P6. Investigating Cannabidiol Biotransformation by Cytochrome P450 Enzymes and Implications for Cannabidiol-Induced Liver Injury

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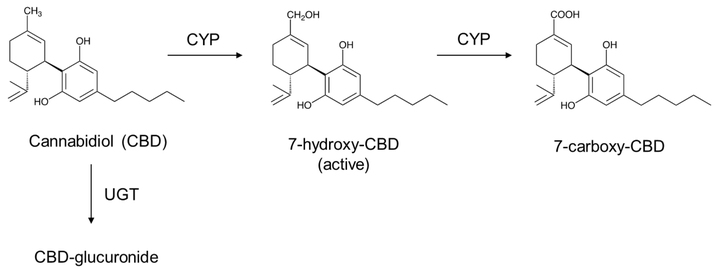
ABSTRACT

Cannabidiol (CBD) is a naturally occurring, non-psychotoxic phytocannabinoid derived from cannabis. CBD has become a popular ingredient in many consumer products and was recently FDA-approved as Epidiolex® (CBD oral solution) to treat seizures associated with Lennox-Gastaut syndrome and Dravet syndrome. Epidiolex® has been associated with dose-dependent hepatotoxicity in clinical trials. This risk is increased with co-administration of valproate, another anti-epileptic drug known to cause liver injury. The mechanism of CBD-induced liver injury remains unknown. CBD is metabolized primarily by cytochrome P450 (CYP) 2C19 and CYP3A4, and by UDP-glucuronosyltransferases. The goals of this study were to characterize the roles of P450 enzymes in CBD metabolism with respect to generation of the active metabolite 7-hydroxy-CBD (7-OH-CBD) and major inactive metabolite 7-carboxy-CBD (7-COOH-CBD), and to investigate the impact of *CYP2C19* genotype on CBD metabolism in human liver microsomes (HLM). We also examined the effect of co-treatment with the nonselective P450 inhibitor 1-aminobenzotriazole and valproate on CBD metabolite generation in sandwich-cultured human hepatocytes (SCHH). CYP2C19 was found to sequentially metabolize CBD to 7-OH-CBD and 7-COOH-CBD. Although CYP3A was found to have a significant role in CBD clearance and 7-COOH-CBD formation, CYP3A was not involved in 7-OH-CBD generation. In addition, individual HLM from CYP2C19 poor metabolizers were found to have significantly higher 7-COOH-CBD generation compared to CYP2C19 rapid and ultrarapid metabolizers. In SCHH pre-treated with 1-aminobenzotriazole, formation of hydroxylated CBD metabolites was reduced, and CBD-glucuronide formation was increased. Co-treatment with both CBD and valproate in SCHH did not significantly alter the metabolic profile of CBD. Further studies are needed to determine the mechanisms of CBD-related hepatotoxicity and the interaction between CBD and valproate.

OBJECTIVES

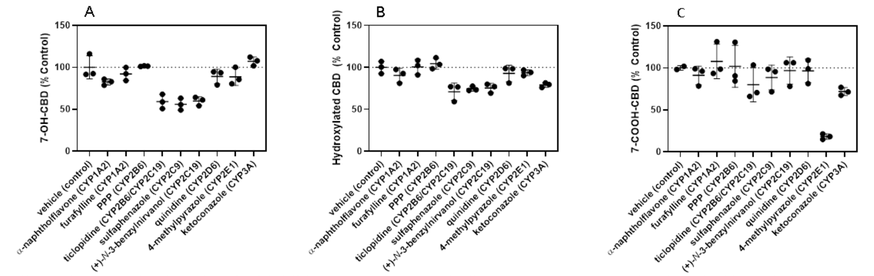
HYPOTHESIS: Variations in CBD metabolism may influence the adverse effect profile and hepatotoxicity observed in patients taking Epidiolex® (cannabidiol).



RESEARCH QUESTIONS:

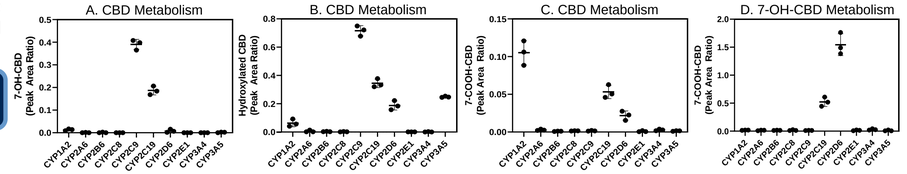
- What are the relative P450 enzyme contributions to CBD metabolism?
- How does CYP2C19 variation impact CBD metabolism?
- What is the metabolic profile of CBD in primary human hepatocytes?

P450 ENZYME CONTRIBUTIONS TO CBD METABOLISM



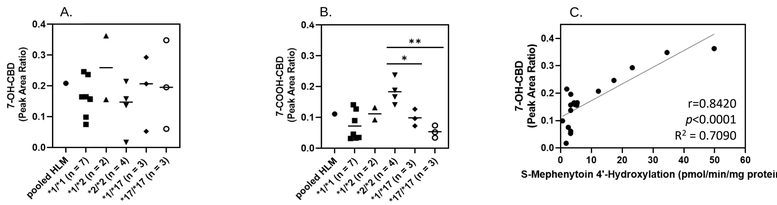
Effect of P450-selective chemical inhibitors on CBD metabolism. CBD (1 µM) was incubated with pooled HLM (0.2 mg/mL) for 10 min. Formation of 7-OH-CBD (A), hydroxylated CBD (B), and 7-COOH-CBD (C) was measured in the presence of P450-selective chemical inhibitors and compared to vehicle control incubations. Bars represent the mean ± SD of a single experiment conducted in triplicate. PPP = phenyl-piperidinyl propane.

CBD METABOLISM BY RECOMBINANT P450 ENZYMES



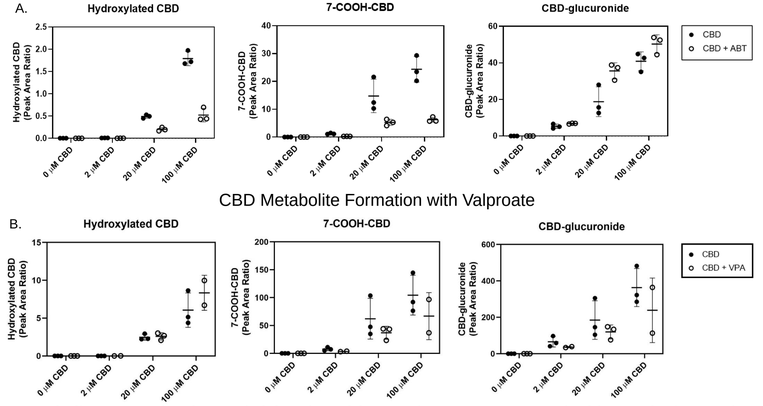
CBD and 7-OH-CBD metabolism by recombinant P450 enzymes. CBD (1 µM) (A-C) and 7-OH-CBD (2 µM) (D) were incubated with P450 Supersomes™ (20 pmol/mL) for 10 min. Metabolites were measured using LC-MS/MS analysis and expressed as a peak area ratio with respect to internal standard, cannabidiol-d_9. Bars represent the mean ± SD of a single experiment conducted in triplicate.

CBD METABOLISM & CYP2C19 ACTIVITY



CBD metabolite formation and correlation with CYP2C19 activity in individual *CYP2C19*-genotyped HLM. CBD (2 µM) was incubated with individual *CYP2C19*-genotyped HLM (0.2 mg/mL protein) for 5 min. Formation of 7-OH-CBD (A) and 7-COOH-CBD (B) was measured as a peak area ratio with respect to internal standard (cannabidiol-d_9). Formation of these metabolites was compared to CYP2C19 activity measured by 4´-hydroxymephenytoin formation (C). Data points represent the mean and bars represent the median metabolite formation of triplicate experiments. Mean metabolite formation was compared across poor metabolizers (*CYP2C19*2/*2*), rapid metabolizers (*CYP2C19*1/*17*), and ultrarapid metabolizers (*CYP2C19*17/*17*), using one-way ANOVA with Tukey's multiple comparisons test (* = p<0.05, ** = p<0.01).

CBD METABOLISM IN HUMAN HEPATOCYTES



CBD metabolite generation in sandwich-cultured human hepatocytes treated with 1-aminobenzotriazole and valproate. CBD (0, 2, 20, and 100 µM) was incubated with sandwich-cultured human hepatocytes for 24 h in the presence and absence of the nonselective P450 inhibitor 1-aminobenzotriazole (1 mM) (A) and valproate (1 mM) (B). Metabolite generation was measured in hepatocyte media collected after 24 h and was determined as a peak area ratio with respect to internal standard (cannabidiol-d_9). ABT = 1-aminobenzotriazole, VPA = valproate.

CONCLUSIONS & FUTURE DIRECTIONS

- CBD active metabolite formation is correlated with CYP2C19 activity.
- P450 inhibition influences the metabolic pathway of CBD in human hepatocytes.

FURTHER RESEARCH QUESTIONS

- Is CBD metabolite generation associated with hepatocyte toxicity?
- How does valproate co-administration influence the risk of hepatotoxicity?

FUTURE DIRECTIONS

- Conduct experiments with hepatocyte 3D spheroid cultures to investigate CBD metabolism, toxicity, and drug interactions with valproate.

REFERENCES

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