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Application of Stable Isotope Method in Study Bioavailability and Bioequivalence of Highly Variable Drugs and Formulations

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ABSTRACT

The stable isotope method has been used successfully in bioavailability studies for highly variable drugs that have extensive first-pass metabolism and exhibit large intra-subject variation in clearance. Recently this technique has been extended to bioequivalence studies where labeled solution is intravenously infused in both occasions while test and reference formulation are administered. This report discusses the advantages and disadvantages of the stable isotope method and its application in bioavailability and bioequivalence studies of highly variable drugs and drug delivery systems.

Key words : Stable isotope method, Bioavailability, Bioequivalence, Highly variable drugs and formulations.

INTRODUCTION

The concept of bioavailability (BA) was developed in the early 1960s when the same active drug ingredient, in the same dose, but formulated in either similar or different products might not have the same therapeutic and/or toxicological properties. This finding prompted numerous efforts to establish definitions and guidelines for experimental protocols, specification for analytical methods, calculation of specific pharmacokinetic parameters and statistical procedures for bioavailability studies. These guidelines have also been extended to bioequivalence (BE) where bioavailability of two similar formulations are compared. The guidelines and criteria for evaluating BA/BE of drugs with relatively small variability are complete and unequivolcal. However, assessing of BA/BE for drugs and formulations exhibit large variability remains problematic. Demonstrating BA/BE for such drugs and formulations using the usual study designs, data analyses, and decision criteria is expensive because they require large numbers of subjects.

High drug variability can be introduced either by the drug, formulation, or both. Highly variable drugs often have high hepatic clearance with high first pass effect or are extensively metabolized. The variability can affects the formulation even though the formulation itself may perform well. On the other hand, the formulation can also cause high variability. An enteric-

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coated tablet displays high variability due to the large inter-and intra-subject variation in gastric emptying. The onset of drug absorption varies greatly due to the enteric coating and physiological conditions but clearly not by the drug itself. The variability of enteric-coated tablet have been effectively handled by other methods[1,2] and will not be discussed this report. Another highly variable formulation is the transdermal drug delvery systems. In this case, variability is due to analytical limitations associated with inherent high variability in measuring extremely low drug concentration and thus calculation of pharmacokinetic parameters.

BASIC CONCEPTS OF BA/BE ASSESSMENT

The bioavailability assessment includes determining the fraction of an oral dose that reaches the systemic circulation. The assessment is based on the clearance concept. The clearance of a drug after intravenous injection, Cl(iv), and oral administration, Cl(oral), can be determined by the following equations:

$$Cl(iv)=\frac{Dose(iv)}{AUC(iv)} \qquad (1)$$

$$Cl(oral)=\frac{F\ Dose(oral)}{AUC(oral)} \qquad (2)$$

Where F is the fraction of the administered dose reaching the systemic circulation, or absolute bioavailability, and AUC is the area under the plasma concentration-time curve to time infinity. Rearranging Equations 1 and 2,

$$F=\frac{AUC(oral)\ Dose(iv)\ Cl(iv)}{AUC(iv)\ Dose(oral)\ Cl(oral)} \qquad (3)$$

If equal doses are administered and assuming that clearance remains constant in the same individual between treatments, then Equation 3 can be simplified to:

$$F=\frac{AUC(oral)}{AUC(iv)} \qquad (4)$$

Therefore, F can be estimated by the ratio of AUC after oral and iv administration.

After oral administration of the same drug as a reference standard and as a test formulation, their Cls are:

$$Cl(ref)=\frac{F(ref)Dose(ref)}{AUC(ref)} \qquad (5)$$

$$Cl(test)=\frac{F(test)\ Dose(test)}{AUC(test)} \qquad (6)$$

By the same reasoning, the relative bioavailability, F(rel), can be derived:

$$F(rel)=\frac{F(test)}{F(ref)}=\frac{AUC(test)}{AUC(ref)} \qquad (7)$$

Where F(test) and F(ref) are the absolute bioavailabilities after oral administration of the test and reference formulations, respectively.

For highly variable drugs, the assumption that Cl remains constant in the same individual between the reference and test formulations may not be valid. In this case, the equation for relative bioavailability is:

$$F(rel)=\frac{AUC(test)\ Cl(ref)}{AUC(ref)\ Cl(test)} \qquad (8)$$

CONVENTIONAL METHODS FOR CORRECTING VARIABILITY

There are a few methods for correcting BA/BE estimates for specific sources of variability; most are designed to correct for variations in clearance based on the modification of Equation 8.

1. β Correction Method: A simple but often overlooked method is the classic "β correction"[3] where Cl is substiuted with β*Vd where β is the terminal rate constant and Vd is the apparent volume of distribution. Assuming that the Vd is constant within the same subject between treatment, β(ref) and β(test) can be used instead of Cl(ref) and Cl(test) in Equation 8 as the measure of the extent of absorption. For a drug with monoexponential disposition, β is propor-

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tional to Cl, thus "β correction" corrects for changes in clearance. For a drug with more complex disposition, β is no longer exactly proportional to Cl, but the approach still provides an approximation.

2. Renal Correction Method: Another method is the "renal clearance correction"[4] which substitutes Cl with renal and nonrenal clearances. Assuming that the nonrenal clearance is constant within the same subject between treatments, the renal clearance after test and reference formulations can be used instead of Cl (ref) and Cl(test) in Equation 8. This method is most useful when a significant fraction of the drug is excreted unchanged in urine.

Many highly variable drugs exhibit a large extent of systemic and presystemic metabolism. The only method which corrects for this source of variability is the stable isotope method. This procedure involves simultaneous administration of an isotope labeled drug and a test formulation. Both the isotope labeled drug and the unlabeled drug from the test formulation are subject to approximately the same extent of metabolism. This approach has successfully reduced the mumber of subjects required for BA evaluation.

APPLICATION OF STABLE ISOTOPE TECHNIQUE TO BA/BE STUDIES

To assess the bioavailability of a drug which displays high inter- and intra- subject variability, a fairly large number of subjects is necessary to achieve the statistical power and a prohibitive number of subjects may be needed to support the assumption of constant clearance over time for a conventional crossover study. The most effective approach to the BA assessment of highly variable drugs is to use the simultaneous stable isotope method where a reference drug containing an isotope (e.g. ^{15}N, ^{13}C or ^{2}H) is administered together with the formulations to be studied. Typically, the isotope drug is administered either as an iv injection, iv infusion, or as an oral solution.

Absolute bioavailability studies involve iv-stable isotope labeled solution as the reference standard and an unlabeled test formulation. Early successful stories include N-acetylprocainamide and methadone[5,6]. Both drugs exhibit considerable variability in pharmacokinetic parameters between and within subjects. Stable isotope technique reduced the variability of both drugs and provided results that were not achievable using conventional techniques.

The stable isotope method has also been used to determine the absorption kinetics and absolute bioavailability of a transdermal formulation. Determining the bioavailability of a transdermal formulation is difficult because very low amounts of drug are delivered and the exact absorbed dose is generally not known. Recently, the stable isotope technique was used to study the absorption of the nicotine transdermal system[7]. Deuterium-labeled nicotine was infused intravenously during the 24 hr application of a transdermal system in cigarette-abstinent smokers. The exact daily systemic dose (19 mg) and the absolute bioavailability (82%) of nicotine was determined. In addition, the rate of nicotine absorption and its absorption-time profile were estimated.

Relative bioavailability studies generally involved a coadminstration of an oral stable isotope solution as the reference standard. This method has been used with many drugs including verapamil[8] and maprotiline[9]. In the maprotiline study, the bioavailability of the test tablet can be demonstrated by using only 3 subjects although 6 subjects were used in the study.

The use of the stable isotope technique has not been extended to BE studies until recently. The first study reported involved two imipramine tablet formulations[10]. They were given in two treatment phases with simultaneous equivalent doses of stable isotope d_2-imipramine solutions given in both phases. This study design permitted clearance calculation based on the labeled solutions assuming absolute bioavailability of solution is constant between treatments. Another recent study compared an experimental

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transdermal nitroglycerine system relative to a commercial transdermal system[11]. This study was also a crossover design with two treatment phases. At each phase when the transdermal formulation was applied, a simultenous stable isotope ^{15}N-nitroglycerine solution was infused intravenously for 16 hr. Temporal plasma clearance changes during two transdermal system applications were determined by their respective iv infusion. The changes in clearance were the cause of the substantial fluctuation in plasma concentration of nitroglycerine. The absolute bioavailability and the absorption rate for both systems were also determined. In both studies, the number of subjects required to demonstrate BE was drastically reduced compared to conventional study design.

DISCUSSION

This report illustrates the potential use of the stable isotope method in BA/BE studies for highly variable drugs and formulation. The method has provided accurate results using minimal subjects and resources. In the past, application of the stable isotope technique in pharmaceutical research was limited by a lack of enriched synthetic intermediates and instrumentaion to accurately measure of isotopic enrichment. It is only in the last decade these problems have been addressed adequately, making stable isotope labeling a practical and economical tool. The development of the quadrupole mass spectrometer coupled to gas or liquid chromatography enabled most laboratories to measure isotope enrichment. This instrument, combines with computer control and data reduction, has permitted a high degree of automation capable of handling high sample volumes.

The initial step of stable isotope bioavailability study is the synthesis of labeled drug. It is important to place the isotope in a molecular position which minimizes the possibility of its involvement in the metabolic process. This reduces the probability of loss of the label druing the study and minimizes any isotopic effects. Following synthesis and characterization of the isotope labeled compound, a small animal study should be performed to ascertain that no untoward effects exist as a result of the synthesis and labeling.

The use of the stable isotope mehthod in BA/BE assessment also has some disadvantages. One of the most apparent is the potential for dose-related kinetic changes. The labeled drug is coadministered in a dose equal to that of the test formulation. This results in test conditions differing from those in a conventional crossover design because the subjects are exposed to higher doses of drug. The investigators must determine that the added labeled drug does not introduce a kinetic artifact which is not related to bioavailability but rather the overall larger drug dosage. It has been observed, particularly with drugs exhibiting large first-pass effects, that the rapidly absorbed labeled drug in solution is metabolized differently than the slower absorbed solid form. Therefor, evaluation of the method's true potential must await broader application and careful statistical evaluation of the outcomes of the experimental results.

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安定性同位素方法在高變數藥物及劑型之生體可用率及生體相等性之應用

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摘　　要

安定性同位素方法在生體可用率之應用已被肯定，尤其是在高度變數藥物(如高度第一次肝代謝及高度代謝)的應用，最近這方法已被應用在生體相等性的決定，當決定兩個非靜脈注射的劑型的生物相等性時，可在每次給藥時同時用靜脈注射安定性同位素之藥物，此方法可決定每次給藥時之清除率，本報告討論安定性同位素方法之優點和缺點，及其在高度變數藥物之劑型(如皮膚貼劑)在生體可用率及生體相等性之應用。