TCP Transl Clin Pharmacol
2017;25(2):53-58
http://dx.doi.org/10.12793/tcp.2017.25.2.53

TUTORIAL

The stable isotope method for determining absolute bioavailability

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Keywords
Pharmacokinetics,
Stable isotopes,
Absolute bioavailability,
Patient studies

pISSN: 2289-0882
eISSN: 2383-5427

The bioavailability of a drug is usually assessed in healthy subjects. However, it is reasonable to expect that significant alterations in bioavailability may occur in actual patients with different diseases or in individuals belonging to special populations. Relatively few studies have been conducted to examine this possibility. The stable isotope method is well suited to compare absolute bioavailability in patients and healthy subjects. Studies in which this method was used indicate that significant changes in the bioavailability of some drugs are particularly likely in patients with advanced liver disease and in those whose splanchnic blood flow is reduced. The expectation is that bioavailability in neonates, children, and pregnant women may also differ from that in non-pregnant adults.

Introduction

Steady state drug concentrations are determined only by the absorbed dose and drug elimination clearance rate. Although considerable emphasis has been placed on characterizing the effects of disease on drug elimination, relatively little attention has been given to the impact of disease on drug absorption. In fact. the FDA guidance on conducting bioavailability and bioequivalence studies recommends that they be performed in healthy subjects where possible.[1]

The stable isotope method for measuring absolute bioavailability was introduced more than 40 years ago and remains a "gold standard" for measuring bioavailability because it eliminates the washout period inherent in the traditional crossover design used in bioavailability studies.[2] Because it provides detailed pharmacokinetic data at a single point in time, the stable isotope method is particularly suitable for studying drug bioavailability in various disease states and in special populations. Review of the results of 10 patient studies provides at least a preliminary indication of the patient groups in whom bioavailability is likely to vary from that measured in healthy subjects.

Background

The development of mass spectrometry and the identification of stable isotopes had a common origin in 1919 when Ashton built the first mass spectrometer to identify the two stable isotopes of neon.[3] However, it was not until 1935 that Schoenheimer and Rittenberg studied the conversion of stable isotope labeled linoleic acid to stearic acid and first used stable isotope labeled physiological compounds to elucidate pathways of intermediary metabolism.[4] In 1950, Maynert and Van Dyke were the first to use a stable isotope labeled drug to explore its possible metabolic pathways.[5] Oral doses of ^{15}N-labeled pentobarbital were administered to dogs. About 60% of the ^{15}N dose was excreted in the first 24 hours but less than 2% of this consisted of parent drug. Gas chromatography was the first separation technique to be directly coupled to mass spectrometers.[6] This greatly enhanced the utility of mass spectrometry and followed the development of molecule separators that enabled effluent from the gas chromatograph column to be transferred from the above atmospheric pressure of the gas chromatograph effluent to the high vacuum within the mass spectrometer.[7] This was accomplished by creating a small gap in the separator across which molecules of interest could pass, whereas a vacuum pump removed most of the much lighter helium carrier gas when it entered the gap.

In 1968, Hammar, Holmstedt, and Ryhage were the first to use gas chromatography-mass spectrometry to identify a drug and its metabolites in human plasma.[8] They also demonstrated that the analytical sensitivity of conventional mass spectrometry could be increased by focusing the mass spectrometer on only two or three prominent mass spectral ions from the analyte. They accomplished this by switching the accelerating voltage of a magnetic sector mass spectrometer to bring the selected ions into focus and termed this technique "mass fragmentography". This technique is now commonly referred to as "selected ion monitoring" and continues to form the basis for most quantitative mass spectrometric applications. Unfortunately, the

magnetic field that was varied to obtain a full mass spectrum had too much hysteresis for it to be switched rapidly enough for selected ion monitoring. Furthermore, the accelerating voltage could be varied over only a limited range and only two or three high voltages could be selected at a time. Subsequently, it was shown that a quadrupole mass spectrometer did not have these limitations and could be easily modified for selected ion monitoring.[9] For that reason, quadrupole-based mass spectrometers continue to be the primary ones used for quantitative work. These advances made it possible to introduce in 1975 the stable isotope method for assessing the absolute bioavailability of a drug.[2]

Clinical Studies

Between 1975 and 2015, thirty-one published studies were identified that were conducted using stable isotope labeled drugs to assess absolute bioavailability. Most of these were carried out in healthy subjects and appeared to be incorporated in the development program of the studied drug. In addition, specially focused studies were conducted to evaluate nifedipine absorption from four intestinal segments,[10] the effect of administration rate on verapamil absorption,[11] and the comparable absolute bioavailability of three transdermal nitroglycerin formulations.[12] Additional special studies reported the absolute bioavailability of phenytoin in patients under steady-state conditions, in order to avoid dealing with its non-linear kinetics,[13] the absorption of different isomers of verapamil,[14] and the absence of a chronopharmacologic effect on the absolute bioavailability of a modified formulation of verapamil.[15]

Of particular interest are ten studies that were conducted either in patients with a particular disorder or in the special populations of elderly, neonatal, or pregnant patients (Table 1). Six of the comparisons were made to healthy subject studies in which the stable isotope method was used but only three of these were done concurrently with the patient study.[16,17,21] The most significant change was the increase in bioavailability that was reported in patients with hepatic cirrhosis.[16] This was attributed to the intrahepatic and extrahepatic shunting that accompanies this condition. When compared to healthy subjects, bioavailability more than doubled in liver disease patients and, combined with a 50% reduction in elimination clearance, was estimated to increase steady state plasma concentrations by five-fold if the same verapamil dose had been given to both groups of study subjects. In one study, slow gastric emptying with increased gastric absorption was suggested as the cause for the apparent increase in the bioavailability of cibenzoline in patients with impaired renal function.[17] However, this difference was not statistically significant, perhaps because only eight patients were included in each study group. In the study of patients with heart disease,[20] absorption of N-acetylprocainamide (NAPA) was reduced to a mean of 78% from the mean of 92% reported separately for four healthy subjects.[2] In four of the studies, there was no difference in bioavailability between the patient and control groups.[18,21,24,26] The conclusion reached in the comparison study for methadone[22] is particularly suspect because the bioavailability for the control group was estimated from the pharmacologic response of cancer patients who were receiving methadone to relieve chronic pain.[23]

Special populations were the subject of four studies. Two of these focused on elderly patients.[24,26] Of particular note is the study of phenytoin absorption in three neonates.[28] Absolute bioavailability was 100% in two of them but only 50% in the patient with the youngest gestational age. A comparator study in healthy neonates was not conducted and probably was not feasible. A study of calcium carbonate absorption in pregnant patients was conducted to compare the absolute bioavailability of a conventional formulation with an extended release formulation in which microencapsulation was designed to release calcium at pH ≥ 5.5.[29] Three different doses were examined but results are shown only for the 500 mg dose that is recommended by the WHO. Each patient served as her own control for only one of the three doses. It was found that the extended release formulation had reduced bioavailability. Unfortunately, the study was not designed to include a comparison of calcium carbonate absorption in pregnant and non-pregnant women.

Feasibility of Implementation

The feasibility of implementing the stable isotope method for determining bioavailability depends on both the advantages and disadvantages of the technique and on certain technical considerations. The method has obvious advantages over the conventional two-stage crossover method. Only a single study and set of blood samples is required, thus shortening study time, minimizing between study variation, and increasing patient comfort and safety. In addition, both labeled and unlabeled drug concentrations can be measured from only a single set of blood samples. Finally, the reduction in between-test variation enables statistically valid results to be obtained with fewer subjects. Eichelbaum estimated that use of the stable isotope method in bioavailability studies can reduce the number of subjects needed by at least 50%.[30] In an FDA-sponsored study, Hect et al. estimated that 28 to 36 subjects would be needed to establish a clinically significant difference in the relative bioavailability of two commercially available formulations of imipramine if the crossover method were used. However, only 4 to 6 subjects would be needed if a solution of a stable isotope standard were administered orally together with each formulation.[31]

Two major disadvantages to stable isotope methods that have been cited in the past are the need for mass spectrometric analysis and the high cost of purchasing stable isotope labeled drugs or the chemical intermediates needed for their synthesis.[32] However, suitable analytical instrumentation is now more generally available and the cost of stable isotope labeled compounds has decreased as the number of competing suppliers has increased. In addition, the cost of these studies to the pharmaceutical industry could be amortized if optimal use were made

Table 1. SUMMARY OF COMPARATIVE STUDIES IN PATIENTS

CLINICAL CONDITION	DRUG	BA RESULT	METHOD	REF.
LIVER DISEASE				
	Verapamil	P 52%, H 22%	SI	16
KIDNEY DISEASE				
	Cibenzoline	P 90%, H 83%	SI	17
	Nitrendipine	21%	SI	18
		23%	SI	19
HEART DISEASE				
	NAPA	P 78%	SI	20
		H 92%	SI	2
EPILEPSY				
	Phenytoin	P 100%, H 100%	SI	21
ADDICTS				
	Methadone	Addict 79%	SI	22
		Cancer 50%?	Response	23
ELDERLY				
	Verapamil	S 14% R 38%	SI	24
		S 16% R 38%	Conventional	25
	Propafenone	Extensive 30% Poor 83%	SI	26
		Extensive 30% Poor 81%	SI	27
NEONATES				
	Phenytoin	50 - 100%	SI	28
		No comparator		
PREGNANCY				
	Ca-carbonate	Non-EC 22% EC 3%	SI	29

* Abbreviations: H = healthy subjects, P = patients, BA-bioavailability, Ref.-reference number, S&R-enantiomers, Extensive and Poor metabolizers, EC-enteric coated, SI-stable isotope method. Healthy subject results are listed on the same line for concurrent studies and on the line below for studies done prior to or after the patient studies.

of stable isotope methodology for other applications throughout early drug development. Browne estimated that this could result in significant reductions in time as well as in the overall costs of these studies.[33]

A technical consideration is that the choice of a stable isotope labeled compound is limited by requirements regarding the selection and positioning of the label. It is critically important to examine the drug's mass spectral fragmentation pattern to make sure that the fragment ion selected for monitoring actually contains the isotope label. Deuterium, ^{13}C and ^{15}N labels are usually chosen for the isotope labeled drug that is administered to study subjects but the choice of isotope and the number isotope atoms that can be incorporated during synthesis may be limited. Deuterium labeling is generally the least expensive and easiest to perform. However, deuterium can alter enzymatic reactions if placed at or near a reaction site, and exchanges with water if linked to oxygen or nitrogen atoms.[34] Therefore, drugs labeled with ^{13}C and ^{15}N are preferred as they do not have these problems and their behavior replicates that of the test drug. It is generally desirable to include two or more labels in synthesizing these compounds to avoid interference from the natural abundance of stable isotopes in the test drug.[35] However, the contributions of the unlabeled and isotope labeled drugs to a monitored mass spectral peak can be separated by deconvolution if this interference cannot be avoided.[36]

With respect to the safety of stable isotope administration,

Klein and Klein point out that with respect to effects on enzymatic, cellular, and physiologic processes, stable isotopes can be subdivided into two categories: deuterium and those that involve all the other elements found in an organism.[37] The relative mass difference between hydrogen and deuterium results in a marked difference in the reactivity of their chemical bonds, and this accounts for deuterium's biological effects. Nonetheless, there is a wide margin of safety and the enrichment of total body water with deuterium may be as high as 1% to 2% without adverse consequences. This level of deuterium enrichment is never attained in pharmacokinetic studies and the stable isotopes of carbon, oxygen, and nitrogen can be administered without any safety concerns.

A final concern is the regulatory status of these studies. The FDA has now included guidance to help determine whether certain kinds of human research can be conducted without an IND.[38] A section on "cold isotopes" is included that exempts stable isotope studies provided that certain conditions are met. Unfortunately, there is a stipulation that the administered doses of the labeled compounds should not have a "clinically detectable pharmacological effect in humans". Since all organic compounds contain stable isotopes, this stipulation seems unwarranted. It might clarify thinking in this regard to refer to stable isotope labeled compounds simply as "stable isotope enriched" rather than as "cold".

Clinical Perspective

Based on the limited number of results shown in Table 1, it appears that disease-related decreases in drug absorption are likely to be greatest in patients with liver disease. In the verapamil study conducted by Somogyi et al., the combined effects of increased absorption and reduced elimination clearance were shown to have a *multiplicative* rather than an *additive* effect that would necessitate a five-fold reduction in oral dose to provide these patients with the same exposure as healthy subjects.[16] Although bioavailability studies are not recommended in the FDA guidance on pharmacokinetic studies in patients with impaired liver function, they would seem to be desirable, particularly for drugs like verapamil that normally have extensive first pass hepatic metabolism.[39]

The cause of reduced NAPA absorption in patients with heart disease suggests that reduced splanchnic blood flow could impair the bioavailability of at least some drugs. As shown in Figure 1, the extent of NAPA absorption was markedly reduced in both patients and one healthy subject when the rapid intercompartmental clearance (CL_F) between the intravascular space and splanchnic organs fell below an apparent threshold of 1.1 L/min. According to the following equation, CL_F in this case is a function of both the compartmental permeability coefficient-surface area product (P • S) and blood flow (Q)

$$CL_F = Q\,(1\text{-}e^{\text{-}P\cdot S/Q})$$

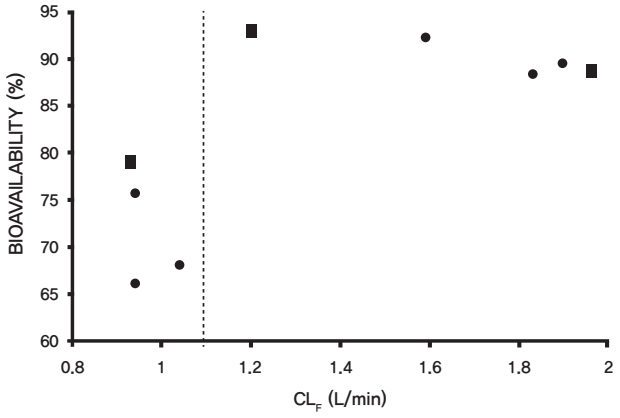

Figure 1. Relationship between bioavailability and CL_F as an indirect indicator of splanchnic blood flow. Absorption of NAPA was reduced in both healthy subjects (■) and patients (■) with CL_F less than 1.1 L/min (p = .0286, Fisher's exact test). (Data from Strong et al. (2) and Atkinson et al. (20)).

so CL_F is to some extent an indicator of splanchnic blood flow when it represents transfer from the intravascular space to splanchnic organs. Reduced splanchnic blood flow has previously been thought to account for slow and incomplete absorption of procainamide[40] and has been shown in animals to reduce bioavailability.[41] So this explanation is plausible but requires further study.

In patients with renal disease, the increase in cibenzoline absolute bioavailability was relatively modest and not readily explained.[17] In addition, there was no difference in nitrendipine bioavailability between renal disease patients and healthy subjects.[18,19] Epilepsy, and age did not affect drug absorption and the non-pharmacokinetic evaluation of methadone absorption in the cancer patient controls was methodologically flawed.

Neonates and children are special populations in which few formal pharmacokinetic studies have been reported. However, Malik has demonstrated that pharmacokinetic studies are feasible even in neonates.[28] In future studies, the stable isotope method can utilize dried blood spot sampling to provide a complete pharmacokinetic analysis with only one set of microsamples.[42] A thorough characterization of pharmacokinetics in pregnant women requires separate studies during each trimester with a comparator study usually conducted in the postpartum period. Although Roth studied pregnant women only in the third trimester, he demonstrated the clinical feasibility of using the stable isotope method in these women even though calcium carbonate obviously differs from most drugs.[29]

Based on only limited data, it can be concluded that the stable isotope method of determining absolute bioavailability is feasible from the technical standpoint and is likely to efficiently characterize differences from normal absorption, particularly in patients with liver disease or reduced splanchnic blood flow. It is also the optimal way to characterize drug absorption in neonates, infants, and pregnant women.

Acknowledgements

Nothing to declare

Conflict of Interest

Nothing to declare

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