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Scientific and Standardization Committee Communication
The Design and Analysis of Pharmacokinetic Studies of Coagulation Factors

On behalf of the Subcommittee on Factor VIII and Factor IX of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis

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Abstract

We have previously published guidelines for the conduct of pharmacokinetic studies of coagulation factors (Thrombosis and Haemostasis 66:384-386, 1991). However, changes in the clinical and regulatory environments since then have necessitated a complete rewrite of these procedures to reflect the importance of factors other than VIII and IX. In addition, we now address important issues of sample size calculation for demonstrating bioequivalence of preparations, outcome variables other than half-life, continuous infusion, and prophylaxis. These suggestions should provide a template for clinical researchers, as well as others evaluating pharmacokinetics in their patients with hemophilia.

INTRODUCTION

In 1991 we published a set of guidelines for the determination of half-life and recovery in studies of Factor VIII and IX.[1] These recommendations were necessarily simple and focused primarily on very basic aspects of these trials, as it was unclear at that point in time as to the general level of sophistication required by all users of the guidelines. However, it did become clear that our primary goal of providing a comparable way of conducting pharmacokinetic studies was being met, as most of these trials of the past several years have followed the principles we recommended.

Nonetheless, because of changes in regulatory requirements for the conduct of these studies and the increased level of complexity that has resulted, we realized that a complete revision of the original guidelines was needed. Furthermore, the need for guidelines to cover factors other than VIII or IX was clear. At a recent specialized committee meeting in London of the Factor VIII/Factor IX Committee we proposed such a new standard. This was formally presented at the ISTH meeting in Washington and was duly reviewed and discussed. This paper is a result of the conclusions of that meeting.

We emphasize again as we did when the original guidelines were published that the approaches recommended here are primarily intended for clinical trials of factor concentrates that are being compared to other, similar preparations. Although we have now introduced proposals for continuous infusion and prophylaxis that may be appropriate, studies on individual patients for diagnostic or therapeutic purposes may not

necessarily be relevant to what is discussed here. This also points out that the central focus of these guidelines is on the pharmacokinetics of coagulation factors after a single bolus infusion.

DESIGN

A. Control Preparation

Unlike the original guidelines, we are now stressing the need for a control preparation. Moreover, the control should be of the same type as the test product if such a preparation exists. For example, a recombinant Factor VIII product would be compared with another recombinant Factor VIII or a plasma-derived material compared with another plasma-derived product. Clearly, however, this will not always be possible (for example, with the first recombinant product of its kind, e.g. recombinant Factor VIIa). Under these circumstances, the control will have to be chosen as is deemed appropriate.

B. Sample Size

The original guidelines proposed a very simple notion of sample size assuming the study goal was one of estimation of pharmacokinetic parameters. As a result, a suggestion of 12 patients was made. This, unfortunately, was overly simplistic. Most pharmacokinetic studies have as their goal, the establishment of statistical bioequivalence between the test and control preparations. Therefore, the sample size should be based on statistical considerations. Of course, this requires a definition of bioequivalence. One such criterion that has been frequently used in the literature is the notion that the test material result should be within 80 to 120% of that for the control preparation.[2] While statistically this may not be optimal in all senses, it has the advantage of being relatively simple and, therefore, interpretable.

To implement this criterion, we need to focus on one particular aspect of the possible pharmacokinetic outcomes and we discuss this below. Regardless of that choice, the sample size can be determined from the following standard formula:[2]

$$n \geq (t[\alpha/2, 2n-2] + t[\beta, 2n-2])^2 (cv/20)^2$$

where t [x,y] is the upper x^{th} percentile of the Student's t-distribution with y degrees of freedom, α is the chosen significance level (size of the Type 1 error), β is the chosen size of the Type 2 error, and cv is the coefficient of variation (namely its standard deviation divided by its mean) of the chosen primary pharmacokinetic parameter. Notice that the formula would have to be evaluated iteratively as n appears on both size of the inequality. Thus, the sample size to be used would be the smallest one that satisfies the inequality.

To illustrate, suppose that CV=30%, α=.05 and β=.20, then approximately 40 subjects would be required in the study. (All receiving both preparations [see below]). Some may find this number out of the question. Therefore, we point out that if the study is intended

for descriptive rather than strict comparative purposes, then a minimum of 12 subjects would still be sufficient (based on our previous arguments[1]).

C. Outcome Variables

Historically, the terminal (β-phase) half-life and in vivo recovery have been the focus of pharmacokinetic studies. However, in traditional pharmacokinetics, the key parameter is the area-under-the-time-versus-concentration curve (AUC) determined using the trapezoidal rule,[3] and this is what we now recommend for the primary outcome variable, both for sample size determination and analytical purposes (notably a view now held by many regulatory agencies).

The specific AUC referred to is the area from time 0 (baseline) to the last time point of measurement (discussed below for the different possible factors).

There are many other possible outcomes that can and should be considered and these include:

a) AUC $_{0\text{-}24hr}$ (u.h/mL), for Factor VIII only.
b) AUC $_{0\text{-}\infty}$ (u.h/mL), where the terminal phase portion of the time versus concentration curve is extrapolated to the time or x-axis.
c) AUMC ($v.h^2$/mL), area under the first moment curve.
d) Half-life (both and β-phase, if two phases are in evidence for the given data set)
e) Incremental recovery (determined as the peak level in the factor in the first hour after the infusion and reported as [u/dL]/[u/kg])
f) Mean residence time[4,5] (hrs); the half life can be evaluated according to the formula: half-life = mean residence time/ 1.443
g) Clearance[4,5] (mL/h/kg); the amount of plasma made free of the drug per unit of time.
h) Volume of distribution – steady state[4,5] (mL/kg)
i) C_{max} (u/mL), the maximum concentration of factor achieved.
j) T_{max} (h), the time at which the maximum factor concentration was achieved.

D. Need for Patient Crossover

We re-emphasize the need in these experiments for a crossover design, i.e. that each subject serve as his own control. Each patient would then receive both study preparations (test and control) in a pre-determined random order of infusions.

Because of the concern of a carryover effect between infusions, a minimum period of five half-lives of the factor under consideration should be maintained between the administration of the two preparations. However, because we now believe it is acceptable to allow for prophylactic infusion of the factor between test infusions in order to prevent breakthrough bleeding in the patient, the main requirement before administering the second study material is that the factor level has returned to baseline for the subject.

We also point out that no more than two months should elapse between the two study infusions. This is to ensure comparability of the patient's clinical situation in both circumstances.

E. Clinical Status of the Patient

The patient should have moderate to severe hemophilia, i.e. a factor level of less than 0.05 u/ml at baseline. They should be in a non-bleeding state. For Factor VIII and IX patients, there should not be a detectable inhibitor (<0.6 Bethesda units) at the initiation of the study infusions. There are no restrictions on HIV status, but HIV positive subjects must be asymptomatic and not on anti-retroviral treatment. There are no restrictions on liver disease with the exception that hemophilia B patients would be excluded if their PT is less than 70% of the lower limit of normal or their INR is greater than 1.3. Finally, all individuals should be at least 12 years of age. (Due to the amount of sampling involved, children less than 12 years of age should be excluded, although we discuss below the possibility of considering younger individuals in limited evaluations).

F. Dosage

The goal in dosing the subject is to achieve a plasma concentration of the factor under study of about 1 u/mL (with the exception of Factor VIIa). Under these circumstances the dosages recommended are: Factor VIII – 25-50 u/kg, Factor IX – 50-75 u/kg. For recombinant Factor VIIa, 90 μg/kg is recommended based on clinical experience. For other factors, the dose will need to be titrated to achieve the concentration requirement.

The dose given in any instance should be based on the labelled potency of the product. To account for possible lot variability, more than one lot of product should be used in the study. These lots should be chosen at random from those available.

Since the infusion time may have an effect on the outcome (M. Morfini, unpublished data), it is recommended that the infusion be completed within 15 minutes of initiation.

G. Potency Assessment

1. Assay method. For Factor VIII, either the one stage or chromogenic assay should be utilized on the plasma samples and the same approach must be employed for both sets of results. For Factor IX, the one-stage assay should be used, while for Factor VII, a one-stage assay using a calibrated thromboplastin reagent is appropriate. For other factors, the assay of choice would be made on the basis of the available scheme for that factor.

   Samples should, in all instances, be tested in duplicate to reduce the influence of assay variability. Each assay should include a minimum of three working dilutions as is part of good assay design.[6]

2. Standards. A concentrate standard might be used in the assay of Factor VIII, and this would be prediluted in severe hemophilic plasma (with physiologically normal levels of vonWillebrand's factor [vWF] – at least 0.9 u/mL) down to a concentration of about 1 u/mL. Recent work by Christine Lee and colleagues[7] has suggested that this concentrate material be equivalent to the infused preparation, but this has not been universally accepted. Therefore, the concentrate may be the usual in-house standard. On the other hand, a 20 donor pool is acceptable as a replacement and is necessary for all factors other than Factor VIII. It should be standardized against the appropriate WHO Plasma Standard for the factor in question, which is available from National Institute for Biological Standards and Control (Dr. Trevor Barrowcliffe), Blanche Lane, S. Mimms, Potters Bar, Hertfordshire EN6 3QG, England. Use of the same plasma pool for more than 6 months would require a recalibration.

3. Instrumentation/reagents. The same recommendations as noted previously[1] are still appropriate.

4. Working dilutions. The assay working dilutions should be made in a maximum 1% (v/v) albumin buffer and at least three working dilutions should be used.

5. Substrate. The same recommendations as noted previously[1] are still appropriate.

6. Analytical methodology. The standard parallel line bioassay method should be used in calculating potency[6]. Only statistically valid assays within the context of this system should be used in the calculations.

H. Time Points for Patient Sampling

In order to adequately assess the pharmacokinetics for both the presumed phases of uptake and utilization, i.e. initial rapid equilibration between the intra and extravascular spaces followed by the (relatively) slow biological elimination, careful selection of the time points for blood sampling is required. For all factors, sampling at the following times are needed: baseline (pre-infusion), 10-15 minutes (times refer to the interval after the completion of the infusion), 30 minutes and one hour. Beyond that, the recommended times depend on the factor being infused:

1. Factor VIII: additional points to include 3,6,9,24,28, and 32 hours post-infusion; a 48 hour sample is optional provided the patient was given at least 50 u/kg

2. Factor IX: additional points to include 3,6,9,24,48 and 50 hours post-infusion; a 72 hour sample is optional provided the patient was given at least 75 u/kg

3. Factor VII: additional points to include 2,4, and 8 hours post-infusion

4. Other factors: five additional points out to 2-2.5 half-lives of the factor.

Note that we have tried to select points that would not be terribly inconvenient for the patient or the study staff, but we recognize that some variation about the recommendations is inevitable. For that reason, it is very important to record the exact time post-infusion that the sample was taken and use these precise values in the analysis.

It is recommended that all blood samples be taken from the arm contralateral to the site of the infusion.

I. Continuous Infusion

Any clinical study of continuous infusion should be performed only on elective surgical procedures. Prior to the surgery, a pharmacokinetic analysis on each individual (as described in these guidelines) should be performed to obtain, in particular, an estimate of clearance. The initial infusion rate should then be based on the clearance as follows:

Clearance (mL/h/kg) x desired steady state level (u/mL) = infusion rate (u/h/kg)

J. Prophylaxis

Prophylaxis is typically performed in children under five years of age. To determine a rough idea of their pharmacokinetics prior to initiation of prophylaxis (as it pertains to Factor VIII or IX), the following limited number of time points for sampling are suggested: baseline, 1 hour, 10 hours, 24 hours and 48 hours. This will allow for a determination of in vivo recovery and time to return to baseline at a minimum (although it is theoretically possible to perform formal pharmacokinetic calculations on these data).

ANALYSIS

A. Area-under-the-curve (AUC)

As noted previously, the area-under-the-time-versus-concentration curve should be the primary outcome variable for the pharmacokinetic studies. This is calculated using the trapezoidal rule[3] and leads directly to the determination of related parameters such as mean residence time, clearance and volume of distribution based on the model-independent (non-compartmental) method.[4,5]

B. Half-life

The determination of the traditional half-life parameter requires the specification of a particular statistical model relating time after infusion to the factor concentration, i.e. the so-called model-dependent method.[8] Typically, the approach used is based on a simplistic physiological model such as the biexponential (derived from a two-compartmental paradigm). This analysis requires the availability of a non-linear regression program [9,10] However, a method has been presented[11] and recently[12] updated

that reduces the calculations by employing a series of simple linear regressions. In any event, the half-life can be determined from the slope of the regression function for the phase of interest (α or β). The key point, however, is that the results from a pharmacokinetic study should be subjected to a rigorous statistical analysis in order to fit the regression function and thereby determine half-life, rather than use a simplistic subjective technique such as graphical analysis (which is difficult to reproduce). To facilitate the calculations, software are available on request from the cited authors (see "Analysis", section F below)

C. In Vivo Recovery

Recovery should be determined from the peak factor level that occurs in the first hour post-infusion. This figure should be reported as an incremental value, i.e. after subtracting the baseline (pre-infusion) level, and then reported on a per-dosage basis as (u/mL)/(u/kg). The practice of calculating expected recovery and incorporating this figure into the calculations in order to obtain a percentage of expected should be discontinued. (This type of analysis is typically based on results published more than 30 years ago using the early factor concentrates. It has become increasingly clear that more modern preparations may show markedly different results).

D. Crossover Analysis

Since the design of most pharmacokinetic studies involves a crossover between preparations, a proper statistical analysis of the primary outcome variable (typically AUC) should be performed first to determine whether any treatment by period interaction exists and then to assess whether bioequivalence exists between the preparations under consideration. With respect to the initial point, an appropriate analysis of variance model should be employed[13] and an assessment of the treatment by period interaction (i.e. whether the difference between the preparations is consistent regardless of the order of administration) made. If there is a statistically significant interaction, there are several recourses for handling the data, but the simplest approach is to analyze only the data from the first period. The error mean square from the analysis of variance is then used to compute two one-sided t-tests to assess bioequivalence as defined in the Design section.[2]

E. Confidence Intervals

We strongly recommend that confidence intervals or alternatives, e.g. root-mean-square error, be used to indicate the precision of all estimates reported.

F. Computer Software

Note that the Pharmacokinetics Repeated Doses software (PKRD $^{5.03}$), suitable for both model-independent and compartmental model kinetic analysis as well as for dosage provision for bolus or continuous infusion administration, can be downloaded from the web site of the Italian Society of Hospital Pharmacy (SIFO): http://members.xoom.com/labsifo. Software for the robust regression approach is available by e-mail from the first author at martin_lee@baxter.com.

DISCUSSION

These guidelines provide a basis for the conduct and analysis of pharmacokinetic experiments with coagulation factor concentrates in hemophiliacs. While standards have been available for several years, we have now provided methodology which is scientifically and statistically more rigorous and also complies with the requirements of various regulatory authorities. Furthermore, we have recognized the need for evaluation of preparations other than Factor VIII and IX, and have expanded the guidelines accordingly. Once again, we emphasize the need for uniformity in the conduct of these studies in order to provide a basis for the comparison among trials. These revised standards should continue to provide that basis.

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