LA, LF, LY, WR, YR, or RR (0 to 20 μ M) for up to 24 hours. EC50s from cell viability (CellTiter Glo 2.0; Promega Corp.) studies were used to determine the cytotoxic potency of each MC congener. The rank order for cytotoxic potency was LY>LW>LA>WR>LF>LR>YR>RR. HH were exposed to each congener at respective EC50 (≤ 0.2 μ M) concentrations (as determined in viability studies) for 2 or 4 hours. After the exposure period, HH were assayed for ROS, GSH (Promega Corp.) or PP2A activity (PP2A Immunoprecipitation Phosphatase Assay; MilliporeSigma). ROS and GSH were unchanged in HH exposed to LR, LW, LA, LF, LY, WR, YR, or RR; however, PP2A activity was inhibited by each congener. The increased inhibition of PP2A activity correlated with decreased cell viability. This study shows that the more hydrophobic MC congeners caused a greater decrease in cell viability, compared to the more hydrophilic congeners. Correlations in cell viability and PP2A activity inhibition suggest that PP2A inhibition could be an important mechanism of cytotoxicity in HH exposed to low concentrations of MCs. Coupled with kinetic data on MC uptake and distribution, our results can provide an enhanced understanding of the potential toxic effects produced by these congeners *in vivo*. [This abstract does not represent EPA policy.]

P117 - IMPACT OF CULTURE CONDITIONS ON FUNCTIONAL EXPRESSION OF MAJOR DRUG METABOLIZING ENZYMES AND TRANSPORTERS IN LONG-TERM UPCYTE® HEPATOCYTE CULTURE

Michelle Schaefer¹, Gaku Morinaga², Asami Saito², Akiko Matsui², Gerhard Schänzle¹, Daniel Bischoff¹, Klaus Klinder¹, and Roderich D. Süßmuth³

¹Boehringer Ingelheim Pharma & Co. KG, Germany, ²Nippon Boehringer Ingelheim Co. Ltd, Japan, ³Technische Universität Berlin, Germany

Second-generation upcyte® human hepatocytes (UHH) have been evaluated as a potential novel source of hepatic cells (1,2,3) addressing the needs of robust compound screening within discovery DMPK including assay complexity, costs and supply of pre-characterized cells. Ideally merging the phenotypical functions of primary human hepatocytes (PHH) with the convenience of immortalized cell lines, UHH have been investigated as short-term monolayer and two-dimensional (2D) long-term cultures for exhibiting a matured hepatic phenotype and for delivering reliable estimates of hepatic metabolic clearance for compounds of low metabolic turnover (4). Metabolic profiling as well as studies to assess the functionality of major hepatic transporters (5) revealed a post-plating modulation of major cytochrome P450 enzymes (P450s) and sinusoidal solute carrier (SLC) transporters requiring cell differentiation in absence of proliferation inducing cytokines. Primarily founded on functional readouts, results of experiments were substantiated by relative mRNA gene expression as well as by protein expression data, applying a modified quantitative targeted absolute proteomics (QTAP) approach. In order to mitigate observed P450- and OATP-related deficiencies of UHH, investigations under modified *in vitro* culture conditions have been expanded. In short, functional expression of P450s, primarily of CYP3A4, were found significantly increased under optimized conditions.

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P118 - REACTION PHENOTYPING OF LOW TURNOVER COMPOUNDS THROUGH PERSISTENT INHIBITION OF CYTOCHROMES P450 IN LONG TERM CULTURES WITH HUMAN HEPATOPAC

Sheri Smith, Donald Tweedie, Michael Lyman, and Karsten Menzel
Merck&Co. Inc., USA

Human HEPATOPAC® co-cultures were used to assess cytochrome P450 (CYP) reaction phenotyping (CRP) for low turnover compounds. Engineered to maintain enzyme and transporter activity over several weeks, Human HEPATOPAC is a favorable system for evaluating CRP for low-turnover compounds. CRP approaches for the polymorphically expressed enzymes, CYP2C9 and CYP2C19, were evaluated using chemical inhibitors and genotyped human hepatocytes. Human hepatocytes were treated daily for the duration of the incubations with 15 μ M tienilic acid or 0.5 μ M S-(+)-N-3-benzylnirvanol, selective inhibitors of CYP2C9 and CYP2C19, respectively. Incubations with tienilic acid or S-(+)-N-3-benzylnirvanol resulted in $\geq 80\%$ reduction in CYP2C9 or CYP2C19 enzyme activity, respectively. Tolbutamide and voriconazole, prototypical slowly- metabolized compounds, were used to validate the approaches. Tienilic acid

significantly decreased tolbutamide Clint greater than 80%. S-(+)-N-3-benzylirvanol decreases voriconazole Clint by greater than 30%. Consistent with regeneration of CYP enzymes during the long term incubations, CYP enzyme activity is gained back following a single addition of inhibitor. This observation requires replenishing the chemical inhibitor on a daily basis to maintain a persistent inhibitory effect. Daily addition of the inhibitor leads to a slight accumulation of the inhibitor by 2- to 2.5-fold over 7 days and exhibits no impact for the inhibitory selectivity with other CYP isoforms. A second approach explored polymorphisms in CYP enzymes using poor metabolizers (PM) from genotyped CYP2C9 and CYP2C19 hepatocyte donors. PM HEPATOPAC® co-cultures were also evaluated with the probe substrates, tolbutamide and voriconazole. Low formation of the oxidative metabolite 4'-OH-diclofenac and > 2-fold reduction in tolbutamide CLint were observed for CYP2C9 PM human hepatocytes. The decrease in CYP2C19 activity and voriconazole Clint with respect to Extensive Metabolizer (EM) is similar to that caused by the strong inhibitor, S-(+)-N-3-benzylirvanol of the considered pathway in EM. The utility of the two CRP methods was further supported by compelling data demonstrating differences in CYP2C19 mediating metabolism of voriconazole for adults and children, as reported in the literature (Yanni et al 2010). These two CRP strategies using Human HEPATOPAC® co-cultures provide discovery teams with an important tool to quantitatively assess contributions of CYPs for low metabolism compounds and define the potential risk for drug-drug interactions.

P119 - DRUG-METABOLIZING ACTIVITY AND CYTOCHROMES P450 INDUCTION IN HUMAN HEPATOCYTES FROM TK-NOG CHIMERIC MICE WITH HUMANIZED LIVERS

Shotaro Uehara¹, Yuichiro Higuchi¹, Nao Yoneda¹, Hiroshi Yamazaki², and Hiroshi Suemizu¹

¹Central Institute for Experimental Animals, Japan, ²Showa Pharmaceutical University, Japan

Human hepatocytes are useful as *in vitro* model for studying drug metabolism and drug-mediated cytochrome P450 (P450) induction. However, the continuous and copious use of fresh or cryopreserved human hepatocytes from the same donor is not possible or limited. The herpes simplex virus thymidine kinase-cDNA-transgenic NOG (TK-NOG) mice transplanting commercially available cryopreserved human hepatocytes (Hu-Liver mice) are attracted as a useful experimental animal for predicting drug metabolism and pharmacokinetics in humans, and also important tool as a source for supplying human hepatocytes. In this study, we evaluated P450-mediated drug oxidation activities, P450 mRNA expression, and drug-mediated P450 inducibilities in human hepatocytes from Hu-Liver mice (Hu-Liver cells) cultured for 4 weeks. The large numbers of human Hu-Liver cells (more than 1×10^7 cells/mouse) isolated from Hu-Liver mice by the collagenase perfusion method. Hu-Liver cells were cultured on collagen-coated dishes for 4 weeks using in commercially available medium for long-term maintenance of cryopreserved plateable human primary hepatocytes. The rate of human albumin production in Hu-Liver cells was maintained during the culture period. Midazolam 1'-hydroxylation activities (CYP3A probe activity) in Hu-Liver cells were stably maintained through culture period, and CYP3A4 mRNA expression level in Hu-Liver cells after 4 weeks of culture was comparable to that of the original level. In propafenone metabolism, the 5-hydroxypropafenone and the glucuronide conjugate (human major metabolic pathway) produced with faster rate than the 4'-hydroxypropafenone and the glucuronide conjugate (rodent major metabolic pathway) in Hu-Liver cells, similar to human hepatocytes. Midazolam 1'-hydroxylation activities and CYP3A4 mRNA in Hu-Liver cells induced by typical CYP3A4 inducers, including rifampicin, phenobarbital, rifabutin, dexamethasone, carbamazepine, nifedipine, bosentan, and ritonavir. These results indicated that Hu-Liver cells have similar characteristics to human hepatocytes with respect to drug oxidation activities and P450 induction. Thus, Hu-Liver cells can be potentially valuable *in vitro* tool for drug metabolism and drug-mediated P450 induction studies.

P120 - IN-VITRO EVALUATION OF PRIMARY ANIMAL HEPATIC FUNCTION USING A 3D-SPHEROID CULTURE SYSTEM

Julia Tritapoe, Sujoy Lahiri, Michael Millett, Theresa Nguyen, Mark Powers, and David Kuninger

Thermo Fisher Scientific, USA

The conventional method of culturing primary hepatocytes in a 2-dimensional monolayer (2D) presents limitations in the study of hepatic biology, liver function, and drug induced hepatotoxicity. Traditional 2D hepatocyte cultures rapidly de-differentiate resulting in the loss of hepatic specific function in approximately 5 days. While there are data to support that culturing primary human hepatic spheroids is a sustainable and robust *in vitro* model, limited information is provided for 3D animal spheroids for their use in drug discovery. It is our hypothesis that growing animal hepatocytes in 3D spheroid cultures will more accurately reflect *in vivo* liver biology and maintain the liver functions for a longer period of time in comparison with the traditional 2D culture. Rat (Sprague Dawley) and Mouse (CD-1) hepatocytes from Thermo Fisher Scientific were plated for spheroid qualification following the recommended hepatocyte spheroid protocol from Thermo Fisher Scientific using Hepatocyte Thaw Medium and primary hepatocyte thawing and plating supplements. Nunclon™ Sphera™ 96U-well plates were used to plate between 375 and 6000 cells/well, which were left undisturbed in a 370C incubator. On day 5, spheroid formation was visually evaluated and half of the media was exchanged to Williams Medium E with primary hepatocyte maintenance supplements. During week 1, the volume of the 3D spheroids was measured and in weeks 1-3 the following were assessed: cell viability (as indicated by ATP synthesis), albumin production, bile canaliculi

formation (as indicated by CFDA staining) and phase I metabolic activity. Our data indicate that 3D spheroids primary rodent hepatocytes formed by Day 5, and the methods described above showed that 3D rodent spheroids were viable and functional for up to 3 weeks. In conclusion, we have provided a simple and reliable method to culture 3D spheroid rodent primary hepatocytes and have provided data to support the formation and characterization of 3D rodent spheroids for up to 3 weeks. Similar to 3D spheroid human primary hepatocytes, our data indicate that animal 3D spheroid cultures are an accurate and sustainable *in vitro* model of hepatocyte function that maintain hepatic functions for a longer period of time in comparison with traditional 2D cultures.

P121 - EARLY DEVELOPMENT OF IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY IN AN IMPAIRED IMMUNE CHECKPOINT MOUSE MODEL

Alison Jee and Jack Uetrecht

University of Toronto, Canada

Background: Most idiosyncratic drug reactions (IDRs) appear to be caused by an adaptive immune response to a drug or its metabolites. For example, carbamazepine causes a variety of IDRs including toxic epidermal necrolysis (TEN), liver injury, aplastic anemia, agranulocytosis, and a lupus-like syndrome. Carbamazepine-induced TEN appears to be caused by cytotoxic CD8+ T cells and is associated with HLA-B*1502. Hypothesis: The adaptive immune response leading to an IDR is preceded by an innate immune response that is not HLA-dependent. The innate immune response may occur in most patients and animals and resolve with immune tolerance. Methods and Results: We studied the immune response to carbamazepine in our impaired immune tolerance model. Carbamazepine was administered in food at 0.5% (w/w) for one week and 0.8% thereafter. A slight increase in serum ALT was observed at week 2 and peaked at week 3, indicating liver injury. To observe the immune cells involved in the liver injury, we evaluated cell isolates from the spleen and the liver by flow cytometry. At one week, an increase in Ly6CintLy6G+ cells, cell surface markers characteristic of myeloid-derived suppressor cells, was observed in the spleen. By immunohistochemical staining, T-cell regions (periarteriolar sheaths) and B-cell regions (marginal zones) appeared reduced in the treated spleens while the red pulp was expanded by erythropoietic cells. No significant changes in non-resident immune cells were detected in the liver. At week two, increased T cells were observed in the liver. There was also slight increase in inflammatory monocytes (Ly6Chi) in the spleens of treated animals. Conclusions: The subclinical innate immune response to a drug may predict its IDR risk. We have begun to characterize the early phase of the immune response to carbamazepine in our mouse model of idiosyncratic drug-induced liver injury and have observed the involvement of innate immune cells. Further work is required to phenotype these cells to determine whether they have immunostimulatory or immunosuppressive function. We will also phenotype the resident macrophages of the spleen and liver, as these phagocytes likely also play a role in the initiation of the innate immune response.

P122 - COMBINATION OF DATA-DRIVEN AND MECHANISM BASED APPROACH FOR PREDICTION OF HUMAN INTESTINAL ABSORPTION

Koichi Handa, Seishiro Sakamoto, and Shin Umeda

TEIJIN PHARMA LIMITED, Japan

It is important to predict an intestinal absorption ratio (F_a) precisely at an early stage in discovery of orally available drug since it directly influences its efficacy. If a compound with potentially low human F_a is selected as a drug candidate in non-clinical stage, it might make difficult to develop without some technologies for special formulation at the later stage, which results in not only costing much but reducing the possibility of approval as new drug. To predict F_a , two methods, GUT framework (GFW) proposed by Sugano et. al. [1], and machine learning (ML) are known. In GFW, F_a of a drug is estimated with an equation based on a mechanism of human intestinal absorption (HIA) using its dose, solubility, membrane permeability, and dispersion. The experimental values for these *in vitro* parameters are needed to predict F_a accurately. However, most of these are generally not available at the early stage. On the other hand, ML is an approach using a dataset of observed F_a of many drugs in human [2, 3]. In these ML approaches, the dose information for each drug was ignored. But it is considered to be a critical defect because F_a could change dose-dependently by the change of solubility, membrane permeability and dispersion. In this study, we combined the GFW and ML to compensate for each defect to predict F_a more accurately at the early stage. Firstly, we used the published data of 460 drugs with chemical structures, F_a and dose amounts in human [1]. The key parameters of GFW (D_o , dose number; D_n , dispersion number; P_n , permeation number) were calculated by BioavailabilityDesign MiniTM 1.2 using each actual dose and the calculated values of solubility and membrane permeability from ADMET Predictor™ 9.0 (AP). The structural descriptors of each compound were also calculated by AP. Thus, we constructed ML models with random forest in R3.4.4. We compared three models; GFW, ML with only structural descriptors (ML Model 1), and ML with combination of structural descriptors and GFW parameters (ML Model 2). For model evaluations, the dataset was split into training (80%) and test (20%), then R2-value and RMSE were calculated for training dataset, 10-fold cross validation using training dataset, and test dataset. As a result, we could construct ML Model 2 with lower RMSE and higher R2-value than GFW and ML Model 1. In conclusion, we developed the more accurate prediction method of human F_a by the combination of data-driven ML and

mechanism based GFW which does not need any experimental data. This could be a powerful tool to discover an orally available drug effectively.

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P123 - ASSESSING THE PREDICTIVE PERFORMANCE OF *IN SILICO* GENERATED BINDING PARAMETERS FOR VARIOUS NATURAL PRODUCT CONSTITUENTS

James Nguyen, Dandan Tian, Rakshit Tanna, and Mary Paine
WSU College of Pharmaceutical Sciences, USA

Many patients, particularly those with chronic illnesses, often supplement their pharmacotherapeutic regimens with botanical and other natural products (NPs), raising concern for adverse NP-drug interactions. A common pharmacokinetic mechanism underlying NP-drug interactions includes inhibition of drug metabolizing enzymes by the NP, leading to increased systemic drug concentrations and potential adverse effects. Accurate prediction of the magnitude of these interactions using static or dynamic models requires robust input parameters, including binding parameters that are often obtained *in silico*. The objective of this study was to compare *in silico* generated fraction unbound (*f_u*) with experimentally determined *f_u* for 12 different NP constituents in human liver microsomes (HLM) and plasma to assess the predictive performance of two modeling and simulation platforms. The *f_u* for each NP constituent (0.5 or 1 μ M) in HLM (0.4 or 0.05 mg/mL) and plasma was determined via equilibrium dialysis using 96-well plates and 6-8 kDa semipermeable membranes. After a 6-hour incubation at 37C, an aliquot (80 μ L) was collected from each side of the membrane and analyzed by LC-MS/MS. *f_u* was calculated by dividing either the peak area ratio (analyte/internal standard) or concentration of NP constituent in the receiver compartment to that in the donor compartment. Observed values were compared with those predicted via Simcyp® and GastroPlus™ using a correlation plot. The ratio of *in silico* generated to observed *f_u* values was assessed in subgroups of low (*f_u* >0.80), moderate (*f_u*, 0.20-0.80), and high (*f_u* <0.20) binding constituents using a forest plot. Average observed (\pm SD) *f_u* for the tested NP constituents ranged from 0.48 \pm 0.02 to 1.00 \pm 0.07 in HLM and from 0.018 \pm 0.002 to 0.59 \pm 0.06 in plasma. *In silico* generated values ranged from 0.61-0.99 in HLM and from 0.02-0.75 in plasma. The ratio of *in silico* generated to observed values ranged from 0.73-1.11, 0.37-2.08, and 0.44-6.29 for low, moderate, and high binding NP constituents, respectively. The observed extent of binding of NP constituents to plasma proteins was generally higher than that to microsomal proteins, consistent with values generated *in silico*. Both modeling and simulation platforms consistently predicted *f_u* values for low binding NP constituents to within 25% of observed values, suggesting that *in silico* generated *f_u* values are reasonable estimates for low binding constituents. However, predictive performance diminished for moderate and high binding constituents. Continued comparisons of observed and *in silico* generated *f_u* values for additional NP constituents will form a database that can be used to develop predictive models of *f_u* using a 'learn and confirm' approach.

P124 - MOLECULAR DETERMINANTS OF THE INHERENTLY SLOW ENTRY OF NKTR-181 INTO THE BRAIN

Aleksandrs Odinecs, Werner Rubas, Myong Lee, Laurie Vanderveen, Michael Eldon, Ute Hoch, Jonathan Zalevsky, and Stephen Doberstein
Nektar Therapeutics, USA

Introduction: NKTR-181, an investigational new molecular entity (NME) opioid analgesic, is a selective μ -opioid receptor agonist providing effective chronic pain relief with a lower potential for abuse. Although rate of movement across the blood brain barrier (BBB) is not the sole determinant of abuse potential, it is understood that rapid entry into the central nervous system (CNS) is an important factor in the overall attractiveness of a drug as a target for abuse. Therefore, it is our hypothesis that drugs with inherently slow CNS uptake could have lower abuse potential and improved safety. Here we describe the effects of PEGylation of the morphinan pharmacophore in NKTR-181 on BBB permeability. Methods: Brain uptake rates of NKTR-181, its structural analogs 6 α -mPEG1-7-oxycodol, and oxycodone were compared using different experimental approaches: *in vitro* (Caco-2 monolayers, a surrogate for the BBB where P-gp is expressed in the luminal membrane of the endothelial cells), *in situ* (rat brain perfusion study), and *in vivo* (IV administration to rats and to *mdr1a* (-/-) P-gp knockout (KO) and *mdr1a* (+/+) WT mice). Artificial neural network ensembles (ANNE) regression modeling was conducted using ADMET Predictor 9.0 (SimulationPlus). Relative sensitivity of BBB uptake rate parameters was evaluated for the following molecular descriptors: sum of absolute values of the charges on each atom of a molecule (ABSQ), number of rotatable bonds, number of hydrogen bond acceptors, molecular volume, topological polar surface

area (TPSA), and Moriguchi octanol-water partition coefficient (MLogP).

Results: Comparison of experimentally determined brain uptake rates shows that NKTR-181 uptake is significantly slower than oxycodone uptake: 38-fold in Caco-2 monolayers (A to B), 70-fold and 25-fold in rats *in situ* and *in vivo*, respectively, and 93-fold in *mdr1a* (+/+) WT mice. Because NKTR-181 is a substrate for P-gp, we assessed the impact of P-gp inhibition on the brain uptake rate. While inhibition of P-gp in Caco-2 cells with verapamil, or administration of NKTR-181 to *mdr1a* (-/-) P-gp KO mice increased uptake, NKTR-181 uptake rates remained 11-fold and 18-fold lower, respectively, than those of oxycodone. ANNE regression modeling identified an increased ABSQ value, a higher number of rotatable bonds, and a higher number of hydrogen bond acceptors as predominant factors in NKTR-181's reduced intrinsic BBB permeability after conjugation of the morphinan pharmacophore with a single 6 PEG chain.

Conclusions: The combination of several physicochemical properties, inherent to the molecular structure of NKTR-181, reduces its intrinsic membrane permeability and is a core factor in the slow CNS uptake of this drug candidate.

P125 - PREDICTING PKA USING A COMBINATION OF QUANTUM AND MACHINE LEARNING METHODS

Peter Hunt, Layla Hosseini-Gerami, Tomas Chrien, and Matthew Segall

Optibrium Limited, United Kingdom

The dissociation of a proton from a heteroatom has a significant influence on the charge distribution and interactions of a molecule. These influence many important molecular properties, including binding to target and off-target proteins, absorption, distribution, metabolism and excretion (ADME) and pharmacokinetic (PK) properties such as solubility, tissue or cellular distribution and permeability. Therefore, the ability to predict the propensity of a molecule to lose or gain a proton in water is crucial for the development of new chemical entities with desirable PK, ADME and binding properties. We describe a method for prediction of the acid dissociation constant (pKa) of a heteroatom that combines quantum-mechanical (QM) descriptors, calculated using the semi-empirical AM1 method, with machine learning to generate an accurate quantitative structure-activity relationship (QSAR) model. The QM descriptors capture the geometric and electronic properties of the environment of site of (de)protonation, in the context of the whole molecule. This provides greater discrimination between potentially acidic or basic sites and greater transferability than simple fragment descriptors. The resulting model achieves a coefficient of determination of greater than 0.9 and a root-mean-square error less than one log unit on an external test set containing both mono- and multi-protic compounds. We also present results and comparisons with other methods for published benchmarking sets.

P126 - OBSERVED VS. PREDICTED EFFECTS OF PROTEIN BINDING: IMPROVING *IN VIVO* PREDICTIONS OF HEPATIC CLEARANCE, INTRACELLULAR CONCENTRATION AND IC50 VALUES

Kenneth Brouwer, Jonathan Jackson, Ronald Laethem, and Robert St. Claire III

BIOIVT, USA

Protein binding is often measured in the drug development process, as it is assumed that the free or unbound drug that is responsible for its pharmacological effect. Protein binding is measured in a separate experiment using an *in vivo* relevant matrix, and the fraction bound (fb) is then used to relate total drug concentrations to unbound (free) drug concentrations and used in modeling software to predict various pharmacokinetic parameters, including hepatic accumulation, intracellular concentration (ICC) and intrinsic clearance. Binding measurements (equilibrium dialysis, ultra-centrifugation) measure only the extent of binding and not the binding kinetics (i.e. on and off rates). *In vivo* it is the rate limiting step - the slowest process that determines the overall rate of a process. If hepatic uptake and intracellular concentration are dependent on the free concentration, then, parameters generated from experiments performed in the absence and presence of protein should be equal when adjusted for protein binding. Sandwich-cultured rat hepatocytes and B-CLEAR® technology were used to determine the ICC and biliary clearance (Cl_b) for 10 compounds (taurocholate, telmisartan, methotrexate, valsartan, DPDPE (1 and 10 μM), digoxin, pitavastatin, rosuvastatin, and pravastatin) in the presence and absence of a physiological concentration of bovine serum albumin (4% BSA). In a separate experiment IC₅₀ values for inhibition of CYP2C9 and CYP3A4 metabolism by fluconazole and ketoconazole, respectively using midazolam as the probe substrate in Transporter Certified™ human hepatocytes in sandwich culture were determined in the presence and absence of 4% BSA. The predicted value for Cl_b was over predicted by greater than 2-fold (Methotrexate, 2.8X, and DPDPE - 1 μM, 2.3X), and under predicted for pitavastatin (2.7X), rosuvastatin (3.1X), and telmisartan (92X). Likewise, the ICC was over predicted for valsartan (2.1X), and underpredicted for pitavastatin (3.2X), rosuvastatin (2.2X), and telmisartan (90X). For the other compounds evaluated (pravastatin, digoxin, DPDPE - 10 μM, and taurocholate), predicted values were not different from the observed values. Normalization of the IC₅₀ values for CYP2C9 inhibition by fluconazole resulted in an over prediction by 1.9X, while the IC₅₀ values for inhibition of CYP3A4 by ketoconazole were under predicted by 257X. If active transport processes are involved in hepatic uptake, intracellular concentrations (bound and free) can differ greatly from the concentration outside of the cell, and the slowest process (dissociation from protein or uptake clearance) will limit the hepatic uptake. The lack of agreement between observed and predicted values may be due to measurement of the extent and not the affinity of the binding. If the dissociation rate of the

drug off of the protein is much greater than the uptake rate, protein binding may not be a limiting factor. Addition of physiologic protein concentrations to *in vitro* systems may improve predictions of a drugs *in vivo* properties and effects.

P127 - PREDICTION OF BRAIN DISPOSITION IN RAT AND HUMAN FROM *IN VITRO* DATA: CORRECTION OF PASSIVE PERMEATION

Johan Nicolaï, **Hélène Chapy**, Eric Gillet, Kenneth Saunders, Jean-Marie Nicolas, Hugues Chanteux, and Anna-Lena Ungell
UCB Biopharma, Belgium

Background. Development of CNS drugs is generally considered to be complex and associated with many possible pitfalls. One of the major hurdles is to accurately predict the extent of brain exposure also referred to as the unbound brain to plasma concentration ratio ($K_{puu,brain}$). The $K_{puu,brain}$ is mainly determined by the interplay between passive diffusion and active transport across the blood-brain barrier (BBB). P-gp is the main efflux pump expressed at the BBB to which numerous drug show interaction.

It is therefore vital to CNS-targeting drug development programs to assess the impact of P-gp on the brain disposition of the drug candidates.

Aim. The aim of this work was thus to validate a P-gp (Mdr1a or MDR1) transfected LLC-PK1 cell line to predict rat and human $K_{puu,brain}$.

Methods. We evaluated a set of 48 UCB compounds and 12 commercially available compounds with different P-gp efflux clearance and available *in vivo* $K_{puu,brain}$ data, in a bidirectional transport assay in Mdr1a-transfected LLC-PK1 cells. A smaller set of 5 compounds with reported human $K_{puu,brain}$ values were tested in the MDR1-LLC-PK1 model. The *in vitro* passive permeability (P) and *in vitro* CL_{efflux} (J_{max}/K_m) were determined using a three-compartmental PBPK model (SIVA®; Simcyp In Vitro Data Analysis) and subsequently extrapolated to *in vivo* (rat and human) passive and efflux clearance using physiological parameters (Deo et al., 2013) and accounting for the *in vitro* vs. *in vivo* difference (i) in P-gp expression and (ii) passive clearance. The K_{puu} for each compound was calculated ($K_{puu,brain} = CL_{passive\ in\ vivo} / (CL_{passive\ in\ vivo} + CL_{efflux\ in\ vivo})$) and compared with the observed values.

Results. *In vitro* data on Mdr1a-transfected LLC-PK1 cells failed to accurately predict the rat $K_{puu,brain}$. Since passive diffusion is the other key determinant of the $K_{puu,brain}$, we verified whether the *in vitro* passive permeation was predictive of *in vivo* observations. Using the same method, we determined the passive clearance (*in vitro* and extrapolated to *in vivo*) for a set of 10 passively diffusing compounds. These results compared with *in vivo* passive permeation clearance from in situ brain perfusion, showed a 10-fold difference. Accounting for this difference in passive permeation clearance between *in vitro* and *in vivo* markedly improved the prediction of $K_{puu,brain}$ for rat and human from 67% to 25% of compounds outside the 3-fold deviation lines and 4 out of 5 compounds were in the 2-fold deviation line, respectively.

Conclusion. With the currently proposed method we can extrapolate *in vitro* data from P-gp overexpressing cells to *in vivo* rat or human $K_{puu,brain}$. This is the first report to determine and apply a correction factor for passive permeation to improve IVIVE of cerebral P-gp-mediated transport.

P128 - *IN VITRO* TO *IN VIVO* EXTRAPOLATION (IVIVE) FOR LOW INTRINSIC CLEARANCE COMPOUNDS

Gaurab KC and Sujal Deshmukh
NIBR, USA

In vitro systems have been long used to determined metabolic clearance of compounds. Liver subcellular fractions such as microsomes, S9, cytosol and suspended hepatocytes have the ability to screen for metabolic instability of new chemical entities (NCE) and help optimization and development activities. However, these assays often fail to provide an accurate metabolic response to predict *in vivo* metabolic fate of low turnover compounds. *In vitro* incubation with suspended hepatocytes could only be performed for few hours, to avoid loss in cell viability and activity of drug metabolizing enzymes. Unlike suspended hepatocytes, plated hepatocytes have been shown to have sustained enzyme activity and cell viability in prolonged incubation.

The aim of our study is to evaluate the plated hepatocytes system to determine intrinsic clearance (Cl_{int}) of compounds known to have low metabolic turnover and assess the predictability of human *in vivo* clearance. The selected low turnover compounds will be incubated in plated hepatocytes in 96 well format. Aliquots will be taken at different time points and the compound disappearance will be analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to determine intrinsic clearance (Cl_{int}) of these compounds. The generated *in vitro* Cl_{int} values will be used to predict *in vivo* clearance using the well-stirred model. Data will be presented on the validation and optimization of incubation conditions for plated hepatocytes using positive controls for specific enzyme isoforms. Additionally, prediction of *in vivo* clearance using Cl_{int} from plated hepatocytes by *in vitro* to *in vivo* extrapolation (IVIVE) for a set of clinical compounds known to have low intrinsic metabolic clearance will be presented.

P129 - *IN-VIVO* TRANSPORTER-MEDIATED HEPATIC CLEARANCE OF ROSUVASTATIN IN HUMANS COULD BE BETTER PREDICTED USING TRANSPORTER-EXPRESSING CELLS THAN HEPATOCYTES

Vineet Kumar¹, Kazuya Ishida¹, Laurent Salphati², Cornelis E. C. A. Hop², Christopher Rowbottom³, Guangqing Xiao³, Yurong Lai⁴, Anita Mathias⁴, Xiaoyan Chu⁵, W. Griffith Humphreys⁶, Mingxiang Liao⁷, Beáta Tóth⁸, Nóra Szilvásy⁸, Scott Heyward⁹, and Jashvant D. Unadkat¹

¹University of Washington, USA, ²Genentech, Inc., USA, ³Biogen Idec, USA, ⁴Biogen Idec, USA, ⁵Gilead Sciences, Inc., USA, ⁶Merck & Co. Inc., USA, ⁷Bristol-Myers Squibb Company, USA, ⁸Takeda Pharmaceuticals International Co., USA, ⁹SOLVO Biotechnology, Hungary, ⁹BioIVT, USA

Purpose: Prediction of transporter-mediated clearance (CL), during drug development, remains a challenge. To date, hepatocytes have been used with varying success to predict *in-vivo* transporter-mediated hepatic CL of drugs. We hypothesized that such prediction could be improved using transporter-expressing cells and LC-MS/MS-based quantitative proteomics (hereafter referred to as the relative expression factor, REF, approach). We tested the above hypothesis by comparing the *in-vivo* hepatic uptake CL of rosuvastatin (RSV) with that predicted using the REF approach (CL_{*in-vivo*,pred,cells}) and human hepatocytes (CL_{*in-vivo*,pred,hep}). Methods: OATP1B1/1B3/2B1 and NTCP-expressing cells (0.5 million cells/well in 24-well poly-D-lysine coated plate) were grown for 24 hours in low or high glucose Dulbecco's Modified Eagle's media prior to conducting the [3H]RSV uptake study. The cryopreserved human hepatocytes were plated (0.35 million hepatocytes/well in 24-well collagen coated plates) for 5 hours (PH) or 4 days (sandwich-cultured, SCHH) or suspended (SH) before conducting the [3H]RSV uptake study. Then, at various time points, transporter-mediated [3H]RSV (30 nM) uptake into transporter-expressing cells, SH, PH or SCHH was determined in standard or sodium-free HBSS buffer (to eliminate NTCP activity) in the presence or absence of a pan-OATP/NTCP inhibitor bromosulphophthalein (200 μM). The total or plasma membrane hepatic transporter abundance in these cells was determined by LC-MS/MS proteomics with or without cell-surface biotinylation. *In-vivo* RSV hepatic uptake clearance in humans was predicted using the REF approach or the human hepatocyte models and compared with the published hepatic CL of RSV after RSV IV administration (assumed to be the rate-determining step in the *in-vivo* RSV hepatic CL). Results and conclusion: [3H]RSV uptake clearance into OATP1B1-, OATP1B3-, OATP2B1- and NTCP-expressing cells was 6.68, 7.27, 3.29, and 13.65 μL/min/pmol of plasma membrane transporter, respectively. [3H]RSV uptake CL into SH, PH and SCHH (OATP-, NTCP-mediated and passive diffusion) was 11.7±5.1 (6.3±4.9, 4.8±0.5, 0.58±0.24), 15.4±7.4 (8.9±7.8, 6.1±1.1, 0.3±0.2), and 10.07±2.85 (5.70±2.96, 3.86±0.91, 0.67±0.57) μL/min/mg protein, respectively. The predicted CL_{*in-vivo*,blood} by the REF approach, SH, PH, and SCHH was 303.2±100.6, 138.9±53.8, 175.2±71.2, and 121.5±31.3 mL/min, respectively. The predicted RSV hepatic uptake CL by the REF approach, SH, PH, and SCHH was about 36, 16, 21 and 14% of the observed CL_{*in-vivo*,blood} (850.5 ml/min), respectively. Both the REF approach and hepatocyte models significantly underpredicted the RSV CL_{*in-vivo*,blood}, but the REF approach performed better than the hepatocyte models. Interestingly, [3H]RSV uptake CL into all the hepatocyte models was successfully predicted using the REF approach. Recently, human serum albumin has been shown to increase (2.48-fold) RSV uptake CL into human hepatocytes. When adjusted for this increase, the REF approach successfully predicted hepatic CL_{*int*,*in-vivo*,pred,cells} (631.8±154.6 ml/min) within 2-fold of the observed value. This successful *in-vitro* to *in-vitro* and *in-vitro* to *in-vivo* extrapolation of [3H]RSV hepatic uptake clearance using the REF approach shows the promise of the REF approach. This work was supported in part by the Simcyp Grant & Partnership Scheme and University of Washington Research Affiliate Program on Transporters (UWRAPT) funded by Genentech, Biogen, Gilead, Merck, Bristol-Meyers Squibb, Pfizer, and Takeda.

P130 - EVALUATION OF POTENTIAL EARLY-LIFE SENSITIVITY USING LIFE-STAGE PHYSIOLOGICALLY BASED PHARMACOKINETICS MODELING WITH *IN VITRO* TO *IN VIVO* EXTRAPOLATION

Pankajini Mallick, Marjory Moreau, Patrick McMullen, Salil Pendse, and Miyoung Yoon
ScitoVation, USA

The adsorption, distribution, metabolism, and excretion (ADME) properties of a compound can have a significant impact on its toxicity. Children differ in susceptibility towards harmful effects of environmental chemical exposures due to their development—a dynamic process with many physiologic, metabolic, and behavioral aspects. Using animal studies to infer human early life-stage sensitivity has several limitations, mainly due to species differences in both physiological and biochemical changes during maturation. Especially, metabolism enzymes and their ontogeny patterns can significantly differ between species. *In vitro* systems offer the advantage of studying these processes in isolation; the challenge is to integrate observations into a system representing the intact organism so that they can be appropriately interpreted in the context of human exposure. Physiologically-based pharmacokinetic (PBPK) models integrate information on the properties of the chemical and of physiology into a mathematical modeling framework, an approach that allows quantitatively bridging of *in vitro* and *in vivo* data to explore the key mechanisms dictating the pharmacokinetics. Thus, the purpose of our study was to develop a life-stage PBPK model incorporating age-appropriate and human-relevant *in vitro* metabolism data to predict age-specific internal exposure and to evaluate potential sensitivity. The model compound is rapidly detoxified via hepatic metabolism, and the potential for developmental changes in physiology and ontogeny of drug

metabolizing enzymes (DMEs) that could result in age-related differences in internal exposure in target tissues was considered. We utilized metabolism data from recombinant DMEs support the IVIVE PBPK-based parameterization for different ages. Our human life-stage PBPK model combined knowledge of (1) developmental changes in DMEs, (2) physiological processes occurring during development, and (3) age-specific chemical exposure levels. The PBPK model is coded in R. This IVIVE-PBPK model predicted adult *in vivo* kinetics of the model compound, demonstrating successful characterization of *in vitro* experimental system and PBPK model structure/parameters. The model simulated maximum target concentrations (C_{max}) following single oral exposures for different ages and showed that the differences in internal exposure for both the target tissue and blood are less than 2-fold in early childhood compared to adults. Thus, our IVIVE-PBPK model described the disposition a model compound at different ages and provided valuable insights into age-related differences in target concentrations that can be used to support safety assessments for early ages.

P131 - OPTIMIZATION AND VALIDATION OF RODENT CHEMICAL KNOCK OUT MODEL FOR P-GLYCOPROTEIN USING THE SELECTIVE INHIBITOR, VALSPODAR, AND APPLICATION TO INTERNAL CUT-OFF VALUES AND CALIBRATION OF MDCK-MDR1 CELL LINE

Christopher Rowbottom, Alicia Pietrasiewicz, Elvana Viezaj, Taras Tuczkewycz, Richard Grater, Sudarshan Kapadnis, Doug Burdette, and Daniel Qiu
Biogen, USA

P-glycoprotein (P-gp, MDR1) is a high capacity, broad specificity efflux transporter located in many regions of the body (e.g. BBB, intestine, kidney, liver). Positioned in the plasma membrane of endothelial cells at the blood brain barrier (BBB), P-gp actively pumps xenobiotics and endogenous compounds from the brain, limiting brain exposure. Poor brain penetration of tool compounds that are P-gp substrates hinders Proof of Biology (POB) in early drug discovery. A P-gp specific chemical knock out model was developed to aid in rodent POB. These data also inform P-gp mediated efflux cut-off values, scaling of *in vitro* P-gp efflux ratios to *in vivo* exposure and brain distribution. To advance these three objectives, we have optimized and applied a rodent P-gp valsopodar chemical KO model which has demonstrated the potential utility while identifying a number of significant challenges to the systematic application of this approach. Formulation, C_{max} -based dose tolerability, and route of administration are a few of the challenges and areas for optimization. Using 3 probe substrates for P-gp and a dose titration of valsopodar, we determined that a four hour constant infusion by jugular vein cannula (JVC) greater than 4 mg/kg at 10 mL/kg provided significant and saturable inhibition of P-gp mediated efflux meanwhile minimizing unwanted side-effects. Once the minimal necessary plasma exposure was determined from the titration study, IV bolus regimens were assessed (5 and 3 mg/kg, 1mL/kg every 2 hours, respectively) to reach the same exposure and efficacy. The optimized P-gp KO with valsopodar proved very effective at quantifying the *in vivo* impact of P-gp at the rat BBB in relation to the efflux ratio determined *in vitro* in MDCK-MDR1 cells. This calibration helped to quantify the *in vivo* efflux ratio in rodent brain exposure (Kpuu) of weak to moderate substrates allowing for translational scaling to predicted human exposure based upon proteomic expression of P-gp and BCRP. This approach has proven very successful in triaging compounds with weak efflux and moderate permeability and predicting the dose needed to achieve *in vivo* free brain exposure sufficient for efficacy based upon *in vitro* potency.

P132 - CONTRIBUTION OF FETAL LIVER AND PLACENTA TO MATERNAL-FETAL OXYCODONE DISPOSITION

Sara Shum and Nina Isoherranen
University of Washington, USA

Oxycodone is an opioid pain medication commonly prescribed to pregnant women in the United States. Its use during pregnancy is known to cause neonatal abstinence syndrome in the newborn. Despite its common use, disposition of oxycodone in pregnant women and their fetuses is not known. Oxycodone is mainly metabolized by CYP3A4/5 to noroxycodone, a major metabolite, and by CYP2D6 to oxymorphone, an active metabolite, in adult liver. Based on the abundance of CYP3A7 in the fetal liver, we hypothesized that fetal liver metabolizes oxycodone and plays an important role in protecting the fetus from oxycodone exposure. To test this hypothesis, we measured the metabolism of oxycodone by recombinant CYP3A7. As expected, noroxycodone but not oxymorphone was formed by recombinant CYP3A7. We also tested oxycodone metabolism in recombinant CYP19, which has been shown to be abundantly expressed in the placenta. Both noroxycodone and oxymorphone were formed by CYP19 at a ratio of ten to one and the formation clearance of noroxycodone by CYP19 was 25% of that by CYP3A7. Together these data suggest that formation of noroxycodone by CYP3A7 will be the main fetoplacental metabolic pathway of oxycodone. To determine the importance of CYP3A7 in maternal-fetal disposition of oxycodone, we characterized the metabolism of oxycodone in human fetal liver S9 fractions (FLS9) and human fetal liver microsomes (FLM) prepared from 18 donors. The donor livers were from healthy pregnancies and ranged in gestational age from 85 to 137 days. As predicted from the recombinant enzyme data, only noroxycodone was detected as oxycodone metabolite in incubations with FLS9 and FLM. Additionally, the measured overall depletion of oxycodone in FLS9 was quantitatively accounted for by the formation of noroxycodone, confirming that noroxycodone formation is the major metabolic pathway for oxycodone in fetal liver. Over 80% of noroxycodone formation in pooled FLS9 was inhibited by either pre-incubation with 50 μ M of troleandomycin or 1 μ M of ketoconazole, selective

inhibitors of CYP3A, demonstrating that noroxycodone formation is mediated by CYP3A7 in fetal liver. Furthermore, the CYP3A7 mRNA expression in the 18 fetal livers correlated positively with noroxycodone formation which further supports the major role of CYP3A7 in noroxycodone formation and oxycodone metabolism in human fetal liver. Collectively, these findings are consistent with previously reported data showing that CYP3A7 is the major fetal liver drug metabolizing enzyme. The overall *in vivo* clearance of oxycodone by the fetal liver was predicted from the measured noroxycodone formation clearance in the 18 fetal livers, and the fetal liver extraction ratio was predicted to be low (ER = 0.015). This suggests that fetal liver metabolism likely plays a minor role in protecting the fetus from oxycodone exposure. Overall, our study shows that fetal liver metabolizes oxycodone to noroxycodone predominantly by CYP3A7 and that the highly abundant CYP19 in placenta may also contribute to oxycodone metabolism during pregnancy.

P133 - EVALUATING STABILITY OF HUMAN MONOCLONAL ANTIBODY IN CULTURED RAT HEPATOCYTES WITH A SURROGATE PEPTIDE APPROACH

Nadya Galeva, Reed Murbach, Krystal Gilligan, Kevin Westland, **Seema Muranjan**, and Joanna Barbara Sekisui XenoTech, USA

In vitro cell based assays are performed throughout the drug development process. Probing cytotoxicity and stability of a drug candidate at relevant concentrations in cell culture are of interest in pharmaceutical research. We evaluated the stability of a human monoclonal antibody (mAb) in cultures of primary rat hepatocytes using enzymatic protein digestion and LC-MS quantitation of surrogate peptides. To demonstrate applicability of the surrogate peptide approach to *in vitro* assays for biopharmaceuticals, cryopreserved cultured rat hepatocytes were treated with human SiLuTMLite SigmaMAb at 1, 10, 100 and 200 µg/mL, and incubated in MCM+ medium at 37 °C for up to 48 hours. Measurements of lactate dehydrogenase (LDH) release into the medium, as a result of the damage to the plasma membrane, were performed on the spent media collected at 24 and 48 h post-treatment. Spent media for mAb stability assessments was collected at 0, 0.25, 3 and 24 h on the last day of treatment. Sample preparation for quantitative LC-MS analysis was performed using tryptic digestion. Stable Isotope labeled SiLuTM SigmaMAb was used as an internal standard. The LDH measurements demonstrated no toxicity to the cultured hepatocyte test system incubated with human mAb over the range 0.007 to 1.4 µM. In order to assess the stability of the test compound, five unique tryptic peptides (three peptides from the light chain and two peptides from the heavy chain) were selected as surrogate peptides for LC-MS analysis based on an *in silico* protein sequence examination for SiLuTMLite SigmaMAb. A quantitative LC-MS method was developed and optimized for the five peptides on a Xevo TQ-S tandem quadrupole mass spectrometer. The performance of the method was shown to be adequate for system suitability. All surrogate peptides showed signal linearity over the range of 0.1 to 100 µg/mL. Data obtained over the duration of the experiment were consistent between the five surrogate peptides showing little or no substrate loss, establishing stability of human SiLuTMLite SigmaMAb in cultured rat hepatocytes over the incubation period. An approach to determining therapeutic protein stability in cultured primary hepatocytes using LC-MS analysis of surrogate peptides was established.

P134 - EVALUATION OF CRISPR-CAS9 AND CRISPR-CAS13 IN MODULATING DRUG METABOLIZING ENZYMES

Lisa Chen¹, Jiabin Qiu¹, Michael Mohutsky¹, Richard Moulton¹, Anne Pak¹, Anthony Borel¹, Jeff Alberts¹, Celia Ochoa², Cydney Martell³, Jayakumar Surendraddoss⁴, Kathleen Hillgren¹, and Yingyin Guo¹

¹Eli Lilly and Company, USA, ²Department of Biomedical Engineering, IUPUI, USA, ³Kalamazoo College, USA, ⁴The Centre for Drug Research and Development (CDRD), Canada

It is challenging to conduct *in vitro* to *in vivo* extrapolation for non-cytochrome P450s (CYP) drug metabolizing enzymes or transporters partly because selective drug inhibitors to discern the specific contribution to drug disposition are lacking. Clustered regularly interspaced short palindromic repeats (CRISPR) gene editing has recently gained rapid development to become a powerful technology with promising capabilities for targeted gene deletions. However, it is unknown how CRISPR may modulate drug metabolizing enzyme(s) activities in expanded primary hepatocytes (Upocyte) and primary hepatocyte co-culture (HµREL®) that maintain important metabolic and transport activities. As a result, the study objectives were to examine the efficiency and specificity/off-target effects of CRISPR in modulating expression and activities of drug metabolizing enzymes in these cell models at 1) DNA level, and 2) RNA level using CRISPR-Cas13 by comparing with siRNA technology. First, CRISPR-Cas9 was used to target the cells at the DNA level. Here, a SpCas9 and dual gRNA plasmid approach was utilized to conduct targeted deletion of UGT1A subfamily in Upocyte hepatocytes. Green fluorescent protein (GFP) was also co-transfected by nucleofection, which allowed fluorescence-activated cell sorting to collect GFP positive cells and eventually develop 120 visible single cell derived colonies. Among the four clones that were able to grow robustly, one was cultured and measured for enzyme activity and demonstrated approximately 90% lowered UGT1A1 metabolic activity as measured by estradiol glucuronide formation compared to the wild type. This clone had one deletion allele of UGT1A by PCR characterization of genomic DNA, and deep sequencing is ongoing to confirm its knockout status. Second, using CRISPR-Cas13 to target the cells at the RNA level, HµREL® HumanPool™ hepatic co-cultures were treated with pAAV-Cas13-gNT (non-targeting control) or AAV-Cas13- CYP3A4 for 7 days and then measured for enzyme activity by metabolite formation and mRNA expression levels by RT-qPCR. No cell deaths were

observed in all infection conditions as measured by CellTiter-Glo® luminescent assay. The non-targeting control infection did not change CYP3A4 mRNA or activity. Furthermore, relative to the non-targeting control, CRISPR-Cas13-CYP3A4 AAV infection has led to approximately 30% reductions ($p < 0.05$) in both CYP3A4 activity as measured by 1'-OH midazolam formation, and CYP3A4 mRNA levels in H μ Rel cells. Within all the other genes measured under the same conditions, i.e. CYP3A5, CYP2E1, CYP2B6, UGT1A1, UGT2B7 and AOX1, CRISPR-Cas13 has only led to significant alterations in gene expression for CYP2E1 and UGT2B7. By contrast, siRNA methodology, though showing more robust reduction of CYP3A4 mRNA, had more off-target effects as demonstrated by altered mRNA expression in the non-targeting siRNA transfection and significant changes in mRNA expressions of additional metabolizing enzymes following siRNA-CYP3A4 treatment. Overall, our data supports improved specificity by CRISPR/Cas-13 mediated knockdown. However, caution still needs to be taken to understand specificity and potential off-target issue when using CRISPR-generated cell models. With the continued improvement in its efficiency and appropriate cell model systems, CRISPR technology may become a useful tool in understanding roles of drug metabolizing enzymes and transporters in drug disposition.

P135 - OPTIMIZATION OF NON-PARENCHYMAL AND HEPATOCYTE CO-CULTURE MODEL

Casey Cossis and Kara Shumansky
Novabiosis, USA

Drug-induced liver injury is one of the main reasons a novel drug compound does not get approved for use. When a drug compound encounters the liver and is metabolized, more than just hepatocytes can play a role. The liver is made up mainly of hepatocytes, but also contain non-parenchymal cells such as Kupffer cells, stellate cells, sinusoidal endothelial cells, cholangiocytes, and a few other cell types. The combination of all the cell types beyond just hepatocytes in culture can provide a very useful *in vitro* research tool in the ADME/Tox field. The co-culture composed of hepatocytes and non-parenchymal hepatic cells create a more realistic liver environment as opposed to a hepatocyte culture alone. Novabiosis explored the co-culture conditions with the goal of optimizing the co-culture to give the longest lasting culture with the highest functionality. First, different ratios of non-parenchymal cells to hepatocytes were investigated. This included the ratios of 1 hepatocyte: 3 Kupffer cells: 1 Stellate cells: 4 sinusoidal endothelial cells: 1 cholangiocyte, 2 hepatocytes: 4 Kupffer cells: 2 Stellate cells: 6 sinusoidal endothelial cells: 1 cholangiocyte, and 1 hepatocyte: 4 Kupffer cells: 4 Stellate cells: 4 sinusoidal endothelial cells: 2 cholangiocytes. While it is hard to predict the exact cell composition in the liver, it has been estimated that Kupffer cells make up a majority of the non-parenchymal cells, followed in quantity by sinusoidal endothelial cells, immune cells, biliary cells such as cholangiocytes, and finally Stellate cells. It was of interest to explore if these percentages were truly preferred by hepatocytes in cell culture or if the exact composition did not contribute to functionality. The hepatocytes were first plated and cultured for 24 hours before the non-parenchymal cell types were added. Cultures were maintained out to at least 5 days. Functionality of the Kupffer cell lots used in culture was determined prior to their addition to the culture by the treatment of LPS and measurement of IL-6 and TNF- α production. Functionality of the hepatocytes was measured by ATP production, albumin secretion, and CYP1A2 (Omeprazole 50 μ M; SigmaAldrich O104), 3A4 (Rifampicin 10 μ M; SigmaAldrich R3501), and 2B6 (Phenobarbital 1mM; SigmaAldrich P1636) expression. The ATP production was determined using the Luminescent ATP Detection Assay Kit (abcam ab113849). Albumin secretion was measured using the Human Albumin ELISA Kit (abcam ab108788). An optimized co-culture model could potentially aid in the prediction of DILI side effects from novel drug compounds.

P136 - OPTIMIZATION OF 3D LIVER SPHEROID CULTURE CONDITIONS OF INDIVIDUAL HEPATOCYTE DONORS AND POOLED HEPATOCYTE DONORS

Casey Cossis and Kara Shumansky
Novabiosis, USA

Spheroids have proven to be an extremely useful *in vitro* model in ADME/Tox research. There is a need for more realistic hepatic models such as spheroids, due to the large amount of costly drug candidate withdraws. Spheroid culture conditions are tested in spheroids from both individual hepatocyte donors and pooled hepatocyte donors in order to determine optimal conditions for the highest functionality. It is hypothesized that spheroid formation will be optimal in a 10% FBS supplemented culture medium, seeded at 1 – 4,500 cells per well in a 96-well plate. In this study, 3D spheroids were created using Novabiosis cryopreserved primary human hepatocytes from individual donors and 5-pooled donors. Here, various seeding densities and compositions of culture mediums are tested, with differing proportions of FBS and other common culture medium additives such as dexamethasone, glutamax, pen/strep, and insulin. The initial plating mediums are altered to measure the effects on hepatocyte functionality as well. Functionality of the hepatocytes was measured by ATP production, albumin secretion, and CYP1A2 (Omeprazole 50 μ M; SigmaAldrich O104), 3A4 (Rifampicin 10 μ M; SigmaAldrich R3501), and 2B6 (Phenobarbital 1mM; SigmaAldrich P1636) expression. The ATP production was determined using the Luminescent ATP Detection Assay Kit (abcam ab113849). Albumin secretion was measured using the Human Albumin ELISA Kit (abcam ab108788). Hepatocyte spheroid cultures were also characterized for their transporter activity for transporters OCT, BSEP, NCTP, and OATP. All spheroid formation was achieved utilizing ultra-low

attachment microplates (Corning™ 3474). An optimized spheroid model will provide an improved testing model for novel drug compounds.

P137 - A NOVEL METHOD FOR THE ISOLATION OF NR8383 LYSOSOMES AND MEASUREMENT OF INTRALYSOSOMAL DRUG CONCENTRATIONS

Laura Francis¹, David Hallifax¹, Andrew Harrell², and **Aleksandra Galetin**¹

¹University of Manchester, United Kingdom, ²GlaxoSmithKline, United Kingdom

Lysosomes are acidic intracellular organelles that can extensively sequester basic lipophilic drugs as a result of pH and membrane partitioning, and therefore may significantly influence subcellular drug concentrations. Various *in vitro* methods are currently used to examine lysosomal drug sequestration; however, such methods typically lack the ability to accurately and sensitively quantify drug concentrations directly within the lysosome. To address this, this work aimed to develop a novel method to isolate lysosomes by magnetic purification from lysosome rich NR8383 cells, and use the isolated lysosomes in intralysosomal drug accumulation studies followed by LC MS/MS analysis to quantify intralysosomal drug concentrations. The developed method resulted in isolated lysosomes of high yield and purity, with negligible contamination by other cellular components based on enzymatic and electron microscopy analysis (4.6 fold enrichment in the lysosomal enzyme β N acetyl glucosaminidase, and 3,000, CV=19%), but concentration dependent drug accumulation could not be achieved under the current experimental conditions. Method optimisation highlighted sensitivity of imipramine KpLysosome values to the volume of isolated lysosomes and buffer conditions used. Application of the developed method to assess lysosomal drug sequestration was investigated using 11 selected drugs with a broad range of physico-chemical properties. These accumulation studies resulted in KpLysosome values ranging from 4,600-7,600 for procainamide to fluoxetine, respectively; a partial, but non significant, trend between KpLysosome and LogP/LogD7.4 values was apparent. The acidic drug diclofenac also produced high KpLysosome values, most likely caused by pH dependent solubility driven accumulation, under these experimental conditions. The novel method developed shows the ability to isolate functional lysosomes of high yield and purity, and can further be adapted to other cell lines. The use of magnetically isolated lysosomes provide a promising new methodology for identifying lysosomotropic compounds and quantification of the extent of lysosomal drug sequestration in drug development.

P138 - DEVELOPING A NOVEL 3-DIMENSIONAL SMALL INTESTINAL-LIKE MODEL FOR XENOBIOTIC SCREENING

Paulus Jochems¹, Jeroen van Bergenhenegouwen², Anne Metje van Genderen¹, Sophie Eis¹, Livia Wilod Versprille¹, Harry Wichers³, Prescilla Jeurink², Johan Garssen¹, and Rosalinde Masereeuw¹

¹Utrecht University, Netherlands, ²Nutricia Research, Netherlands, ³Wageningen Food & Biobased Research, Netherlands

The small intestine forms an important barrier between the external and internal environment. For decades Caco-2 cells cultivated on Transwells™ (TW) are considered the gold standard as *in vitro* model to study the small intestinal permeability. However, shortcomings of this system include a lack of 3-dimensional tube-like structure, cell differentiation and absence of shear stress. In our model, we seeded Caco-2 cells on polyether sulfone hollow fiber membranes providing a tube-like construct allowing cells to proliferate and differentiate. We investigated different hollow fiber membrane sizes, cell seeding densities, extracellular matrix (ECM) coating and shear stress durations to set the optimal microenvironment and culture conditions. Barrier formation was evaluated via inulin-FITC leakage assay and immunofluorescent staining of tight junction molecule, zonula occludens-1 and cell differentiation via staining for goblet cell marker Mucin-2 (MUC2). To highlight some of our findings, we found coating to be essential in inducing cell differentiation into the goblet cell phenotype and shear stress induced the formation of villi-like structures. After the microenvironment was determined cellular differentiation was studied more elaborate showing enterocyte-like differentiation via alkaline phosphatase activity and positive stainings for Paneth cells (Lysozyme), enteroendocrine cells (Chromogranin A) and stem cells (LGR5+). To further establish our model, a direct comparison between the TW and bioengineered intestinal tubules was made comparing inulin-FITC leakage. No significant difference was detected, showing the capability of our model to study intestinal permeability. Finally, to validate and show applicability of the bioengineered intestinal tubules, we exposed our model to toxin A (ToxA) produced by the *Clostridium difficile* bacteria. *Clostridium difficile* infections are one of the most common healthcare associated infections worldwide and the toxins secreted are well-known barrier disrupters. In line with literature, ToxA disrupted the intestinal barrier and showed a slight increase MUC2 expression in our bioengineered intestinal tubules. Altogether, we developed a reproducible 3-dimensional Caco-2 model with increased cell differentiation and improved morphology. Our model more closely mimics intestinal physiology than regular, 2D cultures, hence provides a promising tool in xenobiotic research.

P139 - OPTIMIZATION OF HEPARG WORKFLOW FOR USE IN 3D SPHEROID MODELSMichael Millett, Michael Connolly, Juan Martin Silva, Mark Kennedy, and David Kuninger*Thermo Fisher Scientific, USA*

Growing evidence suggests that cells cultured as 3-dimensional (ie. 3D) spheroids exhibit more *in vivo*-like cellular properties than standard monolayer cultures which underscores their potential as more physiologically relevant *in vitro* culture systems. The hepatic cell line HepaRG, is a well-established model for studying drug metabolism and toxicity, and there have been numerous reports indicating its utility as a 3D model. However, these 3D protocols primarily recapitulate the standard monolayer (ie. 2D) workflow which is based on the dynamic temporal metabolic profile of cytochrome P450 (CYP) enzymes. Since gene expression profiles and the behavior of cells is frequently different in 2D and 3D cultures, we hypothesized that the commonly used HepaRG spheroid culture protocol has not been optimized. To address this, we compared the temporal profile of basal CYP3A4 activity in 2D and 3D HepaRG cultures to determine if cells may function differently between these culture formats. HepaRG monolayers were grown on Collagen I coated plates; 3D spheroids were formed in Nunclon Sphera 96-well U-bottom plates. Basal CYP3A4 activity in 2D and 3D culture was measured every 24hrs for 10 days and normalized to cell viability. Consistent with published data, our HepaRG cultures formed spheroids in 2-3 days of culture and the size of the spheroid was directly proportional to the number of cells seeded. Preliminary data also confirmed that 2D HepaRG cultures had high CYP3A4 activity in the first 24hrs of culture with a subsequent reduction that slowly recovers to peak activity levels at Day 6 of culture that remained relatively constant for the study period. Interestingly, these spheroid cultures had significantly higher basal CYP3A4 activity (>2 orders of magnitude) compared to 2D cultures. During the aggregation phase of the spheroid culture (ie. Day 1-2) CYP3A4 activity was elevated and reached peak levels at Day 3, when spheroids are fully formed. From Day 3-10, HepaRG spheroids showed relatively constant levels of CYP3A4 activity. In conclusion, these results suggest that the temporal profile of commonly studied metabolic enzymes is different between HepaRG monolayer and spheroid cultures. Moreover, the difference in HepaRG spheroids peak activity levels suggest that the spheroid culture method offer more flexibility for experimental design with potentially shorter culture times.

P140 - EXPANSION OF *IN VITRO* DRUG METABOLISM ASSAY CAPACITY AND EFFICIENCY WITH HIGH THROUGHPUT SCREENING USING AUTOMATED LIQUID HANDLINGAndrew Schofield, Bruce Zhu, Christopher Welsh, and Chuong Pham*Alliance Pharma, Inc., USA*

To improve the efficiency of drug discovery, efforts continue towards high through screening (HTS) as a strategy to quickly and effectively move drug candidates through development. Automated liquid handling platforms offer a programmable system for increasing the capacity and turnaround of assays routinely completed manually. Here we describe our efforts to develop methods on the Hamilton MicrolabSTAR automated liquid handling platform. First, our goal was to evaluate and place into production automated methods for basic drug discovery assays including metabolic stability and protein binding. Upon these basic programs we would build more sophisticated enzyme kinetics schemes. We designed a web based portal with a user friendly design to generate parameters for the Hamilton program for each *in vitro* assay. We executed the method for metabolic stability and protein binding with several commercially available compounds with results consistent with known literature values. These results lead us to believe that the Hamilton MicrolabSTAR is an appropriate platform to develop HTS methods for our routine *in vitro* assays.

P141 - QUANTITATIVE AND MECHANISTIC COMPARISON OF FRACTION UNBOUND FROM LIVER HOMOGENATES AND HEPATOCYTES AT 4°C AND APPLICATIONS IN K_{PUU} DETERMINATIONKeith Riccardi¹, Sangwoo Ryu¹, David Tess¹, Roshan Patel², Rui Li¹, and Li Di¹¹Pfizer Inc, USA, ²Spectrix Analytical Services LLC, USA

Fraction unbound values obtained from liver homogenates with equilibrium dialysis (f_u , homo) or hepatocyte partitioning coefficient at 4°C ($K_{pcell,4°C}$) are both frequently used to estimate free drug concentrations and unbound partition coefficients (K_{puu}) of the liver.

To understand the differences and similarities between the two methods, a set of 29 structurally diverse acids and zwitterions with fraction unbound spanned over 3 log units was used for the comparison. The results showed that fraction unbound values from the two methods were well correlated. Both f_u , homo and $K_{pcell,4°C}$ are measures of binding to proteins/lipids in the liver or hepatocytes but differ on pH-gradient impacts of cytosol to organelle permeabilities. Liver homogenization destroys subcellular structures necessitating a mathematical correction of pH-gradient permeability impacts not required of the $K_{pcell,4°C}$ method as organelles remain intact. Additionally, both approaches assume that permeabilities of non-neutral species are negligible, thus negating the impact of membrane potentials. As the liver fraction unbound values from both methods are highly correlated and can be interconverted mathematically, fraction unbound measurement using liver homogenates is recommended over hepatocytes at 4°C due to low cost, high reproducibility and

more amendable to high throughput format. The fraction unbound values were also applied to calculate liver K_{pu} and most acids showed enhanced liver uptake.

P142 - EVALUATION OF FRACTION UNBOUND ACROSS FIVE SPECIES AND SEVEN TISSUES AND THE IMPACT OF LOW TEMPERATURE

Sangwoo Ryu, Karen Atkinson, Jonathan Novak, Woodrow Burchett, David Tess, Li Di, Christopher Keefer, George Chang, Gregory Steeno, Roshan Patel, and Keith Riccardi
Pfizer, USA

Free drug concentration is critical for *in vivo* efficacy and development of pharmacokinetic (PK) /pharmacodynamic (PD) relationships of drug candidates [1]. Knowing the free drug concentration (C_u) in tissue is essential for the success of drug discovery projects. This is typically achieved by measuring total tissue concentration (C_t) *in vivo* in preclinical species and fraction unbound (f_u) *in vitro* ($C_u = C_t * f_u$). f_u of homogenates for liver, brain, muscle, heart, lung, adipose and kidney tissue are often measured in various species under the assumption that species have different binding properties. However, this assumption has not been verified. Recent studies of f_u in rat liver and rat brain homogenates have shown to have good correlation against respective tissues in mouse, human, dog, and monkey [2, 3]. This suggested that binding in other tissues such kidney, lung, adipose, muscle, and heart might be independent of species. In this study, we evaluated the f_u values of homogenates for kidney, lung, adipose, muscle, and heart tissues of human, monkey, dog, rat and mouse using 80 commercial drugs with different physicochemical properties. Equilibrium dialysis in 96-well format was used to determine f_u for this study. The results showed that kidney, lung, adipose, muscle, and heart f_u is independent of species. Further inspection into the data showed there was a correlation between species type and showed rat liver can be used as a surrogate for all different tissue types regardless of species. Although there is a scaling factor for a few tissue types, this approach greatly reduces tissue binding studies in drug discovery, reduces cost and saves time and resources. The effect of low temperature (4 °C) on plasma protein binding and tissue binding was evaluated for the first time using a large set of structurally diverse compounds covering a wide range of physicochemical properties and fraction unbound values. These results show that temperature has little effect on plasma protein binding and tissue binding and that the measured binding values at 4 °C are equivalent, on average, to those at physiological temperature (37 °C).

P143 - EVALUATION OF SINGLE-USE RAPID EQUILIBRIUM DIALYSIS [RED] DEVICE FOR AUTOMATED OCTANOL-WATER PARTITIONING (LOGD7.4) ASSAY TO ASSESS THE LIPOPHILICITY OF DRUG COMPOUNDS

Adrian Sheldon, Sarah Meloche, and Jakal Amin
Charles River Labs, USA

Lipophilicity (hydrophobicity) is an important factor to determine during the initial stages of drug discovery for new therapeutic compounds since lipophilicity has a major influence on ADME properties and the general suitability of drug candidates. Octanol-water partitioning via the shake-flask method is a well-established method for determining the lipophilicity of new chemical entities (NCEs). Measuring the distribution coefficient (LogD7.4) of drug compounds via single-use ThermoFisher Rapid Equilibrium Dialysis [RED] units provides a more compact system than the shake-flask method and is more easily scalable for high-throughput applications in early drug discovery. The RED units contain a semi-permeable cellulose membrane that has been shown to not break down in the presence of non-polar solvents such as octanol. This unit should allow for optimal interaction between the two phases and for diffusion of any compounds toward their more preferred thermodynamic environments. The objectives of our investigations were to demonstrate the suitability and performance of the RED units for this assay, and to determine the optimal equilibration time for the RED units which would enable high-throughput approaches for determining the lipophilicity of NCEs. Therefore, we evaluated ten marketed approved drugs having diverse LogD7.4 values using the RED device for the octanol-water partitioning procedure. The lipophilicity of these known representative drugs was measured by sampling the polar phase (1X PBS) at varying time points up to 26 hours at room temperature and analyzing by LC-MS/MS. We evaluated the effect of spiking and sampling from both solvent compartments to identify an optimal approach; the percent recoveries were also assessed. LogD7.4 calculations were performed by comparing signal intensities normalized against the internal standard (peak area ratios) at the experimental time versus time zero. The results indicate that equilibrium is achieved between the chambers of the RED units following dialysis for 18 hours, so an overnight incubation is convenient. Since the RED device can be successfully used to perform octanol-water partitioning assays and both chambers are easily accessible for pipetting, this assay approach is not only a suitable alternative to the shake-flask method but is also very amenable to be scaled up for screening purposes using lab automation.

P144 - MECHANISTIC INSIGHTS INTO THE HUMAN ADME OF A THERAPEUTIC CANDIDATE**Herve Aloysius**, Megan Turski, Steve Bowlin, Cody Fullenwider, and Amin Kamel*Takeda, USA*

The ADME properties of compound A were evaluated in preclinical species and humans to support its advancement to candidate selection. Compound A is mainly hydrolyzed in the gut to afford hydrolytic metabolites which are further metabolized to a disproportionate circulating metabolite (MET), and others converted to phase 2 metabolites. PK studies in rats, dogs and monkeys suggest that the systemic exposure of compound A in humans will be low. Despite the projected low systemic exposure of Compound A, there is persistent interest in understanding species differences in the source of formation, fate and anticipated human exposure (gut, liver and systemic) of the disproportionate metabolite to better assess its coverage in preclinical toxicological studies. Moreover, the back-up discovery program is optimizing for molecules with low potential of circulating metabolites.

To enable the development of compound A and a back-up molecule with a differentiated metabolic profile, we set out to: 1) characterize the metabolism of compound A to gain early insights into its human ADME using LC/MS/MS 2) establish a reliable *in vitro* system to prioritize molecules for further *in vivo* testing.

Compound A and MET were orally dosed to rats, dogs and monkeys to characterize the absorption profile and hepatic extraction. Preliminary results indicate that MET is formed in the gut and subsequently absorbed; additionally, portal vein studies suggest that MET isn't metabolized in the liver significantly, and thus it is hypothesized that formation of MET occurs in the gastrointestinal tract. Because plateable enterocytes are costly and challenging to implement, cultured hepatocytes were effectively used as a surrogate *in vitro* system to further delineate the enzymes involved in formation of MET in the gut as well as to prioritize analogs of compound A in back-up efforts. Ultimately, these efforts aided in our understanding of the metabolic fate of Compound A and impacted the design and screening strategy for the back-up discovery program

P145 - IN VITRO ABSORPTION AND METABOLISM OF 7-O-SUCCINYLMACROLACTIN A IN HUMAN**Soo Hyeon Bae**¹, Jae Hoon Shim¹, Jung Bae Park¹, Phyu khin Phyu¹, and Soo Kyung Bae²¹*Korea Institute of Radiological & Medical Sciences (KIRAMS), South Korea*, ²*The Catholic University of Korea, South Korea*

7-O-Succinylmacrolactin A (SMA), a macrolactin generated by *Bacillus polyfermenticus* KJS-2, is known to exert antibiotic activities against vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* as well as antitumor effects. The aim of this study was to evaluate absorption and metabolism of SMA *in vitro* and to suggest possible metabolic pathway of SMA. For elucidating metabolism of SMA, metabolic stability in microsomes and then reaction phenotyping study under human cDNA-expressed cytochrome P450s (CYPs), UDP-glucuronosyltransferases (UGTs), and carboxylesterases (CESs) were conducted. With these samples, metabolic pathway of SMA was suggested by analyzing liquid chromatography/ion trap mass spectrometer. We also investigated *in vitro* absorption of SMA using wild-type and P-glycoprotein (P-gp)- and breast cancer resistance protein (BCRP)-overexpressed MDCKII cell line. Most of SMA was hydrolyzed to form its active metabolite, macrolactin A (MA), and a minor portion of SMA was metabolized by UGT1A3. MA was further metabolized by UGT2B7 or oxidized by CYP3A4/5. Glucuronidation of MA by UGT1A1, UGT1A4, UGT1A9 occurred minimally. In addition to metabolism, SMA was not a substrate of P-gp and BCRP. From these results, we concluded that SMA was extensively hydrolyzed by CES and further metabolized by CYPs and UGTs. Moreover, since the major metabolite of SMA, MA, has pharmacological effect and forms extensively by CES, the concentration of MA as well as SMA should be considered for drawing accurate dose-response relationships of SMA.

P146 - A HIGH CAPACITY AND HIGH THROUGHPUT AUTOMATED METHOD FOR THE GENERATION OF RADIO-PROFILING DATA TO SUPPORT ADME STUDIES**Anthony Barros Jr**, Van Ly, Lisa Christopher, Punit Marathe, and Ramaswamy Iyer*Bristol-Myers Squibb, USA*

Radiolabeled ADME studies are routinely conducted in drug discovery and development to understand the disposition of compounds. These studies with either ³H or ¹⁴C labeled compounds provide an understanding of the overall metabolism and excretion of drugs in animals and humans. The analysis usually involves working with plasma, urine, feces, bile and tissue matrices wherein the samples are extracted for all radioactive-related material and then analyzed by LC/MS-Radioactivity detection. The radioactive detection involves collection of fractions into 96-well luma plates followed by drying of the samples for detection. We have developed a high capacity and high throughput method for the automated generation of radioprofiles from 96 well luma plates through a joint collaboration with LEAP Technologies and Perkin Elmer. A LEAP Technologies Dual Collect PAL fraction collector with up to 48 luma plate capacity was developed and combined with a customized Perkin Elmer Twister III robotic arm and MicroBeta2 plate reader system with up to 114 luma plate capacity. This utilization of state of the art automation will enable metabolism and pharmacokinetics (MAP) scientists to generate radioprofiling data in an efficient and timely manner to support preclinical and clinical ADME studies.

P147 - STRUCTURAL ELUCIDATION OF THE MAJOR HUMAN METABOLITES OF VILOXAZINE**Eleanor Barton**¹, Ray Cooke¹, Dylan Williams¹, and Chungping Yu²¹Pharmaron UK, United Kingdom, ¹Supernus Pharmaceuticals, Inc, USA

Supernus Pharmaceuticals, Inc (Supernus) is currently developing extended-release viloxazine (also referred to as SPN-812V) as a treatment for attention deficit hyperactivity disorder (ADHD). Pharmaron UK has worked closely with Supernus to determine the metabolic pathway of viloxazine following oral administration to humans. Plasma samples from subjects dosed with ¹⁴C labelled viloxazine were analysed using high resolution, accurate mass, liquid chromatography mass spectrometry in order to identify the major circulating metabolites. It was determined that the major circulating metabolites of viloxazine are formed through hydroxylation followed by glucuronidation. Metabolite profiling indicated that there were two major components (to be referred to as P1 and P2) which accounted for 10%, or greater, of the circulating radioactivity. P2 was identified as unchanged SPN-812V. P1 was identified to have a nominal molecular weight of 429; based on the accurate mass and product ion data the proposed transformation was hydroxylation followed by glucuronidation. The same component was also observed in human urine (referred to as U1). From the high resolution accurate mass data alone there was insufficient information to identify the exact position of the hydroxylation and subsequent glucuronidation. To confirm the position of the glucuronidation it was necessary to first focus on the site of hydroxylation. The strategy to identify the position of the transient hydroxyviloxazine metabolite (prior to glucuronidation) involved synthesis of the four possible hydroxyviloxazine metabolites coupled with deconjugation of the glucuronide metabolite, in a concentrated urine sample, to produce the hydroxyviloxazine metabolite. A chromatography method was developed by Pharmaron UK to separate the four possible isomers of hydroxyviloxazine. The deconjugated urine sample was subsequently analysed alongside the synthesised metabolite reference standards in order to confirm the exact isomeric structure. A representative plasma sample was also analysed on the same method to confirm that P1 and U1 were indeed the same isomer. Metabolite P1/U1 was confirmed to be 5-hydroxyviloxazine glucuronide.

P148 - IN VITRO MECHANISTIC INVESTIGATION STUDIES TO UNDERSTAND THE METABOLIC FATE OF COMPOUND 1**Steve Bowlin**

Takeda, USA

In vitro mechanistic metabolism studies have been conducted to understand the pharmacokinetics and metabolism of Compound 1. Early *in vivo* studies indicated that Compound 1 undergoes initial hydrolysis of an ester bond to M1 with release of its corresponding acid derivative (M3), followed by a second amide hydrolysis to form M2. Initial amide hydrolysis of Compound 1 and subsequent ester hydrolysis resulted in the formation of M4 and M2, respectively. *In vitro* studies including incubation of Compound 1 in plasma and hepatocytes and subsequent LCMS analysis proved challenging and resulted in no detection of Compound 1 or any of its expected metabolites. Methods: The need to understand the metabolic fate of Compound 1 triggered investigative studies and method development including pH optimization, extraction efficiency of Compound 1 and metabolites, matrix effect studies, and optimization of the LC gradient and MS ionization modes. Structure elucidation of metabolites was carried out on an AB Sciex TripleTOF 5600 LC/MS/MS system using positive and negative ESI modes and full scan TOF-MS with Mass Defect Filter triggered MS/MS acquisition. Preliminary Results: Preliminary results under the newly developed methods proved very successful and established IVIVC of the metabolic fate of Compound 1. Incubation of Compound 1 in plated human hepatocyte cultures for 4 days resulted in the formation of all metabolites detected in *in vivo* (M1, M2, M3 and M4) with initial ester hydrolysis representing the major metabolic pathway. Oral dosing of Compound 1 to rats indicates formation of M2 in the gut, which is well absorbed, with limited parent compound found in circulation. *In vitro* metabolite identification studies support the metabolic profiling conducted in monkey and rat plasma following oral dosing of Compound 1. The results of these metabolism studies have contributed to understanding Compound 1 exposure in the intestine and in plasma, and are being used for chemical design of back up molecules.

P149 - ACQUIREX - A NEW DATA ACQUISITION STRATEGY FOR METABOLITE PROFILING ON AN ORBITRAP ID-X TRIBRID MASS SPECTROMETER**Kate Comstock**¹, Qian Ruan², and Kenneth Matuszak¹¹Thermo Fisher Scientific, USA, ²Bristol-Myers Squibb, USA

Metabolite identification and structural elucidation of drugs warrant the collection of high-quality tandem mass spectra. For the analysis of in-vivo samples with a complex biological matrix, there is a significant challenge in identification of low abundant metabolite ions and triggering of MS/MS or MS_n scans without prior knowledge and prediction of metabolic pathways. However, manually excluding background and including relevant features is too laborious, and still no guarantee of low abundant metabolite identification.

In this study, Orbitrap ID-X Tribid MS AcquireX, a novel data-dependent acquisition workflow, was used to assess its capability for in-vivo metabolite identification.

AcquireX data acquisition workflow carries out real-time background subtraction and method updating. As a result, the updated method only triggers MS and MSn of potential metabolites without user intervention and preexisting knowledge. Methods: Seven selected drugs (Ticlopidine, Buspirone, Nefazodone, Montelukast, Omeprazole, Ranitidine, and Timolol) at 10 μ M were incubated with rat and human liver microsomes with glutathione trapping for 30 minutes. Control incubations of microsomes without the addition of the drugs were used for background subtraction.

The data analysis was conducted on an LCMS system consisting of Vanquish Flex UHPLC with DADA detector and Orbitrap ID-X Tribrid MS with AcquireX data acquisition workflow.

Hypersil C18 column (2.1X50 mm 1.9 μ m) was used. Mobile phases A and B were H₂O/5mM NH₄OAc/0.02% Formic Acid, and ACN respectively. Data were processed using Compound Discoverer 3.0 and Mass Frontier 8.0 software.

Preliminary Result: A set of model drugs were selected for their representative chemical structures and a variety of metabolic pathways, including oxidation, dealkylation, dehalogenation, hydrolysis, and conjugation. The complex mixtures of metabolites were generated by extensive metabolism in rat liver microsomes and moderate metabolism in human liver microsomes. Complete metabolite profiles were elucidated in these systems by using a traditional data-dependent acquisition on Orbitrap ID-X Tribrid MS.

Further experiments were conducted by spiking the metabolite mixtures into rat and human plasma using ID-X MS AcquireX data-acquisition workflow, to assess AcquireX's capability to identify low abundant metabolites present at high matrix background, which often failed triggering of MS₂ scan using the traditional data-dependent analysis due to high background interference.

The model drug metabolites were analyzed using ID-X AcquireX background exclusion workflow. The workflow follows the acquisition logic in real time automatically without user intervention: it builds a background exclusion list using full scan HRAM data of matrix sample, updates acquisition method by inserting the background exclusion list, then the updated method conducts real-time background subtraction, only triggering MS/MS and MSn of potential metabolites.

The results show, compared with traditional data-dependent analysis, that AcquireX data acquisition workflow real-time background subtraction and method updating effectively excluded the matrix background and increased the metabolite identification by triggering MS/MS and MSn of low abundant and sometimes significant metabolites. Coupled with data processing software "Compound Discoverer 3.0" and Mass Frontier 8.0, AcquireX data acquisition workflow is well suited for metabolite profiling in drug discovery and development, both in-vitro and in-vivo.

P150 - INCREASED IDENTIFICATION OF LOW ABUNDANT METABOLITES UTILIZING AUTOMATIC BACKGROUND EXCLUSION AND SPECIFIC FILTERING FEATURES OF ORBITRAP ID-X TRIBRID MASS SPECTROMETER

Kate Comstock¹, Kai Wang², and Kevin Coe²

¹Thermofisher Scientific, USA, ²Janssen R&D, USA

Introduction: The identification of metabolic products is an essential step in the drug development process, as their potential pharmacologic activity or toxicity need to be understood. The routine analysis of drug metabolites relies on the sensitive detection of components in complex matrices. In addition, molecular formula information alone is often insufficient, as confident metabolite identification requires fragmentation data. Ideally, fragmentation data is acquired on all relevant metabolites together with full scan spectral data in a single injection.

Here we present an investigation of drug metabolites using an Orbitrap Tribrid MS with fast polarity-switching, automatic background ions subtraction workflow, isotope intensity-filtering, and other real-time decision-making features to improve the data acquisition quality by detecting and triggering MSn of low-abundant metabolites.

Methods: Diclofenac, nefazodone, troglitazone, tienilic acid and flutamide were selected as model compounds for this study. The compounds and a blank 'no drug' sample were incubated with human liver microsomes in the presence of GSH and NADPH for 0h, 0.5h or 1h.

The LCMS analyses were performed on a Vanquish Horizon Binary UPLC system with variable wavelength detector (VWD) coupled to an Orbitrap ID-X Mass Spectrometer. Chromatographic separation was achieved using a linear gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile on Hypersil GOLD column (2.1X150 mm, 1.9 μ m).

High resolution full scan and MS/MS data were collected in a traditional data-dependent analysis (DDA) with polarity switching and AcquireX data acquisition workflow.

Preliminary Data: The model compounds, which are known to undergo multiple metabolic routes including formation of reactive metabolites trapped by glutathione (GSH), were selected for a variety of reasons such as poorly-ionizable ions by ESI in positive ion mode, isotope pattern, and diverse metabolic pathways. By using the Orbitrap ID-X's dedicated small molecule data acquisition tools, such as AcquireX background exclusion workflow for MS/MS precursor selection, isotope-pattern triggered precursor selection and polarity switching, high-quality MS and MS/MS fragmentation data was acquired successfully even for low abundance and "unexpected" metabolites in a single injection without the need to create inclusion lists and reinject samples. Successful acquisition of MS/MS fragmentation data for all metabolites is important because it is crucial for confident metabolite assignment.

The generated mass spectral data was processed by Compound Discoverer 3.0 software and corresponding metabolites

were detected and annotated. In combination with in-silico fragment ion search (FISh) scoring, the in-silico generated fragmentation data could be used to facilitate localization of oxidation and other biotransformations to specific parts of the molecule particularly for different product isomers.

P151 - BIOACTIVATION OF HALOGENATED AROMATIC DRUGS INTO QUINONE METABOLITES AS PRECURSORS TO DRUG-INDUCED HEPATOTOXICITY

Mary A. Davis¹, Shraddha Thakkar², Grover P. Miller¹, and Tyler Hughes³

¹University of Arkansas for Medical Sciences, USA, ²National Center for Toxicological Research, USA, ³Washington University at St. Louis, USA

Halogens are increasingly utilized in drug development, yet their potential impacts on toxic risk through enzymatic bioactivation remain understudied. It is assumed that halogens suppress metabolism of molecules due to well-studied trends in chemical reactivity. These trends indicate halogenated drugs are less likely to undergo bioactivation into reactive metabolites like quinones and thus, pose a decreased toxicity risk. Nevertheless, the list of halogenated molecules inducing hepatotoxicity continues to grow with the introduction of new molecular entities indicating knowledge of metabolism and bioactivation for halogenated molecules is insufficient for assessing toxic risk. We hypothesize that the presence, type, and location of halogens on a drug aromatic ring impacts the chemical step toward reactive oxidative product formation (V_{max}) based on electronic effects, while enzyme specificity and affinity (K_m) determine the concentration-dependent conditions in which the reaction occurs. We are testing this hypothesis with a novel integration of bioinformatic, computational, and experimental approaches to (1) identify labeled hepatotoxic drugs and drug families as susceptible to metabolism into a reactive quinone, (2) experimentally assess metabolic bioactivation potential and (3) mine data sources such as electronic health records for evidence supporting metabolic activation of halogenated drugs into hepatotoxins. As a first step, we extracted 259 halogenated compounds from the FDA DILIrank database. Computational modeling was performed with our Xenosite deep-learning model for these compounds to identify bioactivation likelihood into quinones. Of these 259 compounds, 13 compounds were selected for further study due to their high model predictions and to their ambiguous participation in DILI according to DILIrank. Meclofenamate was the highest predicted ambiguous compound and was supplemented by structurally similar N-aryl acetic acid derivatives with varied degrees of DILI risk (diclofenac, aceclofenac, lumiracoxib, bromfenac, etc.) through DILIrank and the NIH Inxight drug database. We have developed methodologies to adduct quinone metabolites using dansyl glutathione and have established a sensitive HPLC-UV/Vis method to fluorescently detect these metabolites with isomeric specificity for the N-aryl acetic acid drug family. Future work will establish trends across drug classes that link metabolism and quinone formation, ascertain reaction kinetics, and identify responsible cytochromes P450 to understand the role of halogens in metabolic clearance and bioactivation contributing to drug-induced liver injury (DILI). These findings will provide critical insights on the impact of halogenation to quinone formation as a precursor to DILI and thus, provide a foundation for better risk assessment in drug discovery and development.

P152 - ESTABLISHMENT OF HEPATOCYTE MODEL FOR POOR METABOLIZER USING GENETICALLY MANIPULATED HUMAN IPS CELLS

Sayaka Deguchi, Tomoki Yamashita, Keisuke Igai, Kazuo Takayama, and Hiroyuki Mizuguchi
Graduate School and School of Pharmaceutical Sciences, Osaka University, Japan

Cytochrome P450 family 2 subfamily C member 19 (CYP2C19), in liver, plays important roles in terms of drug metabolism. It is known that CYP2C19 poor metabolizers (PMs) lack CYP2C19 metabolic capacity. Thus, unexpected drug-induced liver injury or decrease of drug efficacy would be caused in CYP2C19 substrate-treated CYP2C19 PMs. However, it is difficult to evaluate the safety and effectiveness of drugs and candidate compounds for CYP2C19 PMs because there is currently no model for this phenotype. Here, using human iPS cells and our highly efficient genome editing and hepatocyte differentiation technologies, we generated CYP2C19-knockout human iPS cell-derived hepatocyte-like cells (CYP2C19-KO HLCs) as a novel CYP2C19 PM model for drug development and research. The gene expression levels of hepatocyte markers were similar between WT HLCs and CYP2C19-KO HLCs, suggesting that CYP2C19 deficiency did not affect the hepatic differentiation potency. We also examined CYP2C19 metabolic activity by measuring S-mephenytoin metabolites using LC-MS. The CYP2C19 metabolic activity was almost eliminated by CYP2C19 knockout. Additionally, we evaluated whether clopidogrel (CYP2C19 substrate)-induced liver toxicity could be predicted using our model. Unexpectedly, there was no significant difference in cell viability between clopidogrel-treated WT HLCs and CYP2C19-KO HLCs. However, the cell viability in clopidogrel- and ketoconazole (CYP3A4 inhibitor)-treated CYP2C19-KO HLCs was significantly enhanced as compared with that in clopidogrel- and DMSO-treated CYP2C19-KO HLCs. This result suggests that CYP2C19-KO HLCs can predict the clopidogrel-induced liver toxicity. We succeeded in generating CYP2C19 PM model cells using human iPS cells and genome editing technologies for pharmaceutical research.

P153 - THE NATURAL PRODUCT 2-METHOXY TRANS-CINNAMALDEHYDE IS A TIME DEPENDENT INHIBITOR OF THE NICOTINE AND LETROZOLE METABOLIZING ENZYME CYTOCHROME P450 2A6

Michael Espiritu, Michael Larkin, Heather True, Jeannine Chan, Brendan Stamper, and John Harrelson
Pacific University, USA

Previously we have shown that the dietary phenylpropanoid trans-cinnamaldehyde (CA) is a metabolism dependent inhibitor of the nicotine metabolizing enzyme cytochrome P450 2A6 (CYP2A6) and that the route of inhibition is through abstraction of a formyl hydrogen radical, which contributes to destruction of the heme. This process leads to substantial inhibitory effects on nicotine metabolism *in vitro*, indicating promise to serve as a lead compound for treating nicotine addiction. From this data we hypothesized that natural product analogs of CA might have a similar effect on CYP2A6. Here we investigate another component of cinnamon oil, 2-methoxycinnamaldehyde (2MCA). We explored 2MCA and its effect on CYP2A6 by elucidating the possible mechanisms of inhibition, binding affinity, hepatocyte toxicity, and interactions with both nicotine and letrozole *in vitro*. Time dependent inhibition (TDI) of CYP2A6 by 2-MCA was initially measured using nicotine as a substrate in human liver microsomes (HLM) with cytosol. Inhibition was also determined in HLM using letrozole as a substrate since CYP2A6-mediated metabolism is the primary route of clearance for this aromatase inhibitor, which is used to treat breast cancer. Spectral binding curves were generated using recombinant CYP2A6 to better understand the binding of 2-MCA to CYP2A6. To evaluate the mechanism of inactivation, heme loss was measured by LC-MS/MS in comparison to controls without inhibitor, using recombinant CYP2A6 and rat P450 reductase. 2-MCA hepatocellular toxicity was evaluated in TAMH cells (transforming growth factor- α transgenic mouse hepatocytes) and a fluorescent resazurin assay. 2-MCA inhibited nicotine metabolism in a time dependent manner with an inactivation rate of 0.1002 min^{-1} ($\pm 0.0080 \text{ min}^{-1} \text{ SE}$), considerably higher than the rate measured previously for CA. Letrozole metabolism was markedly inhibited (activity loss = $78\% \pm 7\% \text{ SD}$) after a 9 minute preincubation with 2-MCA ($80 \mu\text{M}$), compared to a NADPH-only control. Spectral binding revealed type I binding with a spectral disassociation constant of $1.57 \mu\text{M}$ ($0.053 \mu\text{M SE}$). Heme loss (46% decrease in comparison to negative controls) was observed with $80 \mu\text{M}$ 2-MCA after a 5 minute incubation, indicating heme destruction is at least partially responsible for CYP2A6 inactivation. The LC50 for 2-MCA was $152 (\pm 0.017 \mu\text{M SD}) \mu\text{M}$, which was similar to TCA ($p = 0.495$ using a t-test). This work furthers our previous studies on TCA by highlighting a second natural product from cinnamon oil that inactivates nicotine metabolism, thus providing a potent alternative to TCA, which could serve as a smoking cessation agent. In addition, we have described a second natural product within cinnamon that interacts with letrozole, providing further evidence that high cinnamon exposure could contribute to an herb-drug interaction and variability in letrozole plasma concentrations.

P154 - ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF THE NOVEL ANTIEPILEPTIC DRUG CENOBAMATE: MASS BALANCE FOLLOWING ORAL ADMINISTRATION IN HEALTHY MALE SUBJECTS

Kelli J. Glenn, Stephen Greene, Hong-Wook Kim, Susan M. Melnick, and Laurent Vernillet
SK Life Science, Inc., USA

Background: Cenobamate (YKP3089; C₁₀H₁₀O₂) is a novel antiepileptic drug in development for treatment of partial-onset seizures. Cenobamate has a potentially unique dual mechanism of action, in that it may enhance fast and slow inactivation of sodium channels, with preferential inhibition of the persistent component, and may be a positive allosteric modulator of gamma-aminobutyric acid (GABA-A) receptors. Examination of absorption, distribution, metabolism, and elimination of new drugs is critical to determine the risk-benefit profile of the parent drug and its metabolites.

Design/Methods: This single-center, phase 1, open-label, study in 6 healthy Caucasian male subjects (mean age [range], 23 [20-27] years) was conducted to assess the mass balance of cenobamate following a single oral dose of ¹⁴C-labeled cenobamate (50 μCi ; 400 mg). Primary objective was determination of percent radioactivity excreted in urine and feces. Secondary objectives included determining pharmacokinetic parameters from total radioactivity and cenobamate in plasma, identification of cenobamate metabolites in plasma, urine, and feces, and exploring whether cenobamate partitions into red blood cells (RBCs). Blood, urine, and fecal samples were collected predose and from 0 to 312 hours postdose. HPLC with mass spectrometry, radio flow-through detection, and scintillation counting were used for radio-quantification.

Results: Throughout the study, mean total radioactivity concentration equivalents in plasma were greater than in whole blood (WB), with peak values of 11.6 and 7.0 $\mu\text{g/mL}$ in plasma and WB at 1.5 hours postdose. In plasma, mean cenobamate radioactivity concentration equivalents were similar to total radioactivity until both reached peak concentration (11.9 vs. 11.6 $\mu\text{g/mL}$) between 1.25-1.5 hours postdose. The extent and rate of exposure to total radioactivity in plasma and WB were both ~66% greater in plasma versus WB. Urinary excretion was the primary route of elimination, with 88% of administered radioactive dose recovered in urine and 5.2% in feces. The major radioactive component in plasma was cenobamate (>98% of total radioactivity AUC); the only metabolite detected in plasma was M1, a direct N-glucuronide metabolite (<2% of total radioactivity AUC). Metabolite analysis revealed cenobamate and 8 metabolites (M1, M2a, M2b, M3, M5, M6, M7, M11) in urine, with M1 as the major radioactive component (mean cumulative recovery=37.7%) followed by M2b and M7 (means, 17.5% and 7.9%). Cumulative recovery of cenobamate in

urine was 6.4%, and <5% for all other metabolites. Mean total cumulative recovery in feces was 3.5%, with M1 as the major radioactive component (1.8% recovery) and 0.5% cenobamate recovered. Blood/plasma ratios for both AUC_{0-∞} and C_{max} were ~60% and mean erythrocyte transfer ratio and erythrocyte/plasma partition coefficients were <15% throughout the study, both of which indicate that cenobamate is mainly confined to plasma with limited penetration of cenobamate and its metabolites into RBCs.

Conclusions: Oral radiolabeled cenobamate was absorbed quickly and almost completely. Most of the cumulative dose of total radioactivity was recovered in combined urine and feces. Unchanged cenobamate was the primary component circulating in plasma. Cenobamate and its metabolites were almost exclusively excreted in urine and had limited penetration into RBCs. Sponsored by SK Life Science, Inc.

P155 - EARLY LIFE EXPOSURE TO ENVIRONMENTAL CONTAMINANTS (BDE-47, TBBPA, AND BPS) PRODUCED PERSISTENT GUT DYSBIOSIS IN ADULT MALE MICE

Matthew Gomez¹, Julia Yue Cui¹, Alexander Suvorov², Haiwei Gu³, and Xiaojian Shi³

¹University of Washington, USA, ²University of Massachusetts Amherst, USA, ³Arizona State University, USA

Gut microbiome is increasingly recognized as a pivotal player in toxicological responses, and dysbiosis may worsen chemical-induced adverse outcomes such as inflammation, metabolic syndrome, and cancer. Early life exposure to environmental contaminants may produce long-lasting toxicities in adulthood and little is known to what extent early life exposure to environmental toxicants modulate the gut microbiome beyond childhood. Therefore, this study tested the effect of perinatal exposure to 3 human health relevant environmental contaminants (BDE-47, TBBPA, and BPS), on the composition and functions of the gut microbiome of perinatally exposed adult male mice. CD-1 mouse dams were orally exposed to vehicle (corn oil, 10ml/kg), BDE-47 (0.2ml/kg), TBBPA (0.2ml/kg), or BPS (0.2ml/kg) once daily from gestational day 8 to the end of lactation (postnatal day 21). Feces from male pups were collected at 12-weeks of age (n=14-23/group). Microbial DNA was isolated, subjected to 16S rDNA sequencing, and analyzed using QIIME. Microbial biomarkers for each chemical exposure were predicted using LefSe. Microbial functions as well as key taxa that drive the functional changes were predicted using PICRUST and FishTaco, respectively. To validate the functional shifts in gut microbiome, human health relevant microbial-derived short chain fatty acids (SCFAs) in fecal samples were analyzed using gas chromatography-mass spectrometry (GC-MS). None of the 3 chemicals markedly altered the overall richness of the gut microbiome in adult male pups. However, principle coordinate analysis showed a distinct separation among different exposure groups, and especially between BPS and vehicle exposure groups. The BDE-47, TBBPA exposure groups overlapped slightly, but both remained separated from the vehicle cluster. A total of 73 taxa were persistently altered by at least 1 chemical exposure, among which 12 taxa were commonly regulated by all 3 chemicals. Most notably, there was a persistent decrease in many taxa of the Clostridia class in the Firmicutes phylum by early life exposure to all 3 chemicals, whereas many taxa in the Bacteroidia class of the Bacteroidetes phylum were persistent up-regulated. The most representative microbial biomarkers for each exposure condition were Clostridiales for vehicle, S24-7 for BDE-47, Rikenellaceae for TBBPA, and Lactobacillus for BPS. Regarding SCFAs, BPS downregulated acetic acid, whereas TBBPA upregulated propionic acid and succinate. Together these observations suggest that early life exposure to these human health relevant environmental contaminants produce persistent gut dysbiosis in adult male offspring, leading to functional shifts that may play important roles in regulating certain diseases of the host.

P156 - IN VITRO METABOLISM OF BARDOXOLONE, AN ACTIVATOR OF NRF2 VIA REVERSIBLE COVALENT MODIFICATION OF KEAP1: FORMATION OF BARDOXOLONE EPOXIDE AND NOVEL METABOLIC PATHWAY FOR OXIDATIVE DENITRILATION VIA REDUCTIVE ADDITION OF GLUTATHIONE

Amin Kamel

Takeda, CA, USA

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a master transcription factor that generates the cell's primary antioxidant and cytoprotection defense mechanisms. Under normal conditions, the cytoskeletal protein, Kelch-like ECH-associated protein 1 (Keap-1), works as the repressor of Nrf2 by binding to the N-terminal Neh2 domain of Nrf2. Bardoxolone methyl (BARD-Me) is alpha-cyanoenone derivative of oleanolic acid that activates Nrf2 by reversible covalent modification of Cys151 of Keap1.

BARD-Me undergoes glutathione (GSH) adduct formation via direct nucleophilic attack at the beta-carbon of the alpha,beta-unsaturated carbonyl fragment. The addition of the highly electron withdrawing nitrile group to the alpha-carbon render the alpha,beta-unsaturated ketone more susceptible to nucleophilic attack by GSH and can effectively stabilize a negative charge converting BARD-Me to reversible covalent inhibitor without the potential liabilities of irreversible covalent adduct formation.

The metabolic fate of BARD-Me was studied in different *in vitro* systems and the potential metabolic formation of its epoxide metabolite was assessed. Results showed that BARD-Me undergoes epoxide formation and can potentially react with thiols irreversibly. The epoxide of BARD-Me was therefore chemically synthesized and its metabolic fate was studied *in vitro*. Structure elucidation of metabolites was carried out on an AB Sciex TripleTOF 5600 LC/MS/MS system using

positive ESI mode and full scan TOF-MS with Mass Defect Filter triggered MS/MS acquisition. In addition to other metabolic pathways, BARD-Me epoxide undergoes novel metabolic pathway; epoxide reduction and oxidative denitration. This novel metabolic pathway proceeds via nucleophilic attack of the thiol group of GSH at each of the two C-atoms of the epoxide as evident by the formation of two regioisomers of the hydroxyl metabolite (beta-hydroxy sulfide). BARD-Me bears no H-atom at the alpha-carbon of its alpha,beta-unsaturated carbonyl fragment and therefore cannot undergo oxidative denitration without the formation of the epoxide metabolite. Oxidative denitration proceeds via nucleophilic attack of the thiol group of GSH at C-atom of the epoxide to the cyano group to form a cyanohydrin metabolite which spontaneously decomposes to release HCN and the corresponding carbonyl metabolite. Further experiments to assess the metabolic fate of BARD-Me epoxide are ongoing.

P157 - ASSESSMENT OF DE-FLUORINATION OF [18F]MEFWAY IN LIVER FRACTIONS

Byoung Soo Kim, Jae Yong Choi, Jae Eun Ju, and Tae Hyun Choi
Korea Institute of Radiological and Medical Sciences, South Korea

Purpose: [18F]Mefway has been developed for imaging the serotonin 1A receptors in the brain. De-fluorination of F-18 labeled PET probes *in vivo* obstructs the exact quantification of receptor density due to a spillover effect, in which radioactivity in the skull can be also detected in brain regions nearby. This study was performed to measure the degree of de-fluorination of [18F]mefway in rat and human liver fractions.

Methods: To distinguish the radioactivity of [18F]mefway from that of free [18F]fluoride, solid phase extraction method was applied using anion-exchange cartridges (Sep-Pak Light QMA carbonate, Waters). The cartridges were activated prior to use by passing with 1 mL of ethanol followed by 1 mL of distilled water. The radioactivity recovery efficiency of the cartridge was evaluated with [18F]mefway and [18F]fluoride before the incubation. *In vitro* metabolism studies were performed using rat liver microsome and S9 fraction, and human liver microsome and S9 fraction. [18F]Mefway (60 MBq/mL, 2.5 μ L) was added to the incubation mixtures. The microsomal incubation mixture consists of 0.1 M KH₂PO₄ (pH 7.4, 147.5 μ L), 0.1 M MgCl₂ (25 μ L), and 50 μ L of 5 mg/mL rat or human liver microsome. And the S9 incubation mixture consists of 0.1 M KH₂PO₄ (pH 7.4, 147.5 μ L) and 50 μ L of 10 mg/mL rat or human liver S9 fraction. After pre-incubation of the mixture for 3 min in a shaking water bath at 37°C with 40 rpm agitation, the reaction was initiated by adding 25 μ L of 10 mM NADPH or 50 μ L of Phase II cofactors. A portion (20 μ L) was withdrawn prior to and 5, 10, 30 min after incubation and mixed with acetonitrile (60 μ L). The supernatant (20 μ L) was loaded onto an activated cartridge. The cartridge was eluted with 5 mL of 20% acetonitrile. The radioactivity in cartridge and eluent was separately counted on a scintillation counter. The de-fluorination ratio was estimated considering the radioactivity recovery efficiency of the cartridges.

Results: The radioactivity recovery efficiency of the anion-exchange cartridges for the separation of [18F]mefway from that of free [18F]fluoride was over 97% in all liver fractions. The mean de-fluorination ratios at 0 and 30 min after incubation in rat liver microsome were -0.1 and 25.9%, and the ratios after incubation in human liver microsome were 14.2 and 39.9%. The mean de-fluorination ratios at 0 and 30 min after incubation in rat liver S9 fraction were 0.5 and 1.5%, and the ratios after incubation in human liver S9 fraction were 13.6 and 64.7%.

Conclusion: De-fluorination assay method was well established using an anion-exchange cartridge. [18F]Mefway was more stable in rat liver fractions than in human against de-fluorination *in vitro*. Interestingly, free [18F]fluoride departed from [18F]mefway by pre-incubation without cofactors in human liver fractions. It is thought that other enzymes are involved in de-fluorination aside CYPs and Phase II enzymes. Identification of major enzymes for de-fluorination of [18F]mefway was in progress.

P158 - QUALITATIVE METABOLITE PROFILING OF DRUG CANDIDATES IN HUMAN LIVER MICROTISSUE SYSTEMS IN SUPPORT OF DRUG DISCOVERY PROJECTS

Lloyd King, Emre Isin, and Reiner Class
UCB, Belgium

Use of 3D cell culture systems, such as human liver microtissues, for use in cell toxicity assessments and/or metabolite profiling of new chemical entities (NCEs), has been gaining impetus in the recent years (1,2). This is due to an increasing recognition of these systems as a more physiologically relevant and robust platform (3,4) compared to the more common 2D systems, such as plated or suspended cell formats. The ability of these 3D cell cultures to remain both viable, phenotypically relevant and non-differentiated (5) over an extended period of time has resulted in their use as an integral part of hepatotoxicity assessment strategies by pharmaceutical companies. However, from a biotransformation perspective, we are interested in whether the human liver microtissue systems, with their potential to provide metabolite profile data as a result of chronic exposure to NCEs, can be used to improve our ability to identify potential unique human metabolites *in vitro* and thereby provide valuable predictability of finding these metabolites in human systemic circulation. This is of interest considering the drawback of the current 2D systems that have been shown to fail to generate human specific metabolites which require multi-step biotransformation pathways and/or longer exposure in metabolising systems(6). To exemplify the value of utilising 3D cell systems in drug discovery, metabolite profiling data obtained from

incubations of commercially available compounds, such as Rosiglitazone, Clozapine & Rimonobant, in 3D human liver microtissue assays will be shown. To date, the data obtained through LC-HRMS analysis of the supernatants alone, from single spheroid incubations (comprising ~ 1500 cells), across a range of substrate concentrations, over a period of 7 days, have shown that they are capable of generating some, though not always all human relevant metabolites. The results of these ongoing studies are highlighting the improvements in the tools available to generate clinically relevant metabolite profiles earlier in the drug discovery process than has been achieved previously.

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P159 - A NOVEL HYDROLYTIC ACTIVATION PATHWAY FOR AN ESTER-TYPE PRODRUG IN HUMAN PLASMA: CONTRIBUTION OF α 1-ACID GLYCOPROTEIN VARIANT

Kenta Kono¹, Yukina Fukuchi¹, Rui Fujimura², Yuka Nakamura³, Kenichi Nunoya¹, Haruo Imawaka¹, Hiroshi Watanabe², and Toru Maruyama²

¹ONO Pharmaceutical Co., Ltd, Japan, ²Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan,

³School of Pharmacy, Kumamoto University, Japan

Background and Objectives: ONO-2160 is a newly developed ester-type prodrug of levodopa and is efficiently hydrolyzed in human plasma. We previously elucidated the novel hydrolytic activation process for ONO-2160 in plasma in which α 1-acid glycoprotein (AGP), one of the major drug binding proteins in plasma, was responsible for this hydrolysis via the formation of an intermediate metabolite by relay-type role sharing with human serum albumin (HSA). Although AGP exists as a mixture of F1*S and A variants in human plasma, it is not clarified whether AGP variant is involved in the hydrolytic activation for ONO-2160 and where this hydrolytic reaction takes place. Therefore, we evaluated the difference in the hydrolyzing activity on ONO-2160 between F1*S and A variants to elucidate the mechanism of the esterase-like activity of AGP.

Methods: ONO-2160 was incubated with human plasma, HSA, AGP, F1*S variant, or A variant at 37°C to evaluate the hydrolyzing activity on ONO-2160 in human plasma. ONO-2160 was also incubated with selective ligands of F1*S variant or A variant, or non-selective ligands in AGP solution to evaluate the ligand inhibitory potency on the metabolism.

Results and Discussion: The Km value for ONO-2160 hydrolysis by AGP was smaller than that by HSA, and the intrinsic clearance per mL of plasma (CL_{int,p}) of ONO-2160 in AGP solution was about 3-fold that in HSA solution and was almost equivalent to that in human plasma. Accordingly, we concluded that ONO-2160 was mainly hydrolyzed by AGP in human plasma. The CL_{int,p} in F1*S variant solution was 50-fold that in A variant solution, and F1*S variant exhibited similar CL_{int,p} and Km value to those of AGP. Moreover, the hydrolysis of ONO-2160 in AGP solution was inhibited by the ligands that bind to F1*S variant, and the ligand inhibitory potencies correlated with ligand binding association constants to F1*S variant. These findings suggest that among AGP variants, F1*S variant is predominantly contributing to the hydrolytic activation of ONO-2160 in human plasma, and this hydrolytic reaction takes place in the ligand binding site of F1*S variant.

Conclusion: ONO-2160 is mainly hydrolyzed by AGP in human plasma, and F1*S variant is responsible for the esterase-like activity of AGP, not A variant. To our knowledge, this is the first report that AGP has esterase-like activity. These findings would be useful for drug development of the ester-type prodrugs.

P160 - IN VITRO METABOLISM OF ENDOSULFAN SULFATE IN HUMAN LIVER MICROSOMES, S9 FRACTIONS AND HEPATOCYTESHwa-Kyung Lee¹, **Jeong-Han Kim**¹, Tae Yeon Kong², Won-Gu Choi², Ju-Hyun Kim³, and Hye Suk Lee²¹Seoul National University, South Korea, ²The Catholic University of Korea, South Korea, ³Yeungnam University, South Korea

In general, xenobiotics undergo extensive metabolic transformations in living organisms through various metabolic reactions [1]. In this study, we investigated the metabolism of endosulfan sulfate in human liver preparations by non-targeted metabolite profiling method using a liquid chromatography-high resolution mass spectrometer (LC-HRMS). Endosulfan sulfate is a major oxidized metabolite of endosulfan. Endosulfan is a broad-spectrum chlorinated cyclodiene insecticide and is rapidly metabolized by oxidation or hydrolysis products to endosulfan sulfate, endosulfan alcohol, endosulfan ether, endosulfan lactone and endosulfan hydroxyether [2]. In the present study, we revisited endosulfan sulfate metabolism in human liver preparations and identified several novel metabolites. In human liver microsomes, S9 fractions and hepatocytes, endosulfan sulfate was metabolized to endosulfan diol monosulfate and unknown metabolite M1 as phase I metabolites. Endosulfan diol monosulfate is a known metabolite of endosulfan sulfate in microbial metabolism studies [3], and M1 was predicted as (1,4,5,6,7,7-hexachloro-3-formylbicyclo[2.2.1]hept-5-en-2-yl)methyl hydrogen sulfate by identification of the structure using HRMS spectrum. In addition, endosulfan sulfate-glutathione (GSH) conjugate was identified as phase II metabolite in all three human liver preparations. In human liver S9 fractions and hepatocyte, different form of endosulfan sulfate-GSH conjugate was also detected. GSH bound to endosulfan sulfate was not identified as tripeptide form but was identified as dipeptide form of cysteine and glycine. M1-GSH conjugate was detected only in human hepatocyte incubation, and the GSH form of that conjugate was identified as mono-peptide form of cysteine. Therefore, in human liver S9 fractions or hepatocyte, it is presumed that the metabolic reactions of GSH conjugates have further progressed than human liver microsomes. There were no other phase II metabolites detected in the three human liver preparations. As a result of metabolic stability test over time in human hepatocytes, endosulfan sulfate was maintained over 90% level for up to 2 hours, so it was considered to be relatively stable.

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P161 - QUANTITATIVE METABOLIC PROFILING OF SLOWLY CLEARED CHEMICALS IN IN VITRO MODELS INCLUDING 3D LIVER SPHEROID AND HEPATOPAC AND HEPATIC CLEARANCE PREDICTIONS FROM IN VITRO DATA USING GASTROPLUS PBPK MODELLING FOLLOWING TOPICAL APPLICATION**Mi-Young Lee**¹, Sue Martin¹, Juliette Pickles¹, Annabel Rigarsford¹, Ian Sorrell¹, Krisztina Herédi-Szabó², Bernadett Kalapos-Kovács², and Richard Cubberley¹¹Unilever R&D, United Kingdom, ²Solvo Biotechnology, Hungary

The transition to a pathway-based approach for risk assessment requires better understanding of exposure science. This includes an understanding of the bioavailability of chemicals in the human body and definition of toxicologically-relevant approaches that permit the estimation of internal doses in humans. Physiologically-based biokinetic (PBBK) modelling is recognised as a useful tool to achieve this, simulating whole-body toxicokinetic profiles by integrating chemical-independent (physiological) and chemical-dependent (absorption, deposition, metabolism and excretion, (ADME)) parameters. We present an evaluation of how well current non-animal *in vitro* methods can accurately predict human biokinetics of topically applied compounds. Hepatic metabolism data was generated in different *in vitro* model systems using S9, microsomal fractions and primary human hepatocytes (suspensions, 2D plated culture, relay method, liver spheroid, and micropatterned co-cultured systems) with the aim to determine the most appropriate system in which to study low clearance compounds.

Early prediction of clearance in humans is often challenging, in particular for low-clearance compounds. Widely used microsomes or hepatocytes suspensions only allow short term metabolism studies due to a rapid decline in the activity of drug metabolizing enzymes in these model systems over time (i.e. in the hepatocyte assay enzyme activity declines within 4 hours).

Recent development of 3D cell culture models as well as the micropatterned co-culture model (MPCC, HepatoPac) allow their use in a wide range of biological applications and may potentially provide a useful tool for improving intrinsic

clearance predictions of slow clearance chemicals. The aim of this work was to assess the potential application of 3D cellular models (such as InSphero's primary liver microtissue and the Ascendances's HepatoPac model) for prediction of CL_{int} for low clearance compounds in longer duration incubation conditions, in comparison to other *in vitro* systems. We have tested a range of substrates including CYP probe drugs, such as diclofenac and tolbutamide and measured substrate depletion and metabolite profiles of each by LC-MS/MS. Rates of metabolism have been compared with those from other assays (e.g. hepatocyte suspension, 2D culture). Predictions of plasma profiles following topical application were generated using Gastroplus 9.0 PBPK software for each test chemical. The predicted plasma profiles predicted from both microtissue and HepatoPac correlated well with published clinical studies.

The combination of *in vitro* data generation and *in silico* PBPK modelling showed good accuracy for predicting human plasma maximum concentration and AUC values within an order of magnitude for all slowly cleared compounds. The micropatterned hepatocyte model showed promise for measuring the clearance of chemicals that are metabolised too slowly to be detected using a suspension assay.

P162 - FURTHER CHARACTERIZATION OF HEPATOPAC – NON-TARGETED METABOLITE-PROFILING OF 7 CHEMICALS INCLUDING SLOW CLEARANCE CHEMICALS AND METABOLISM-MEDIATED TOXICITY.

Mi-Young Lee¹, Richard Cubberley¹, Zsuzsanna Gáborik², Bálint Molnár², Krisztina Herédi-Szabó², and Bernadett Kalapos-Kovács²

¹Unilever R&D, United Kingdom, ²Solvo Biotechnology, Hungary

Long-term *in vitro* liver models have been widely explored for human hepatic metabolic clearance prediction and comparison of slow clearance chemicals. Recently, several peer-reviewed journals have reported the superiority of the HepatoPac® system compared to other models- HepG2, HepaRG™, iPS, PHH in suspension. HepatoPac® is micropatterned co-cultured hepatocytes with 3T3 stromal cells, which has been engineered to possess extended cell viability (typically up to 6 weeks) with retention of *in vivo*-like hepatocyte functions in culture (e.g. albumin secretion, urea synthesis, drug metabolizing activities, CYP induction, transporter activities).

To determine intrinsic clearance of slow clearance compounds, HepatoPac® allows metabolic stability assay of test chemicals over 7 or more days without changing the media. However, there are remaining questions: potential background noise from 3T3 stromal cell and dose responses of HepatoPac in long term culture.

Here we report the result of non-targeted metabolite identifications of 7 chemicals (diclofenac, imipramine, dextromethorphan, nicotine, salicylic acid, tolbutamide and R/S-warfarin) incubated in HepatoPac® culture from a single donor over 7 days. In addition, 8 concentrations (Minimum 0.14 microM, maximum 15000 microM) of 7 test chemicals (including amiodarone as a control) were incubated in HepatoPac® over 1, 5 and 7 days without changing the media. The assay was repeated with two donors. Hepatic function (urea, albumin) and cell toxicity (ATP and GSH) were monitored. In brief, the results demonstrate that prominent glucuronidation activity in HepatoPac® (i.e. salicylic acid metabolism) was observed over time, yet neglectable levels were measured in 3T3 stromal cells. Dose-response data from one of the single donor, showed metabolism-driven toxicity and metabolism driven detoxification in HepatoPac® in two test chemicals.

In summary, HepatoPac® is robust *in vitro* model to predict clearance rate with confidence for slow clearance chemicals and a potentially useful model to provide information on metabolism-driven effects (such as toxicity).

P163 - DETERMINATION OF INTRINSIC CLEARANCE OF LOW TURNOVER DRUGS USING HuREL HEPATOCYTE CO-CULTURES

Anantha Ram Nookala, Michael Fitzsimmons, Daniel Albaugh, Donald L. McKenzie, and **Gang Luo**
Covance Laboratories Inc., USA

Low turnover drugs offer the advantages of lower doses and dosing frequencies and thereby improve patient compliance. Estimating *in vitro* intrinsic clearance of low turnover drugs using traditional hepatocyte suspensions, however, has been challenging due to limited duration of metabolic activities. In addition, identifying metabolites from *in vivo* matrix may be challenging because of low quantities of metabolites formed. HuRel hepatic co-culture of hepatocytes and stromal cells provides a suitable test system that is able to quantitatively predict the *in vivo* clearance from *in vitro* experiments. In this present study, four low turnover drugs (warfarin, meloxicam, tolbutamide and verapamil) were incubated at 1 µM with HuRel hepatic co-cultures prepared from rat, dog, monkey and human. Samples were collected after incubation for 0, 24, 72, and 168 hours and analyzed by liquid chromatography coupled with a quadrupole tandem mass spectrometer with electrospray ionization. The *in vitro* hepatic intrinsic clearance (CL_{int}) was calculated from drug metabolism half life and scaling factors including hepatocyte density in co-culture, hepatocyte yield, and liver to body ratio. The CL_{int} values obtained in this study were 2.54, 4.39, 7.94, and 42.1 mL/min/kg for warfarin, meloxicam, tolbutamide and verapamil, respectively. The values reported by others using the same model were 2.53, 1.79, 4.84, and 33.9 mL/min/kg for warfarin, meloxicam, tolbutamide and verapamil, respectively. In addition, *in vivo* hepatic clearance (CL_{hep}) was estimated from CL_{int} corrected with hepatic blood flow and free fraction in plasma. The values obtained in the present study were 0.0339, 0.0217, 0.287, and 1.39 mL/min/kg for warfarin, meloxicam, tolbutamide and verapamil, respectively. The *in vivo* CL

values reported in literature were 0.045, 0.15, 0.17, and 15 mL/min/kg for warfarin, meloxicam, tolbutamide and verapamil, respectively. Metabolites generated in the system were also characterized. The unique human metabolites 9R,11S-warfarin alcohol and 9S,11S-warfarin alcohol were identified for S-warfarin. These results indicate that H9rel hepatic co-culture is able to serve as a robust model for predicting CL_{int} of low turnover drug candidates with greater chances of identifying metabolites formed from longer incubation times.

P164 - HUMAN METABOLISM OF [14C]-BENZO[A]PYRENE AFTER ORAL MICRO-DOSING AS DETERMINED BY UPLC-ACCELERATOR MASS SPECTROMETRY

Monica Maier¹, Lisbeth Siddens¹, Sandra Uesugi¹, Ted Ognibene², Kenneth Turteltaub², and David Williams¹

¹Oregon State University, USA, ²Lawrence Livermore National Laboratory, USA

Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon classified as a class 1 human carcinogen. Following metabolic activation by cytochrome P450 (CYP) and epoxide hydrolase BaP can be transformed to the 7,8-dihydrodiol-9,10-epoxide (BaPDE), a mutagen and carcinogen. The risks associated with BaP exposure relates to the extent and profile of metabolism and the yield of BaPDE compared to other metabolites with minimal toxicity. Little or no information is available on the *in vivo* metabolism of BaP following a defined, environmentally relevant, dose. To determine if BaP underwent significant metabolism in humans an oral micro-dose of 46 ng (5 nCi) of [14C]-BaP was administered and metabolite profiles in plasma extracts over the following 72 hours were determined by UPLC-accelerator mass spectrometry (250 kV). We hypothesized that the parent BaP at this dose would be not be rapidly metabolized and would be detectable at the 1 hour time point. Somewhat surprisingly, we found very little parent compound in plasma even at the earliest time points. This is consistent with extensive hepatic and/or intestinal first-pass metabolism, though the observed metabolite profiles varied between the participants examined. At this time BaP metabolites could not be specifically identified although the elution profile suggest that the compounds are primarily tetrols, diols, and quinones. An ongoing dose-response study will add speciation to the metabolic profiles. This study is the first to directly assess BaP metabolism in humans at environmentally relevant levels of exposure, however it is limited by a small sample population and a single dose paradigm which is inconstant with likely human exposure. This study was supported by PHS grants P42ES016465, K.C. Donnelly Supplement P42ES016465, R01ES028600 and T32ES07060 and NIH grant P41 GM103483.

P165 - IMPROVED ON-LINE ACCELERATOR MASS SPECTROMETRY TECHNIQUES FOR THE ANALYSIS OF ENVIRONMENTAL CHEMICALS AT ULTRA-LOW EXPOSURE LEVELS

Michael Malfatti¹, Ted Ognibene¹, Erin Madeen², Benjamin Stewart¹, David Williams², Graham Bench¹, and Kenneth Turteltaub¹

¹Lawrence Livermore National Laboratory, USA, ²Oregon State University, USA

Accelerator mass spectrometry (AMS) is the method of choice for quantitation of small amounts of carbon-14 labeled biomolecules and metabolites and has been used for decades to measure exposures to biologically-relevant quantities of environmental chemicals. Despite exquisite sensitivity, important limitations of AMS are its low throughput and inability to provide structural information about the analyte. Here we describe a novel user interface that allows for the on-line AMS detection of analytes using a liquid-sample moving wire and UPLC separation, together with a new coupled analysis method, Parallel Accelerator and-Molecular Mass Spectrometry (PAMMS), that provides simultaneous AMS and MS analysis of analytes. These new techniques greatly improves sensitivity and throughput, as well as, provide structural information of analytes. Here we present on-line AMS analysis of the toxic polyaromatic hydrocarbon (PAH) dibenzo[def,p]chrysene, which has allowed for the first *in vivo* data set describing pharmacokinetics in humans of a high molecular weight PAH. Previously, human *in vivo* metabolism studies of potentially toxic PAHs were not possible due to health risks from the dosage concentrations necessary for detection by traditional instruments. Additionally, preliminary proof-of-principle experiments demonstrating the utility of PAMMS for the measurement of small molecules (amino acids) are presented. Applications of PAMMS and the on-line moving wire interface include drug and toxicant metabolism and pharmacokinetics, toxicant exposure analysis, and metabolic pathway analysis experiments.

This work was performed at the Research Resource for Biomedical AMS which is operated at LLNL under the auspices of the U.S. Department of Energy under contract DE-AC52-07NA27344 and National Institute of General Medical Sciences, Biomedical Technology Research Resources under grant number P41GM103483. Portions of this work was funded by PHS grants P42ES016465, K. C. Donnelly Supplement P42ES016465-S1, P41GM103483, P01CA90890, and T32ES07060 (to E.P.M.).

P166 - THE SUDOXICAM FAMILY: IDENTIFYING HOW THIAZOLE STRUCTURE DETERMINES BIOACTIVATION RELEVANCEDustyne Barnette¹, Mary A. Davis¹, Michael Ward², S. Joshua Swamidass², and **Grover P. Miller¹**¹University of Arkansas for Medical Sciences, USA, ²Washington University - St Louis, USA

Biologically active thiazoles are common to natural products and drugs earning the label of “privileged scaffold”; however, the motif can undergo bioactivation and then elicit hepatotoxicity such that thiazoles also classify as structural alerts. In the latter case, epoxidation of the 4,5 carbon-carbon double bond leads ultimately to a reactive thioamide that forms potentially toxic protein adducts. The role of molecular structure in determining whether thiazoles undergo harmful bioactivation or harmless elimination remain unknown. We hypothesize that thiazole bioactivation depends solely on reactivity of the thiazole ring, yet its relevance depends on competing non-bioactivation pathways leading to detoxification. We are testing this hypothesis by assessing the bioactivation and overall metabolism of a family of sudoxicam molecules. Sudoxicam is a nonsteroidal anti-inflammatory drug that readily undergoes thiazole bioactivation and was withdrawn from the market due to severe hepatotoxicity. By contrast, meloxicam exhibits much less toxic risk, despite possessing only the addition of a methyl group on the thiazole ring. The hydroxylation of this substituent may reflect a possibly competitive detoxification pathway, yet there are no reported kinetics to demonstrate the relative significance of metabolic pathways. In this study, we employed parallel desktop modeling and benchtop experimental arms to determine the impact of thiazole substituents on competing pathways for sudoxicam bioactivation and detoxification. For a rapid but qualitative analysis, we introduced a wide range of electron-donating and withdrawing groups to the sudoxicam thiazole ring and predicted their impact on bioactivation using a deep neural epoxide model created previously by our group. Preliminary studies revealed three features: (1) sudoxicam was already highly susceptible to bioactivation despite absence of substituents; (2) electron donating groups had minimal effect on bioactivation, while electron withdrawing groups decreased the outcome; and (3) substituent effects located at C5 were more pronounced than at C4. General model findings were consistent with previous experimental studies indicating lower ring electron density disfavored epoxidation as well as the C5 position being a “soft” spot in reactivity. Current modeling efforts are exploring the relevance of competing pathways and predictive outcomes for thiazole bioactivation on FDA-reported drug-induced liver injuries (DILIrank). For experimental studies, we identified a sudoxicam test family using Tanimoto similarity scores to extract related commercially available molecules reported in eMolecules. Their study required development of novel fluorescence-based LC approaches to label and quantitate metabolic intermediates including the thiazole epoxide, subsequent diol, and degradative metabolites, i.e. the dicarbonyl and thioamide. These successful efforts made possible on-going steady-state kinetic studies on the metabolism of sudoxicam family members through bioactivation and detoxification pathways. Knowledge of these mechanistic details will provide insights for understanding the differences in toxic outcomes as determined by the substituent. Findings from this study will be transformative in advancing an understanding of what governs thiazole bioactivation along with the value of modeling and experimental approaches to foster the design of better, safer drugs.

P167 - CHARACTERIZATION OF DIFFERENTIAL METABOLIC ROUTES OF TIENILIC ACID AND ITS 3-THIOPHENE ISOMER WITH ION MOBILITY ENABLED MASS SPECTROMETRYLauren Mullin¹, Giorgis Isaac¹, Ian Wilson², Muireann Coen², Nathan Anderson¹, and Robert Plumb¹¹Waters Corporation, USA, ²Imperial College, United Kingdom

Tienilic acid (TA) is a uricosuric diuretic found to induce immune-mediated hepatotoxicity in patients, while its 3-thiophene isomer (TAI) exhibits direct hepatotoxic effects^{1,2}. Metabolite identification is a critical step in understanding these differential mechanisms of toxicity. High resolution mass spectrometry (HRMS) is a powerful tool to elucidate metabolite structures, and recent advances in coupling HRMS to ion mobility separation (IMS) represent a valuable enhancement to the metabolite identification workflow. IMS-enabled HRMS experiments provide a further dimension of discrimination and allow collision cross section (CCS) measurements of ions to be made. The aim of this study is to demonstrate this approach in the analysis of urine collected at 2 h, 6 h and 24 h time points, from rats dosed with TA and TAI in multiple animals for each treatment. Urine was also collected from rats dosed with blank vehicle and analyzed in this sample set. Metabolite identification of metabolites in the urine samples indicated hydroxylation for both TA and TAI, though occurring in higher relative abundance for TA at all three time points. Major conjugated metabolites for TA included glucuronidation and acetylation. TAI treatment produced unique cysteine metabolites, indicative of the production of reactive species, not seen in TA treated animals. Further investigation of numerous relatively minor metabolites found in the samples will also be discussed. Identifications were supported by the presence of common fragments resulting from loss of the thiophene and carboxylic groups, as well as the expected isotope distribution patterns of the double chlorinated TA and TAI molecules and the previously mentioned product ions. IMS-derived CCS experimental values were also compared with theoretical values obtained through machine-learning models, providing an avenue for further metabolite identification support.

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P168 - METHODS TO DETECT AND ENRICH TRIMETHOPRIM PROTEIN ADDUCTS IN BIOLOGICAL MATRICES**Whitney Nolte**, Barry Preuett, and Jennifer Goldman

Children's Mercy Hospital, USA

Background: Bactrim, the combination antibiotic trimethoprim-sulfamethoxazole (TMP-SMX), is an efficacious and broadly prescribed drug for the treatment of infections. Unfortunately, Bactrim has a high rate of idiosyncratic adverse drug reactions (IADRs), which has almost exclusively been attributed to SMX due to its well-characterized reactive metabolites and protein binding. More recent data suggests that TMP has been overlooked as a cause of these IADRs as TMP can also be bioactivated to reactive intermediates capable of reacting with thiols and potentially proteins [1]. Further studies in which radiolabeled TMP was incubated *in vitro* with human liver microsomes provided evidence of substantial protein binding by TMP metabolites similar to SMX [2]. N-acetyl-cysteine-TMP adducts were detected in the urine of patients taking TMP-SMX, indicating that bioactivation of TMP occurs *in vivo* [3]. Previous in-house studies using anti-TMP antiserum detected circulating TMP protein adducts in patients taking TMP-SMX. However, methods to detect and characterize individual protein adducts *in vivo* need to be established. Here we describe the development of methods to enrich and detect TMP-protein adducts in biological matrices. Methods: In-house data indicates that the circulating TMP benzylic alcohol metabolite (C α -OH-TMP) can react with thiols including N-acetyl-cysteine and human serum albumin (HSA). To investigate whether we could detect TMP adducts in human plasma, we incubated human plasma *in vitro* with C α -OH-TMP to generate modified proteins. The samples were analyzed by western blotting and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect and identify protein adducts. Methods to enrich TMP adducts from biological samples were also investigated. We immobilized partially purified TMP antiserum onto beads, and used these beads to enrich TMP-modified peptides from tryptic digests of HSA incubated with C α -OH-TMP. The enrichment fractions were analyzed by LC-MS/MS. Results: We observed modification of plasma proteins by C α -OH-TMP by western blotting and by LC-MS/MS. Incubation of human plasma with the C α -OH-TMP metabolite resulted in covalent modification of HSA on the Cys34 residue. Using beads with immobilized TMP antiserum, we were able to selectively enrich TMP-modified peptides from tryptic digests of TMP-modified HSA. Conclusions: We report that C α -OH-TMP can react with plasma proteins, and that these modified proteins can be detected by LC-MS/MS and western blotting. We also demonstrated a method to enrich TMP-modified peptides from tryptic digests. Further work is required to determine whether these methods can be applied to patient samples to detect and measure specific TMP-modified proteins *in vivo*. Quantification of TMP-modified proteins *in vivo* has the potential to yield mechanistic understanding of Bactrim IADRs and to identify prospective biomarkers of IADR development.

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P169 - PYRROLIZIDINE ALKALOID DNA ADDUCT FORMATION AND THE INTERPLAY BETWEEN UPTAKE, METABOLISM, AND EFFLUX IN RAT SANDWICH CULTURED HEPATOCYTES**Cindy Obringer**, Cathy Lester, John Troutman, Mike Karb, Yan Xu, Pete Stoffolano, Catherine Mahony, and Ken Wehmeyer

Procter & Gamble, USA

1,2-unsaturated pyrrolizidine alkaloids (PAs) are hepatotoxic compounds produced in some flowering weeds that can contaminate crops used for food or herbal medicines. The liver is a primary organ of toxicity which involves metabolic activation and formation of pyrrolizine reactive intermediates known to bind to proteins, glutathione and DNA. DNA adducts have been associated with liver tumor formation. Current regulatory approaches to risk assessment take a precautionary approach and assume all 1,2-unsaturated PAs display the same toxicity as the most potent PAs such as lasiocarpine or riddelliine (EFSA, 2011, 2017). *In vitro* measurements of metabolic kinetics and DNA adduct formation in rat hepatocytes have previously been used to inform intrinsic relative PA toxicity. To give further mechanistic insight into potency differences between a structurally diverse series of 8 PAs, intracellular and extracellular PA concentrations were measured to investigate the interplay between metabolism, cellular uptake (active and passive) and potential efflux, including excretion into the bile pocket using plated male Sprague-Dawley rat sandwich culture hepatocytes (rSCHs). Using this model, we have previously correlated DNA adduct levels with parent loss in the extracellular matrix to derive a ranking of intrinsic relative potency. After incubating the PAs at a target concentration of 100 μ M for 6 hours, the intracellular to extracellular PA concentration ratio ($K_p = C_{\text{cell}}/C_{\text{media}}$) ranged from 0 to 3.5. We find that lasiocarpine and riddelliine (two of the more potent PAs) display low K_p values. The high metabolic clearance and relatively high DNA adduct formation for these two compounds at 100 μ M suggest that metabolism is the primary clearance pathway determining the intracellular concentration for these PAs, since very little or no unmetabolized compound was detected in

the bile pocket. Lycopsamine and indicine display higher total intracellular than extracellular concentrations with $K_p > 1$. Relatively slow substrate depletion rates for these PAs suggest that uptake into the hepatocyte may determine the intracellular concentration, however the effects of binding cellular targets cannot be ruled out. K_p is close to 1 for europine, echimidine, monocrotaline and heliotrine suggesting that the rate and extent of uptake may be similar to the rate and extent of elimination through metabolism and efflux. These data demonstrate that differences exist in the hepatocellular concentrations of PAs which is consistent with the differences observed previously in intrinsic relative potency. The overall trend in the observed K_p values showed good agreement with phys-chem properties that are known to influence permeability mechanisms (ionization) and metabolism (hydrophilicity). Recovery of intact (unmetabolized) PA in bile canaliculi was less than 2% for all PAs tested indicating that biliary excretion is a minimal elimination pathway for the PAs tested in this study. The current data provides knowledge of the overall influence of physical-chemical properties on hepatocellular disposition, metabolic activation potential and toxicity for use in non-animal chemical risk assessment.

P170 - MICROTRACER BIOAVAILABILITY, BIOTRANSFORMATION MECHANISMS, AND EXCRETION OF THE COVALENT BTK INHIBITOR ACALABRUTINIB IN HUMANS

Terry Podoll¹, Paul G. Pearson², Jerry Evarts³, Tim Ingallinera³, Elena Bibikova⁴, Hao Sun⁵, Mark Gohdes⁶, Stephen Byard⁷, Kristen Cardinal⁶, Mitesh Sanghvi⁸, and J. Greg Slatter³

¹IV/PO, LLC, USA, ²Pearson Pharma Partners, USA, ³Acerta Pharma (former employee), USA, ⁴Acerta Pharma, USA, ⁵Covance (former employee), USA, ⁶Covance, USA, ⁷Arcinova, United Kingdom, ⁸Xceleron, a Pharmaron Company, USA

Acalabrutinib (CALQUENCE®) is a highly selective, potent, orally administered, targeted covalent inhibitor (TCI) of Bruton tyrosine kinase (BTK) that received accelerated approval for relapsed/refractory mantle cell lymphoma by the United States Food and Drug Administration (FDA) in October 2017. It features a unique 2 butynamide electrophile that has relatively low reactivity. A [¹⁴C]acalabrutinib microtracer absorption, distribution, metabolism, and excretion (ADME) study was conducted in healthy human subjects, with analysis by accelerator mass spectrometry (AMS).¹ The human ¹⁴C microtracer bioavailability arm revealed moderate intravenous clearance (39.4 L/h) and an absolute bioavailability of 25.3 ± 14.3% (n = 8). Absorption and elimination of acalabrutinib following a 100 mg ¹⁴C microtracer acalabrutinib oral dose were rapid, with maximum concentration reached in <1 h and elimination half-life values <2 h. Low concentrations of radioactivity persisted longer in the blood cell fraction and the PBMC subfraction (enriched in target BTK) relative to plasma. Total radioactivity recovery was 95.7 ± 4.6% (n = 6), with 12.0% of dose in urine and 83.5% in feces. [¹⁴C]acalabrutinib was metabolized to over three dozen metabolites, with primary metabolism by cytochrome P450 (CYP) 3A-mediated oxidation of the pyrrolidine ring, thiol conjugation of the butynamide electrophile, and amide hydrolysis. A late-eluting, +16 Da major metabolite circulated at concentrations higher than parent drug and oxidation regiochemistry could not be determined by LC MS/MS. *In vitro* biosynthesis and preparative HPLC generated a pure sample of the metabolite for characterization by nuclear magnetic resonance (NMR) spectroscopy. NMR revealed a pyrrolidine ring-opened ketone, designated ACP 5862. The structure of ACP 5862 and a less abundant -2 Da peak, a dehydropyrrolidine, M25, inferred a common carbinolamide intermediate. Both metabolites retained the butynamide electrophile present in acalabrutinib. *In vitro* studies on the inhibition of BTK and related Tec and Src kinases revealed that ACP-5862 was active against BTK with similar selectivity and potency to acalabrutinib.² Novel enol thioethers from the 2 butynamide electrophile arose from glutathione and/or cysteine Michael additions and were subject to subsequent hydrolysis to a β ketoamide and downstream metabolites that were predominantly observed in feces. Simple *in vitro* degradation studies on the enol thioethers revealed potential for thiol catalysis of the hydrolysis reaction. Acalabrutinib's highly selective, covalent mechanism of action, coupled with rapid absorption, novel biotransformation, and near-complete excretion enables high and sustained BTK target occupancy following twice daily administration.

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P171 - HEPATIC METABOLISM OF BENZALKONIUM CHLORIDE DISINFECTANTS BY CYP2D6 AND CYP4 FAMILY MEMBERS

Ryan Sequin, Josi Herron, Joseph Dempsey, and Libin Xu
University of Washington, USA

Introduction: Benzalkonium chlorides (BACs) are common quaternary ammonium compounds used as disinfectants and preservatives in various cleaning solutions, medical products, and the food processing industry. A wide range of toxicity has been reported for BACs *in vitro* and in animal models and we recently discovered that BACs are potent inhibitors of

cholesterol biosynthesis. Despite a high likelihood for systemic exposure, human metabolism of BACs has never been studied. The chemical structures of BACs [alkyl dimethyl benzyl ammonium chloride] contain an even-numbered carbon chain of varying length (C8 to C18). Here, we examined metabolism of the most commonly used BACs (C10, C12, C14, and C16) by conducting *in vitro* human metabolism studies and an *in vivo* mouse study. The major goal of this work is to elucidate hepatic mechanisms of BAC metabolism using human liver microsomes (HLM) and recombinant cytochrome P450 (CYP) isoforms. Additionally, we report on the detection of BAC metabolites in the kidney of an orally exposed mouse. Methods: Depletion of BACs and formation of BAC metabolites was monitored by LC-MS in incubations with HLM and recombinant CYP isoforms. Isoform-specific CYP inhibitors were used in HLM incubations to identify the relevant CYP isoforms. A selection of BAC metabolites was synthesized for structural confirmation. Time-course and kinetic studies of BAC metabolism in HLM were also completed. Mouse *in vivo* work: C57BL/6J mice were orally dosed with BACs (120 mg/kg/day) over 28 days under IACUC approved protocol. Tissues were then dissected, extracted, and analyzed for BACs and BAC metabolites by LC-MS. Results: In HLM metabolic stability assays, BAC consumption was rapid and NADPH-dependent. Approximate *in vitro* half-lives were 1, 3, 6, and 15 minutes for C10, C12, C14, and C16 BACs, respectively. We found recombinant CYP4F2 and CYP2D6 were both active in metabolizing BACs. Synthesis of *w*-hydroxy, (*w*-1)-hydroxy, (*w*, *w*-1)-dihydroxy, and carboxylic acid C10-BAC authentic standards confirmed these metabolites are formed in HLM. Application of HET0016 and quinidine, specific inhibitors of CYP4 and CYP2D6, revealed *w*-hydroxylation was CYP4-mediated and (*w*-1)-hydroxylation was CYP2D6-mediated. Michaelis-Menten parameters (V_{max} , K_m) for C10-BAC metabolism were 365 pmol/min/mg, 0.31 μ M for *w*-hydroxylation and 126 pmol/min/mg, 0.15 μ M for (*w*-1)-hydroxylation. Finally, analysis of kidney tissue from a BAC-exposed mouse demonstrated *in vivo* formation of the same BAC metabolites detected in HLM. Conclusions: We provide the first evidence that BACs are extensively metabolized by CYP enzymes in HLM. CYP2D6 and CYP4 isoforms were primarily responsible for the depletion of BACs in HLM leading to (*w*-1)- and *w*-hydroxylated metabolites followed by further sequential oxidations of the alkyl chain. Detection of these same metabolites in kidney extract of a BAC-exposed mouse lends *in vivo* relevance to these results. The identification of CYP4s and CYP2D6 as the primary enzymes metabolizing BACs suggests that certain human special populations may be more susceptible to BAC exposure as both enzymes are highly polymorphic.

P172 - I-SCREEN: AN EX VIVO HUMAN MICROBIOME PLATFORM TO STUDY MICROBIOME INDUCED REVERSE METABOLISM OF METABOLITES BACK TO PARENT

Evita van de Steeg¹, Frank Schuren¹, Irene Nooijen¹, Steven Erpelinck¹, R. Scott Obach², and Wouter Vaes¹

¹TNO, Netherlands, ²Pfizer, USA

The role of the gut microbiome in the metabolism of xenobiotics and is increasingly recognized. It has been demonstrated that gut microbiota can directly metabolize xenobiotics into active, inactive or toxic metabolites, thereby influencing pharmacokinetics, efficacy and toxicity profiles of prescribed drugs. We have previously shown metabolism of selected drugs by pooled human microbiota (Van de Steeg et al 2018). Here we show the fate of drugs that are being metabolized in the liver with the metabolites subsequently exposed to microbiota in the colon environment. The metabolic capacity of gut microbiota was investigated by incubating 9 drug metabolites (5 glucuronides, 2 N-oxides, 1 sulphate and 1 sulfamic acid), in i-screen using pooled human colonic microbiota. Experiments were run for 24 hours under fully anaerobic conditions, with samples taken at 0, 6 and 24h, followed by LC-HRMS analysis of the samples. Previously we have shown that out of 12 parent drugs 5 were metabolized in the i-screen platform mimicking the colon environment. Here we show that 9 metabolites derived from 8 different parent drugs, are readily metabolized by intestinal microbiota in a colonic environment. For all 9 metabolites metabolism could be detected in 24 hours, with 4 metabolites being completely metabolized within 6 hours. In three cases the parent drug generated in the experiment was also shown to be subject to further metabolism within the 24h timeframe. Microbiome free incubations confirmed absence of metabolism for 6 metabolites, for 3 metabolites some parent compound at t=0 or in cell free incubations could be detected. In conclusion, we have shown that human intestinal microbiota not only is able to metabolize parent drugs which enter a colonic environment, but also can metabolize parent drugs which have been first metabolized in the liver and re-enter the GI-tract through enterohepatic cycling. In the colonic environment these metabolites are amenable to microbial metabolism thereby generating parent drugs or metabolites thereof in the colon, which can subsequently enter the circulation via intestinal absorption.

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P173 - TIME-RESOLVED METABOLOMICS REVEALS DYNAMIC ALTERATIONS OF ENDOBIOTICS IN MOUSE LIVER AFTER ISONIAZID EXPOSUREPengcheng Wang¹, Madhav Sachar², Jie Lu², Amina I. Shehu², Xiaobo Zhong³, Xiaochao Ma², and **Junjie Zhu**¹¹University of Maryland School of Pharmacy, USA, ²School of Pharmacy, University of Pittsburgh, USA, ³School of Pharmacy, University of Connecticut, USA

Isoniazid (INH) is a first-line antituberculosis drug, but it causes liver injury frequently by unclear mechanisms. Time-resolved metabolomics, which involves comprehensive characterization of dynamic alterations of endogenous and exogenous metabolites, provides a unique platform to explore the underlying mechanism of drug-induced liver injury. In the current study, we used a time-resolved metabolomics approach to investigate the time course effects on INH-induced liver metabolome alterations and liver injury. We found that INH disrupted multiple hepatic endobiotic metabolic pathways in the liver in a time-dependent manner, including the accumulation of linoleoyl-L-carnitine, oleoyl-L-carnitine, heme, and NAD and the depletion of pyridoxal-5-phosphate at the early time points (0.25 and 0.5 h) and the accumulation of protoporphyrin IX accumulated at late time points (4 and 8 h). These endobiotic metabolic pathways play critical roles in hepatocellular metabolism and functions, and disruption of these pathways may contribute to INH hepatotoxicity. These observations highlighted the critical role of time course in profiling INH-induced liver metabolome alterations and liver injury. These results highlighted the potential application of time-resolved metabolomics in studying the mechanism of drug toxicities.

P174 - ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF TROPIFEXOR, A POTENT FXR AGONIST FOR THE TREATMENT OF NASH, FOLLOWING A SINGLE ORAL 1 MG DOSE OF [14C]TROPIFEXOR IN HEALTHY MALE SUBJECTS**Lydia Wang-Lakshman**¹, Zhuang Miao¹, Jessie Gu¹, Subhajit Choudhury², Elizabeth McNamara¹, Markus Walles³, Ralph Woessner³, Gian Camenisch³, and Jin Chen¹¹Novartis Institute for Biomedical Research, USA, ²Novartis Healthcare Pvt. Ltd., India, ³Novartis Institute for BioMedical Research, Switzerland

Tropifexor (NVP-LJN452) is a highly potent and selective nonsteroidal non-bile acid farnesoid X receptor (FXR) agonist for the treatment of nonalcoholic steatohepatitis (NASH). Its absorption, distribution, metabolism, and excretion was studied following a single 1-mg oral dose of [14C]tropifexor to four healthy male subjects. The rate and route of excretion of [14C]tropifexor-related radioactivity was determined as well as pharmacokinetic profiles of tropifexor and of total radioactivity in plasma. The key biotransformation pathways and clearance mechanisms of [14C]tropifexor in human were elucidated. Mass balance was achieved with approximately 94% of the administered dose recovered in excreta through the 312 hr collection period. Fecal excretion of tropifexor-related radioactivity played a major role (~65% of the total dose) while urinary excretion played a slightly minor role (~29% of the total dose). After oral administration of 1 mg [14C]tropifexor to human subjects, parent tropifexor reached C_{max} of 33.5 ng/mL with a median T_{max} of 4 hours and eliminated with a T_{1/2} of 13.5 hr in plasma. Unchanged tropifexor was the principal drug-related component found in the plasma (~92% of total radioactivity). Two minor oxidative metabolites, M11.6 and M22.4, were observed in circulation, at ~2% and ~5% of the total drug radioactivity exposure, respectively. Tropifexor was eliminated predominantly via metabolism with > 68% of the dose recovered as metabolites in excreta. Oxidative metabolism appeared to be the major clearance pathway of tropifexor as the majority of the radioactivity observed in human excreta consisted of oxidative metabolites. Primary phase I oxidative pathways included: 1) oxidative O-dealkylation; 2) oxidation at the phenyl cyclopropyl isoxazole moiety; 3) oxidation at the benzothiazole and fused ring structure. Metabolites containing multiple oxidative modifications and/or glucuronidation to oxidative products were also observed in human excreta. The involvement of direct glucuronidation to tropifexor clearance was not ruled out by the study, as previous *in vitro* and non-clinical *in vivo* studies suggested its contribution to overall tropifexor metabolism. Due to instability of glucuronide metabolites in the GI tract, the contribution of glucuronidation could not be estimated in the present study.

P175 - URINARY ESTROGEN DERIVATIZATION AND HIGH-RESOLUTION LC-MS ANALYSIS TO DETERMINE MODULATION OF ESTROGEN METABOLISM IN WOMEN RESULTING FROM USE OF BOTANICAL DIETARY SUPPLEMENTS**Alan Wong** and Richard van Breemen
Oregon State University, USA

Menopause is the natural decline of estrogen levels for all women in their late 40s to early 50s. It can cause long-lasting health effects, including loss of bone density and increased risk of cardiovascular disease.[1] Hormone therapy is the conventional treatment used to manage symptoms such as hot flashes and vaginal dryness; however, prolonged use of hormone therapy might increase risks of heart disease, stroke, blood clots, and breast cancer. In particular, the increased breast cancer risk has been associated with 4-hydroxyestradiol, an estrogen metabolite formed by P450 1B1, 1 which can form an ortho quinone and cause oxidative DNA damage through redox cycling.[2] Approximately 70% of American peri-

and post-menopausal women have reported the consumption of botanical dietary supplements to manage menopausal symptoms.[3] We hypothesize that most botanical dietary supplements used by women to manage menopausal symptoms are safe, but some may pose risk through the modulation of estrogen biosynthesis and metabolism.[4,5] We carried out post-analysis of urine from our completed phase I and phase II clinical trials of hops (*Humulus lupulus*) and red clover (*Trifolium pratense*) in peri- and post-menopausal women with the goal of measuring the trace levels of estrone, estradiol, and their metabolites. This requires development of a highly-sensitive liquid chromatography-high resolution mass spectrometry method to identify changes in estrogen levels before and after consumption of the botanical dietary supplement for 14-days. This analytical method utilized dansyl chloride derivatization and differential isotope labeling to enhance sensitivity and improve quantitation.[6,7] For 16 women in a phase II clinical trial receiving a hop supplement, we measured a reduction in the levels of 4-hydroxyestradiol, a genotoxic metabolite formed via 4-hydroxylation of estradiol by P450 1B1. No changes were observed for other analytes, including 2-hydroxyestradiol, another estrogen metabolite which is formed from estradiol by P450 1A1. These data support our hypothesis that some botanical dietary supplements can selectively affect the modulation of estrogen metabolism.

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P176 - BINDING PROTEINS HAVE ENZYME SPECIFIC EFFECTS ON XENOBIOTIC METABOLISM

King Yabut and Nina Isoherranen

University of Washington, USA

Lipophilic, poorly water-soluble drugs are assumed to be highly protein bound in systemic circulation, but whether they bind to intracellular soluble binding proteins such as lipid binding proteins (iLBPs) in the cytosol has not been well characterized. The free drug hypothesis states that only unbound drug accesses receptors and metabolic enzymes. However, cytochrome P450 (CYP) enzymes in the ER membrane are believed to accept their substrates from the lipid membrane. Free concentrations of drug in the cytosol primarily impact substrate access to the CYP by affecting drug concentrations in the ER membrane. Based on this model, we hypothesized that iLBPs will decrease free concentrations of substrates in solution, and therefore decrease metabolic rates of xenobiotics via decreasing substrate concentrations in the ER membrane. The goal of this study was to determine whether, according to this model, product formation by each CYP would be affected to the same degree through the universal impact of binding proteins on the drug concentrations in ER membrane. This study used albumin, FABP5, and CRABP as model iLBPs and Δ^9 -tetrahydrocannabinol (THC) and all-trans-retinoic acid (atRA) which have been shown to bind to the above iLBPs as model substrates. CYP2C9, CYP3A4, CYP2C8, CYP2C19 and CYP26A1 were studied as the model CYPs, and the metabolite formation by each of these CYPs was measured in the presence and absence of the iLBPs. THC metabolite formation was nearly abolished in the presence of FABP5 and albumin with both recombinant CYP3A4 and CYP2C19, but only modestly affected with recombinant CYP2C9, demonstrating an enzyme specific effect of iLBPs. A CYP specific effect was also found with atRA as a substrate. CRABP abolished 4-OH-atRA formation by recombinant CYP3A4 and CYP2C8 but not by CYP26A1. The enzyme specific effects were confirmed in incubations in human liver microsomes (HLMs). In the presence of 0.1% albumin the apparent K_m of 11-OH-THC formation by CYP2C9 decreased, but the K_m for 9 α ,10 α -epoxy-hexahydrocannabinol (EHHc) formation by CYP3A4 increased in comparison to no binding protein control incubations. Furthermore, the product ratio of 11-OH-THC/EHHc increased by over 2-fold in the presence of albumin and FABP5, suggesting that the iLBPs specifically favor substrate delivery to CYP2C9 in comparison to CYP3A4. Similarly, with atRA as a substrate, presence of binding proteins appeared to specifically target atRA for metabolism by CYP26A1. In the absence of CRABP the formation of 4-OH-atRA was significantly inhibited by CYP3A4 inhibitors troleanomycin and ketoconazole. However, the presence of CRABP eliminated the metabolism by CYP3A4 in HLMs based on the decreased 4-OH-atRA formation in the presence of CRABP and lack of inhibition of the remaining 4-OH-atRA formation by CYP3A4 inhibitors. This suggests that CYP26 is the major contributor to 4-OH-atRA formation in the presence of CRABP, and that CRABP preferentially channels atRA to CYP26. Taken together, our data suggests that binding proteins may alter the

metabolism of xenobiotics via preferential interactions with CYPs and that enzyme specific effects of binding proteins should be considered when assessing fractional contributions from metabolic enzymes to substrate clearance.

P177 - COMPARATIVE METABOLISM OF [14C]-BMS-986165 IN MICE, RATS, MONKEY, AND HUMANS

Ming Yao, Xiaomei Gu, John Brailsford, Janet Caceres Cortes, Ramaswamy Iyer, and Wenying Li
Bristol-Myers Squibb, USA

The metabolism and disposition of [14C]BMS-986165, (Chemical name: 6-(cyclopropanecarboxamido)-4-((2-methoxy-3-(1-methyl-1H-1,2,4-triazol-3-yl)phenyl)amino)-N-(methyl-d3)pyridazine-3-carboxamide), a highly selective and potent small molecule inhibitor of receptor-mediated tyrosine kinase 2 (Tyk2) activation, were investigated in mice, rats, monkeys, and humans after a single oral administration. Plasma, urine, and feces were collected at various times or time intervals up to 316 hours post-dose. Biotransformation profiles in each pooled matrix were obtained after analysis by high-performance liquid chromatography (HPLC) with radioactivity detection and mass spectrometry (MS). In plasma, the parent compound was the major circulating component, accounting for 87.7%, 75%, 91% and 43% of total radioactivity AUC in mice, rats, monkeys, and humans, respectively. In human plasma, metabolite formed via cyclopropyl carboxamide hydrolysis of BMS-986165 (M7), N-demethylation of BMS-986165 (M13), and direct glucuronide conjugation of BMS-986165 (M6) represented approximately 24%, 11% and 7% of total radioactivity respectively, based on AUC_{0-24h}. There was adequate coverage of the two major circulating metabolites, M7 and M13, in animal plasma. The fecal excretion was a major elimination pathway for [14C]BMS-986165-derived radioactivity, accounting for >72% of the dose in intact animals and 52% in humans. Urinary excretion of radioactivity accounted for <20% of the dose in animals and 53% in humans. BMS-986165 was a major component in feces and urine of all species. M7 was a prominent metabolite in excreta of mice, rats, and humans. M13 was a prominent metabolite in excreta of mice, rats, monkeys, and humans. M6 was a prominent metabolite in monkey and human excreta. Metabolite profiles were qualitatively similar in all species (mice, rats, monkeys, and humans) and there were no unique human metabolites. Overall, BMS-986165 is eliminated via multiple clearance pathways in humans and animals, including metabolism, renal excretion, and fecal excretion.

P178 - OPTIMIZATION OF SAMPLE PROCESSING FOR METABOLISM STUDIES OF TARGETED COVALENT INHIBITORS (TCI)

Chenghong Zhang, Dian Su, Shuguang Ma, Cyrus Khojasteh, Marcel Hop, Donglu Zhang, and Richard Zang
Genentech, USA

Targeted covalent inhibitors (TCI) are designed to form a covalent bond between the TCI electrophile (also known as warhead) on the ligand and a nucleophilic center of the targeted protein. Acrylamide-containing drugs such as afatinib and ibrutinib are recent examples of approved covalent inhibitor drugs targeting the specific cysteine thiol in the active site of the target. Our hypothesis is: their intrinsic electrophilic reactivity creates additional challenges in sample preparations for metabolism and pharmacokinetic studies. Therefore, acrylamide-containing market drugs were selected to compare metabolite profiles in seven cross species liver hepatocytes under conditions of sample-drying with immediately or delayed injection versus direct injection without sample-drying step. The results demonstrated that different metabolite profiles may occur under varied sample treatment conditions depending on the reactivity of warhead. For very chemically reactive warhead extensive reversible reactions of labile products with nucleophiles were observed under drying-condition. Also, during delayed injection periods, continually chemical reactions with nucleophiles were also observed, especially in concentrated hepatocytes samples. To our knowledge, this kind of evaluation has not been paid attention in the pharmaceutical industries previously. Based on these results, we have optimized for the ADME assays. It could provide high quality data during drug discovery stage. A recommendation was made to allow for *in vitro* or *in vivo* ADME sample analysis for TCI programs.

P179 - CELL-TYPE SPECIFIC METABOLISM OF ISONIAZID IN THE LIVER

Junjie Zhu, Jie Lu, Hung-Chun Tung, Wen Xie, and Xiaochao Ma
University of Pittsburgh, USA

Isoniazid (INH) is a front-line drug for the treatment of tuberculosis, but it can cause liver damage by unknown mechanisms. INH metabolism and immune response have been implicated in INH hepatotoxicity. The current work explored INH metabolism in primary mouse hepatocytes (HP) and the liver-resident macrophages (Kupffer cells, KC). As expected, we determined the formation of acetylisoniazid (AcINH) in HP where N-acetyltransferase (NAT) is highly expressed. In addition, we uncovered the adduct of β -nicotinamide adenine dinucleotide (NAD⁺) with INH in KC but not in HP. The formation of INH-NAD adduct is dependent on cluster of differentiation (CD) 38 protein. We verified that CD38 is highly expressed in KC but not in HP. Moreover, we identified a new metabolite as AcINH-NAD adduct, indicating that AcINH produced in HP is transferred to KC where AcINH can be further metabolized by CD38 to form AcINH-NAD. In

summary, the current work illustrated cell-type specific metabolism of INH in the liver. Future studies are warranted to determine the impact of cell-type specific metabolites of INH in cell-cell communications in the liver and its contribution to INH hepatotoxicity.

P180 - PHARMACOKINETIC STUDY OF A RECOMBINANT HUMAN PLACENTAL ANTICOAGULANT PROTEIN DERIVATIVE IN RHESUS MONKEYS

XiaoXia Zhu, ZhiYun Meng, Ruolan Gu, Hui Gan, Zhuona Wu, and Guifang Dou
Beijing Institute of Radiation Medicine, China

Background: Recombinant human placental anticoagulant protein derivative (rHAP) is an effective anticoagulant and anti-thrombosis agent, which was conjugated with Gly-Pro-Arg-Pro tetrapeptide, 20 peptide of the C-terminal hirudin and the annexin-V protein together. Aims: To evaluate the pharmacokinetics properties of rHAP in rhesus monkeys and to support its further clinical study. Methods: 12 rhesus monkeys, both sexes, were randomly assigned to three groups. The pharmacokinetics were evaluated after single i.v. administration (1/3 rHAP dosage for bolus first and followed by 2/3 dosage i.v. drip in 60min) of 0.1mg•kg⁻¹, 0.5 mg•kg⁻¹ and 2.5 mg•kg⁻¹ of rHAP in rhesus monkeys respectively (n=4, 2 female and 2 male). The plasma rHAP was determined by a validated enzyme linked immunosorbent assay (ELISA) method. The pharmacokinetic parameters were calculated using the Phoenix WinNonlin 5.2 software with non-compartmental models. Results: The half-life (t_{1/2}) were 16.5±12.0, 6.1±1.2 and 6.1±2.3 h, the area under the concentration-time curve (AUC_{last}) were 371±148, 7211±1857 and 39371±12463 ng•mL⁻¹•h, the clearance (Cl) were 0.24±0.07, 0.07±0.02 and 0.07±0.02 L•h⁻¹•kg⁻¹, and the apparent volume of distribution (V_d) were 6.15±5.68, 0.63±0.19 and 0.62±0.31 L•kg⁻¹, for 0.1mg•kg⁻¹, 0.5 mg•kg⁻¹ and 2.5 mg•kg⁻¹ group respectively. Conclusions: For the dosage between 0.5 mg•kg⁻¹ and 2.5 mg•kg⁻¹ of rHAP in rhesus monkeys after i.v. administration, the pharmacokinetic characteristics was linear, for the amplification of AUC_{last} was in conformance with the amplification of dosage (0.5: 2.5), and the t_{1/2} was not changed obviously. The reason why the C_{max} and the AUC_{last} were especially much low while the t_{1/2} was relatively long was probably because the proportion of the rHAP conjugated with its receptor at low dose was relatively higher than the case of middle and high dose.

P181 - ACCOMPANYING TOXICOKINETIC STUDY OF A RECOMBINANT HUMAN ANNEXIN V DERIVATIVE IN RHESUS MONKEYS

Ruolan Gu, Zhiyun Meng, **Xiaoxia Zhu**, Hui Gan, Zhuona Wu, and Guifang Dou
Beijing Institute of Radiation Medicine, China

Background: Recombinant human annexin V derivative (rHAP) is an anticoagulant protein by conjugated annexin V with the Gly-Pro-Arg-Pro tetrapeptide, and the 20 peptides of the C-terminal hirudin to enhance the antithrombin and antifibrin activities together. Aims: To evaluate the accompanying toxicokinetics properties of rHAP in rhesus monkeys and to support its further clinical safety study. Methods: 18 rhesus monkeys, both sexes, were randomly assigned to three groups, and were i.v. administration (1/3 rHAP dosage for bolus first and followed by 2/3 dosage i.v. drip in 60min) of 0.5mg•kg⁻¹, 2.0 mg•kg⁻¹ and 8.0 mg•kg⁻¹ of rHAP respectively (n=6, 3 female and 3 male) once a day for 4 weeks. The plasma rHAP was determined by a validated enzyme linked immunosorbent assay (ELISA) method. The toxicokinetic parameters were calculated using the Phoenix WinNonlin 5.2 software with non-compartmental models. Results: From the dosage 0.5 mg•kg⁻¹ to 8.0 mg•kg⁻¹ of rHAP in rhesus monkeys after i.v. administration, the pharmacokinetic characteristics was linear, for the amplification of AUC_{last} was approximately in conformance with the amplification of dosage (1: 4:16). No sexual difference was found for all the three dose groups for the main kinetic parameters (P>0.05). After once a day for four weeks, the AUC_{last} of 16 rhesus monkeys were increased obviously, the ratio between last administration and first administration was about 1.2-21.0:1. Conclusions: For rhesus monkeys i.v. administered of rHAP once a day for four weeks between 0.5 mg•kg⁻¹ to 8.0 mg•kg⁻¹, there was a trend of accumulation of rHAP *in vivo*, which indicated the accumulation possibility in clinical study and needs to be considered when designing the dosage regimen.

P182 - APPROACH FOR THE INVESTIGATION OF THE BIOTRANSFORMATION OF OLIGONUCLEOTIDE THERAPEUTICS IN BIOLOGICAL MATRICES USING SPE, FOLLOWED BY LC/UV/HRMS

Heasook Kim-Kang and David Heim
Q² Solutions, USA

Oligonucleotide-based drugs became an important class of therapeutics under investigation within the pharmaceutical industry in the treatment of various disease states, with the potential of exhibiting high specificity with gene targeted therapies. Understanding the biotransformation and disposition of new therapeutics is essential for drug development. Unlike small molecule drugs, the biotransformation of oligonucleotide drugs mainly consists of cleavage of the phosphodiester bonds between nucleic acids by nucleases, however, due to their structures similar to biomolecules, large molecular weights as well as the tendency to form multiple-charged ions, it poses different challenges from small molecule

therapeutics. In current study, two 21-mer oligonucleotides, one with phosphodiester (Oligo 1) and the other composed of a mixture of phosphodiester and phosphorothioate bonds (Oligo 2), were used for the investigation. The compounds were individually incubated in a pure 5'-exonuclease (from *Crotalus adamanteus* venom) to generate a mixture of hydrolysis products, which served as working standard mixtures. The incubation mixtures were processed through an SPE procedure using Clarity OTX cartridges to remove enzymes and aliquots of the final solutions were spiked into control plasma, urine, or feces from humans. The spiked biological matrices (plasma and urine) were diluted with loading buffer and subjected to SPE procedure. Spiked feces were first digested with protease prior to the dilution with loading buffer. The final eluate was evaporated to near dryness, followed by reconstitution in a mixture of mobile phases (TEA and HFIP, pH 8). The final samples were analyzed by HRMS (Thermo QE HF) equipped with PDA detector using OST C18 column and the collected data were used to optimize mass spectral parameters and the search strategy for drug related metabolites. A template spreadsheet was prepared to calculate the predicted hydrolysis products and/or fragment ions with different charge states. The final samples were also analyzed using Thermo LTQ Orbitrap to evaluate any advantage offered by CID-MS/MS. The approach provided qualitatively representative samples by providing similar profiles of the spiked hydrolysis products before and after sample work up, from which a reliable metabolite identification strategy was developed, utilizing characteristic fragment ion filtering at high SID, UV absorptivity of oligonucleotides, and an optimized chromatography. The two oligonucleotides were then incubated for 7 hrs in fresh human serum (known to contain both 3'- and 5'-exonucleases) at 10 μ M to generate more diverse hydrolysis products, subjected to SPE procedure, and the final samples were analyzed by LC/UV/MS. Then the established search strategy was used to detect and identify the metabolites. Oligo 2, while stable in the pure enzyme used, yielded a mixture of metabolites after incubation in human serum. The results confirmed the manufacturer's note that mono- to tetramers are not retained in the OTX cartridge. Thus, to evaluate those small hydrolytic products, the unretained load eluate may need to be handled in the same way as small molecule drugs and the recovered extract may need to be analyzed using a different type of LC column. Other critical items which require attention will be also presented.

P183 - EXPOSURE TO CADMIUM MODULATES THE COMPOSITION OF SHORT CHAIN FATTY ACID-PRODUCING MICROBIOTA IN AN ALZHEIMER'S DISEASE MOUSE MODEL

Angela Zhang, Megumi Matsushita, Liang Zhang, Zhengui Xia, and Julia Yue Cui
University of Washington, USA

Exposure to cadmium, a toxic heavy metal found in sources such as industrial waste and contaminated foods, is highly detrimental to human health. Specifically, cadmium-induced neurotoxicity is implicated in several neurodegenerative diseases such as Alzheimer's disease (AD). Previous research has shown that AD patients have aberrant hepatic expression of drug-processing genes. Furthermore, there is growing evidence that the gut microbiome plays an important role in drug metabolism; the gut-liver axis may affect the efficacy or the toxicity of numerous medications for AD. Although research continues to investigate the roles of cadmium exposure and the gut microbiome in neurodegenerative diseases separately, little is known about the interaction between cadmium and the gut microbiome in the pathogenesis of AD. Apolipoprotein (ApoE) is involved in the transport of lipids and cholesterol in the body. While ApoE3 is the wild-type allele, the E4 variant is a major risk factor for late-onset sporadic AD in humans. In this study, we used humanized ApoE3/4 knock-in mice models in order to test our hypothesis that ApoE4 mice are more susceptible to cadmium-induced gut dysbiosis and dys-regulation of hepatic drug metabolism. Fecal 16S rDNA sequencing, serum lipid metabolomics and hepatic transcriptomics were performed in ApoE3 and ApoE4 mice orally exposed to vehicle, a low dose (0.6 mg/L), or a high dose (3 mg/L) of cadmium in drinking water for 14 weeks ($n = 4$ or 5). Cadmium had the largest effect on alpha diversity (Shannon index) for ApoE females compared to ApoE males, regardless of genotype. Genotype, however, had the most prominent effect on beta diversity (Bray-Curtis), followed by gender, regardless of differing doses of cadmium. Cadmium-exposed ApoE4 males had the most prominent dysbiosis, evidenced by increased short chain fatty acid (SCFA)-producing *Akkermansia muciniphila* and *Prevotella*, but decreased *Clostridium cocleatum*, *Peptostreptococcaceae*, and *Bacteroides ovatus*. Increased *Prevotella* and *Akkermansia muciniphila* suggests a possible compensatory response to cadmium exposure. Decreased counts of *Bacteroides* species and *Clostridium cocleatum*, both producers of lactate coincided with the significant decrease of serum lactate levels in cadmium-exposed ApoE4 males. To note, lactate deficit has been previously linked to neuronal damage in AD mice models. In regards to the host transcriptome, several genes involved in drug metabolism such as cytochrome P450s and sulfotransferases were down-regulated in ApoE4 females. In summary, this study is among the first multi-omics investigation to provide evidence that the neurotoxicant cadmium produces compositional and functional changes in the gut microbiome, as well as dys-regulation of host drug metabolism in carriers that are genetically susceptible to Alzheimer's disease.

P184 - AN EVALUATION OF PROBE SUBSTRATES AND INHIBITORS OF ALCOHOL DEHYDROGENASE, ALDEHYDE DEHYDROGENASE AND ALDEHYDE OXIDASE ENZYMES FOR USE IN REACTION PHENOTYPING INVESTIGATIONS

Vicky Birks, Sophie Hayo, Richard Cole, and Michael Hall
Pharmaron UK Ltd., United Kingdom

Metabolism can directly impact upon the pharmacokinetics and thereby pharmacodynamics and toxicological effects of drugs. Consequently, the identification of the enzymes involved in the metabolism of a drug, through the technique of reaction phenotyping can be of great clinical value and represent significant issues in drug development and toxicity. Screening techniques to improve drug candidate stability and reduce the potential for drug-drug interactions (DDIs) via cytochrome P450 (CYP) mechanisms have led to an increasing number of new drug candidates being metabolised by non-CYP pathways. The European Medicines Agency, US Food and Drug Administration and Japanese Ministry of Health, Labor and Welfare DDI guidance documents recommend that if an investigational drug undergoes significant *in vivo* metabolism that is not mediated by a major CYP enzyme, then it is probably a substrate for other enzymes and the sponsor should determine which these are.

The use of probe substrates and selective inhibitors as positive controls to confirm the activity of the enzymes being investigated is an essential part of the reaction phenotyping process. In this investigation, ethanol, 4-nitrobenzaldehyde and zaleplon were assessed as potential probe substrates of alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and aldehyde oxidase (AO) enzymes, respectively, in human hepatocytes and human liver S9 fraction (ADH and ALDH only). In addition, the ability of 4-methylpyrazole (ADH), disulfiram (ALDH), hydralazine (AO) and raloxifene (AO) to inhibit these enzymes was evaluated.

ADH activity was measured spectrophotometrically by monitoring NADH formation from NAD in the presence of ethanol. ALDH activity was measured by the formation of 4-nitrobenzoic acid and monitored by HPLC-UV or LC-MS/MS. AO activity was assayed by the formation of 5-oxo-zaleplon and quantified by LC-MS/MS.

The activity of these enzymes was confirmed in human hepatocytes and human liver S9 fraction (ADH and ALDH only). The inhibitor 4-methylpyrazole (ADH) elicited 94% inhibition of NADH formation in human liver S9 fraction and 89% inhibition in human hepatocytes. Disulfiram (ALDH) elicited 88% inhibition of 4-nitrobenzoic acid formation in human liver S9 and 92% inhibition in human hepatocytes. Hydralazine and raloxifene caused 90% and 17% inhibition, respectively of AO in human hepatocytes.

In summary, incubation conditions and analytical techniques for the determination of the activity and inhibition of ADH, ALDH and AO were determined. These assays should prove useful in the reaction phenotyping of investigational drugs.

P185 - CHEMOPROTECTIVE ROLE OF ALDEHYDE OXIDASE AND XANTHINE OXIDOREDUCTASE IN A MODEL ORGANISM

Douglas Clarke

University of Huddersfield, United Kingdom

Although aldehyde oxidase (AO) and xanthine oxidoreductase (XOR) are classed as phase I detoxication enzymes as they catalyse the oxidation of a diverse number of xenobiotic N-heterocycles and aldehydes that are drugs and environmental pollutants however definitive proof of their chemoprotective function has not been assessed in any organism to date. The aim of this study was to use the model organism *Drosophila melanogaster* to determine the *in vitro* and *in vivo* detoxicative functions of AO and XOR using wild type, mutant and transgenic animals with a variety of xenobiotics. HPLC and spectrophotometric assays demonstrated that the *Drosophila* AO and XOR orthologs catalysed the oxidation of a similar wide range of aldehydes and N-heterocyclic compounds as their mammalian counterparts indicating this was a good animal model. AO and XOR mutant *Drosophila* were characterised by DNA sequencing, enzyme assays, and cellulose acetate electrophoresis. This demonstrated genetic mutations were responsible for a complete deficiency of the AO and/or XOR. Administration of drugs and foodstuffs that are XOR substrates to these mutant *Drosophila* demonstrated that several methylated xanthines were toxic to the XOR-deficient strains in comparison to wild type animals. Similarly N-heterocyclic pollutants and drugs that are AO substrates were significantly more toxic to AO-deficient strains. P-element transformation of the mutant strains with wild type genes resulted in rescue of the chemoprotective effect. This study therefore provides *in vivo* experimental proof that both AO and XOR are detoxication enzymes for the first time.

P186 - PH DEPENDENT CES1 MEDIATED HYDROLYSIS OF VIXOTRIGINE IN HUMAN LIVER MICROSOMES

Joshua Johnson, Michael Rooney, and Chungang Gu

Biogen, USA

Carboxylesterases (CES) are a family of enzymes that catalyze the hydrolysis of ester bonds to the corresponding carboxylic acid and alcohol subunits. They can also hydrolyze amides to carboxylic acids. Vixotrigine, (2S,5R)-5-(4-((2-fluorobenzyl)oxy)phenyl)pyrrolidine-2-carboxamide, is a state- and use dependent Nav1.7 blocker that also inhibits other

voltage-gated sodium channels, in development for the treatment of various neuropathic pain conditions. Human ADME data of [¹⁴C]-vixotrigine including chemical structures of the parent drug and its metabolites reported at the 21st North American Meeting of ISSX in 2017 (Woodward et al, 2017) indicated the conversion of the amide to the carboxylic acid metabolite (M14) as a major *in vivo* metabolic pathway. While M14 is formed to a significant extent in human hepatocyte incubations, incubations in human hepatic microsomes (HLM) and S9 fractions yield only trace levels of M14, making it challenging to identify the metabolizing enzyme(s) responsible. For example, this would almost have excluded CES1 that is mainly present in HLM from being the likely enzyme. In this study, various chemical inhibitors specific for hydrolytic enzymes were tested with Vixotrigine in human hepatocyte incubations to identify the enzymes responsible for vixotrigine hydrolysis. The formation of M14 in human hepatocytes was diminished by the CES inhibitor Bis(4-nitrophenyl) phosphate (MilliporeSigma, St Louis, MO) in a concentration dependent manner, suggesting that the reaction was catalyzed by CES. This was confirmed by incubating Vixotrigine with recombinant human CES1 and CES2 (Corning, Corning, NY), showing a higher formation rate of M14 by CES1 than CES2. While exploring the disconnect between reactions using hepatic subcellular fractions that should contain CES1 and CES2 performed at pH 7.4 and hepatocytes, it was observed that the formation of M14 in HLM was pH dependent with the optimal pH at pH 8.5-9, rationalizing the low turnover to M14 of vixotrigine observed in the hepatic subcellular fractions at pH 7.4. The pH dependence of other known CES1 substrates was also tested in this study. All *in vitro* incubation samples were analyzed using LC-high resolution MS (Vanquish UHPLC coupled with a Fusion Lumos Tribrid Orbitrap mass spectrometer, Thermo Fisher Scientific, Waltham, MA).

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P187 - STRUCTURE-ACTIVITY RELATIONSHIP OF ATORVASTATIN DERIVATIVES FOR METABOLIC ACTIVATION BY HUMAN CARBOXYLESTERASE

Kenta Mizoi¹, Masato Takahashi², Sachiko Sakai², Masami Haba², Masakiyo Hosokawa², and Takuo Ogihara¹

¹Takasaki University of Health and Welfare, Japan, ²Chiba Institute of Science, Japan

[Purpose] Human carboxylesterases (hCESs) play an important role in metabolism of many ester-drugs. The majority of hCESs that have been identified are either hCES1, expressed in the liver and lung, or hCES2, expressed in the small intestine and kidney. The substrate specificities of hCES1 and hCES2 are significantly different. The hCES1 isozymes hydrolyze substrates with a small alkoxy group and a large acyl group. In contrast, the hCES2 isozymes hydrolyze substrates with a large alkoxy group and a small acyl group.1) We undertook a comprehensive study of the structure-activity relationship for metabolic activation of synthesized atorvastatin derivatives (esters, thioesters, amides, and lactone) by hCESs in order to identify candidate prodrugs and obtain basic data for prodrug design. [Method] We synthesized 31 kinds of atorvastatin derivatives using a Fischer esterification reaction, a condensation reaction, or a transesterification reaction.2) The synthesized atorvastatin derivatives were subjected to hydrolysis by hCESs. The amount of metabolic form was analyzed by HPLC. [Results and Discussion] The susceptibility of substrates to hCES1 was greatly affected by steric hindrance. In the case of aliphatic esters, the hydrolysis rate decreased as the ester moiety became more crowded. The hydrolytic activity of hCES2 was influenced by the electron density at the ester: the hydrolysis rates of halogen-containing aliphatic esters were clearly increased compared with those of the corresponding aliphatic esters. There was no significant correlation between hCES1 and hCES2. The result indicates that methyl ester, ethyl ester, allyl ester, and ethyl thioester were selectively hydrolyzed by hCES1, whereas fluorine-containing esters and aromatic esters were hydrolyzed not only by hCES1, but also by hCES2. We found that the susceptibility of these substrates to hCES1 was influenced not only by the size of the acyl group and alkoxy group, but also by the degree of steric crowding around the alkoxy group. On the other hand, the susceptibility to hCES2 increased with a decrease in the electron density around the alkoxy group of the substrate. These findings should be useful in prodrug design for controlling metabolic activation.

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P188 - EFFECTS OF RNA EDITING ON EXPRESSION AND FUNCTION OF HUMAN ALDO-KETO REDUCTASE FAMILY 1 MEMBER C (AKR1C)

Koki Morita, Keito Amai, Masataka Nakano, Tatsuki Fukami, and Miki Nakajima
Kanazawa University, Japan

Adenosine-to-inosine RNA editing, catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes, can affect amino acid sequence, splicing, or microRNA targeting. We previously reported that there is a large interindividual variation (220-fold) in ADAR1 protein expression in the human liver samples, and that ADARs regulate the expression of drug-metabolizing cytochrome P450s. However, it is still unknown whether non-P450s are also regulated by ADARs. In our preliminary study, it was revealed that aldo-keto reductase 1C1, 1C2, 1C3, and 1C4 (AKR1Cs) account for ~45 % of the total expression of carbonyl reductases in the human liver. AKR1Cs catalyze NADPH-dependent reduction of xenobiotics such as an anthracycline anti-cancer drug, doxorubicin (DOX) and an opioid antagonist, naloxone, as well as endobiotics such as progesterone. In the present study, we sought to clarify the possibility that ADARs affect the expression and reductase activity of AKR1Cs. To investigate whether ADARs affect human AKR1Cs expression, ADAR1 or ADAR2 in HepG2 cells were knocked down by transfection of siRNA. The knockdown of ADAR1 resulted in increases in AKR1C1, 2, 3, and 4 mRNA levels, and that of ADAR2 resulted in an increase in AKR1C3 mRNA level. In addition, it was revealed, by Western blotting using an anti-AKR1C1 antibody that recognizes AKR1C1, 2, and 3 proteins, that the knockdown of ADAR1 significantly increased AKR1Cs protein level. These results indicated that ADARs negatively regulate AKR1Cs expression. Consistently, naloxone and DOX reductase activities in HepG2 cells were increased by the knockdown of ADARs. Because the anti-tumor drug DOX is metabolized to doxorubicinol, of which cytotoxicity is 10% of the parent drug, mainly in the liver, our results indicated that ADARs have a role to prolong the anti-tumor effect of DOX by down-regulation of AKR1Cs. By a luciferase assay using a reporter plasmid containing the promoter region of AKR1C1, it was revealed that ADAR1 down-regulates transactivity of AKR1C1, and that the promoter region from -196 to -82 is responsible for the regulation. Collectively, we found that ADAR1 negatively regulates the expression of AKR1Cs by suppressing the transcription of AKR1Cs. This finding strongly supports the fact that the regulation by ADARs is one of the mechanisms affecting hepatic drug metabolism potencies.

P189 - BIOINFORMATIC ANALYSIS OF COMMON NON-SYNONYMOUS SINGLE NUCLEOTIDE VARIANTS IN CYTOCHROME P450 PHARMACOGENES

Cheng Shoong Chong, Vachiranee Limviphuvadh, and Sebastian Maurer-Stroh
A-Star Bioinformatics Institute, Singapore

Common non-synonymous SNVs (nsSNVs) present in the genes of certain members of the cytochrome (CYP) P450 enzyme superfamily are known to cause differential drug metabolism that may result in toxicity or a diminished therapeutic response. Although CYP P450 pharmacogenetics have been intensively investigated, the boom in sequencing data from next generation sequencing technology have resulted in an increase in common novel variants that are yet to be studied. Here, we aim to discover unidentified common nsSNVs that are possibly deleterious to enzyme function by analyzing the exome sequencing data of 60,706 individuals from the Exome Aggregation Consortium (ExAC) database in all CYP P450 genes listed in three pharmacogenomics (PGx) databases, namely the Pharmacogene Variation Consortium (PharmVar), Pharmacogenomics Knowledgebase (PharmGKB) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) repositories. Specifically, for each gene, a list of nsSNVs was extracted from ExAC and upon completion of this step, the lists were consolidated and filtered to identify common missense nsSNVs (>0.5% minor allele frequency globally or ≥1% in a sub-population). To determine if clinical or functional annotations exist for a given common nsSNV, it was searched against the three pharmacogenomics databases. Variant pathogenicity predictors (SIFT Aligned, PolyPhen2 and Evo-D) were then used to analyze the variants' functional consequences on the associated P450 enzymes along with *in silico* 3D structure analysis using Yasara. In total, 8408 nsSNVs in 35 CYP P450 genes from 14 CYP families were obtained. After filtering, 204 common nsSNVs remained and of this, approximately 51% (n=104) have been annotated by at least one pharmacogenomic (PGx) database with 29 variants being clinically actionable (CPIC-listed). For the other 49% (n = 100) of unannotated variants, 77 of them were predicted to be damaging to protein function by at least one predictor. Notably, we found 5 common nsSNVs that are unknown to the three PGx databases in two CYP P450 genes (CYP2D6 and CYP2C19) that are highly involved in drug metabolism. Three of the five were predicted to be deleterious to function by all three tools. It was also observed that two of them (CYP2D6 A449D and CYP2C19 A173V) were found to be respectively enriched in the Latino and South Asian sub-populations with minor allele frequencies of 5.9% and 4.2%. 3D structure analysis suggests that residue 449 is located at the meander region of CYP2D6 while residue 173 is at helix E of CYP2C19. To conclude, we have provided an overview of the current state of knowledge of common nsSNVs in CYP P450 enzymes that are relevant in drug metabolism and have identified previously unknown gene variants that are potentially damaging to protein function. Further biochemical characterization will be needed to determine variant-specific effects of these candidates on enzyme function.

P190 - THE EFFECT OF CYP2C9 AND CYP2C19 GENOTYPE ON THE PHARMACOKINETICS OF PF 04965842, A JAK1 INHIBITOR IN CLINICAL DEVELOPMENT

Martin Dowty, Xin Yang, Jian Lin, Jonathan Bauman, Angela Doran, Theunis Goosen, Linda Wood, Jillian Johnson, Christopher Banfield, Pankaj Gupta, and Bimal Malhotra
Pfizer Inc, USA

Understanding cytochrome P450 (CYP) isoform assignment and phenotypic variation is important in predicting efficacy and safety responses of new chemical entities. PF-04965842 (N-{cis-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)cyclobutyl]propane-1-sulfonamide) is a Janus kinase 1 (JAK1) selective inhibitor in clinical development for atopic dermatitis. *In vitro* phenotyping of PF-04965842 metabolism in human hepatocytes has assigned fraction metabolism (fm) values for CYP2C19 of 0.53, for CYP2C9 of 0.30, for CYP3A4 of 0.11, and for CYP2B6 of 0.07. An ad hoc analysis of the impact of CYP genotype on the clinical pharmacokinetics (PK) of PF-04965842 was subsequently undertaken, with emphasis on the highly polymorphic CYP2C9 and CYP2C19 isoforms. Results to date from more than 100 subjects from 4 phase 1 studies, indicated that PF-04965842 area under the curve (AUC) values in subjects identified as reduced and elevated CYP2C19 and CYP2C9 metabolizers (CYP2C19*1/*2, *2/*3, *2/*2, *1/*17, *2/*17, *17/*17; CYP2C9*1/*2, *1/*3, *3/*3) were within the approximate 4-fold range of AUC values seen in wild-type CYP2C9*1/*1 and CYP2C19*1/*1 patients. Consistent with these clinical results, the *in vitro* intrinsic clearance of PF-04965842 in genotyped dual, poor metabolizer, CYP2C9/2C19 human liver microsomes was approximately 46% of that of extensive metabolizers, indicating a maximal approximate 2-fold variation in AUC based on CYP2C9/2C19 genotype. These results highlight the importance of obtaining genotype information in PK studies of drugs metabolized by highly polymorphic CYP isoforms, and, in the case of PF-04965842, CYP2C9/2C19 genotypes are not expected to be a significant covariate of systemic exposure or therapeutic response.

P191 - THE CYP2D6 'ENHANCER' SNP: LONG-RANGE LINKAGE ANALYSIS AND IMPACT ON ACTIVITY IN HUMAN LIVER TISSUE

Andrea Gaedigk, Erin C. Boone, Jean C. Dinh, Roger Gaedigk, Ryan F. Lata, Robin E. Pearce, Wendy Y. Wang, Neil A. Miller, Vincent S. Staggs, and J. Steven Leeder
Children's Mercy Kansas City, USA

Background: A distant SNP (rs5758550 A>G) located 116kb downstream of the CYP2D6 gene locus has been reported to impact the function of this important drug metabolizing enzyme, leading to a proposal that rs5758550, also known as the CYP2D6 'enhancer-SNP', be incorporated into the Activity Score system to more accurately predict phenotype (Wang et al 2015, HMG 24:1556). We have computationally/statistically phased genotype data including rs5758550 from >3000 subjects and found that rs5758550 is not present on all CYP2D6*2, and is present on subsets of other allelic variants. We also observed substantial differences among ethnicities. To unequivocally establish rs5758550-coding region haplotypes by direct means, rather than statistical analysis, and determine the impact of these long-range haplotypes on activity *in vitro*, we established a digital droplet PCR method called Drop-Phase. Methods: To optimize Drop-Phase, high-molecular weight genomic DNA was isolated with different kits and linkage between rs16947 C>T within CYP2D6 and SNPs at distances of 25kb, 75kb and 116kb (rs5758550) determined using Drop-Phase (adapted from Reagan et al. 2015, PLoS One 4:e0118270). In essence, if both SNPs interrogated in a reaction are linked, i.e. are on the same strand of DNA, a statistically significant amount of droplets are positive for both signals. In addition to blood and tissue samples, a set of Coriell DNAs was tested. Drop-Phase was established and validated on samples previously genotyped including rs16947 and rs5758550. We also phased the 'enhancer-SNP' using publicly available 10X Genomics (www.10xgenomics.com) linked-read data from the Illumina HiSeqX-PGx Cohort (<https://github.com/Illumina/Polaris/wiki/HiSeqX-PGx-Cohort>) as proof-of-concept. CYP2D6 activity using dextromethorphan (DM) and atomoxetine (ATX) as substrates was determined in human liver microsome samples (n=143). Results: Results were most consistent for samples isolated with the MegaLong™ kit (G-Biosciences). Linkage between rs16947, the 25kb and 75kb SNPs and rs5758550 was successfully established for Coriell samples. Subsequent Drop-Phase analysis on selected samples heterozygous for both, rs16947 and rs5758550 allowed us to unequivocally map the enhancer-SNP. While rs5758550 'G' was most commonly found on CYP2D6*2, we confirmed its presence on other haplotypes including CYP2D6*1, *5, *17 and *35 as predicted by computational phasing against >3000 samples. Drop-Phase data for a small set of samples were also consistent with long-range 10X Genomics phasing. Preliminary regression analyses, using computationally phased diplotypes, revealed that the 'enhancer-SNP' accounts only for negligible variability in ATX product formation beyond activity score alone (48% vs. 46%). For DM, inclusion of rs5758550 increased the accounted variability from 7% to 9%. Conclusions: We have established a ddPCR-based method, Drop-Phase, which links SNPs over long distances that cannot be covered by conventional methods. Our preliminary analyses suggest that overall, variability of *in vitro* CYP2D6 activity is not appreciably impacted by rs5758550. However, before definitive conclusions regarding the impact of the 'enhancer-SNP' on CYP2D6 activity can be drawn, analyses must be performed using rs5758550/coding region haplotypes that have been experimentally established. Considering the uncertainty of the impact of the distal 'enhancer-SNP' on CYP2D6

activity *in vitro*, it appears premature to incorporate rs5758550 into the activity score to translate genotype into phenotype to guide drug therapy.

P192 - IDENTIFICATION OF GENETIC DETERMINANTS FOR CENTRAL PAIN SENSITIZATION IN FIBROMYALGIA PATIENTS

Yvonne Gloor¹, Médéric Mouterde², Alain Matthey¹, Estella Poloni², Jocelyne Chabert¹, Aurélien Simona¹, Luc Bovet¹, Marie Besson¹, Valerie Piguet¹, Christine Cedraschi¹, Gisèle Pickering³, Eva Kosek⁴, Georg Ehret¹, and **Jules Desmeules**¹

¹Geneva University Hospitals (HUG), Switzerland, ²University of Geneva, Switzerland, ³University of Clermont Auvergne, France, ⁴Karolinska University Hospital, Sweden

Aims: Fibromyalgia syndrome (FMS) covers a spectrum of chronic pain conditions characterized by widespread pain and increased sensitivity to nociceptive stimulus or tenderness. Central sensitization is thought to be one of the key mechanisms underlying FMS. This process can be described as a loss of the natural balance between transmission of pain stimuli to the CNS and the central pain inhibitory feedback mechanisms. Central pain can be measured using the Nociceptive Flexion Reflex (NFR) or RIII threshold. While familial aggregation could suggest a potential genetic component in FMS development, isolation of genetic determinants has proven difficult due to the multi-factorial nature and complexity of the syndrome.

Methods: We used a customized Infinium CoreExome-24 BeadChip from Illumina to genotype 555'356 human genetic polymorphisms in 284 FMS volunteers and searched for genetic determinants of lowered NFR threshold. The NFR value was corrected for medication while FM diagnosis, age, gender and cohort of origin were used as co-variables.

Results: All samples call rates exceeded 99 % and the genotype completeness exceeded 99% in 97.8% of the SNPs. Following quality control, 98.1% of the SNPs were cleared for further bioinformatics analysis using the nociceptive flexion reflex (NFR) as a primary stratification determinant.

Conclusions: In summary, the genome-wide characterization of our cohort will be used to confirm suspected genetic predisposition and identify new genetic determinants of FMS based on objective measurements of central sensitization. Further bioinformatics analysis will focus on candidate gene approaches as well as subsets of neurological expressed SNPs. Follow-up work combining genomic, transcriptomic and proteomic techniques will aim to gain a better understanding of the various patho-physiological mechanisms underlying the disease and allow the development of optimized personalized treatments for FMS patients. International collaborations will allow replication of our findings in two independent cohorts.

Finally, increasing the discrimination between different mechanisms underlying FMS will allow a better adjustment of personalized treatments.

P193 - PERSISTENT REGULATION OF HEPATIC NON-CODING RNAs FOLLOWING NEONATAL EXPOSURE TO BPA, BDE-99, AND PCBs

Joe Lim¹, Joseph Dempsey¹, Hans-Joachim Lehmler², James MacDonald¹, Theo Bammler¹, Terrance Kavanagh¹, and Julia Yue Cui¹

¹University of Washington, USA, ²University of Iowa, USA

Persistent environmental contaminants such as bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are commonly found in the environment and associated with many human diseases. It is increasingly recognized that there is a sensitive developmental time window during which exposures to toxicants may have a life-long impact on disease risk. There is growing evidence that non-coding RNAs (ncRNAs) such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) are involved in the regulation of many protein-coding genes (PCGs) as well as other distinct functions, such as histone modifications. The liver is an essential organ for xenobiotic metabolism and nutrient homeostasis, and altered liver function may result in increased susceptibility to diseases such as fatty liver disease and cancer. Here, we hypothesized that neonatal exposure to these environmental contaminants persistently dysregulates ncRNAs which may contribute to the persistent dysregulation of PCGs. Two-day-old male and female C57BL/6 mice were supralingually exposed to corn oil or BDE-99 (57mg/kg), or the Fox River Mixture (PCB mixture, 30mg/kg), once daily for three days. Ribosomal RNA depleted whole transcriptome RNA-Seq and small RNA-Seq were conducted of livers in 5- and 60- day-old mice. Whole transcriptome RNA-Seq data were analyzed using Hisat2 and Cufflinks, and small RNA-Seq data were analyzed using STAR and edgeR. Differentially expressed (DE) lncRNAs were paired with DE PCGs, and the degree of base-pair fit was analyzed using LncTar. Differentially expressed miRNA data were linked to putative PCG targets using miTarBase. 111 (male) and 51 (female) lncRNAs were differentially regulated in adulthood following neonatal exposure to BPA, BDE-99, and PCBs. The expression of lncRNA H19 was persistently up-regulated by BPA and BDE-99. Within the imprinted Dlk1-Dio3 cluster, which expresses genes in a parent-specific manner and is known to promote liver tumors, BPA and BDE-99 persistently increased the maternal allele-specific lncRNAs Meg3 and Rian. However, PCGs from the paternal allele (Dlk1, Rtl1, and Dio3) remained lowly expressed. Examples of differentially expressed miRNAs in the Dlk1-Dio3 cluster include Mir493, 370, 154, 485, and 541 which are

associated with tumorigenesis. Gene regulatory networks, predicted targets of the miRNAs, and the degree of base-pair fit between lncRNAs and PCGs among the chemical-exposed groups showed potential interactions between the dysregulated non-coding RNAs and PCGs. Enrichment of the predicted targets included drug and lipid metabolism, as well as cell cycle. Our results demonstrate that neonatal exposure to these environmental toxicants persistently dysregulates the liver transcriptome in adulthood, possibly due to liver epigenome reprogramming as well as dysregulation of non-coding RNAs.

P194 - LIGAND-FREE ESTROGEN RECEPTOR ALPHA (ESR1) AS MASTER REGULATOR FOR THE EXPRESSION OF CYP3A4 AND OTHER CYTOCHROME P450S (CYP) IN HUMAN LIVER

Danxin Wang¹, Rong Lu², Grzegorz Rempala³, and Wolfgang Sadee³

¹University of Florida, USA, ²University of Texas Southwestern Medical Center, USA, ³The Ohio State University, USA

CYP3A4 is the predominant drug metabolizing enzyme in the liver, displaying large inter-person variability of unknown causes. CYP3A4 transcription is controlled by hepatic transcription factors (TFs), but how TFs dynamically interact remains uncertain. We hypothesize that several TFs form a regulatory network with nonlinear, dynamic, and hierarchical interactions. To resolve complex interactions, we have applied a computational approach for estimating Sobol's Sensitivity Indices (SSI) under generalized linear models, to existing liver RNA expression microarray data (GSE9588) and RNAseq data from GTEx, generating robust importance ranking of TF effects and interactions. The SSI based analysis identified TFs and interacting TF pairs, triplets, and quadruplets involved in CYP3A4 expression. In addition to known CYP3A4 TFs, ESR1 emerges as key TF with the strongest main effect and as the most frequently included TF interacting partner. Model predictions were validated using siRNA/shRNA gene knockdown and CRISPR-mediated transcriptional activation of ESR1 in biliary epithelial Huh7 cells and human hepatocytes in the absence of estrogen. Moreover, ESR1 and known CYP3A4 TFs mutually regulate each other. Detectable in both male and female hepatocytes without added estrogen, the results demonstrate a role for unliganded ESR1 in CYP3A4 expression, consistent with unliganded ESR1 signaling reported in other cell types. Added estrogen further enhances ESR1 effects. We propose a hierarchical regulatory network for CYP3A4 expression, directed by ESR1 through self-regulation, cross regulation, and TF-TF interactions. We also demonstrate that ESR1 regulates the expression of other CYP enzymes, supporting broad influence of ESR1 on xenobiotics metabolism in human liver.

P195 - A NOVEL LC-MS/MS METHOD FOR PIVKAI1 QUANTIFICATION: CHARACTERIZATION OF WARFARIN INDUCED ALTERED PROTHROMBIN DES-CARBOXYLATION

Abdul Basit¹, Joanne K. Estergreen¹, Daniel E. Sabath¹, David Veenstra¹, Scarlett Hopkins², Bert Boyer², Allan Rettie¹, Kenneth Thummel¹, and Bhagwat Prasad¹

¹University of Washington, USA, ²Oregon Health & Science University, USA

Background and Objectives: Warfarin is a narrow therapeutic index anticoagulant drug and its use is associated with significant bleeding adverse events. Currently, the international normalized ratio (INR) is the most commonly used biomarker to monitor warfarin efficacy and adverse effects. However, because INR is a functional assay, it is prone to technical variability due to effect of method of blood collection, temperature, and sample handling time. Moreover, there is a time lag between warfarin dose and the initiation of the therapeutic response, depending on the half-life of coagulation factors. Warfarin inhibits γ -carboxylation of key clotting factors resulting in the build-up of des-carboxy prothrombin (also referred to as PIVKAI1) in the plasma. Indeed, PIVKAI1 has been suggested as a potential serological biomarker of warfarin efficacy (1). The conventional assay for measuring PIVKAI1 is ELISA-based and requires the use of a monoclonal antibody. In addition, the available ELISA assay does not identify the position and the number of des-carboxylation sites in prothrombin (2). Here we report a targeted proteomics-based LC-MS/MS assay for the simultaneous quantification of PIVKAI1 and active prothrombin in human plasma. The method was validated and applied to predict warfarin induced changes in INR.

Methods: Surrogate peptides for the quantitative analysis of carboxy and des-carboxy prothrombin were selected using an *in silico* approach. A precise and cost-effective plasma albumin depletion method utilizing 0.1% trifluoroacetic acid in isopropanol was optimized. The extracted proteins were reduced, alkylated and digested by trypsin. A robust LC-MS/MS assay was developed and validated. Finally, carboxy and des-carboxy peptides were quantified in 10 μ L plasma from control (n=12), low dietary vitamin K (n=12), and warfarin (n=12) treated individuals. Data were acquired using Analyst 1.6 and analyzed using Skyline software. **Results:** Prothrombin recovery from plasma was 95%. The LC-MS/MS assay was linear ($r^2 = 0.98$) with a dynamic range of 10 – 1000 ng/ μ L. The assay inter-day precision was within 10%. The surrogate peptides representing total prothrombin (SGIE[C]QLWR, TATSEYQTFNPR, and ELLESTIDGR) and carboxy prothrombin (ANTFL[E][E]VRK) were negatively correlated with INR in the warfarin treated samples ($r = 0.51$ and $r = 0.77$, respectively, $p < 0.0001$). Consistent with this, descarboxy prothrombin peptides (GNLER and ANTFLEEVK) positively correlated ($r = 0.75$, $r = 0.69$ respectively, $p < 0.01$) with the INR values. A strong negative correlation ($r = 0.99$ and $r = 0.78$, $p < 0.0001$) was observed with carboxy peptide (ANTFL[E][E]VRK) and descarboxy peptides (GNLER and ANTFLEEVK). A statistically significant reduction in total prothrombin and carboxy prothrombin ($p < 0.001$) was observed in the warfarin

treated subjects as compared to the control and the vitamin K deficient subjects. Conclusion: To the best of our knowledge, this is the first cost-effective LC-MS/MS assay for the simultaneous quantification of total, carboxy and des-carboxy prothrombin in plasma. Carboxy (ANTFL[E][E]VRK) and des-carboxy peptides (GNLER/ANTFLEEVRK) of prothrombin may serve as novel biomarkers of vitamin K status to facilitate safe and effective use of warfarin during oral anticoagulant therapy.

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P196 - INVESTIGATING THE TISSUE DISTRIBUTION OF [14C]-PRUCALOPRIDE FOLLOWING A SINGLE ORAL DOSE IN RATSDevin Welty and Zhen Lou*Takeda Pharmaceutical Company Limited, USA*

Prucalopride is a selective, high-affinity agonist of the 5-hydroxytryptamine-4 (5-HT₄) receptor and is indicated for the treatment of adult patients with chronic constipation. In humans, prucalopride is rapidly absorbed and extensively distributed, reaching the maximum plasma concentration 2-3h after a single 2mg oral dose. Three studies investigated the tissue distribution of prucalopride in rats after a single oral dose. ¹⁴C-labelled prucalopride at 5mg base-equivalent(eq.)/kg was administered to male (n=5) and female (non-pregnant rats [seven groups of 3] and pregnant rats [n=4; dose administered on day 18 of gestation]) SPF Wistar rats. Total radioactivity (TR) in various tissues was measured over time (up to 96h). In female rats, the area under the concentration curve for up to 0-8h (AUC_{0-8h}) for TR was determined and the AUC_{0-8h} tissue:plasma ratio calculated. Additionally, the mean tissue concentration of unchanged drug (UD) was measured over time (up to 48h). The highest mean TR in male, non-pregnant female and pregnant female rats was detected in the pancreas (8.9µg-eq./g within 30 min of dosing; below the limit of detection [LOD] after 24h), the liver (12.5µg-eq./g within 20 min of dosing; 0.3µg-eq./g after 96h) and the small intestine (15.6µg-eq./g within 30 min of dosing; below the LOD after 24h), respectively. The highest mean concentration of UD in non-pregnant female rats was detected in the small intestine (10.1µg-eq./g within 20 min of dosing; below the LOD after 24h). UD was not measured in male or pregnant female rats. The lowest mean TR was detected in the brain of male (0.12µg-eq./g within 30 min of dosing), non-pregnant female (0.21µg-eq./g within 1h of dosing) and pregnant female rats (0.21µg-eq./g within 30 min of dosing), respectively. TR in the brain was below the LOD after 2, 24 and 8h of dosing, respectively. In non-pregnant female rats, the highest AUC_{0-8h} and AUC_{0-8h} tissue:plasma ratios of TR were observed in the small intestine (75.3 and 31.3, respectively); the lowest values were observed in the brain (0.85 and 0.35, respectively). Findings were similar for UD. In pregnant female rats the highest AUC_{0-8h} and AUC_{0-8h} tissue:plasma ratios of TR were observed in rectal (mucosa) and salivary gland tissue (122 and 20.5, respectively for both tissues); lowest values were observed in the brain (0.33 and 0.17 [AUC_{0-2h}], respectively). AUC was not calculated for male rats. Taken together, these data show that high concentrations of a single oral dose of prucalopride were detected in the pancreas, liver and small intestine and that very low concentrations were detected in the brain (~0.05% of the dose in non-pregnant female rats). In addition, mean concentrations of TR and UD in the brain were below the LOD within 24h of dosing, further suggesting that prucalopride is not unduly retained in this tissue.

P197 - ABSTRACT WITHDRAWN**P198 - ABSTRACT WITHDRAWN****P199 - HILIC UPLC-MS/MS METHODS FOR THE ASSESSMENT OF HYDROXYNORKETAMINES PHARMACOKINETICS IN MOUSE PLASMA AND BRAIN**Amy Q. Wang¹, Kylie M. Konrath¹, Jaclyn N. Highland², Lacey M. Riggs², Patrick J. Morris¹, Craig Thomas¹, Todd D. Gould², and Xin Xu¹¹National Institutes of Health, USA, ²University of Maryland School of Medicine, USA

(R,S)-ketamine (ketamine) exerts rapid antidepressant effects in treatment-resistant patients when administered at subanesthetic doses. However, its use is limited by its dissociative side effects and abuse potential. Ketamine undergoes extensive metabolism to norketamine, hydroxynorketamines (HNKs) and dehydronorketamine. HNKs are formed through the hydroxylation of the cyclohexyl ring of norketamine at various locations. In animal models, (2R,6R)-HNK and exerts antidepressant-like behavioral effects without the sensory dissociation, ataxia, and abuse liability of ketamine (1-2). It is possible that additional HNKs contribute to ketamine's antidepressant-like effects, potentially with even greater potency or a more favorable pharmacokinetics (PK) profile than (2R,6R)-HNK. The objective of this study is to develop accurate, sensitive and selective UPLC-MS/MS methods and assess PK and brain exposure of HNKs in mice. The quantitative bioanalysis of HNKs in biological matrices has several challenges, including hydrophilicity, low molecular weight, and

endogenous interference. With these challenges in mind, HILIC UPLC-MS/MS methods for the quantification of HNKs in plasma and brain samples have been developed. The developed methods have a linear calibration range of 5.0 - 5000 ng/mL using protein precipitation sample preparation in 96-well plates. The optimized HILIC UPLC-MS/MS methods provided good chromatographic retention for HNKs, separated analyte from the endogenous interference, reduced the sample preparation and analysis time, and increased sample throughput. The peak width was 2 sec with 2 min gradient. The developed HILIC UPLC-MS/MS methods were utilized in preclinical PK/PD studies to assess PK and brain distribution of HNKs in healthy male and female CD-1 mice. HNKs readily crossed the blood-brain barrier, with brain to plasma AUC ratios between 0.7-1.3. HNKs had elimination half-lives ranging from 0.5-0.9 hr in the plasma and 0.3-0.8 hr in the brain.

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P200 - EVALUATION OF LEVORNIDAZOLE PHOSPHATE PHARMACOKINETICS AND PHARMACODYNAMICS FOR EARLY DOSING OPTIMIZATION

Minji Wei and Pu Zhang

Institute of Clinical Pharmacology, First Hospital, Peking University, China

Levornidazole, the levo isomer of ornidazole, has shown anti-anaerobic activity similar to ornidazole but with less central neurotoxicity. levornidazole phosphate, a prodrug of levornidazole, is developed mainly to improve the solubility of levornidazole in the injection solution. Pharmacokinetic studies in healthy volunteers show the transformation is quick and the pharmacokinetic parameters are comparable to that of levornidazole. However, there is discrepancy among countries regarding the infusion time. The purpose of this study is to evaluate the optimal infusion time recommended for further clinical studies. Methods: Pharmacokinetic (PK) parameters from two phase I studies in healthy volunteers (24 volunteers) were estimated using compartment analysis. The pharmacodynamic (PD) target was a ratio of 15 between the unbound peak concentration (fC_{max}) over the minimal inhibitory concentration (MIC) of the liable bacteria. Based on the mean and SD of PK parameters, we simulated several dosing regimens by Monte Carlo method and analyzed the fC_{max}/MIC ratio for MICs from 0.125-4mg/L. Results: A two-compartment model was used. Regarding the probability to achieve a fC_{max}/MIC of greater than 15, the 1g (based on levornidazole) iv for 20min regimen was optimal to achieve the PK/PD target in 90% of patients for a MIC of 1mg/L, which is the MIC₉₀ of levornidazole against most anaerobes. Recently, an exploratory clinical study in patients with pelvic inflammatory disease was used to compare the efficacy of two levornidazole phosphate dosing groups (2g daily, 1g every 12h) and one active control(levornidazole 1g every 12h). In this trial, all the iv time was 30-60min. Clinical efficacy rates for the three groups were 91.11%, 86.36% and 80.85%, respectively but with MIC₉₀ less than 0.8mg/L, Conclusions: Simulation results favor daily dosing and with a short infusion time.

P201 - SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS EFFECTS OF MULTIPLE-DOSE ADMINISTRATION OF FOTAGLIPTIN BENZOATE-DIPEPTIDYL PEPTIDASE-4 INHIBITOR IN TYPE 2 DIABETES MELLITUS

Min Wu¹, Ji Chen², Haigang Sun², Hong Zhang¹, Xiaojiao Li¹, Yanhua Ding¹, and Cuiyun Li¹

¹The First Hospital of Jilin University, China, ²Shenzhen Salubris Pharmaceuticals Co.,Ltd, China

Dipeptidyl peptidase-4 (DPP-4) inhibitors are used for the treatment of type 2 diabetes mellitus (T2DM) with a new action mechanism, which are secreted by the digestive tract along with dietary intake to stimulate insulin secretion. Fotagliptin benzoate, a novel DPP-4 inhibitor which was developed by Salubris Pharmaceuticals Co. Ltd. (Shenzhen, China) using the strategy of structure-based drug design, is under clinical development for the treatment of T2DM. This study assessed the pharmacokinetics (PK), pharmacodynamics (PD) and tolerability of fotagliptin in Chinese patients with T2DM. In this randomized, double-blind, placebo-controlled study, 14 patients with T2DM were assigned to receive a single oral dose of fotagliptin 24mg or placebo (10:4 ratio) once daily for 14 days. PK and PD profiles were assessed on days 1 and 14. Tolerability assessments were conducted throughout the study. Fotagliptin was rapidly absorbed, and median T_{max} values were about 1.5 hours after both single- and multiple-dose. Half-life (t_{1/2}) of fotagliptin was estimated to be 27.36~45.13h hours at steady-state, and is potentially suitable for a dosing interval of one day or longer. Plasma fotagliptin concentration appeared to be maintained at a steady state after 12 day doses. The AUC_{0-t} of fotagliptin and its metabolite (M1 & M2-1) over 144 h post-dose were 803.46±71.25, 105.05±26.87, and 61.67±45.14 ng•h/ml, respectively,

C_{max} were 103.022±20.19, 5.84±1.34, 4.81±3.46 ng/ml, respectively. The accumulation rate ranges of fotaliptin and its metabolite (M1 & M2-1) for AUC were 1.02~1.31, 1.08~1.91 and 1.05~1.77, respectively. The Max DPP-4 inhibition effect of fotaliptin occurred at 1hr after dosing, reaching more than 95% inhibition, and gradually declined to at least 60% at 96 hrs. The time of duration for the rates of DPP-4 inhibition more than 80% were 23.50h and 24.0h on Day1 and Day 14, respectively. The geometric mean point estimates for inhibition of DPP-4 activity (AUEC_{0-24h}) following the first dose and last dose with T2DM groups were higher than that of placebo groups (P<0.001). The concentrations of GLP-1 were higher on day 1 and 14 than those at the corresponding time point of baseline (day-1) and the increment was higher than that of the placebo group. AUEC_{0-24h} for rates of DPP-4 inhibition was significantly correlated with AUC_{0-24h}. The mean fasting plasma glucose level at baseline was similar to that on Day 1 and Day 14 before dose. The adverse events were mostly mild or moderate in intensity. There were no hypoglycemia episodes, serious adverse event, or discontinuation due to adverse event. Fotaliptin exhibited favorable pharmacokinetics profiles in Chinese patients with T2DM, and was well tolerated. Treatment of fotaliptin can achieve high inhibition of DPP-4 and increased plasma GLP-1 concentration.

P202 - POPULATION PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF EPTIFIBATIDE IN THE INHIBITION OF PLATELET AGGREGATION IN HEALTHY CHINESE SUBJECTS

Qinmei Li, **Hong Zhang**, Min Wu, Jingrui Liu, Cuiyun Li, and Yanhua Ding
The First Hospital of Jilin University, China

Hypothesis: Glycoprotein (GP) IIb-IIIa inhibitors could prevent platelet aggregation. Eptifibatide, a GP IIb-IIIa inhibitor, has been safely and successfully used for the treatment of non-ST-segment elevation acute coronary syndromes and in patients undergoing percutaneous coronary Intervention. However, eptifibatide has not been widely used in Chinese patients, and to date, the dose- response relationship has not been systematically evaluated for the proper selection of dose regimens. Objective: The present study aimed to evaluate the pharmacokinetic (PK) properties and its antiplatelet aggregation activity (pharmacodynamics, PD) of eptifibatide in healthy Chinese subjects. Methods: Eptifibatide (180 µg/kg) was administered by two bolus injections 10 min apart, followed by a 2.0 µg/kg/min infusion to 24 h from the Caucasian label. The eptifibatide PK and antiplatelet aggregation activities were evaluated using nonlinear mixed-effects modeling and noncompartmental analysis. Safety assessments included adverse events, hematology, and biochemistry tests. Results: Twelve Chinese healthy subjects were enrolled and completed the study. Steady-state concentrations were achieved at 0.5 to 24 h post-dose. The mean t_{1/2} was 148.19 min. The effective inhibition of platelet aggregation occurred at 3 min after starting dosing to 4h after infusion termination. Eptifibatide concentrations and the antiplatelet aggregation activities were fitted with a three-compartment model linked with an E_{max} model. The typical clearance and IC₅₀ values were 0.111 L/min and 155 ng/mL, which were higher and lower than those at Caucasian, respectively. No significant covariates were found. If only the PK result and the target concentration of 1650 ng/mL to achieve 80% platelet GP IIb/IIIa receptor occupancy were considered, an increase of the dosage is recommended. If PD results (less than 20% platelet aggregation rate occurred at 3 min after starting dosing to 4 h after infusion termination in this study) were considered, the dosage of eptifibatide from the Caucasian label should not be changed. Three mild adverse events were detected in the study. Conclusions: Eptifibatide displays high sensitivity and excellent tolerability in healthy Chinese subjects. The dosage of eptifibatide from the Caucasian label can effectively inhibit platelet aggregation.

P203 - MATHEMATICAL PHARMACOKINETIC MODELING TO INTERPRET METABOLIC PATHWAY OF DECURSIN, DECURSINOL ANGELATE AND DECURSINOL

Go-Wun Choi¹, Yong-Bok Lee², and **Hea-Young Cho**¹

¹CHA University, South Korea, ²Chonnam National University, South Korea

Angelica gigas Nakai (AGN) has active pharmaceutical ingredients of decursin (D), decursinol angelate (DA), and their metabolite of decursinol (DOH). D and DA are known as isomers and they are rapidly metabolized to DOH by liver microsomes. As major active components of AGN, they possess pharmacological and biochemical activities both *in vitro* and *in vivo*, including anti-cancer and neuroprotective activities. Although numerous pharmacology and pharmacokinetics (PKs) works of AGN have been reported, there are no demonstrations to integrate these findings of absorption, distribution, metabolism, and excretion properties of AGN components. In this study, prior knowledge of metabolism is incorporated into mathematical modeling for deepen understanding of PKs of D, DA, and DOH. Healthy male Koreans participated in this clinical study and were given AGN root extract powder contained 0.055 mg of D, 0.184 mg of DA. Plasma concentration of D, DA, and DOH was simultaneously determined using UHPLC-MS/MS. PK analysis was conducted by non-linear mixed effect modeling software using Winnolin® ver 8.0. A model structure was built by two steps approach. Firstly, the plasma-time data of D and DA were fitted into two compartment model and then their metabolite profile was added with accepting prior parameters. The model structure included clearance (CL), inter-compartmental clearance (Q), volume of distribution of central (V) and peripheral (V₂) compartment, absorption rate constant (K_a), and fraction metabolized to DOH (F_m). The model evaluation was performed using objective function value, goodness of fit plots, and parameter accuracy. Total clearance of D (CLD) and DA (CLDA) were 3.17 and 5.42 L/hr, respectively. The hepatic elimination was calculated with a product of total clearance and fraction of metabolized from D

(Fm, D) and DA (Fm, DA) to DOH with values of 0.73 and 0.16. These results are consistent with previously reported data of *in vitro* microsome study which is a large portion of DOH is converted from D than DA. In conclusion, the developed population PK model quantitatively analyzed the disposition of D, DA, and DOH. Especially, key elimination pathway of D and DA was investigated. These findings could be helpful to provide scientific evidence for further development and translational study of D, DA, or DOH as a potential therapeutic agent.

P204 - PHARMACOKINETICS FOR BIOACTIVE COMPOUNDS AFTER ORAL ADMINISTRATION OF SOSIHOTANG SOFT-EXTRACT TO HUMANS

Eun-Jeong Choi¹, Young-Dal Kwon², Yong-Bok Lee³, and **Hea-Young Cho**¹

¹CHA University, South Korea, ²Wonkwang University Gwangju Medical Center, South Korea, ³Chonnam National University, South Korea

Sosihotang is a traditional herbal medicine used to treat various fever and inflammatory diseases. Recently, it was developed into soft-extract to increase patient compliance and is covered by Korean national health insurance. The purposes of this study were to develop determination of baicalin, baicalein and 18 β -glycyrrhetic acid as a metabolite of glycyrrhizin in human plasma using UHPLC-MS/MS and to evaluate the pharmacokinetics (PKs) of these compounds after oral administration of Sosihotang soft extract in humans. Chromatography separation was performed using a Kinetex C18 column (2.1 x 50 mm, 1.7 μ m), and mobile phase was consisted of 0.1% formic acid in water and acetonitrile using gradient elution. The compounds were detected using MRM mode with positive electrospray ionization, and they were extracted by liquid-liquid extraction using methanol and acetonitrile. 12 healthy Korean subjects were employed in a randomized and open-labeled clinical study. The study protocol was approved by the Institutional Review Board of the Wonkwang Oriental Medicine Hospital, Gwangju, Korea (<https://cris.nih.go.kr> No. KCT0003164). Blood samples were collected up to 48 h after administration. The PK parameters such as elimination half-life ($t_{1/2}$), clearance (CL/F) and volume of distribution (Vd/F) were evaluated by non-compartmental and compartmental analysis using WinNonlin® software (version 8.0, Pharsight®, a Certara™ Company). The linear calibration curves were fitted over the concentration ranges of 1-500 ng/mL for 18 β -glycyrrhetic acid, and 0.5-50 ng/mL for baicalin and baicalein, with correlation coefficients greater than 0.994. The inter- and intra-day precision was within 13.64%, and accuracy was between 95.17-110.92% for all compounds. Since glycyrrhizin was mostly metabolized into 18 β -glycyrrhetic acid after absorption, only PKs of 18 β -glycyrrhetic acid was evaluated. The PK model of 18 β -glycyrrhetic acid was well described with the one-compartment model. Baicalin and its metabolite, baicalein, had double site absorption kinetics, which suggested that baicalin and baicalein undergo enterohepatic circulation after they were orally administered to human. The $t_{1/2}$, CL/F and V/F were 8.39 h, 12.22 L/h and 135.18 L for 18 β -glycyrrhetic acid, 9.05 h, 149.65 L/h and 458.97 L for baicalin, and 7.41 h, 73.36 L/h and 1025.01 L for baicalein. In this study, analytical methods using UHPLC-MS/MS were developed and validated for 18 β -glycyrrhetic acid, baicalin and baicalein in human plasma. This study was the first study to evaluate the PKs of three compounds after oral dose of Sosihotang soft extract in Koreans.

P205 - DETERMINATION OF PERMEABILITY ACROSS CACO-2 CELL MONOLAYER AND DEVELOPMENT OF PREDICTABLE EQUATIONS FOR ORAL ABSORBABILITY OF GENERAL CHEMICAL SUBSTANCES

Yusuke Kamiya, Hiroka Takaku, Rio Yamada, and Hiroshi Yamazaki

Showa Pharmaceutical University, Japan

General toxicities of chemical substances have been extensively investigated by repeated doses in rodents after oral administration defined by Organisation for Economic Co-operation and Development (OECD) test guidelines. However, pharmacokinetics of chemicals including absorption after oral administration or internal exposures was not considered in toxicological evaluation. If quantitative prediction of intestinal absorbability of the targeted chemicals could be available, it might help to reduce the time, cost, and numbers of experimental animals. In this study, to estimate oral absorbability for general chemicals, prediction equations were constructed for the permeability coefficients of various compounds experimentally determined on the Caco-2 cell system, which was used widely for *in vitro* assessment of intestinal absorption in the pharmaceutical medicines. Various compounds including drugs, medicines and chemical substances (over 50) showed a wide range of permeability coefficients across the Caco-2 cell sheets. Predicting equations were set up by multiple regression analysis methods using the determined permeability coefficients and estimated physicochemical properties. The predicted permeability coefficients values obtained from the constructed predicting equations showed a high correlation with the measured values ($r \geq 0.77$, $n=56$, $p < 0.001$) under the present conditions. As far as investigated in this study, the predicted values of 15 out of 21 external compounds were close to the measured values within three-fold errors and correlated with measured values ($r \geq 0.69$, $n=21$, $p < 0.001$). These results suggest that proposed multiple regression equations using the physicochemical properties of compounds could predict the permeability coefficients across the Caco-2 cell sheets and also oral absorbability *in vivo* and that are expected to be important information for predicting pharmacokinetics after oral administration of unknown chemical substances and drug candidates.

P206 - PHARMACOKINETIC STUDIES OF ASARININ, β -EUDESMOL, AND WOGONIN IN RATS USING A VALIDATED SIMULTANEOUS UPLC-MS/MS METHODYong-Bok Lee¹, Hea-Young Cho², Seung-Hyun Jeong¹, and Ji-Hun Jang¹¹Chonnam National University, South Korea, ²CHA University, South Korea

The purpose of this study was to develop a method for simultaneous analysis of asarinin, β -eudesmol, and wogonin, which are representative pharmacological components of *Asarum heterotropoides*, *Atractylodes lancea*, and *Scutellaria baicalensis*, respectively, in rat plasma and urine using UPLC-MS/MS. Asarinin, β -eudesmol, and wogonin have anti-angiogenic activity in common [1]. Therefore, they have the potential to be clinically used for cancer cell death and inhibition of metastasis [2]. In addition, they are important ingredients that are frequently combined with other herbal medicines [1, 2]. Although there have been a few reports of single pharmacokinetic (PK) for each of asarinin, β -eudesmol, and wogonin, no *in vivo* PK studies have been reported with simultaneous administration of asarinin, β -eudesmol, and wogonin. Therefore, we thought it necessary to study *in vivo* PK of these compounds. The three components were separated using 5 mM aqueous ammonium acetate containing 0.1% formic acid and acetonitrile as a mobile phase by gradient elution at a flow rate of 0.3 mL/min, equipped with a KINETEX core-shell C18 column. Quantitation of this analysis was performed on a triple quadrupole mass spectrometer employing electrospray ionization technique, operating in multiple reaction monitoring mode. The chromatograms showed high resolution, sensitivity, and selectivity with no interference with plasma and urine constituents. In all analytes, both the intra- and inter-day precisions (CV%) were less than 7.76%. The accuracy was 97.66-107.39% for asarinin, 97.24-102.44% for β -eudesmol, and 97.37-102.86% for wogonin. The developed analytical method satisfied the criteria of international guidance and could be successfully applied to the PK studies including oral bioavailability of asarinin, β -eudesmol, and wogonin after oral and intravenous administration and their urinary excretion rate in oral administration to rats. The results showed that asarinin, β -eudesmol, and wogonin were absorbed rapidly after the oral administration. The T_{max} of oral administration was 2.40 \pm 0.89 h, 0.50 \pm 0.00 h, and 0.40 \pm 0.22 h for asarinin, β -eudesmol, and wogonin, respectively. In addition, the results showed that asarinin, β -eudesmol, and wogonin were eliminated quickly after the IV administration. The t_{1/2} of IV administration was 4.42 \pm 0.31 h, 5.87 \pm 0.62 h, and 5.07 \pm 0.64 h for asarinin, β -eudesmol, and wogonin, respectively. Most of the three compounds were excreted within 12-24 h after oral administration. After oral administration, 0.93 \pm 0.12, 0.96 \pm 0.13, and 6.17 \pm 0.43% of the dose were recovered in rat urine within 36 h, of which unchanged asarinin, β -eudesmol, and wogonin, respectively. Our results in this study are expected to be useful for future related studies such as quantification of concentration in biological samples after administration of the formulation (such as decoction or tablet) or interactions between the components.

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P207 - ASSESSING THE ACCURACY OF PREDICTED HUMAN PHARMACOKINETICS FOR CANDIDATE DRUGSDermot McGinnity¹, Ken Grime², Rasmus Jansson-Lofmark², Rhys Jones¹, Adrian Fretland³, Susanne Winiwarer², Michael Davies¹, and Paul Morgan¹¹AstraZeneca, United Kingdom, ²AstraZeneca, Sweden, ³AstraZeneca, USA

Prior to the first human dose of a novel candidate drug, the pharmacokinetics (PK) of the drug in human are predicted from pre-clinical data, to determine whether the disposition characteristics of the drug correspond to an acceptable risk profile for future development. Complementary pre-clinical prediction methods for the key PK parameters are used; these include estimating the rate of human liver metabolic clearance by scaling from *in vitro* metabolic stability assays, and estimating human volume of distribution from pre-clinical species. The predicted primary PK parameters describing absorption, distribution, metabolism and excretion are integrated using physiologically-based PK modelling to provide a simulated human PK profile and secondary PK metrics such as half-life, and maximum concentration (C_{max}) and area under the curve (AUC) for a given dose. Once clinical PK data are available, the observed human PK are compared against the simulations, providing an opportunity to assess and refine the prediction methods. AstraZeneca have built upon previous analyses that focussed on quantitative comparison of predicted vs observed secondary PK parameters (e.g. Poulin et al (2011), *JPharmSci* 100, 4127), by also including a qualitative evaluation of the impact of the PK predictions on the development of the candidate drug. The combined quantitative plus qualitative assessment was classed as successful (green) if the majority of PK parameters were within 2-fold of predicted; or as unsuccessful (red) if a prediction was >5-fold from the observed value, or led to significant delay/stop in development. We have reviewed the predictions of 117 AstraZeneca compounds that were first dosed in the clinic between 2000 and 2018. Overall, most predictions were accurate, with 64% of the compounds rated green, and there was no bias in the predicted PK parameters towards an under- or over-prediction. Prior to 2011, 58% of drug PK predictions were rated green; since 2011, 83% have been rated green, demonstrating the improvement of methods over time. Analysis of data subsets split by

chemical class shows that predictions are most accurate for basic and neutral compounds, but can be challenging for acidic compounds. The increased accuracy of PK predictions in recent years has been achieved despite an increasing level of chemical diversity, with new modalities joining small molecule drugs in the development portfolio.

P208 - GENERATION OF HIGH-PURITY HUMAN IPS CELL-DERIVED INTESTINAL EPITHELIAL CELLS FOR PHARMACEUTICAL RESEARCH

Kazuo Takayama¹, Ryosuke Negoro¹, Takanori Mori², Yoshiyuki Yamaura², and Hiroyuki Mizuguchi¹

¹Graduate School of Pharmaceutical Sciences, Osaka University, Japan, ²Ono Pharmaceutical Co., Ltd, Japan

To develop an effective and safe orally administered drug, it is important to predict its intestinal absorption rate, intestinal first-pass effect, and drug-drug interactions of orally administered drugs. However, there is no existing model to comprehensively predict the intestinal pharmacokinetics and drug-response of orally administered drugs. In this study, we attempted to generate homogenous and functional intestinal epithelial cells from human induced pluripotent stem (iPS) cells for pharmaceutical research. We generated almost-homogenous Villin- and zonula occludens-1 (ZO1)-positive intestinal epithelial cells by adenovirus vector-mediated forkhead box A2 (FOXA2) and caudal-related homeobox transcription factor 2 (CDX2) transduction into human iPS cell-derived intestinal progenitor cells. The drug absorption rates in human iPS cell-derived intestinal epithelial cell monolayers (iPS-IECM) were highly correlated with those in humans ($R^2=0.91$). The expression levels of cytochrome P450 (CYP) 3A4, a dominant drug-metabolizing enzyme in the small intestine, in human iPS-IECM were similar to those in human small intestine *in vivo*. In addition, intestinal availability in human iPS-IECM (the fraction passing the gut wall: $F_g=0.73$) was more similar to that in the human small intestine *in vivo* ($F_g=0.57$) than to that in Caco-2 cells ($F_g=0.99$), a human colorectal adenocarcinoma cell line. Moreover, the drug-drug interaction and drug-food interaction could be observed by using our human iPS-IECM in the presence of an inducer and inhibitor of CYP3A4, i.e., rifampicin and grape fruit juice, respectively. Taking these results together, we succeeded in generating the human iPS-IECM that can be applied to various intestinal pharmacokinetics and drug-response tests of orally administered drugs. Our human iPS cell-derived intestinal epithelial cells will be commercially available in this summer.

P209 - PREDICTION OF HERB-DRUG INTERACTION BETWEEN SCHISANDRA SPHENANTHERA AND IMATINIB: IN VITRO METABOLISM STUDY COMBINED WITH PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING APPROACH

Jeffry Adiwidjaja¹, Alan Boddy², and Andrew McLachlan¹

¹The University of Sydney, Australia, ²University of South Australia, Australia

Introduction. Long-term use of imatinib is effective in people with chronic myeloid leukaemia (CML) [1]. Due to imatinib administration on a daily basis, patients with CML often seek complementary medicines including Schisandra sphenanthera. This natural product is mainly used for its putative hepatoprotective effect and has been explored for anti-cancer properties [2]. It contains three major bioactive lignans, i.e. Schisantherin A, Schisandrin A and Schisandrol B which are potent cytochrome P450 (CYP)3A modulators with evidence for clinically-significant drug interactions [3,4]. Physiologically-based pharmacokinetic (PBPK) models for these compounds will provide a greater insight into interaction between S sphenanthera and imatinib and allow prediction of the extent of the interaction. **Aims.** This study aimed to develop and verify PBPK models for the three lignans in S. sphenanthera to predict clinical significance of the interaction with imatinib. **Methods.** Imatinib metabolism was investigated in human liver microsomes and recombinant CYP3A4 enzyme in the presence and absence of Schisantherin A, Schisandrin A and Schisandrol B using LC-MS/MS assay for N-desmethyl metabolite. PBPK models for the three lignans accounting for the reversible and mechanism-based inhibition and induction of CYP3A were built in Simcyp Simulator (v.17), verified to clinical pharmacokinetic data and assessed for their capability to predict interactions with midazolam and tacrolimus. The effect of S. sphenanthera on systemic exposure of imatinib, expressed as AUC ratio, was then predicted through PBPK simulation. **Results.** In the current *in vitro* study, Schisantherin A and Schisandrol B, but not Schisandrin A potently inhibited CYP3A4-mediated metabolism of imatinib (K_i of 0.12 and 4.90 μM , respectively). All three compounds showed a strong reversible inhibition on CYP2C8 enzyme with K_i of 0.2-0.6 μM . The verified PBPK models were able to capture the increase of systemic exposure of both midazolam and tacrolimus due to co-administration of S. sphenanthera, consistent with the reported changes in the corresponding clinical interaction study [3,4] (AUC ratio of 2.00 vs 2.05 and 2.43 vs 2.12, respectively). The PBPK simulation suggested that the recommended oral dosage of Schisantherin A (9.4 mg), Schisandrin A (6.8 mg) and Schisandrol B (0.5 mg) three times daily increased imatinib exposure with an AUC ratio of 1.32. **Conclusion.** PBPK models for the key components in S. sphenanthera with a good predictive capability were successfully developed. S. sphenanthera extract at a clinically-relevant dose was predicted to increase imatinib systemic exposure by up to 32%.

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P210 - DEVELOPMENT OF A PHYSIOLOGIALLY BASED PHARMACOKINETIC MODEL TO PREDICT CHANGES IN EXPOSURE OF A CIRCULATING METABOLITE WITH CYP3A4 INDUCTION

Tamara Cabalu, Ka Lai Yee, Yuhsin Kuo, Kerry Fillgrove, Larissa Wenning, and Rosa Sanchez
Merck & Co., Inc., USA

Compound A is predominately cleared by P450 3A4 (CYP3A4) and metabolized to an oxidative metabolite (M-1), which circulates at approximately 17% of the parent exposure. The pharmacokinetics (PK) of Compound A have been shown to be impacted by CYP3A inducers, however, concentrations of M-1 were not monitored in clinical DDI studies. Therefore, a physiologically based pharmacokinetic (PBPK) model was developed for Compound A and M-1, enabling investigation of the change in exposure of M-1 when Compound A is co-administered with CYP3A inducers. Simulations of the effects of strong and moderate CYP3A inducers rifampin and efavirenz demonstrated that although the rate of M-1 formation may increase by around 3-fold following CYP3A4 induction, the extent of M-1 formation was predicted to increase by only 1.2-fold. These findings support that increases in M-1 exposure are primarily influenced by the high fraction metabolized by CYP3A4 and the large amount of M-1 that is already formed even in the absence of induction, rather than by the change in Compound A clearance under induced conditions.

P211 - ITERATIVE DEVELOPMENT AND VERIFICATION OF A MIDDLE-OUT PBPK MODEL FOR ABIRATERONE

Eleanor Jing Yi Cheong, Ting Jian Yap, Ervin Zhi Bin Cheong, Ho Thanh Tu, Ratha Mahendran, Edmund Chiong, Kesavan Esuvaranathan, Christina Li Lin Chai, and Eric Chun Yong Chan
National University of Singapore, Singapore

Abiraterone, administered as prodrug abiraterone acetate (AA), is a first-in-class inhibitor of cytochrome P450 17A1 (CYP17A1), a pivotal enzyme mediating persistent extragonadal and intratumoural androgen biosynthesis in metastatic castration resistant prostate cancer. Significant associations between abiraterone trough concentrations and pharmacodynamic outcomes coupled with demonstration of dose-dependent mineralocorticoid toxicities suggest that minimizing pharmacokinetic (PK) fluctuations is critical to ensuring therapeutic efficacy and safety (1,2). Unfortunately, the PK of abiraterone is complex and immensely susceptible to intrinsic and extrinsic variabilities (2). Consequently, to facilitate the prospective interrogation of untested, albeit clinically relevant scenarios, this study aims to develop and verify a middle-out physiologically-based pharmacokinetic (PBPK) model for abiraterone via combining top-down analysis of clinical data with *in vitro-in vivo* extrapolation (IVIVE)-based approaches. To characterize preabsorptive AA hydrolysis and supersaturation of abiraterone observed *in vivo*, *in vitro* aqueous solubility data, biorelevant measurements as well as supersaturation and precipitation parameters were incorporated into the Advanced Dissolution Absorption and Metabolism (ADAM) model of the Simcyp simulator for mechanistic oral absorption simulation. CYP3A4-mediated N-oxidation and SULT2A1-catalyzed sulfation of abiraterone were subsequently quantified in human liver microsomes (HLM) and human liver cytosol (HLC) respectively. Iterative PBPK model refinement involved evaluation of abiraterone uptake in plated cryopreserved HepaRG cells. Concentration-dependent uptake data measured under initial rate conditions was analyzed via a dynamic two-compartmental model and derived kinetic parameters were applied to inform the permeability-limited hepatic uptake of abiraterone. The developed PBPK model accurately recapitulated the duodenal intraluminal dissolution, supersaturation, and precipitation behaviour of abiraterone after administration of a single 250 mg dose of AA, with simulated mean maximum duodenal concentration (C_{max}) and exposure (AUC) of both AA and abiraterone falling within 2-fold of the reference published data. However, the predicted plasma concentration-time profile of abiraterone assimilating derived unbound intrinsic clearance values (293.3 $\mu\text{L}/\text{min}/\text{mg}$ and 520.3 $\mu\text{L}/\text{min}/\text{mg}$ in HLM and HLC respectively) markedly overestimated clinical data, alluding to the presence of alternative mechanisms governing abiraterone's *in vivo* disposition. Aligned with recent evidence demonstrating abiraterone uptake in organic anion transporting polypeptide (OATP) 1B3-overexpressing prostate cancer cells (3), our findings similarly established active, saturable transport ($J_{max}/K_m, \text{unbound} = 72.0 \mu\text{L}/\text{min}/\text{million cells}$) that accounted for >90% of total uptake in HepaRG cells. Subsequent simulations effectively predicted the observed PK of abiraterone upon single and multiple dosing, exemplifying how a middle-out reverse translation approach was instrumental in uncovering the arcane role of enzyme-transporter interplay in mediating abiraterone's hepatic elimination. Our robust and systematic model verification qualify the PBPK model of abiraterone for extrapolation beyond the clinical trial population in the quantitative and mechanistic management of PK variabilities.

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P212 - PBPK MODELING AND SIMULATION OF MORPHINE IN VIRTUAL NEONATES GENERATED USING ACTUAL PATIENT INFORMATION

Chie Emoto, David Hahn, Joshua Euteneuer, Tomoyuki Mizuno, Alexander Vinks, and Tsuyoshi Fukuda
Cincinnati Children's Hospital Medical Center, USA

Morphine is the most commonly used opioid for acute pain treatment in pediatric patients, including neonates and small infants. This drug is a substrate of organic cation transporter 1 (OCT1) and UDP-glucuronosyltransferase 2B7 (UGT2B7), which contribute to hepatic uptake and metabolism, respectively. The pharmacokinetics of morphine display large variability, and this variability is pronounced in neonates due to the growth and maturation of organ functions. At the present, morphine management in neonates still relies heavily on educated guesswork and prior experience of neonatologists, due to the limited knowledge of the causes of the variability in neonates. In order to tackle this situation, we attempted to integrate and translate existing information related to morphine disposition into clinically actionable practices by using a physiologically-based pharmacokinetic (PBPK) model (1), which was previously developed using Simcyp (Certara UK Limited, Simcyp Division). In this study, the PBPK model-based concentrations of morphine were assessed by comparing observed data in neonates.

The PBPK simulation was conducted in a virtual neonatal subject population, which was generated based on individual, actual patient data such as postnatal age (selected patients with gestational age over 37 weeks), gender, dosing history (body weight-based dosages and number of administrations), and OCT1 haplotype, along with time-dependent developmental changes in pediatric physiology during the administration period. A total of 114 concentrations and data from 32 neonates were used to evaluate the PBPK model-based simulation results.

PBPK model-predicted concentrations showed a reasonable prediction relative to observed concentrations, where the median ratio of predicted-to-observed concentrations was: 0.99 (95% CI, 0.84-1.15) for all OCT1 haplotypes – 0.97 (0.78-1.10) for wild type; 1.03 (0.72-1.38) for heterozygous; and 1.58 (0.55-2.91) for homozygous haplotypes. The model accuracy and precision (i.e. median percentage error and median absolute error, respectively) were higher in the homozygous haplotype of OCT1 compared to the other types. For each type, approximately 60% and 85% of predicted concentrations were within two- and three-fold of observed concentrations, respectively.

Predicted values of the outside two- and three-fold ranges suggest an influence of additional factor(s) underlying this discrepancy. One possible mechanism involves the (patho)physiological differences in hepatic protein expression levels of OCT1 and UGT2B7 during the neonatal period between the study patients and virtual subjects (i.e. settings in the PBPK model). Although barriers still exist to applying these study results to clinical practice, they contribute to the accumulation of system knowledge and improved understanding of how the patient's condition may be leveraged to further improve the predictive performance of PBPK modeling. Further model fine-tuning is expected to be valuable in supporting clinical decisions before starting medications in critically-ill neonates.

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P213 - A PRIORI PREDICTION OF CYP3A VICTIM DRUG-DRUG INTERACTION OF MK-1 AND DILTIAZEM USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING (PBPK): QUALIFICATION USING CLINICAL DATA

Christopher Gibson, Georgy Hartmann, Bennett Ma, Anne Chain, Sean Smith, Mark Forman, Sandip Datta, Thomas Bateman, Ying Li, and Sauzanne Khalilieh
Merck, USA

MK-1 is a BCS Class 1 small molecule drug within the development portfolio of MRL. Nonclinical studies conducted *in vitro* and *in vivo* at the time of candidate nomination led to the hypothesis that MK-1 would be cleared in humans predominantly by Cytochrome P450 (CYP) 3A mediated biotransformation with minor contributions from CYP2C9 and CYP1A2. Studies conducted *in vitro* using human liver microsomes and recombinantly-expressed CYP450's suggested the fraction of MK-1 metabolized (fm) by CYP3A4 ranged between 0.70 – 0.86. A middle-out PBPK model was built which leveraged physical/chemical, non-clinical and early clinical data for MK-1 and was used to simulate the effect of CYP3A4 inhibition and induction on the pharmacokinetics of MK-1. The DDI between MK-1 and 240 mg Diltiazem-XR (a moderate CYP3A inhibitor) was simulated using sensitivity analyses of CYP3A4 fm ranging between 0.70 - 0.95 which was selected

to cover the plausible values observed *in vitro* and to cover a 'worst-case' scenario fm of 0.95 which was higher than what was predicted from the *in vitro* models. Using the range of CYP3A4 fm's the PBPK model predicted geometric mean ratios (GMR) of AUC and Cmax between 2.0 - 3.4 and 1.4 - 2.0, respectively. A clinical DDI study was conducted in n=10 healthy volunteers between MK-1 (2 mg) and Diltiazem-XR (240 mg) and the resulting GMR for AUC and Cmax were 2.0 and 1.3, respectively. The PBPK simulations conducted a priori were remarkably close to the observed data and suggested the fm CYP3A4 of MK-1 is 0.70, a value supported by *in vitro* biochemical data. The agreement between the model simulations (fm=0.70) and the observed DDI clinical data qualifies the MK-1 PBPK model to quantitatively predict victim DDI with other CYP3A4 inhibitors or inducers.

P214 - INVESTIGATING THE IMPACT OF ENZYME CORRELATIONS ON INTER-INDIVIDUAL VARIABILITY IN PHARMACOKINETICS AND DRUG-DRUG INTERACTIONS

Oliver Hatley¹, Udoamaka Ezuruike¹, Janak Wedagedera¹, Ryan Takahashi², Jialin Mao², and Iain Gardner¹

¹Certara UK (Simcyp Division), United Kingdom, ²Genentech, Inc., A Member of the Roche Group, USA

The generation of virtual individuals for physiologically based pharmacokinetic (PBPK) modelling often employs correlations of covariates to generate realistic physiological system parameters and incorporate known sources of inter-individual variability in drug exposure (1). Extensive meta-analysis of cytochrome P450 (CYP) abundances based on western blot analysis have been published and used in mechanistic PBPK models (2). However, blotting has been rarely employed to look at multiple enzyme abundances within the same individual due to the technical challenges of the technique and the large sample requirements. The emergence of LC-MS/MS technologies for quantifying enzyme abundance in biological samples enables multiple enzymes to be measured in the same individual, allowing for relationships between different metabolic enzymes to be investigated. A Cholesky matrix (based on a database from 24 donors (3)) was incorporated into the Simcyp Simulator (from V17) to enable multiple correlations between hepatic CYP enzymes to be described. The aim of this work is to investigate the impact of including enzyme correlations within PBPK models on the simulated pharmacokinetic and drug-drug interaction (DDI) variability for compounds that are substrates for multiple CYP enzymes. In this study, Repaglinide (CYP 2C8, 3A4 and OATP 1B1 substrate) and Sildenafil (CYP 2C9 and 3A4) were selected as investigational compounds. The impact of enzyme correlations on DDIs was investigated using Ketoconazole (strong CYP3A4 inhibitor), and Gemfibrozil and its metabolite (Gemfibrozil 1-O- β -Glucuronide) (CYP2C8/9 & OATP 1B1 inhibitors) in a virtual population of healthy individuals. The power of a simulated study to show a difference between populations of individuals that were the same apart from whether a correlation between CYP enzymes was incorporated or not, was investigated. In addition, a sensitivity analysis was conducted to look at DDI liability in populations with or without enzyme correlations as the fraction metabolised by the inhibited enzyme was varied. The correlation between CYP 2C8 and 3A4 and CYP 2C9 and 3A4 were 0.74 and 0.49, respectively. For Repaglinide, a study power of 80% was observed when using a population size of ~350 individuals. For Sildenafil, the study power was low (~50%), and decreased with increasing CYP2C9 fm. Including enzyme correlations in the PBPK model increased variability in Repaglinide exposure but slightly reduced the predicted DDI with ketoconazole (mean and range) when CYP 3A fm was >0.25. The simulated variability in Sildenafil exposure was also increased when enzyme correlations were considered. The variability in DDI with ketoconazole increased when the CYP 3A4 fm was >0.5. Gemfibrozil DDIs were unchanged by using enzyme correlations for either substrate. Further work will focus on investigating the impact of the multiple-CYP correlation approach for other known substrates of multiple enzymes, and the impact of using larger datasets of enzyme abundance correlations.

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P215 - RISK ASSESSMENT IN HUMANS USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF DIISOBUTYL PHTHALATE AND ITS MAJOR METABOLITE, MONOISOBUTYL PHTHALATE: DEVELOPMENT AND EVALUATION OF MODEL

Yong-Bok Lee¹, Hea-Young Cho², Seung-Hyun Jeong¹, and Ji-Hun Jang¹

¹Chonnam National University, South Korea, ²CHA University, South Korea

Diisobutyl phthalate (DiBP), which belongs to the group of low molecular weight phthalate, is a substance used in the production of objects frequently used in human life [1]. Therefore, humans are widely exposed from the surrounding environment such as water, air, soil, and food. As previously reported in many studies, DiBP is an endocrine disruptor with reproductive toxicity [1-3]. In addition, monoisobutyl phthalate (MiBP), a major metabolite *in vivo* of DiBP, is a biomarker for DiBP exposure assessment and is known as an endocrine disruptor reported to be reproductive toxicity similar to DiBP [4]. Therefore, toxicokinetic studies on DiBP and MiBP that have not yet been reported in detail are needed. The aim of this study is to develop and evaluate physiologically based pharmacokinetic (PBPK) model for DiBP and MiBP in rats and

to extend this to human risk assessment based on human exposure. We conducted this study *in vivo* after the oral or intravenous administration of DiBP in male (0.1-10 mg/kg dose) and female (2 mg/kg dose) rats. The biological samples consisted of plasma, urine, feces, and 11 different tissues. We analyzed the samples using UPLC-ESI-MS/MS method. For DiBP, the tissue to plasma partition coefficient was the highest for thymus followed by kidney. However, for MiBP, it was the highest for gastrointestinal-tract and kidney. The predicted rat plasma, urine, feces, and tissue concentrations using a developed PBPK model fitted well with the observed values. We extrapolated the PBPK model in rats to a human PBPK model of DiBP and MiBP based on human physiological parameters. The reference dose of 0.10 mg/kg/day of DiBP and external doses of 1.478 $\mu\text{g}/\text{kg}/\text{day}$ (children), 1.322 $\mu\text{g}/\text{kg}/\text{day}$ (adolescents), 1.133 $\mu\text{g}/\text{kg}/\text{day}$ (adults), and 1.478 $\mu\text{g}/\text{kg}/\text{day}$ (elderly) of DiBP for human risk assessment were estimated using Korean biomonitoring values. Our study provides valuable insight into human health risk assessment regarding DiBP exposure.

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P216 - DEVELOPMENT AND VERIFICATION OF A LINKED THC/11-OH-THC PBPK MODEL IN HEALTHY SUBJECTS AND EXTRAPOLATION TO PREGNANCY

Jashvant D. Unadkat and **Gabriela Patilea-Vrana**²

¹University of Washington, USA, ²Seattle Genetics, USA

The prevalence of cannabis use among pregnant women is rising. In preclinical studies, the psychoactive cannabinoid, THC and its active hydroxy metabolite, 11-OH-THC, have been shown to cause fetal risks. However, the impact of in utero fetal cannabis exposure to human fetal outcomes is controversial [1]. In order to assess the risks associated with maternal cannabis use, the maternal and fetal cannabinoid (THC and 11-OH-THC) exposure, throughout pregnancy, must be known. Since determining such exposure in the clinic is logistically and ethically difficult, we used PBPK modeling and simulation to predict THC and 11-OH-THC maternal and fetal plasma concentrations throughout pregnancy. To do so, first we extrapolated to *in vivo* our previously quantified *in vitro* mechanistic hepatic THC and 11-OH-THC metabolism data (by CYP2C9/2D6/3A and UGT1A9/2B7) in human liver microsomes [2]. Second, we built a linked THC/11-OH-THC PBPK model in a healthy nonpregnant population. Third, we successfully verified our predictions with the observed THC/11-OH-THC plasma concentration data after THC administration via IV and inhalation to healthy subjects. Inhalation was chosen as the administration route since it is the most popular. The bioavailability (F_{inh}) of THC after inhalation was higher in chronic versus casual cannabis users (F_{inh} = 0.35 and 0.19, respectively). Because THC hepatic clearance is blood-flow limited, THC plasma clearance was not sensitive to modest alterations in intrinsic CYP metabolic clearance (CL_{int}) whereas 11-OH-THC was. Next, the exposure (plasma concentrations and AUC) of THC and 11-OH-THC after THC inhalation were simulated at the end of the first, second, and third trimester. To do so, we adjusted for gestational-age dependent physiological changes, which include increase in CYP2C9/2D6/3A activity and decrease in the blood concentration of plasma protein binding proteins. Simulations showed that during pregnancy, THC plasma AUC did not change but 11-OH-THC plasma AUC decreased up to 1.9 -fold by the end of the third trimester. Furthermore, the maximum decrease of the combined THC and 11-OH-THC exposure was 1.2 -fold. Using the pregnancy THC PBPK model, the observed maternal THC plasma concentrations at term were well-predicted. Lastly, fetal (F) and maternal (M) THC plasma concentrations were simulated at term using our maternal-fetal PBPK in the presence and absence of placental THC efflux and compared with the observed plasma concentration in F/M pairs. These simulations predicted that the fraction of THC effluxed by placental P-gp/BCRP is approximately 90%. In conclusion, we built and verified a linked THC/11-OH-THC PBPK model in a healthy nonpregnant population and demonstrated how this mechanistic physiological and pharmacokinetic platform can be extrapolated to special populations, such as pregnant women. Supported by NIDA P01DA032507.

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P217 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF LIPOSOMAL COMPOUND X - DECIPHERING THE MYSTERY OF LIPOSOMAL DELIVERY

Lian Qian, Aline Barth, Michael Liu, Mohammad Hossain, and Valeriu Damian
GlaxoSmithKline, USA

Despite the enormous excitement and expectation around liposome drug products, evaluating their safety and efficacy is still challenging due to the limited information around their bio-distribution. Here, we developed a mechanistic model to simultaneously describe the bio-distribution of encapsulated and released drug following intravenous administration of liposomal compound X. It's a hybrid model which incorporated an empirical compartment for encapsulated drug, release kinetics from liposomes and a conventional systemic PBPK model for the released drug compound X. Parameters were fitted using total compound X (combined encapsulated and released drug) and released drug X concentrations measured preclinically. First-order kinetics was assumed for the release of compound X from liposomes. The disposition of compound X following its release from the liposomes was described by a blood-flow limited whole-body PBPK model with fourteen organ tissue compartments. Once the mechanistic model was established in multiple preclinical species, a similar human model was constructed for human dose prediction. Both physiologically-based and allometric scaling based translations were used for model parameterization in human. The model not only has the capability to predict total, released and unbound drug concentrations in plasma, but also in tissues (i.e. kidney, skin, heart, and brain) where clinical measurements would be difficult to conduct. This PBPK modeling framework will be used for optimizing liposomal drug delivery systems and estimating doses in early clinical studies.

P218 - QUANTITATIVE PREDICTION OF ORAL DRUG ABSORPTION BY KINETICALLY ANALYZING GASTROINTESTINAL WATER DYNAMICS

Yoshiyuki Shirasaka, Satoru Suzuki, Yuta Funai, Miyuki Takemura, Yuta Tateishi, Kazuki Ichijo, Ren Okada, Marika Ishihara, Rika Oda, Akari Eguchi, Hisanao Kishimoto, and Katsuhisa Inoue
Tokyo University of Pharmacy and Life Sciences, Japan

Almost all the drugs administered orally are absorbed into systemic circulation from the gastrointestinal (GI) tract. Intestinal absorption of drugs from solution may occur via both passive diffusion and drug transporters, and, in addition, may be influenced by metabolic enzymes. Absorption through these mechanisms depends upon the concentration gradient across the membrane and/or saturation property. Therefore, understanding drug concentration in the GI fluid is important to consider absorption characteristics. However, because water volume is altered by the absorption of ingested water and the secretion of biological fluid, drug concentration in GI tract would become complicated (1). Changes in the GI water volume may indirectly influence drug absorption profile by altering drug concentration and absorption kinetics (1, 2). In the present study, we aimed to characteristically analyze water absorption and secretion along the GI tract. In addition, we tried to establish a physiologically-based pharmacokinetic (PBPK) absorption model incorporating GI water dynamics and its effects on oral drug absorption. Studying luminal water volume by means of an in situ closed loop technique with FD-4 (a non-absorbable compound), the remaining fraction of water in rat jejunum and ileum but not colon gradually decreased and reached a steady state at 30 min. However, determination of the remaining fraction of [³H]water revealed that applied water was almost completely disappeared within 30 min in all segments of intestine. When these results were kinetically analyzed, rate constants for GI water secretion were evaluated in the following rank order: jejunum > ileum > colon, implying that water secretion process is the major determinant of the site-dependency of water dynamics. A model-fitting analysis with experimental data was applied to analysis of human GI water dynamics reportedly observed using water-sensitive magnetic resonance imaging (MRI). Furthermore, by incorporating this approach into PBPK absorption model, integrated liquid and intestinal absorbed drug (ILIAD) model was newly-established. The ILIAD model exhibited a consistent simulation result with human plasma concentrations-time profiles of drugs. In conclusion, our simulation approach with ILIAD model incorporating GI water dynamics and its effects on drug absorption would be considered of value in more precisely prediction of oral drug absorption and pharmacokinetics in human. This work was supported in part by Research Grant from Japan Research Foundation for Clinical Pharmacology [2017A13].

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P219 - DEVELOPING A CLASS OF ORALLY ACTIVE DRUGS WITH UNUSUAL *IN VITRO* ADME PROPERTIES: THE ROLE OF PBPK MODELLING IN THE DISCOVERY PROCESS.

Mariarita Stefani, Nicola Cesari, Giandomenico Brogin, Michela Salvadori, and Paola Puccini
Chiesi Farmaceutici S.p.A., Italy

The development of a whole body PBPK (WB-PBPK) model proved to be crucial for the pharmacokinetic screening strategy in the early development of a class of orally active drugs, as the *in vitro-in vivo* correlation (IVIVC) demonstrated some discrepancies and was lacking in a clear identification of the best compounds for the project progression. The approach used, permitted also the reduction of the number of *in vivo* experiments and the number of animals required for the pharmacokinetic evaluation of compounds.

It is well established that the most important *in vitro* parameters affecting oral bioavailability are dissolution, permeability and first pass effect. These parameters were initially evaluated in the discovery process and predicted from aqueous kinetic solubility at pH 7.4, Caco-2 permeability value and microsomal metabolic stability, respectively. We validated the accuracy of the *in vitro* assays comparing the estimated oral bioavailability with the pharmacokinetic parameter calculated from intravenous and oral administration of the compounds to rats.

Low metabolic clearance was targeted to ensure *in vivo* systemic exposure and an appropriate half-life. Most of the considered compounds had very low hepatic extraction, predicted from the microsomal metabolic stability ($Cl_i < 14 \mu\text{L}/\text{min}/\text{mg}$) but resulted in a wide range of systemic clearance when the *in vivo* values were considered ($Cl_{obs} = 10 - 80 \text{ mL}/\text{min}/\text{kg}$). Considering that low extraction ratio compounds have a restrictive elimination, the fraction unbound in plasma was considered essential in the estimation of the systemic clearance and therefore the microsomal metabolic stability was corrected by the plasma protein binding.

Interestingly, an inverse correlation of the solubility with the experimental oral bioavailability was observed: the lower the solubility the higher the bioavailability, suggesting that aqueous kinetic solubility at pH 7.4 underestimated the dissolution of these compounds. The evaluation of the impact of bile salts on compound dissolution was then considered.

We devised in house, a WB-PBPK model using MatLab to predict the plasma concentration-time profiles after intravenous and oral administration to rats using physiological literature parameters and compound *in vitro* data as input. The model incorporated a customized version of the Advanced Compartmental Absorption and Transit model (ACAT), which was able to simulate compound permeability from *in vitro* Caco-2 values and predict the dissolution in the gastrointestinal tracts from kinetic solubility in aqueous buffers, accounting for the presence of bile salts at physiological concentrations.

The use of predicted solubility in the biorelevant media avoided the dissolution tests in FaSSGF, FeSSIF and FaSSIF, that in drug discovery could be too expensive in terms of amount of compound and time required. Moreover, it was possible to theoretically investigate the individual contribution and the interplay of the permeability, metabolism and dissolution on oral bioavailability.

As a result, the bioavailability estimated from the WB-PBPK model correlated very well with the pharmacokinetic parameters calculated from intravenous and oral administration of the compounds to rats and demonstrated the benefit of this model in compound evaluation in drug discovery.

P220 - A MECHANISTIC PBPK/PD MODELING APPROACH TO PREDICT THE EFFECT OF CYP3A4 ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF RACEMIC WARFARIN

Ziteng Wang and Weimin Cai
Fudan University, China

Objectives: Warfarin is one of the most prescribed drugs for venous thromboembolism treatment with large interindividual variability caused by genetic polymorphisms and drug-drug interactions. Previous studies mostly focused on the influence related to CYP2C9 enzyme. According to several *in vitro* studies and case reports, catalytic activity change of CYP3A4 enzyme, which involved in the metabolism of R-warfarin, should also be concerned. **Methods:** To study the effect of CYP3A4 on the pharmacokinetics and pharmacodynamics of the enantiomers, we developed a mechanistic and physiologically based pharmacokinetic/pharmacodynamic model (PBPK/PD) incorporating population-based Simcyp and NONMEM software. PBPK model of racemic warfarin was firstly built and verified within Simcyp by setting S- and R-warfarin as substrate and inhibitor, respectively. Liver total concentrations of both S- and R-warfarin were selected as the output of Simcyp and then used as two covariates for NONMEM. The mechanistic PK/PD model consisted of five differential equations representing the kinetic processes of vitamin K cycle and synthesis and elimination of clotting factors. Prothrombin complex activity (PCA), set as dependent variable, was simulated by the estimation function of NONMEM and then transformed to INR value. **Results:** The effect of rare CYP3A4 loss-of-function mutation was simulated through the combined PBPK/PD model by changing enzyme abundance to zero in Simcyp. Results showed only a slight increase of INR value ($p > 0.05$), suggesting CYP3A4 genetic polymorphisms may not be the main factors leading to bleeding risks. Then, several CYP3A4 inhibitors, including erythromycin, clarithromycin, diltiazem, cyclosporine and cimetidine, were designed for drug interaction simulation based on published papers. Elevated INR values up to 3 after interaction were observed ($p < 0.05$), which were consistent with case reports. Most of the inhibitors strongly slowed down the pharmacokinetics of R-warfarin and enhanced the pharmacodynamics of the enantiomers. **Conclusions:** This

approach of combining PBPK model and mechanistic PK/PD model together could be generalized to further investigate drugs and other factors that may interact with CYP3A4 enzyme and result in clinically significant consequences. It could be another valuable tool for the prediction of rational use of warfarin.

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P221 - UNDERSTANDING THE PHARMACOKINETIC NONLINEARITY OF TABALUMAB BY INCORPORATING TARGET-MEDIATED DRUG DISPOSITION IN A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL

Lian Zhou, Bing Han, David Radtke, and Jennifer Witcher
Eli Lilly Company, USA

Tabalumab, a human monoclonal antibody (mAb) targeting B-cell activating factor (BAFF), exhibited nonlinear pharmacokinetics (PK) in its first-in-human studies (1). The nonlinear PK was observed across the 0.01 to 8.0 mg/kg dose range after a single i.v. (infusion) dose of tabalumab administered to subjects with rheumatoid arthritis or systemic lupus erythematosus (1). The clearance of tabalumab decreased from 2.9 to 0.1 L/day and terminal half-life increased from about 1.6 to 25 days in the studies. Consistent with the observation in humans, the nonlinear PK was also observed across the dose range of 0.1 to 30 mg/kg in monkeys. It was hypothesized that the observed PK non-linearity of tabalumab likely reflects the target-mediated drug disposition (TMDD) due to tabalumab binding to BAFF. To understand this nonlinear PK of tabalumab, a physiologically based pharmacokinetic model (PBPK) was constructed using a population-based simulator (V15, SimCYP, Sheffield, UK). It was assumed that binding to both membrane and soluble forms of BAFF are responsible for the catabolism of tabalumab via receptor-mediated endocytosis. The affinity of tabalumab to the soluble BAFF was determined to be 126 pM in a previous study (2) and the expression level of the soluble BAFF in humans was measured. It was assumed that the membrane-bound BAFF was expressed and bound to tabalumab similarly to the soluble BAFF. Given BAFF is a member of the tumor necrosis factor (TNF) superfamily, the synthesis and internalization rates of BAFF were considered similarly to the simCYP built-in profile for TNF. Binding of tabalumab to FcRn with an affinity of 0.728 μ M was included into the PBPK model to account for the protection of tabalumab from degradation, which prolonged tabalumab half life by recycling endocytosed tabalumab back to circulation. As a result, the PBPK model captured the more than dose proportional increases in exposure, and the prolonged terminal half-life with increase of dose observed in the studies. The predicted concentration-time profiles of tabalumab well described the observed concentration data obtained from subjects with rheumatoid arthritis or systemic lupus erythematosus at doses of 0.01 (n=3), 0.04 (n=2), 0.125 (n=6), 0.5 (n=3), 2.0 (n=5), 8.0 mg/kg (n=3) for 260 days. In conclusion, the PBPK modeling confirmed the hypothesis that the TMDD component of tabalumab was responsible for the nonlinear PK observed in the clinical studies.

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P222 - PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF ATIPAMEZOLE UNRAVELS THE SATURABLE METABOLISM TO ITS NONLINEAR PHARMACOKINETICS ACROSS DIFFERENT SPECIES

Xiaomei Zhuang¹ and **Chuang Lu**²

¹AMMS, Beijing Institute of Pharmacology and Toxicology, China, ²Sanofi Company, USA

Hypothesis: Atipamezole displayed nonlinear pharmacokinetics in rats. When a drug exhibits nonlinear PK in preclinical animal studies, it is crucial to elucidate the mechanism responsible for nonlinear PK to estimate the appropriate human dosage. To address this, we performed physiologically based pharmacokinetic modeling analyses. Methods used: A commercially available PBPK model, Gastroplus was used. Key *in vitro* and *in vivo* data for atipamezole in rats and human were generated. Physicochemical parameters of atipamezole were obtained by ADMETP software. Volume related parameters including protein binding, Rbp, Kp were obtained by *in vitro* and *in vivo* study. Clearance related parameters including Vmax and Km were determined in liver microsomes and recombinant CYPs. These data were crucial to developing, refining and validating a novel bottom-up, top-down and middle-out PBPK model. Supporting data: After intravenous administration of three doses (0.3, 0.9, 3mg/kg) in rats, the systemic exposure increased in a dose-proportional manner in the dose range of 0.3-0.9mg/kg. However, with doses of atipamezole greater than 3mg/kg, the fold

increases in the systemic exposure was greater than dose proportional. The main reason led to nonlinear PK of atipamezole is saturable metabolism induced. Thus, enzyme kinetic parameters are key data to describe the saturable clearance. K_m and V_{max} of atipamezole in liver microsomes of rats and human are 1.2 ± 0.28 , $3.9 \pm 0.7 \mu\text{M}$, 0.05 ± 0.004 and 0.06 ± 0.005 nmol/min/mg(protein), respectively. CYP2A6, 2B6, 2C19 and 2D6 are involved in the biotransformation of atipamezole. K_m and V_{max} of atipamezole in CYP2A6, 2B6, 2C19 and 2D6 are 2.2 ± 0.3 , 0.06 ± 0.01 , 0.3 ± 0.04 , $0.3 \pm 0.04 \mu\text{M}$, 0.3 ± 0.003 , 0.07 ± 0.002 , 0.001 ± 0.0004 , and 0.18 ± 0.01 nmol/min/pmol (CYP), respectively. Results: The main outcome is an accurate PBPK model to capture the nonlinear PK in rats by using middle-out approach where the measured K_m and enlarged V_{max} values were used for the clearance fitting. After transferring the same PBPK model of rats into human, all the measured K_m and V_{max} of atipamezole in CYP2A6, 2B6, 2C19 and 2D6 can successfully capture the clinical PK of atipamezole reported in a literature with the dosage range of 10-100mg per healthy volunteer where the nonlinear PK is not obvious. After increasing the dosage to 1000mg, mild fold increases in the systemic exposure greater than dose proportional began to occur. Conclusions: The PBPK models for atipamezole were successfully established and validated, and the comparison of different species PBPK models identified the saturation of the metabolism process as a major contributing factor for the nonlinear pharmacokinetics. But based on the same mechanism of saturable metabolism, nonlinear PK for atipamezole of rat and human are different.

PP23 - PPB: CONSIDERATIONS FOR SAFETY STUDIES AND DATA INTERPRETATION

Natasa Pajkovic¹, Yuhsin Kuo¹, Amrith Bennet¹, Raymond Gonzalez¹, Laïla Bernard², Frédéric Poignant², and Randy Miller¹

¹Merck & Co., Inc., USA, ²Laboratoires Merck Sharp & Dohme-Chibret, France

Practices with respect to the integration of the extent of plasma protein binding (PPB) when relating drug exposure to *in vivo* safety findings are inconsistent across the industry and across regulatory guidances. While some regulatory guidances suggest use of unbound exposure, they often limit its application to cases where PPB is higher in preclinical species than in human. In this presentation we illustrate how significant differences in PPB across species as well as saturation of PPB may confound interpretation of toxicokinetic data and determination of safety exposure margins. Two structurally similar compounds have been assessed in nonclinical safety studies. In a rising single dose pharmacology study in dogs with Compound 1, adverse effects were noted at the highest dose tested. The total plasma exposures of Compound 1 at this dose, however, were comparable to those at the approximately 4-fold lower dose which had no noteworthy effect. Similarly, in a 4-month dog toxicity study with Compound 2, target organ toxicity was limited to the liver and correlated with elevations in liver enzymes at the highest dose tested. However, there were no adverse effects observed at a dose that was 20-fold lower despite having only 1.5-fold lower total plasma exposure. Both compounds had very high PPB in dogs and demonstrated saturation of PPB at high compound concentrations both *in vitro* as well as in *ex-vivo* samples from the above toxicological studies. Integration of the concentration dependent fraction unbound in plasma across all dose levels revealed dose dependent increases in unbound exposures not apparent based on total plasma exposure. We believe this provides a plausible explanation for the adverse effects observed in these studies at doses having similar total plasma exposures to the no effect doses. Both compounds also exhibited significant species differences in the extent of PPB which was considered in the design of the toxicological studies. This work highlights the importance of evaluating PPB and integrating it into the overall interpretation of toxicokinetic data and safety multiple determination.

PP24 - ISOPROPANOL-TRIFLUOROACETIC ACID EXTRACTION SELECTIVELY DEPLETES ALBUMIN FROM LIVER HOMOGENATE AND PLASMA: APPLICATION IN DMET PROTEOMIC ANALYSIS

Matt Karasu, Abdul Basit, Chris Wolford, and Bhagwat Prasad
University of Washington, School of Pharmacy, USA

Background and objectives: Quantification of drug metabolizing enzymes and transporters (DMET proteins) is important in predicting i) the interindividual variability in drug disposition in humans and ii) interspecies extrapolation of drug metabolism and transport. However, tissue proteome is complex which poses a major challenge in LC-MS/MS analysis of low abundant DMET proteins¹. In this study, we aimed to optimize the depletion of high abundant proteins and enrichment of DMET proteins using a simple and cost-effective isopropanol-trifluoroacetic acid (IPA-TFA)-based extraction method for liver tissue proteomics. We then compared albumin depletion performance of this assay across matrices, i.e., tissue and plasma.

Method: Proteins from liver homogenate (80 μL) were precipitated using ice cold methanol (500 μL). The protein pellet was either re-suspended with ammonium bicarbonate solution (100 mM, 80 μL) for trypsin digestion using an optimized protocol (control) or washed with 400 μL IPA:TFA solution (99:1 v/v) for albumin depletion and DMET protein enrichment (extracted). The washed pellet was dried at room temperature and digested using trypsin. Both control and extracted samples were analyzed using i) untargeted proteomics performed on AB SCIEX 5600 triple TOF and ii) targeted DMET proteomics performed on AB SCIEX 6500 triple quadrupole, both coupled with Waters Acquity UPLC. The data were analyzed using Trans Proteomic Pipeline (TPP) and Skyline. The method was also used for albumin depletion from

human plasma.

Result: 26.4% of proteins identified in untargeted proteomics were observed only in control samples, whereas 3.6% of identified proteins were selectively detected in the IPA-TFA extracted sample. Targeted analysis indicates that ~ 25% albumin is depleted with IPA-TFA extraction in tissue samples. In contrast, >90% of the serum albumin was depleted in plasma samples using this method. Majority of UGTs, SULTs, CES and AOX were enriched by 15-55%, 15-65%, 25-30% and 37%, respectively in liver tissue sample. Although this is a moderate enrichment of DMET proteins, IPA-TFA extraction significantly improved signal-to-noise ratio that could allow precise quantification of low abundant proteins. Conclusion: IPA-TFA extraction method is a potential cost-effective approach for depleting high abundant proteins, i.e., albumin. This method could be applied in DMET tissue proteomics after further optimization. The albumin depletion by IPA-TFA method was more effective in plasma than in liver tissue sample.

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P225 - IDENTIFICATION AND QUANTIFICATION OF CYP AND UGT ENZYMES AND DRUG TRANSPORTERS IN HUMAN KIDNEY CORTEX USING QCONCAT-BASED PROTEOMICS

Zubida Al-Majdoub, **Daniel Scotcher**, Brahim Achour, Jill Barber, Aleksandra Galetin, and Amin Rostami-Hodjegan
University of Manchester, United Kingdom

Quantitative data on key drug metabolizing enzymes and transporters in relevant organs are important for prediction of drug disposition using *in vitro* - *in vivo* extrapolation (IVIVE) as an integral part of physiologically-based pharmacokinetic (PBPK) models. Proteomic techniques are used frequently to measure target proteins in biological systems and qualify the tissue fractions using specific markers.

The aim of this study was to quantify enzymes and transporters in human kidney cortex membrane fractions [1] in order to facilitate IVIVE related to the disposition of drugs in kidney. Using targeted accurate mass and retention time (AMRT) approach and QconCAT as a standard [2] (LC-Q Exactive Orbitrap MSMS system), 6 UGTs, 3 CYPs, 8 transporters, one tight junction protein and one plasma membrane marker were quantified in 20 human kidney cortex samples.

Depletion of unbound mycophenolic acid was used to assess the activity of UGT1A9 and UGT2B7 and the quality of proteomic measurements of these enzymes. Inter-correlation analysis for enzymes and transporters was performed on abundance data generated by targeted proteomics. In addition, global proteomic analysis was applied to evaluate the heterogeneity of kidney proteins by assigning them to their subcellular locations and functional classes using UniProtKB, the Gene Ontology Project, the Human Protein Atlas and PANTHER v14 classification system.

In the 20 kidney samples, UGT1A9 and UGT2B7 expression levels were the highest. Spearman rank order (R_s) indicated a significant correlation between UGT abundance and activity measured by mycophenolic acid clearance for UGT1A9 ($R_s=0.82$, $p=0.03$) and UGT2B7 ($R_s=0.70$, $p=0.02$). Kidney CYP2B6, CYP3A5 and CYP4F2 showed comparable expression (on average 0.18 pmol/mg microsomal protein). Expression levels of SLC47A1 and SLCO4C1 transporters were high (7.75 ± 4.73 ; 6.09 ± 5.08 pmol/mg microsomal protein, respectively) and SLC22A3, SLC51A and SLC51B were quantified for the first time. No within- or cross-family correlations were observed for UGTs and CYPs in kidney, in contrast to strong inter-correlations between transporters: ABCB1/SLC47A1 ($R_s=0.76$, $p=0.003$), ABCB1/SLCO4C1 ($R_s=0.78$, $p=0.003$), SLC47A1/SLCO4C1 ($R_s=0.75$, $p=0.007$), SLC51A/SLC51B ($R_s=0.78$, $p=0.003$). The global proteomic method identified >3000 proteins, 447 of which were classified as functional plasma membrane proteins and 865 proteins were directly involved in metabolism.

In conclusion, our findings provide an overview of the expression profile of important drug metabolizing enzymes and transporters and heterogeneity of kidney cortex membrane fractions. Importantly, 3 transporters (SLC22A3, SLC51A and SLC51B) were measured for the first time. Several inter-correlations between transporters in the kidney were established to support simulation of virtual population of subjects with more realistic distributions of transporter abundances.

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P226 - INVESTIGATING UREMIC SOLUTE-DRUGS INTERACTION USING *IN VITRO* TRANSPORTER ASSAYS

Shih-Yu Chang, Alenka Chapron, Tomoki Imaoka, and Catherine K. Yeung
University of Washington, USA

Renal tubule secretion via transporter proteins plays a critical role in the renal clearance of drugs and endogenous compounds. We hypothesize that competition between accumulating uremic solutes and drugs for proximal tubule

transport results in unpredictable drug exposure in people with renal impairment. The goal of this study was to characterize the potential of prominent uremic solutes, including hippuric acid (HA), indoxyl sulfate (IS), p-cresol sulfate (pCS) and trimethylamine N-oxide (TMAO), to inhibit renal tubule transport. We used penciclovir (PEN), oseltamivir carboxylate (OST) and tenofovir (TEN) in our study as model transporter substrates. We conducted systemic screens of transporter inhibition using *in vitro* assays, including uptake (overexpressed cell lines) and efflux (in-side out vesicle membranes) transporters, to investigate whether uremic solutes and model drugs are potential inhibitors/substrates of renal uptake and efflux transporters involved in tubular secretion (OAT1/2/3, MRP2/4, MATE1/2K, P-gp, BCRP). This systematic study of uremic solute - drug interaction demonstrated that some uremic solutes and drugs (PEN and TEN) might compete and inhibit uptake transporters via OATs and efflux via MRPs transporters. Furthermore, TEN uptake via OAT1/3 was inhibited by HA (IC₅₀= 10.11 to 20.99 μM for OAT1 and IC₅₀= 28.65 to 40.82 μM for OAT3), IS (IC₅₀= 23.03 to 81.18 μM for OAT1 and IC₅₀= 72.31 to 117.4 μM for OAT3), and pCS (IC₅₀= 37.34 to 55.12 μM for OAT1 and IC₅₀= 32.15 to 68.67 μM for OAT3). A clearer understanding of the interactions between drugs and uremic solutes may lead to personalized approaches for the selection and dosing of medications in people with kidney disease.

P227 - PHARMACOKINETICS OF GDC-0810 AND ITS INTERACTION WITH COPROPORPHYRIN I IN THE FIRST-IN-HUMAN, ASCENDING DOSE STUDY IN WOMEN WITH LOCALLY ADVANCED OR METASTATIC ESTROGEN RECEPTOR POSITIVE BREAST CANCER

Kit Wun Kathy Cheung¹, Kenta Yoshida², Sravanthi Cheeti², Buyun Chen², Roland Morley², Iris T. Chan², Srikumar Sahasranaman², and Lichuan Liu²

¹University of California, San Francisco, USA, ²Genentech, Inc, USA

GDC-0810 is an orally bioavailable, selective estrogen receptor (ER) degrader developed for the treatment of ER-positive breast cancer (BC). A first-in-human (FIH) ascending dose study that enrolled 41 female ER+, human epidermal growth factor 2-negative (HER2-) BC patients was conducted to characterize the pharmacokinetics of GDC-0810 and its two major metabolites to inform the recommended Phase 2 dose. Pharmacokinetic profiles were assessed both after single dose and at steady state in all patients. GDC-0810 demonstrated linear pharmacokinetic profiles from 100 mg QD to 600 mg QD. The mean terminal half-life following a 600-mg single dose was at approximately 8 hours under non-fasted condition and minimal accumulation was observed following multiple dosing for both QD and BID regimens. Since GDC-0810 is a potent *in vitro* inhibitor of organic anion transporting polypeptide (OATP) 1B1/3, the kinetic profile of coproporphyrin I (CPI), a promising endogenous biomarker for OATP1B1/1B3, was also analyzed in a subset of the plasma samples collected in the same study (single dose analysis: n = 21; steady state analysis: n =18). CPI exhibited a GDC-0810 dose-dependent increase, suggesting *in vivo* inhibition of OATP1B transporters. To quantitatively predict the magnitude of OATP1B-mediated drug-drug interactions (DDI) with pravastatin, a known OATP1B substrate, the *in vivo* unbound inhibition constant was estimated using a one-compartment model and was then incorporated to a physiologically-based pharmacokinetic model. The model showed some under-estimation of the magnitude of DDI when compared to the results from a clinical DDI study, while prediction had relatively large uncertainty due to the small effect size, limited sample size per cohort and variability in CPI kinetics. Collectively, this study confirmed the utility of CPI to detect OATP1B-mediated DDI of a new molecular entity (NME) as early as the ascending dose phase in drug development. This approach would allow not only early evaluation of the DDI potential of the NME at a wide range of drug exposure but also limit unnecessary drug exposure in dedicated DDI studies that often enroll healthy volunteers.

P228 - ASSAY CALIBRATION TO REFINE PREDICTION OF OATP1B1/1B3 MEDIATED DRUG-DRUG INTERACTIONS (DDI) BASED ON *IN VITRO* UPTAKE TRANSPORT ASSAY DATA

Peter Kovacs, Rucha Sane, Kit Wun Kathy Cheung, Emese Kis, Emile Plise, and **Zsuzsanna Gaborik**
SOLVO Biotechnology, Hungary

According to the draft guidance on drug-drug interaction of The U.S. Food and Drug Administration (FDA) *in vitro* assays used for DDI prediction should be calibrated to refine prediction parameters. Inhibition potency of all investigational drug on the uptake of a known OATP1B1 and 1B3 substrates should be tested *in vitro*, applying a 30 minutes pre-incubation with the drug. Based on the measured IC₅₀ values, R values are calculated according to the guidance. If the R value is ≥1.1 (FDA) or 1.04 (EMA), the investigational drug has a potential to inhibit OATP1B1/1B3 *in vivo*. However, these cut-off values may not be directly translatable to observed DDI magnitude due to the significant lab-to-lab variability of IC₅₀ values for transporters and involvement of multiple transporters in substrate disposition. Therefore, our aim was to calibrate uptake transporter assays to evaluate the *in vivo* prediction value and refine the cut-off criteria if necessary. 26 compounds with available clinical DDI data using rosuvastatin as a victim drug were evaluated for their *in vitro-in vivo* correlation of OATP1B1/1B3 inhibition. IC₅₀ determination was conducted using validated assay systems, and probe substrates of E217βG for OATP1B1 and CCK-8 for OATP1B3. Our results demonstrated that DDI between rosuvastatin and a new molecular entity can be predicted with relatively high level of confidence using E217βG as OATP1B1 substrate in *in vitro* experiments. Using FDA cut-off the true positive rate (TPR) of DDI prediction is 75%, with a false positive rate (FPR) of 46%. EMA cut-off gave a higher sensitivity of 92%, but in parallel the FPR increased to 69%. Using the simplified

calculation proposed by Vaidyanathan et al, with a cut-off value of 0.1 for I_{max}/IC_{50} [1], gave a lower FPR of 46% than R-value calculation with the same level of sensitivity (92%). Incorporating 1B3 contribution weighted with *in vivo* expression or activity data, caused only minor changes in the overall prediction. To further improve the accuracy of the prediction we considered the role of BCRP inhibition by the respective perpetrators. Using a method adapted from Elsby et al [2], predicted *in vivo* AUCR was calculated using the OATP1B1/1B3 and BCRP IC_{50} values for the relevant probe substrates. The sensitivity of the prediction increased, and predicted AUCR values are closer to the *in vivo* AUCR-s. In conclusion, our OATP1B1 assay using E217bG as substrate and $R \geq 1.1$ cut-off yielded acceptable level of sensitivity and FPR. Considering OATP1B1/1B3 and BCRP inhibition holistically increased quantitative prediction accuracy of *in vivo* DDI magnitude.

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P229 - P-GLYCOPROTEIN ACTIVATION MECHANISM BY SNAIL-INDUCED EPITHELIAL-TO-MESENCHYMAL TRANSITION IN LUNG CANCER CELLS

Hiroki Kamioka¹, Takumi Tomono², Kentaro Yano¹, Yukiyoishi Fujita³, Atsushi Fujita³, Ryoichi Onozato³, Misa Iijima³, Shigeru Tsuchida³, Takahiro Arai³, and Takuo Ogihara¹

¹Takasaki University of Health and Welfare, Japan, ²Setsunan University, Japan, ³Gunma Prefectural Cancer Center, Japan

[Purpose] Epithelial-mesenchymal transition (EMT) is known as one of the metastasis mechanisms of cancer cells which is caused by transcriptional factor such as Snail. It has been suggested that EMT is involved not only in metastasis but also in drug resistance, and we previously reported that efflux transporter P-glycoprotein (P-gp), which is thought to be involved in drug resistance, activation and drug resistance is observed in lung cancer cells at EMT induced by Snail.¹) However, the mechanism remains unclear. In this study, we aimed to clarify the influence of Snail-induced EMT on P-gp expression and activity and its mechanism. [Method] Lung cancer cell HCC 827 was transfected with Snail gene to induce EMT. The expression level of P-gp and its membrane expression regulator Moesin, were measured by qRT-PCR and Western blotting. Furthermore, the function of P-gp and cell viability were evaluated using rhodamine 123 (rho123) or anti-cancer drug paclitaxel, which are substrate drugs of P-gp. [Results and Discussion] In Snail-transfected cells, no change was observed in the protein expression level of P-gp. However, in efflux assay using rho123, the excretion of rho123 from the cell was enhanced.

At this time, enhancement of resistance to paclitaxel and increase of expression level of Moesin were observed. In addition, following knockdown of Moesin expression using siRNA, activity of P-gp and resistance to paclitaxel were attenuated. Furthermore, a positive correlation was found between Snail and Moesin expression levels in cancer tissues of lung cancer patients. On the other hand, no correlation was found in normal tissues. It was revealed that resistance to P-gp substrate anticancer drugs is exhibited by increasing the efflux activity of P-gp by increasing the Snail expression level in lung cancer cells. It was suggested that this mechanism involves an increase in the expression level of Moesin.

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P230 - INCREASE IN RENAL EXPOSURE TO IMATINIB DURING CHOLESTASIS IN MICE

Takumi Kawanishi, Hiroshi Arakawa, Yusuke Masuo, and Yukio Kato
Faculty of Pharmacy, Kanazawa University, Japan

[Introduction] Liver plays a central role in elimination of drugs by enzymatic metabolism and biliary excretion. Therefore, once dysfunction of the liver such as cholestasis is induced, systemic elimination of many drugs is delayed due to decrease in the function of metabolic enzymes and/or hepatic transporters. In such condition, on the other hand, renal secretion process for a certain drug is up-regulated possibly to compensate the reduced hepatic elimination of the drugs from the body. Patients with liver dysfunction thus generally show increased risk of drug-induced nephrotoxicity due to increasing renal exposure, although underlying mechanism has not yet been fully clarified. The aim of the present study is to clarify possible renal exposure of imatinib during cholestasis with an aim to clarify which process is involved in the increased renal exposure.

[Methods] Bile-duct above pancreas was ligated to construct bile-duct ligation (BDL) mice. Pharmacokinetic experiments were performed in the BDL mice one day or two weeks after the surgery.

[Results and Discussion] The plasma concentration profile of imatinib after intravenous administration in BDL mice was essentially similar to that in sham-operated mice, whereas the distribution of imatinib to the kidney in BDL mice was 16-times higher than the sham-operated ones. Initial phase renal distribution of imatinib after intravenous administration to the kidney of BDL mice was just twice that in sham-operated ones. Urinary excretion was minimally different between BDL and sham-operated mice, and less than 1% in both group. Uptake of imatinib into kidney slice especially at late phase was increased in BDL mice compared to sham-operated ones, whereas its efflux from the slices was decreased in BDL mice. Thus, up-regulation of uptake transporter and/or down-regulation of efflux transporter in the kidney may be one of the reasons for the increased renal exposure to imatinib in BDL mice, although possible change in intracellular handling may not be excluded.

[Conclusion] Renal accumulation of imatinib was increased in cholestatic mice compared with normal mice. This may represent compensation of reduced hepatic elimination, and both increased renal uptake and decreased renal efflux might be involved as the mechanism.

P231 - STUDY TO ASSESS THE FOOD EFFECT ON PLASMA LEVELS OF EMERGING ENDOGENOUS BIOMARKERS OF TRANSPORTER MEDIATED DRUG-DRUG INTERACTIONS

Amanda King-Ahmad, Brian Rago, Lina Luo, A. David Rodrigues, and Ragu Ramanathan
Pfizer Inc., USA

Drug-Drug interactions (DDIs) can have significant clinical implications for new drugs and are routinely evaluated during development. DDIs involving drug-metabolizing enzymes, such as cytochromes P450 are well documented¹. However, more recently, there has been an increased interest in DDIs involving transporters¹. Currently the paradigm for determining the DDI liability of new drugs uses predefined decision trees from regulatory agencies which rely on *in vitro* data². Using this process, drugs with a potential liabilities are identified and investigated with dedicated clinical DDI studies. This process has historically lead to high false positive rates and costly clinical studies which ultimately return no DDI findings². Recently, the use of endogenous substrates of specific transporters early in clinical trials, as probes of DDIs, has gained traction to reduce the false positive rates and the number of costly clinical trials³. Coproporphyrin-I (CP-I) and coproporphyrin-III (CP-III) have shown promise as two such biomarkers selective for organic anion-transporting polypeptides (OATPs), with plasma AUC changes similar to those observed with statins in dedicated clinical DDI studies⁴. In addition to CP-I and CP-III, known biomarkers of other transporters include N1-methylnicotinamide (NMN) for organic cation transporters (OCT2/MATEs)⁵, and selected bile acids (BAs), also for OATPs⁶. Although CP-I, CP-III, NMN, and BAs have been shown to be biomarkers of transporter mediated DDIs, it is not understood what effect food intake might have on their plasma concentrations. The present study was designed to gain an understanding of how food intake may affect endogenous levels of these biomarkers in humans. Donors who had not been on any medications or supplements for at least 5 days were selected for this study and divided into two groups; a high fat meal group and a high carbohydrate meal group. Plasma samples were collected from each donor fasted and 90-minutes after consumption of the appropriate meal type. Plasma levels of CP-I, CP-III, NMN and selected bile acids were evaluated for these subjects using fit for purpose LC-MS/MS or LC-HRMS assays. Preliminary results indicate that selected bile acids plasma levels vary with food intake, with the direction of change dependent on the meal type. Additional data were pending at the time of abstract submission.

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P232 - PREDICTION OF HEPATIC CLEARANCE AND PARTITIONING IN RAT FOR IMPROVED BSEP INHIBITION RISK ASSESSMENT IN HUMANS

Anil Kolur¹, Andrew Hogan², Shelby Hullett¹, Geri Sawada¹, Michael Mohutsky¹, Kathleen Hillgren¹, Yingying Guo¹, and Bridget Morse¹

¹Eli Lilly & Company, USA, ²Advanced Testing Laboratory, USA

It is imperative to understand the unbound tissue partitioning (K_{Pu,u}) and clearance in the liver for compounds whose disposition are determined by both transporters and metabolism. K_{Pu,u} and clearance of substrates that are transported via the Organic Anionic Transporter Polypeptide 1B1 (OATP1B1) have been generally under-predicted. As a result, the addition of albumin to *in vitro* studies has been suggested in order to improve *in vitro* to *in vivo* extrapolation. In this study, we predicted K_{Pu,u} and *in vivo* clearance of 28 compounds from a diverse chemical space, including substrates of OATP1B1 or Organic Cationic Transporter 1 (OCT1) using rat and human hepatocytes. Rat liver K_{Pu,u} and *in vivo* clearance was obtained via steady state infusion. Uptake in the presence and absence of transporter inhibitors was determined with cryopreserved hepatocytes in suspension with 4% bovine serum albumin (BSA). The unbound fraction was determined for both plasma and 4% BSA, and subsequently used to correct for free active and passive uptake clearance. Metabolic intrinsic clearance was measured via either oxidative or glucuronidation pathways using human and rat liver microsomes. Liver K_{Pu,u} was calculated using various published methods. Using the extended clearance approach as well as total uptake, intrinsic clearance was calculated for all the substrates, and then scaled to *in vivo* clearance using the well-stirred model. In addition, Bile Salt Export Pump (BSEP) inhibition was evaluated using a vesicle system. From evaluation of uptake into human hepatocytes, the addition of albumin increased both the passive and active intrinsic clearance proportionally. However, addition of albumin effectively slowed the reaction to enable accurate measurement of uptake. As a result, K_{Pu,u} was under-predicted while incorporating the active and passive uptake clearances as well as the metabolism. Correspondingly, total uptake better predicted rat and human *in vivo* clearance when compared with the extended clearance method. Appropriate scaling factors were determined from rat *in vitro* and *in vivo* studies, and implemented to accurately scale human *in vitro* clearance to *in vivo* within two-fold of the observed value. This approach was used to estimate human intracellular liver concentrations to incorporate with *in vitro* BSEP inhibition data for better risk assessment of BSEP inhibition *in vivo*.

P233 - QUANTIFICATION OF PRAVASTATIN UPTAKE INTO RAT, DOG, MONKEY AND HUMAN PLATED CRYOPRESERVED HEPATOCYTES IN THE PRESENCE AND ABSENCE OF BOVINE SERUM ALBUMIN

Albert P. Li¹, Maria Ribadeneira², Kirsten Amaral¹, Novera Alam¹, Lili Yao³, and Patricia Schroeder³

¹In Vitro ADMET Laboratories Inc., USA, ²Forma Therapeutics Inc., USA, ³Forma Therapeutics Inc., USA

Transporter-mediated drug uptake is becoming commonly used strategy of drug delivery into the liver. We report here an experimental approach using hepatocytes from multiple species, including human, to evaluate transporter-mediated uptake, with the intention to more accurately predict human hepatic drug clearance. Our goal is to develop *in vitro-in vivo* correlations between *in vitro* hepatocyte and *in vivo* data with the preclinical animal species, followed by extrapolation of human hepatocyte data to human *in vivo*. We report here results with a model uptake transporter substrate, pravastatin, using cryopreserved hepatocytes from SD rat, beagle dog, cynomolgus monkey and human. Pravastatin uptake was evaluated using a plated-hepatocyte uptake assay developed in our laboratory with hepatocytes cultured as monolayer cells in collagen-coated 96-well plates. Uptake was performed in the presence and absence of the uptake transport inhibitor, rifampin, to allow the quantification of diffusion uptake (uptake in the presence of rifampin) and transporter-mediated uptake (total uptake minus diffusion uptake). Hepatocyte uptake was evaluated in protein-free Hepatocyte Incubation Medium (HQM) as well as in medium containing 7% BSA to model plasma protein binding *in vivo*. Upon incubation of the hepatocytes with pravastatin, the treatment medium was removed. The hepatocytes were washed multiple times with protein-free HQM to remove any remaining extracellular drug material. Acetonitrile was then added to extract the intracellular drug fraction followed by quantification of drug concentration by LC/MS-MS. Time- and concentration-dependent uptake of pravastatin and inhibition of uptake by rifampin were observed both in the protein-free and BSA medium. Species differences in pravastatin uptake were observed, with the uptake rate in rat>monkey>dog>human. The calculated passive and active clearance values from hepatocyte uptake data in the presence and absence of BSA were used to estimate *in vivo* clearance values. Our results suggest that quantification of drug uptake in hepatocytes from preclinical animal species and human combined with *in vivo* animal data may aid the estimation of transporter-mediated drug clearance in human *in vivo*.

P234 - USE OF A BILE SALT EXPORT PUMP KNOCKDOWN RAT SUSCEPTIBILITY MODEL TO INTERROGATE MECHANISM OF DRUG INDUCED LIVER TOXICITY

Yutai Li, Raymond Evers, Michael Hafey, Kyeongmi Cheon, Hong Duong, Lisa LaFranco-Scheuch, Alex Tamburino, Kara Pearson, Jose Lebron, Warren Glaab, and Frank Sistare
Merck, USA

Introduction: Inhibition of the Bile Salt Export Pump (BSEP) may be associated with clinical drug-induced liver injury, but it is poorly predicted by preclinical animal models. Here we present the development of a novel rat model using siRNA knockdown (KD) of Bsep that displayed differentially enhanced hepatotoxicity to 8 Bsep inhibitors and not to 3 Bsep non-inhibitors when administered at maximum tolerated doses for 7 days.

Hypotheses: The hypotheses tested in the studies are that: (i) Knockdown of Bsep will reduce the rat liver's normal bile acid (BA) export capacity resulting in enhanced BA levels in liver and plasma; (ii) Such rats will be more responsive, sensitive, and/or susceptible to Bsep inhibition when administered effective doses of Bsep inhibitor drugs and present with an even greater BA response in liver and plasma. This model is expected to enhance our understanding of the utility of mechanistic liver tissue (mRNA and BAs) and translational biomarkers (plasma BAs and a stable label BA tracer) that could inform disruption by drugs of BA homeostasis. Because rats have much less toxic BA profiles as compared to humans, the model may or may not be expected to present a conventional DILI phenotype.

Materials and Methods: Wistar rats were i.v. injected with siRNA targeting rat Bsep on study day 1, then dosed with Bsep inhibitor and non-inhibitor drugs for 6 days. Plasma and liver samples were collected for analysis of drugs and bile acids. The mRNA gene expression levels were also measured using qPCR. Serum clinical chemistry and liver histomorphology were evaluated.

Results: Bsep KD alone resulted in 3- and 4.5-fold increases in liver and plasma levels, respectively, of the sum of the 3 most prevalent taurine conjugated BA (T3-BA), approximately 90% decrease in plasma and liver glycocholic acid, and a distinct BA regulating gene expression pattern, without resulting in hepatotoxicity. Among the Bsep inhibitors, only asunaprevir and TAK-875 resulted in serum transaminase and total bilirubin increases associated with increases in plasma T3-BA that were enhanced by Bsep KD. Benzbromarone, lopinavir, and simeprevir caused smaller increases in plasma T3-BA, but did not result in hepatotoxicity in Bsep KD rats. Bosentan, cyclosporine A, and ritonavir, however, showed no enhancement of T3-BA in plasma in Bsep KD rats, as well as Bsep non-inhibitors acetaminophen, MK-0974, or clarithromycin. T3-BA findings were further strengthened through monitoring TCA-d4 converted from cholic acid-d4 overcoming inter-animal variability in endogenous bile acids. Bsep KD also altered liver and/or plasma levels of asunaprevir, TAK-875, TAK-875 acylglucuronide, benzbromarone, and bosentan.

Conclusions: The Bsep KD rat model has revealed differences in the effects on bile acid homeostasis among Bsep inhibitors that can best be monitored using measures of T3-BA and TCA-d4 in plasma. However, the phenotype caused by Bsep inhibition is complex due to the involvement of several compensatory mechanisms.

P235 - IN VITRO DEVELOPMENT OF FOLATE TRANSPORT AND ENDOCYTOSIS BY PCFT, RFC, and FR α , IN A TRANSWELL SYSTEM

Xuexiang "John" Zhang, Yewei Du, Mark Warren, and Steven Louie
BioIVT, USA

Scientists continue to explore novel approaches to drug discovery and development for cancer therapeutics. Frequently transporters are exploited to target drug delivery to cancer cells. Folic acid (Vitamin B9) transporters remain a viable transporter candidate to explore anti-cancer drugs. Folic acid is needed for de novo synthesis of purines and thymidine for DNA synthesis and repair. Folate or its reduced form is transported by reduced folate carrier (RFC), proton-coupled folate transporter (PCFT), and folate receptor (FR) α, β, γ . FR- α, β isoforms are membrane bound. FR- α on non-cancerous cells is confined to cells crucial for embryonic development, the choroid plexus, and kidneys. However, due to the ubiquitous expression of RFC and PCFT in cancers, folate analogs (antifolates) have been developed for treatment: methotrexate, pemetrexed, pralatrexed are transported into tumor cells by RFC and PCFT. In addition, FR- α is expressed 100-300x higher in carcinomas than healthy cells, making this an ideal target to exploit novel drug therapies. FR- α is over expressed in epithelia carcinomas and can be used as tumor-targeted drug delivery system for small molecule-drug conjugates. In fact, many new, folic acid conjugated cytotoxics can be endocytosed, released in endosome, diffused out to intracellular targets. Here we describe development of *in vitro* folate transporter and receptor assays to potentially support drug targeting. We transiently-transfected PCFT, RFC, or FR α to MDCK cells in 96-well transwells and tested folate and anti-folate probe transport. PCFT was expressed mainly on the apical side and demonstrated both methotrexate and folic acid transport, preferentially at acidic pH. PCFT folate transport showed a 31.7x FOA above mock controls. Bromosulphthalein (BSP) inhibited PCFT folic acid transport with an estimated IC50 value of 78.1 μ M. No folic acid transport was observed by RFC. However, RFC transported methotrexate from the basolateral side and was inhibitable by BSP and pemetrexed with an IC50 value for pemetrexed at 157 μ M. We demonstrated FR α receptor-mediated endocytosis of folic acid from both the apical and basolateral sides, resulting in an intracellular folic acid concentration 10.7x above mock controls. Both pemetrexed and BSP inhibited folic acid endocytosis with IC50 values of 17.5 μ M and 1634 μ M, respectively. Taken

together, we have developed viable *in vitro* tools to support folate transporter drug discovery as well as the potential to develop other receptor-mediated endocytosis/transcytosis such as asialoglycoprotein receptor (ASPGR), transferrin receptor (TfR), low density lipoprotein receptor (LDLR), or potentially any other membrane bound receptor.

P236 - SCREENING OF ENDOGENOUS OCTS SUBSTRATES APPLICABLE FOR PREDICTION OF DRUG INTERACTIONS

Yusuke Masuo, Megumi Kato, Shiori Komura, Takahiro Ishimoto, and Yukio Kato
Kanazawa University, Japan

Organic cation transporters (OCT1/SLC22A1 and OCT2/SLC22A2) are expressed on the sinusoidal membranes of hepatocytes and basolateral membranes of renal proximal tubules, respectively, and involved in hepatic and renal distribution of various substrates. Since patients with SNPs decreasing functional activity of OCT1 showed higher plasma concentrations of certain OCT1 substrate drugs than those with wild-type, OCT1 may be primarily involved in pharmacokinetics of some substrate drugs. Although several tyrosine kinase inhibitors inhibit OCTs *in vitro* and their IC50 values were close to the unbound concentration in circulating plasma in humans, their possible inhibition of OCTs *in vivo* is still largely unknown. Thus, plasma concentration of endogenous OCTs substrates would be useful to estimate the transport activity and the possible occurrence of drug interaction via OCTs *in vivo*. The aim of the present study is to search endogenous OCTs substrates. Plasma and liver samples of mice with or without intravenous administration of OCTs inhibitors, tetrapentylammonium (TPeA; 11.3 mg/kg) and imatinib (100 mg/kg), were comprehensively analyzed by LC-TOFMS. Peaks with significantly different intensity between vehicle- and inhibitor-treated groups were regarded as candidates of endogenous OCTs substrates. Parent and product mass scanning were then performed to obtain chromatogram and MS spectrums, which was then compared with MS spectrums of database and standard compounds. Uptake studies in HEK293 cells expressing OCTs were also performed to examine whether or not the selected compounds are transported by OCT1. According to the untargeted metabolome analysis, plasma concentration of some acylcarnitines in both TPeA- and imatinib treated mice were higher than that in vehicle-treated mice. Since acylcarnitines contain quaternary ammonium cation in their structures, they are possible substrates of OCTs. Actually, higher plasma concentration of acylcarnitines in liver-specific Slc22a1^{-/-} mice was previously reported although minimal information is available on the effect of exogenous administration of OCTs inhibitors on their concentration *in vivo*. Plasma concentration of acylcarnitines, especially hydroxyacylcarnitines and long-chain acylcarnitines, was increased after administration of TPeA and imatinib, but not vehicle alone, in time-dependent manner. Time-dependent uptake of acylcarnitines and hydroxyacylcarnitines was also observed in HEK293/hOCT1 cells, but this uptake was not significantly reduced in the presence of the OCTs inhibitors. Since acylcarnitines can be metabolized to short-chain acylcarnitines in cells, isotope labelled-acylcarnitines should be used to further evaluate transporter-mediated uptake. Thus, acylcarnitines were presumably endogenous OCT1 substrates although the role of OCTs in their tissue distribution should be further evaluated.

P237 - VARIABILITY OF OATP1B1/1B3 *IN VITRO* INHIBITION CONSTANTS AND THE RESULTING IMPACT ON CLINICAL EVALUATION

Savannah McFeely, Tasha Ritchie, and Isabelle Ragueneau-Majlessi
University of Washington, USA

The effect of inhibition of the organic anion transporting polypeptides (OATP) 1B1 and 1B3 has continued to grow in clinical significance and recognition. In the last five years, a significant portion of newly approved drugs in the US have been shown to be inhibitors of OATP1B1 and/or OATP1B3 *in vitro*. For this reason, it is critical to understand the effect of experimental variability on drug interaction predictions and how it impacts the decision for a clinical evaluation. Using the University of Washington Drug Interaction Database (DIDB®, www.druginteractioninfo.org), all studies showing *in vitro* inhibition of OATP1B1 and/or -1B3 were identified and those reporting a Ki or IC50 value retained. Data were further refined by retaining those substrate/inhibitor pairs with at least three unique experiments reporting an inhibition constant. For each pair, a variability ratio (VR, highest value relative to lowest) was calculated. For OATP1B1, cyclosporine/estradiol-17- β -glucuronide (E217 β G) showed the highest variability while rifampin/E217 β G showed the most variability for OATP1B3, with VR of 86.4 and 58.1, respectively. Two experimental factors were found to contribute the most to inhibition constant variability – cell type and preincubation versus co-incubation with inhibitors. When these two conditions were accounted for, the overall variability in the entire dataset was reduced from 12.35 to 5.23. A significant degree of substrate-dependency was also observed. Of the inhibitors evaluated, cyclosporine and rifampin were tested with the largest array of substrates, and interestingly, the highest variability was observed with non-clinically relevant substrates, namely E217 β G, estrone-3-sulfate, and bromosulfophthalein (BSP). For OATP1B1, cyclosporine VR ranged from 3.42 to 86.32 (atorvastatin and E217 β G, respectively) while rifampin VR ranged from 3.85 to 43.64 (atorvastatin and BSP, respectively). When only HEK293 cells and co-incubation were considered, the variability for each inhibitor was reduced to a range of 3.04 – 12.62 for cyclosporine and 3.85 – 7.91 for rifampin. To determine the effect of the observed variability on clinical predictions, R-values (as described in the latest FDA guidance) were calculated for each constant

and the range for each inhibitor determined. Despite significant changes in VR when incubation conditions were accounted for, the resulting R-values did not show a significant shift with respect to the FDA cut-off value for prompting a clinical evaluation ($R \geq 1.1$). For the recommended index inhibitors cyclosporine and rifampin, all calculated R-values were ≥ 1.1 regardless of the *in vitro* conditions. Similar to VR, R-values showed substrate-dependent variability with the fold-change within each substrate/inhibitor pair ranging from 2.34 – 51.15 for cyclosporine and 3.13 – 12.77 for rifampin. This variability, both within the pairs and for the inhibitors overall, was decreased when the two primary sources of variability were accounted for, ranging from 2.34 – 8.58 for cyclosporine and 2.84 – 5.71 for rifampin. These results suggest significant variability in *in vitro* inhibition constants for inhibitors of OATP1B1 and OATP1B3, which translates to variability in R-values, highlighting the importance of standardizing the experimental conditions, including the substrate used.

P238 - FUNCTIONAL INVESTIGATION OF SOLUTE CARRIER FAMILY 35 MEMBER F2 (SLC35F2) AS A DRUG TRANSPORTER IN THE BLOOD-BRAIN BARRIER

Tatsuki Mochizuki¹, Tadahaya Mizuno¹, Kei Higuchi², Yuma Tega², Toshiki Kurosawa², Tomoko Yamaguchi³, Kenji Kawabata³, Yoshiharu Deguchi², and Hiroyuki Kusuhara¹

¹Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan, ²Faculty of Pharma-Sciences, Teikyo University, Japan, ³National Institutes of Biomedical Innovation, Health and Nutrition, Japan

[Purpose] In order to address regulation of drug distribution to the central nervous system, elucidation of drug transport systems in the blood-brain barrier (BBB) is essential. In this study, we have focused on SLC35F2 as a candidate of the novel drug transporter in the BBB because of its high mRNA expression in mouse brain microvascular endothelial cells (BMECs)¹. The objective of this study was to elucidate the localization and function of SLC35F2 in the BBB. [Methods] A surviving inhibitor YM155 was used as a SLC35F2 function probe. YM155 was quantified by LC-MS/MS (QTRAP 5500). The BBB permeability of YM155 was analyzed by *in situ* mouse brain perfusion. We used three *in vitro* primate BBB models; hCMEC/D3 cells, MBT-24 (Pharmaco-cell company) composed of monkey BMECs, rat astrocyte and pericyte, and BMECs derived from human iPS cells (hiPS-BMECs)². SLC35F2 knockout (KO) PC-3, SLC35F2 highly expressing cell, and hiPS-BMECs were constructed using CRISPR/ Cas9 system. [Results and Discussion] SLC35F2 protein expression was detected in mouse/ monkey BBB fraction, hCMEC/D3 cells and hiPS-BMECs by western blot analysis. Immunocytochemistry revealed SLC35F2 was localized on the apical (luminal) membrane of hiPS-BMECs, and not detected in SLC35F2 KO hiPS-BMECs. SLC35F2 KO PC-3 cells was employed to evaluate YM155 uptake mediated by SLC35F2. It was inhibited by famotidine, not by tetraethylammonium and L-carnitine. In *in situ* mouse brain perfusion test, YM155 permeability across the BBB was saturable. However, Slc35f2 KO mice showed an identical permeability to wild type mice. YM155 transport in mouse BBB could be mediated by organic anion transporter 1a4 (Oatp1a4). On the other hand, in hCMEC/D3 cells, YM155 uptake was significantly decreased by famotidine and SLC35F2 siRNA treatment. In MBT-24, YM155 Apical-to-Basal (A-to-B) transport and its intracellular accumulation were markedly decreased by famotidine treatment. In hiPS-BMECs, the inhibition profile of YM155 transport was similar to that of YM155 uptake in PC-3 cells which was mediated by SLC35F2. Crucially, YM155 A-to-B transcellular transport was drastically decreased and hardly detected in SLC35F2 KO hiPS-BMECs. [Conclusions] These results prove that SLC35F2 is localized on the luminal membrane of the BMECs and is a functional drug influx transporter in various primate BBB model cells. There should be species difference in YM155 transport across the BBB between mouse and primates and it may be caused by the difference of OATP transporter expression.

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P239 - BCRP GENE DELETION ALTERS THE GUT MICROBIOME IN MICE

Kamalika Mukherjee¹, Samit Ganguly², Jingjun Lu¹, and Erin Schuetz¹

¹St. Jude Children's Research Hospital, USA, ²The University of North Carolina at Chapel Hill, USA

Breast cancer resistant protein (BCRP) is an important member of the ATP-bound cassette (ABC) family of efflux transporters, known to transport a wide variety of endogenous, dietary and xenobiotic substrates. BCRP plays a significant role in the intestinal secretion of its endogenous substrate uric acid, and loss of function polymorphism of BCRP (Q141K) has been linked to hyperuricemia and gout. Interestingly, it has been recently reported that the intestinal microbiota of healthy humans is significantly different from gout patients, suggesting that an altered gut microbiome might contribute to the systemic pool of uric acid. Therefore, elucidating the potential impact of the uric acid-secreting BCRP

transporter on intestinal microbiota may help us better understand several pathophysiological conditions associated with the gut microbiome especially in a population with BCRP polymorphisms, as well as identify probable side effects associated with orally administered BCRP inhibitors. Our recent findings from an untargeted metabolomics study identified several gut microbiota-related metabolites which were significantly altered in the cerebrospinal fluid (CSF) and plasma of Bcrp-Pgp double knockout rats, compared to wild-type (WT) rats, suggesting that the absence of these transporters might affect the intestinal microbiota, leading to an altered CSF and plasma metabolome in rats. Based on these results, we hypothesized that Bcrp might play an important role in regulating the host gut microbiome. To test this hypothesis, we performed 16S rRNA sequencing of fecal microbiota obtained from WT FVB and Bcrp knockout (KO) male mice (n = 10/genotype) at Microbiome Insights and analyzed microbial diversity / abundance using MicrobiomeAnalyst software. Principal Coordinates Analysis (PCoA) revealed a significant difference in beta diversity or microbial composition (PERMANOVA: $R^2 = 0.39$; $p < 0.001$) between WT and Bcrp KO mice, with hierarchical clustering separating the genotypes into two distinct clusters. The abundance of gut bacteria from all taxonomic levels were found to be significantly altered between WT FVB and Bcrp KO mice ($FDR < 0.05$), with at least one of them being previously reported to be associated with uric acid levels. Interestingly, we observed a trend toward increased concentration of uric acid in the feces of Bcrp KO mice, compared to WT mice. In addition, unbiased Pearson correlation analysis identified several bacteria, among those altered between WT and Bcrp KO mice, that correlated significantly ($p < 0.05$) with the fecal uric acid concentrations. Covariation of microbiota composition with uric acid levels and Bcrp genotype replicated in an independent set of WT and Bcrp KO mice on a C57BL/6J background. In conclusion, our results indicate that Bcrp gene deletion alters the gut microbiome in mice, suggesting an important role of a major intestinal efflux drug transporter in regulating host – microbiome interactions.

P240 - SUBSTRATE RECOGNITION AND CHLORIDE ION DEPENDENT TRANSPORT OF ANGIOTENSIN II RECEPTOR BLOCKERS BY OAT4

Saki Noguchi, Moeko Tobita, Hayumi Atsuta, Rika Kimura, Ayaka Fukumoto, Tomohiro Nishimura, and Masatoshi Tomi
Faculty of Pharmacy, Keio University, Japan

The purpose of this study was to evaluate organic anion transporter (OAT) 4-mediated transport of angiotensin II receptor blockers (ARBs) for considering the potential involvement of OAT4 on the renal elimination. OAT4, which is localized at the apical membrane of the human renal proximal tubules, can export olmesartan (the active metabolite of olmesartan medoxomil), an ARB, against the physiological Cl⁻ gradient. Although some ARBs are partly excreted in the urine, the transporters involved in the renal excretion of ARBs are little understood. Then, since ARBs besides olmesartan may also be substrates of OAT4, we examined the substrate recognition and effect of extracellular Cl⁻ on OAT4-mediated transport of various ARBs. The uptake of ARBs by tetracycline-inducible OAT4 expressing HEK293 cells was quantitated by LC-MS/MS. The uptake of azilsartan (the active metabolite of azilsartan medoxomil), candesartan (the active metabolite of candesartan cilexetil), carboxylosartan (the active metabolite of losartan), and valsartan by tetracycline treated (OAT4-overexpressing) cells were significantly higher than that by the tetracycline untreated (OAT4-uninduced) cells, whereas, OAT4-mediated uptake of irbesartan and telmisartan, whose renal excretion is negligible, were hardly detected in the absence of extracellular Cl⁻. In the absence of the extracellular Cl⁻, OAT4-mediated azilsartan, candesartan, carboxylosartan, and valsartan uptakes were saturable and followed Michaelis-Menten kinetics with Km of 9.9, 26, 13, and 1.7 μ M, respectively. The initial uptake clearance of azilsartan, candesartan, carboxylosartan, and valsartan by OAT4, as estimated by the linear regression, were increased by the removal of extracellular Cl⁻. Furthermore, in terms of these ARBs, OAT4-mediated uptake reached steady state within 30 seconds in the presence of extracellular Cl⁻, that was earlier compared with those in the absence of extracellular Cl⁻. These results may represent that the OAT4-mediated efflux transport of carboxylosartan, candesartan, azilsartan, and valsartan at the steady state were facilitated by the counter transport with extracellular Cl⁻. In conclusion, OAT4 recognizes various ARBs as substrates, and is possible to mediate bidirectional transport of azilsartan, candesartan, carboxylosartan, and valsartan. Each of these ARBs has clearance ratio (the renal clearance divided by product of the plasma protein unbound fraction and the glomerular filtration rate) larger than 1 in humans. Additionally, with respect to candesartan, carboxylosartan, and valsartan, the renal clearance has been reported to account for more than a quarter of total plasma clearance in humans.

P241 - ESTABLISHMENT OF A COMPLETE *IN VITRO* DRUG TRANSPORTER PANEL TO MEET REGULATORY GUIDELINES

Chunying Gao¹, Lu Huo², Christopher Welsh¹, and **Chuong Pham**¹

¹Alliance Pharma, Inc., USA, ²Incyte, USA

Drug transporters play a key role in the absorption, distribution, metabolism, elimination and toxicity (ADME-Tox) of many drugs by controlling the entry or exit of drug molecules into and out of cells. Due to the importance of transporters in these processes and the drug-drug interactions they may contribute to, *in vitro* transporter studies are required by the FDA, EMA, and PDMA regulatory agencies to evaluate the potential interactions between investigational drugs acting as substrates and/or inhibitors of the standard panel of drug transporters. The current panel includes P-gp, BCRP,

OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K; although regulatory agencies are routinely requesting data on additional transporters outside of the current group. Alliance Pharma has established a complete set of cell- or membrane vesicle-based assays to satisfy the studies required by regulatory agencies. The data presented here describes our work to show that transiently transfected HEK293 cells are capable of characterizing transporter specific substrates and inhibitors. For ABC transporters, a membrane vesicle assay (for P-gp and BCRP), Caco-2 bidirectional permeability assay (for P-gp and BCRP), and MDR1/MDCK bidirectional permeability assay (for P-gp) were previously established. For SLC transporters, we present an optimized and validated assay platform based on transiently transfected HEK293 cells that has been built and implemented, and can be used for both substrate/inhibitor screening and Km/IC50 determination.

P242 - *IN VITRO* FUNCTIONAL CHARACTERIZATION OF RARE GENETIC VARIANTS IN HEPATIC BILE ACID AND DRUG TRANSPORTER NTCP

Laura Russell

University of Western Ontario, Canada

Background: Sodium taurocholate co-transporting polypeptide (NTCP) is a membrane-bound transport protein in the liver. NTCP, encoded by the gene SLC10A1, is a central facilitator of hepatic bile acid and drug uptake. Genetic variation in NTCP may affect bile acid circulation and signaling, as well as disposition of drugs including cholesterol-lowering statins. Despite this recognition, transport by NTCP variants remains poorly characterized relative to other transporters. The aim of this study is to assess transport of an endogenous substrate, taurocholic acid, and a drug substrate, rosuvastatin, by rare genetic variants of NTCP. Additionally, *in silico* prediction algorithms will be assessed for accuracy of predicting altered substrate transport.

Methods: Rare (minor allele frequency 75% for seven of 34 variants. Uptake of rosuvastatin was reduced by >75% for 14 of 34 variants. Substrate specificity was observed for one variant, p.G191R, where rosuvastatin uptake decreased by >90% yet taurocholic acid uptake remained similar to wild-type. None of the *in silico* tools used were able to predict the functional effect on substrate transport with 100% accuracy.

Conclusions: Recent advances in technology have yielded large genomic datasets which show the abundance of rare genetic variation in the population. Accordingly, *in silico* models are becoming more critical to identify variants of potential clinical significance. Our study shows that concordance between *in silico* prediction and observed *in vitro* NTCP activity is not sufficiently predictive. Accordingly, caution should be exercised for pharmacogenes while prioritizing variants based on predictive algorithms. Validation through the application of cell-based assays remains the most accurate method to determine the effect of genetic variation on protein function.

P243 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING OF TRANSPORTER-MEDIATED HEPATIC DISPOSITION USING THE IMAGING BIOMARKER GADOXETATE

Daniel Scotcher¹, Sirisha Tadimalla², Adam Darwich¹, Sabina Ziemian³, Kayode Ogungbenro¹, Gunnar Schütz³, Steven Sourbron², and Aleksandra Galetin¹

¹*University of Manchester, United Kingdom*, ²*University of Leeds, United Kingdom*, ³*Bayer AG, Germany*

Physiologically-based pharmacokinetic (PBPK) modelling provides a framework for *in vitro-in vivo* extrapolation (IVIVE) of drug disposition. However, prediction of transporter-mediated processes and tissue permeation remains challenging due to the lack of available *in vivo* tissue data for validation. Gadoxetate is a magnetic resonance imaging (MRI) contrast agent used clinically for hepatic lesion characterisation. As a substrate of organic anion transporting polypeptide 1B1 (OATP1B1) and multidrug resistance-associated protein 2 (MRP2), gadoxetate is being explored as a novel imaging biomarker for hepatic transporter function in context of evaluation of drug-drug interactions and drug induced liver injury [1]. The current study aimed to characterise uptake kinetics of gadoxetate in plated rat hepatocytes and develop a PBPK model to predict gadoxetate *in vivo* plasma and liver exposure. *In vitro* uptake was measured by incubating rat hepatocytes with 0.01 – 10mM gadoxetate for 0.5 – 150 min. Relevant *in vitro* transporter kinetic parameters were derived using a mechanistic cell model [2]. Subsequently, a novel PBPK model was developed for gadoxetate in rat, where liver uptake and cellular binding were informed by IVIVE. Gadoxetate *in vivo* blood, spleen and liver data obtained in the presence (n=9) and absence (n=27) of a single 10 mg/kg intravenous dose of rifampicin [3] were used for PBPK model validation/refinement. *In vitro* gadoxetate uptake affinity constant (Km) obtained in rat hepatocytes was 0.106 mM (n=4 rats), with saturable active transport accounting for 94% of gadoxetate cellular uptake; bidirectional transport, not saturable under current experimental conditions, was minor. The fraction unbound in hepatocytes was estimated to be 0.65. The total (Kp,u) and unbound (Kp,uu) hepatocyte:media partition coefficients were 26.0 and 16.9, respectively. The PBPK model successfully predicted gadoxetate concentrations in systemic blood and spleen and corresponding 2-fold increase in gadoxetate systemic exposure in the presence of rifampicin. In contrast, liver concentrations were under-predicted. Refinement of the PBPK model using the dynamic contrast agent enhanced (DCE)-MRI data enabled recovery of the liver profile, assuming complete and partial inhibition of hepatic uptake and biliary efflux by rifampicin, respectively.

The current study demonstrates utility of imaging data in validating and refining PBPK models for prediction of transporter-mediated disposition; considerations of interpretation of quantitative DCE-MRI data to inform PBPK models are discussed.

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P244 - INVESTIGATION OF INTERINDIVIDUAL VARIABILITY IN KIDNEY DRUG TRANSPORTER EXPRESSION AND FUNCTION BY UTILIZING NON-INVASIVE URINARY EXOSOMES AND ENDOGENOUS BIOMARKERS

Yuanyuan Shi¹, Haeyoung Zhang¹, Jeffrey Testani², J. Steven Leeder³, and Bhagwat Prasad¹

¹University of Washington, USA, ²Yale University School of Medicine, USA, ³Children's Mercy Hospital and Clinics, USA

Background: Kidney drug transporters are critical determinants of secretion and proximal tubular accumulation of drugs, thereby affecting drug clearance and toxicity. Interindividual variability in expression of kidney drug transporters contributes to changes in pharmacokinetics and toxicokinetics of a variety of xenobiotics. While there is a lot of information concerning renal drug transporters in adults, little is known regarding the activity of these transporters in children due to non-availability of tissue samples. Because urine is enriched with endogenous metabolites (potential transporter biomarkers) and extracellular vesicles (i.e., exosomes), we investigated whether these potential biomarkers and transporter proteins can be quantified reproducibly by quantitative metabolomics and proteomics in urine samples from dog and human across age. **Methods:** A novel LC-MS/MS based proteomics method was developed and applied for dog transporter quantification in juvenile and adult dogs (n=3 each). Individual dog and a pooled adult urine samples were used for exosome isolation for proteomics analysis using this method or previously validated human method. A targeted metabolomics method was also developed and validated for determination of eight potential transporter biomarkers, i.e., N-methylnicotinamide, thiamine, taurine, hippurate, glycochenodeoxycholic acid 3-sulfate (GCDCAS), 6 β -hydroxycortisol, estradiol-sulfate, and dehydroepiandrosterone sulfate (DHEAS), for organic cation transporter 2/ multidrug and toxin extrusion protein (OCT2/MATE), OCT2, organic anion transporters 1 (OAT1), OAT1, OAT3, OAT3, multidrug resistance-associated proteins (MRP1/MRP2), and MRP4, respectively, in urine samples from dogs and humans (one adult pool and five 7-10 years old children). Creatinine and etiocholanone glucuronide were also quantified for data normalization. **Results:** OAT1, MATEs, and MRP3 showed significantly higher abundance in adult dog kidneys as compared to that of the juvenile dogs, whereas organic/carnitine cation transporter was modestly lower in adults. Hippurate, an OAT1 endogenous substrate, was higher in urine sample of adult dogs when compared to the juvenile dogs. Although dog urine exosomes could not be isolated due to limited volume, the human exosomes from adult pooled urine (100 ml) showed a quantifiable signal of P-glycoprotein, OCT2 as well as aquaporin 1 and 2. All eight metabolites were reproducibly quantified in pediatric as well as adult human urine samples. Except for DHEAS and 6 β -hydroxycortisol, the median concentration of all other metabolites in pediatric urine was within 50% to 170% of the adult levels (p value >0.05). DHEAS and 6 β -hydroxycortisol levels in children were 5% and 200% of the adult urine levels. High variability was observed in thiamine and N-methylnicotinamide urine levels. Further investigation on quantitative differences in metabolite and exosome transporter levels in larger human sample cohort is ongoing in our laboratory. **Conclusions:** Analysis of urinary exosomes could be a potential approach for predicting transporter function in kidney; however, high volume of urine is required for exosome isolation. These endogenous metabolites could be used as potential biomarkers of renal transporter mediated drug-drug interactions in children.

P245 - INVESTIGATION OF RENAL DRUG SECRETION IN A KIDNEY-ON-A-CHIP MODEL

Simone Stahl¹, Pär Nordell², Tom Nieskens², Katherine S Fenner¹, and Pedro Caetano-Pinto¹

¹AstraZeneca, United Kingdom, ²AstraZeneca, Sweden

Renal drug clearance is the result of filtration, secretion and re-absorption. Transporter proteins expressed at the basolateral and apical membranes of proximal tubule (PT) cells cooperatively mediate active secretion of drugs from the blood into urine. However, the study of renal secretion *in vitro* is severely limited by the lack of physiological models with appropriate expression and activity of transporters as well as adequate formation of tight cell monolayers. In contrast, cells cultured under shear stress conditions in organ-on-a-chip models demonstrate improved tightness and polarization (1,2). We therefore investigated if monolayer formation, transporter expression and function in the Nortis® kidney-chip is improved compared to conventional two-dimensional monolayer (2D) or transwell (TW) cultures.

Cryopreserved human renal proximal tubule epithelial cells (hRPTECs) were cultured on collagen IV-coated 96-well plastic or transwell-filter plates or injected into a Nortis® chip which had been pre-filled with a collagen I matrix and coated with collagen IV. Cells were characterized via immunocytochemistry staining for PT cell markers and gene expression analysis. Cell monolayer tightness and transporter activity were determined using relevant fluorescent or radiolabeled probe substrates in absence or presence of inhibitors. In the kidney-chip, hRPTECs self-assembled into a renal tube, displayed characteristic cobblestone morphology and were impermeable to the leakage marker FITC-Inulin. Zonula-occludens 1 (ZO1) and P-glycoprotein (P-gp) were expressed on the apical domain whilst Na⁺K⁺ATPase, Organic Cation Transporter 2 (OCT2), and Organic Anion Transporter 1 (OAT1) were located on the basolateral membrane demonstrating that the cells polarize in the chip cultures. Transporter mRNA expression levels in the kidney-chip were maintained (OCT2, MRP4) or increased (MATE1, MATE2-K, OATP4C1) compared to 2D and TW cultures whilst some reduction was observed for P-gp. Remarkably, expression of OAT1 was absent in 2D- and TW-cultured cells but recovered in the kidney-chip cultures. Efflux of the P-gp and MRP substrates calcein and CFDA was inhibited in presence of valspodar and MK571, respectively. In the kidney-chips but not in TW cultures, basolateral to apical trans-epithelial secretion of [¹⁴C]-metformin and [³H]-cidofovir was demonstrated which was inhibited by imipramine and probenecid, respectively. In conclusion, hRPTECs cultured in the kidney-chip show polarized drug transporter protein localization and a superior gene expression profile relative to 2D- or TW-cultured cells. Importantly, the kidney-chip demonstrates trans-epithelial transport functionality for the key renal cation and anion secretory pathways which is not detected in conventional TW cultures. Therefore, kidney-chip models represent a novel and improved tool suitable for the study of renal drug secretion *in vitro*.

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P246 - NEPHROTOXICITY IN MATE1, MATE2K and MATE1/2K KNOCKOUT CELL LINES FOLLOWING TREATMENT WITH CISPLATIN USING THE MIMETAS 3D ORGANOPLATE PLATFORM

Toni Steiner and David Thompson
MilliporeSigma, USA

Multidrug and Toxin Extrusion Proteins (MATEs) are expressed on the apical membrane of renal proximal tubule epithelial cells (RPTECs). There are two isoforms of MATE, SLC47A1 (MATE1) and SLC47A2 (MATE2K). MATE1 is expressed in the liver and kidney, whereas MATE2K is expressed only in the kidney. Cisplatin is a chemotherapeutic drug used to treat solid tumors and induces dose-dependent nephrotoxicity in renal proximal tubules. MATEs are involved in the excretion of cisplatin and other organic cation compounds into the urine. Reduced function of MATE transporters has been shown to potentiate cisplatin-induced nephrotoxicity *in vivo*. The objective of this study was to determine the effect of cisplatin treatment on single and double MATE knockout cell lines in the Mimetas 3D OrganoPlate® platform. Control (SA7K), MATE1 (-/-), MATE2K (-/-) and MATE1/2K (-/-,-/-) cells are pseudo-immortalized human RPTECs that were developed from primary cells using COMPOZR Zinc Finger Nuclease technology. Cells were grown as perfused tubules against an extracellular matrix in the 3-lane OrganoPlate®. Antibody staining against ZO-1 (tight junction protein located on cellular borders), acetylated tubulin (located on primary cilia pointing towards the lumen of the tubule) and ezrin (expressed on apical surface) confirmed the correct polarization of the renal tubules. Cells were treated with cisplatin on day six in culture. After 24-hour treatment, media from channels with tubules were harvested. Toxicity of cisplatin was determined by measurement of the amount of LDH activity in media. MATE1 and MATE2K single knockouts did not show a significant increase in LDH activity compared to control; however, a significant increase in LDH activity was observed in MATE1/2K double knockout cells. Based on these data, cisplatin appears to be a substrate for both MATE1 and MATE2K as the single knockout of either MATE1 or MATE2K did not increase toxicity in these cell lines. In contrast, knockout of both MATE1 and MATE2K reduced the ability to excrete cisplatin from this cell line sufficient to cause an increase in toxicity. These RPTEC knockout cell lines were instrumental in demonstrating that while MATE transporters collectively are involved in the efflux of cisplatin from the proximal tubule, cisplatin is not a selective substrate for either MATE1 or MATE2K.

P247 - INDOXYL SULFATE ALBUMIN-FACILITATED TRANSPORT VIA RENAL DRUG TRANSPORTER OAT1 – TRANSLATION FROM MICROFLUIDIC *IN VITRO* SYSTEM TO HEALTHY AND SUBJECTS WITH SEVERE KIDNEY DISEASE

Thom van der Made¹, Michele Fedecostante², Daniel Scotcher¹, Amin Rostami-Hodjegan¹, Rosalinde Masereeuw², and Aleksandra Galetin¹

¹University of Manchester, United Kingdom, ²Utrecht Institute for Pharmaceutical Sciences, Netherlands

Indoxyl sulfate (InS), a highly albumin-bound uremic solute, accumulates in plasma in chronic kidney disease (CKD). InS is cleared via active secretion in the proximal tubule, with important involvement of organic anion transporter 1 (OAT1). In CKD, extent of InS binding to albumin is reduced and this is attributed to changes in albumin concentrations and potentially also posttranslational modifications to the protein. The present study was designed to investigate the impact of healthy human serum albumin (HSA) and CKD-modified HSA on OAT1-mediated InS uptake in a microfluidic system.[1] Secondly, quantitative translation of *in vitro* findings to *in vivo* to predict extent of change in InS CLR in CKD stage IV patients relative to healthy was performed. Conditionally immortalized proximal tubule epithelial cells transduced with OAT1 (ciPTEC-OAT1) were incubated with different concentrations of InS (5 - 200 μ M) in the absence of HSA (HSA-free), presence of HSA or presence of CKD-modified HSA. *In vitro-in vivo* extrapolation (IVIVE) of *in vitro* InS unbound intrinsic clearance (CL_{int}) obtained in ciPTEC-OAT1-microfluidic system was explored.[2] Additionally, consequences of potential differences in scaling factors between healthy subjects and CKD patients were investigated. InS uptake in the presence of HSA resulted in more than 20-fold decrease in K_{mu} and 38-fold increase in CL_{int} compared to HSA-free incubation media. In the presence of CKD-modified HSA, K_{mu} increased 3.9-fold compared to HSA condition; fold-change exceeded differences in InS binding between the two conditions. InS CL_{int} decreased 6.7-fold in the CKD-modified HSA condition (5.1 μ L/min/10⁶ cells) relative to healthy HSA (34 μ L/min/10⁶ cells). Translation of InS *in vitro* OAT1-mediated active secretion predicted 60% decrease in InS renal elimination as a result of CKD, in agreement with the observed data (80%). The findings of this study emphasize the importance of inclusion of albumin in physiologic concentrations in the incubation medium for highly-bound compounds, supporting the hypothesis of albumin-facilitated active transport via OAT1. This study addressed quantitative translation of data generated from novel microfluidic *in vitro* system to a clinically relevant setting and explored the impact of knowledge gaps on accurate prediction of CLR in healthy subjects and in CKD patients.

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P248 - SIMULATIONS OF NONCOMPETITIVE SUBSTRATE TRANSPORT

David Wagner¹, Joanne Wang², and Adrian Fretland¹

¹AstraZeneca, USA, ²University of Washington, USA

Drug uptake transporters including organic anion transporting polypeptides (OATPs) and organic cation transporters (OCTs) mediate the disposition of drugs. Drug-drug interactions with uptake transporters can lead to changes in distribution to the site of action, elimination, or toxicity. *In vitro* inhibition assessments are recommended to predict the potential for clinically relevant drug-drug interactions with OCT2, OATP1B1, OATP1B3, and other uptake transporters. These *in vitro* transport inhibition experiments generally rely on the simple four-state carrier model of transport for interpretation of data. However, growing evidence suggest multiple substrates or inhibitors may bind simultaneously to OCTs and OATPs which violates the assumptions of the 4-state model. The goal of these simulations is to predict specific transport kinetic characteristics of multiple binding sites that can be identified within *in vitro* experiments and identify potential challenges in the prediction of drug-drug interactions with multiple transport binding sites. We simulated zero-trans inhibition of a carrier by a second noncompetitive substrate with and without simultaneous transport (active or inactive SES complex). Expected changes in apparent kinetic parameters (K_m and V_{max}) that can be observed *in vitro* are highlighted. These simulations identify potential situations where a single probe substrate may not be sufficient for characterizing the inhibition of a second substrate. These alternative models of transport can be considered when a four-state carrier model cannot adequately describe interactions and may offer mechanistic insight into substrate dependent inhibition.

P249 - LC-MS/MS BASED BIOANALYSIS METHOD FOR BILIRUBIN GLUCURONIDES: APPLICATION IN UGT1A1 INHIBITION EVALUATION AND COMPARISON WITH DATA OBTAINED USING ESTRADIOL

Chunyan Han, Dong Yang, and Mandy Xu
Pharmaron, China

Endogenous (4Z, 15Z)-bilirubin IXa, the end product of heme catabolism, is eliminated through UDP-glucuronosyltransferases 1A1 (UGT1A1) catalyzed glucuronidation. Inhibition of UGT1A2 might lead to hyperbilirubinemia, thus UGT 1A1 is an important content in DDI potential evaluation of new chemical entities. Estradiol is widely used as a probe for evaluation of UGT1A1 inhibition with estradiol-3-glucuronidation as the target metabolite, but it is not a selective substrate since UGT1A3 is also involved in estradiol-3-glucuronidation. Bilirubin is highly specific to UGT1A1, and is the substrate of the interest. However, current bioanalysis method for bilirubin glucuronides are all HPLC-UV based, and the elution time tend to be long, which is not compatible with instrumental set-up and experimental design of high throughput screening. Besides, bilirubin could form multiple conjugates, but most reported method use bilirubin total glucuronide (the sum of monoglucuronides and diglucuronide) as end point, enzyme kinetics with monoglucuronides and diglucuronide as target metabolites were not fully evaluated. In the present study, we developed robust LC-MS/MS method for bilirubin, bilirubin monoglucuronides and bilirubin diglucuronide. Km values for mono-, di- and total glucuronides were determined in both human liver microsomes and recombinant human UGT1A1 (rhUGT1A1) reaction systems. Inhibition potential of ten compounds (silybin, atazanavir, lopinavir, ritonavir, baicalein, 4-methylumbelliferone, riluzole, carvedilol, daidzein and ketoconazole) on the formation of mono-, di- and total glucuronides were evaluated in both human liver microsome and recombinant human UGT1A1 reaction systems and results were compared with that obtained using estradiol.

P250 - UTILITY OF ION MOBILITY-ENABLED HIGH RESOLUTION MASS SPECTROMETRY AND CCS PREDICTION FOR CHARACTERIZING ISOMERIC METABOLITES IN BIOTRANSFORMATION STUDIES

Richard Clayton¹, Rosalind Southall¹, **Daniel Albaugh**², Joseph Marini², Russell Morthishire-Smith³, Chris Hughes³, Mark Wrona⁴, Lauren Mullin⁴, and Nathan Anderson⁴

¹Covance Laboratories, United Kingdom, ²Covance Laboratories, USA, ³Waters, United Kingdom, ⁴Waters, USA

Biotransformations often lead to a range of isomeric metabolites which can be difficult to differentiate by MS/MS experiments. This is particularly true of glucuronide metabolites where the first fragmentation step simply yields the parent drug or aglycone metabolite structure. Ion mobility separations offer the power to differentiate isomers by their collision cross section (CCS). Calculation of theoretical CCS on the basis of de novo calculations using molecular modelling is complex and computationally intensive. In comparison, machine learning algorithms provide a relatively fast mechanism for estimating CCS values which can be applied to experimental results to differentiate isomeric forms of metabolites. A range of commercially available drug compounds and their glucuronide metabolites were analysed using a Vion IMS Q-ToF mass spectrometer to generate CCS measurements. Glucuronide metabolites were generated via incubation of parent drug with *in vitro* test systems or sourced as reference standards from commercial sources. Replicate measurements of CCS values for denopamine and its two glucuronide metabolites were conducted to benchmark the reproducibility of CCS measurements over time and between laboratories. These values were compared with values predicted by a machine learning model to evaluate the accuracy of prediction. Additional predictions were made and evaluated for a set of glucuronides for which previously measured CCS data were available.

P251 - AHR-MEDIATED EFFECT OF BENZO(A)PYRENE AND SMOKING ON MICRORNA EXPRESSION IN LUNG CANCER

Lyudmila Gulyaeva

Federal Research Center "Basic and Translational Medicine", Russia

Smoking is a crucial risk factor of majority of lung cancers. We analyzed a frequency of somatic mutations in EGFR in men (n=352) and women (n=197) with non-small cell lung cancer (NSCLC) in the Novosibirsk region. The frequency of EGFR mutations was higher in never-smoking men (6%) than in smokers (2.6%). Mutation in EGFR was detected in 35.5% of never-smoking women with lung adenocarcinoma, while it was not found in smoking women. These results confirmed other mechanisms of NSCLC development for never-smokers compared to smokers and also supported the hypothesis of epigenetic mechanisms of lung cancer. Since the main component of cigarette smoke is benzo(a)pyrene (BP), we modeled the long-term effect of BP on the expression of microRNAs in lungs of female and male rats. We proposed that BP binding with aryl hydrocarbon receptor (AhR) might activate target genes, including microRNAs (miRs). With Biostrings program (Bioconductor R package) we searched for promoters of the microRNA host genes and intergenic microRNAs with predicted DRE. We chose microRNAs whose target genes (TargetScan program) are highly expressed in lung according to human proteome atlas. Expression levels of chosen microRNAs: miR-22,-29,-126a,-483 as well their target genes were determined by real time PCR. The small nuclear RNAs U6 and U48 were used as reference genes. The expression levels of CYP1A1 and CYP1B1, classical target genes of AhR, increased 10-20 times

under chronic three-month BP exposure in both rat male and female lungs, indicating the activation of AhR. The expression of oncogenic miR-22 increased 2.5-3 times in the lungs of females and males, while the expression of its target gene PTEN decreased 2-3 times. Expression of miR-29 increased up to 4 times mostly in males with expression decrease of the target gene SLC34A2. In females, on the contrary, miR-29 expression decreased accompanied by an increase of EMP2 gene expression and a decrease of IGF and PTEN gene expression. Expression of miR-126a increased and expression of its target gene ITGA6 decreased in males. In female lungs the expression of miR-126A decreased twofold. The expression level of miR-483 increased 6 times and 3 times in male and female lungs, respectively, whereas expression of its target gene IGF1 significantly decreased in the female lungs. We determined the expression of human orthologs of studied miRs in NSCLC samples (n=17) without mutation in EGFR. The expression level of CYP1B1 increased up to 15 times in adenocarcinoma of smoking patients. Expression levels of studied miRs depended on smoking. Expression of their target genes ITGA6, PTEN and IGF1 significantly (2-6 times) decreased in lung adenocarcinoma of smoking patients. These results favor the role of PTEN, SLC34A2, IGF1, ITGA6, EMP2 and miRNAs regulating their expression in lung carcinogenesis in smokers. This work was supported by Russian Foundation for Basic Research, grant # 18-415-540002.

P252 - EFFECTS OF O,P'-DDT, P,P'-DDT AND ENDOSULFAN ON THE EXPRESSION OF HORMONE-SENSITIVE MICRORNA IN MCF-7 CELLS

Lyudmila Gulyaeva, Tatiana Kalinina, and Vladislav Kononchuk
Federal Research Center "Basic and Translational Medicine", Russia

Dichlorodiphenyltrichloroethane (DDT) and endosulfan are potent organochlorine insecticides which have been used worldwide for agricultural and public health purposes. These pesticides have a long half-life and are accumulated in living beings. Because of these properties, the use of DDT and endosulfan has led to environmental contamination with these compounds. It is well appreciated that DDT and endosulfan have both estrogenic and antiandrogenic properties. The estrogen and androgen receptors (ER and AR) regulate the expression of genes encoding proteins that are involved in the regulation of proliferation, apoptosis, epithelial-mesenchymal transition. One of the well-known targets of ER is the progesterone receptor (PGR). Thus, the effect of these pesticides in tissues with high expression of AR, PGR and ER is of particular concern. In addition, a number of studies have shown that DDT and endosulfan are able to change the profile of microRNA expression. Therefore, in order to better understand the mechanisms of action of these insecticides, the expression of microRNAs in MCF-7 cells treated with estradiol, testosterone, progesterone, o,p'-DDT, p,p'-DDT or endosulfan for 24 hours was measured. MicroRNAs (miRs), putative promoter regions of which contain conservative binding sites for nuclear receptors, such as AR, PGR or ER, were chosen. Expression level of chosen microRNAs was determined by RT-qPCR. The small nuclear RNAs, U44 and U48, were used as reference genes. We revealed the changes in expression levels of miR-190b, -184, -190a, -23a, -423, -216 -127, -194 and -196a in cells after incubation with hormones. MiR-23a, -190b, -184, -23a, -423, -216, -194, and -196a levels also changed in cells treated with DDT or endosulfan. The level of miR-190b increased 1.5-fold under the estradiol and o,p'-DDT treatment. The level of miR-184 and -194 decreased 1.4-fold under the estradiol and endosulfan exposure. The expression level of miR-23a, -216, -196a was 1.3-2-fold lower in cells treated with progesterone and endosulfan. The expression of miR-216 also increased 1.3-fold under the testosterone exposure. The level of miR-423 was lower in testosterone-treated cells, but higher in cells treated with o,p'-DDT. These results confirm the potential involvement of receptors in altering expression of these microRNAs under the endocrine disruptors. The level of miR-483, -200c was also significantly altered in cells treated with studied xenobiotics. The expression of target genes of studied miRs ANKRD30A, OXTR, AKR1C2, ALX4, DUSP4, MMP-8, PAX3, TRPS1 was significantly changed under the xenobiotic exposure. These results confirm that toxic effects of some xenoestrogens like DDT and endosulfan might be associated with altered expression of studied microRNAs caused by changes in AR, PGR or ER activity. This work was supported by Russian Science Foundation, grant # 19-15-00319.

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