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UTILITY OF BIOAVAILABILITY STUDIES IN DRUG DEVELOPMENT

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ABSTRACT

A sequentially designed drug development program considering bioavailability studies in relation to the many disciplines involved in drug development will have the greatest potential for success.

INTRODUCTION

Drug development programs are multi-disciplinary in scope and require systematic coordination of the various disciplines involved to ensure maximum productivity, efficiency, and success. A focal point in such a multi-disciplinary program is the scientific group responsible for defining and evaluating the bioavailability characteristics of a drug.

The term bioavailability has been used to define a variety of scientific endeavors and it is necessary, therefore, to develop a working definition in relationship to its allied disciplines of pharmacokinetics and biopharmaceutics.

Pharmacokinetics is defined as the rate processes associated with the absorption, distribution, metabolism, and elimination characteristics of a drug, e.g., monitoring the drug and its metabolite(s) as a function of time from the moment of administration until virtually complete elimination from the body.

Biopharmaceutics is defined as the relationship of the physicochemical properties of the drug substance per se, and its delivery system, to its absorption into the systemic circulation following all routes of administration except for the intravenous route.

Bioavailability is defined as a measure of the rate and extent of absorption of an administered dose into the systemic circulation. This can be reported in absolute terms or in relative terms by comparison to a standard which may or may not have a known absolute bioavailability. A relative or comparative bioavailability study is also referred to as a bioequivalence study.

The bioavailability of a drug substance is influenced not only by its physicochemical (biopharma-

ceutical) properties, but also by prevailing physiological factors as well. These are best understood by referring to Figure 1 which depicts the pathways in the physiological disposition of a drug. Upon intravenous administration, where the drug is placed directly and completely into the systemic circulation, one achieves instantaneous and complete bioavailability; and the data generated from such a profile represents the primary standard against which one can obtain absolute bioavailability parameters and primary pharmacokinetic information. Following oral administration of a solid dosage form (the most common delivery system), the drug must first dissolve in the gastrointestinal fluids, be transferred across the gastrointestinal mucosa into the mesenteric blood system, and then pass through the liver prior to reaching the systemic circulation. Although the physicochemical properties of the drug influence the dissolution rate and transfer characteristics (permeability) of a drug (as will be discussed later), there are normal physiological factors that may diminish the bioavailability of an orally administered compound.

The drug may either be biodegraded by the gastrointestinal fluids, or by the enzymes in the gastrointestinal mucosa and in the liver prior to reaching the systemic circulation. The net result is incom-

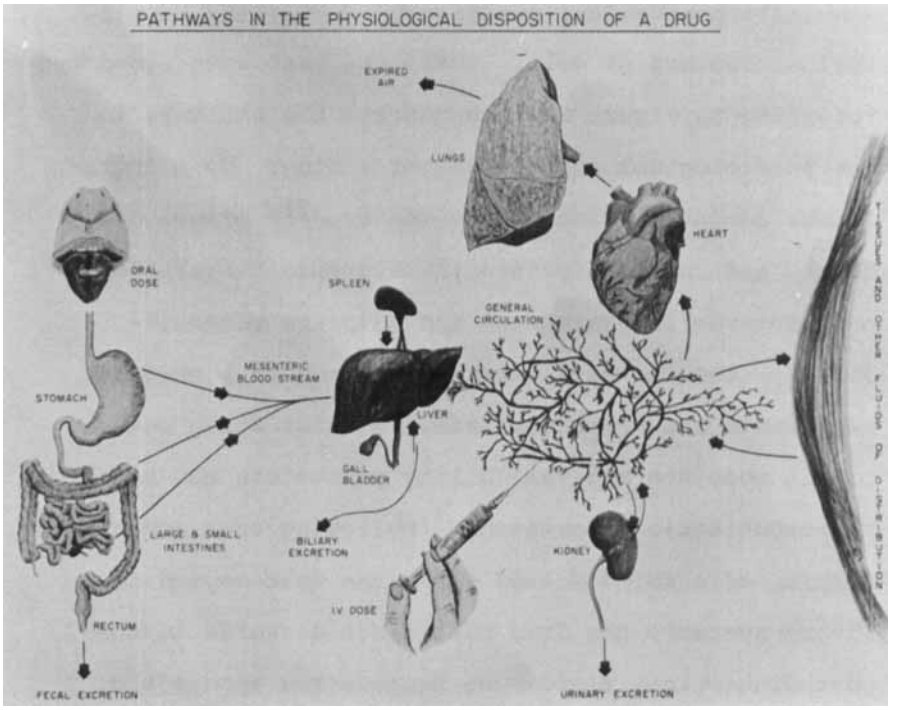


Figure 1. Physiological factors associated with bioavailability

plete bioavailability of the administered drug due to the so called "first pass" metabolism of the drug. The biodegradation products may be pharmacologically active or inactive substances. These physiological events may vary from species to species (relative to their occurrence), and may also vary in rate in the same individual from administration to administration.

In the early stages of drug development when many compounds are being screened, it is not always practicable or economical to perform the appropriate bio-

availability studies on every compound. However, it would be equally imprudent to discard a potentially useful and novel compound, and/or inadvertently accept a potentially toxic compound for further testing because the drug was incompletely or erratically absorbed from its delivery system. Such factors must be defined with a modicum of precision. Consider the absorption-response profiles depicted in Figure 2.

Curve A is the drug level curve of a compound that is rapidly and completely absorbed. Because of the rapid absorption, the pharmacological response should be prompt and reproducible. Curve B results from a formulation of the same drug which is completely absorbed as in case A, however, the rate of absorption is much slower. The pharmacological response will be delayed and more prolonged than A. Although formulation A and B are both absorbed to the same extent as indicated by their respective areas under the curve, they are not bioequivalent because their rates of absorption are different. Curve C also results from a formulation that is completely absorbed, however, absorption occurs at such a slow rate that no pharmacological response is observed. The level of drug is always below the minimum effective level required for a pharmacological response. Curve D indicates incomplete absorption from its for-

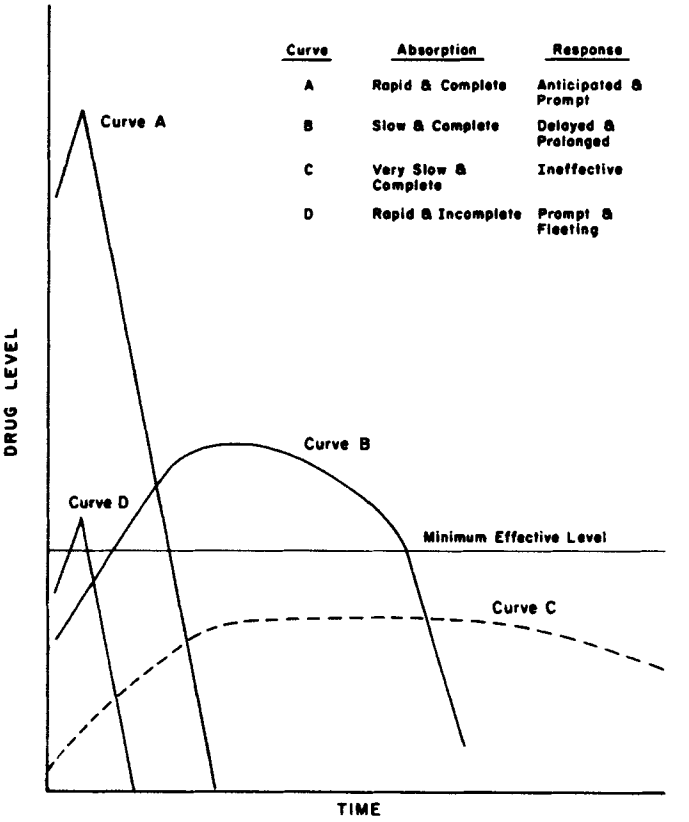


Figure 2. Bioavailability considerations in relation to pharmacological response.

mulation, but unlike formulation C, formulation D does elicit a pharmacological response, which is rapid but fleeting.

One can well imagine the potential confusion within a group of investigators each working with the same chemical entity administered in a variety of delivery systems. Therefore, the following presenta-

tion details the utility of bioavailability testing as part of both preclinical and clinical drug development studies so that much trial, error, and confusion can be replaced by quantitative precision and scientific accuracy. This requires a multi-disciplinary effort in which relevant biopharmaceutical and pharmacokinetic properties can be adequately investigated, and potential problem areas corrected at an early stage of drug development and/or avoided entirely (1).

BIOAVAILABILITY CONSIDERATIONS

The flow diagram depicted in Figure 3 presents a scheme of the multi-disciplinary interactions that occur in the early stages of drug development in which a lack of appropriate bioavailability information may be detrimental to the overall success of the program.

Bioavailability considerations in drug development could actually begin with the synthetic chemist when initiating a new substance. In working with a series of compounds which exhibit a potential for dissolution rate limited absorption, one might consider synthesizing a more water soluble derivative or salt to overcome this problem; or a derivative in which aqueous solubility would increase with an in-

Figure 3a. Organization of a bioavailability oriented multi-disciplinary drug research program.

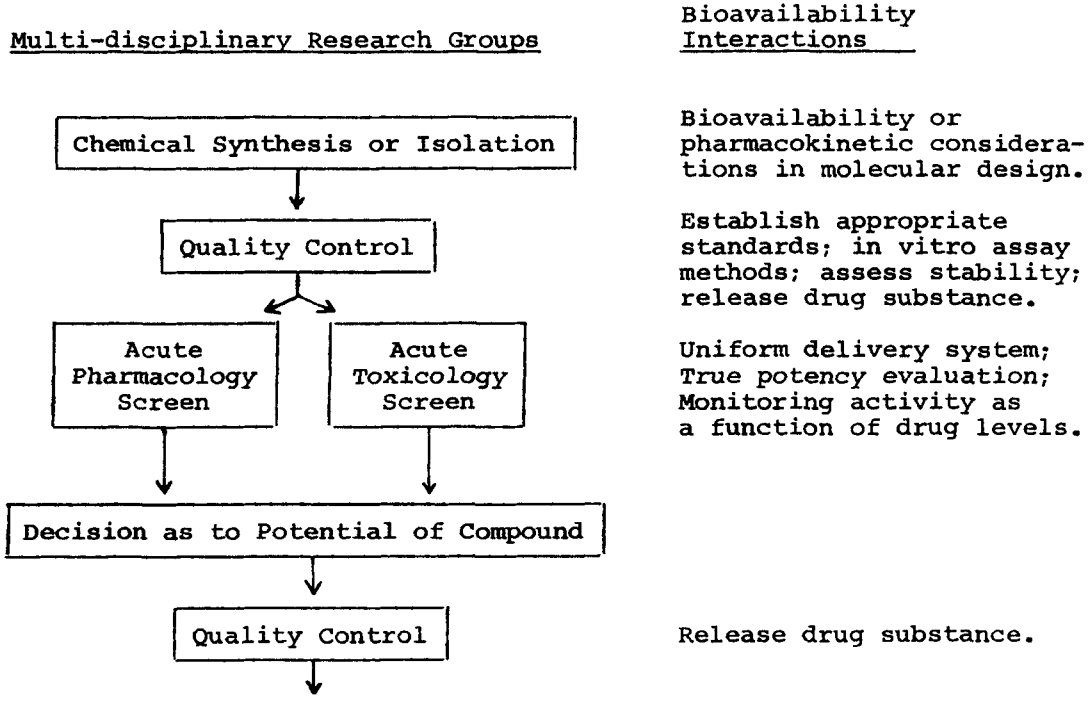


Figure 3b (cont.). Organization of a bioavailability oriented multi-disciplinary drug research program.

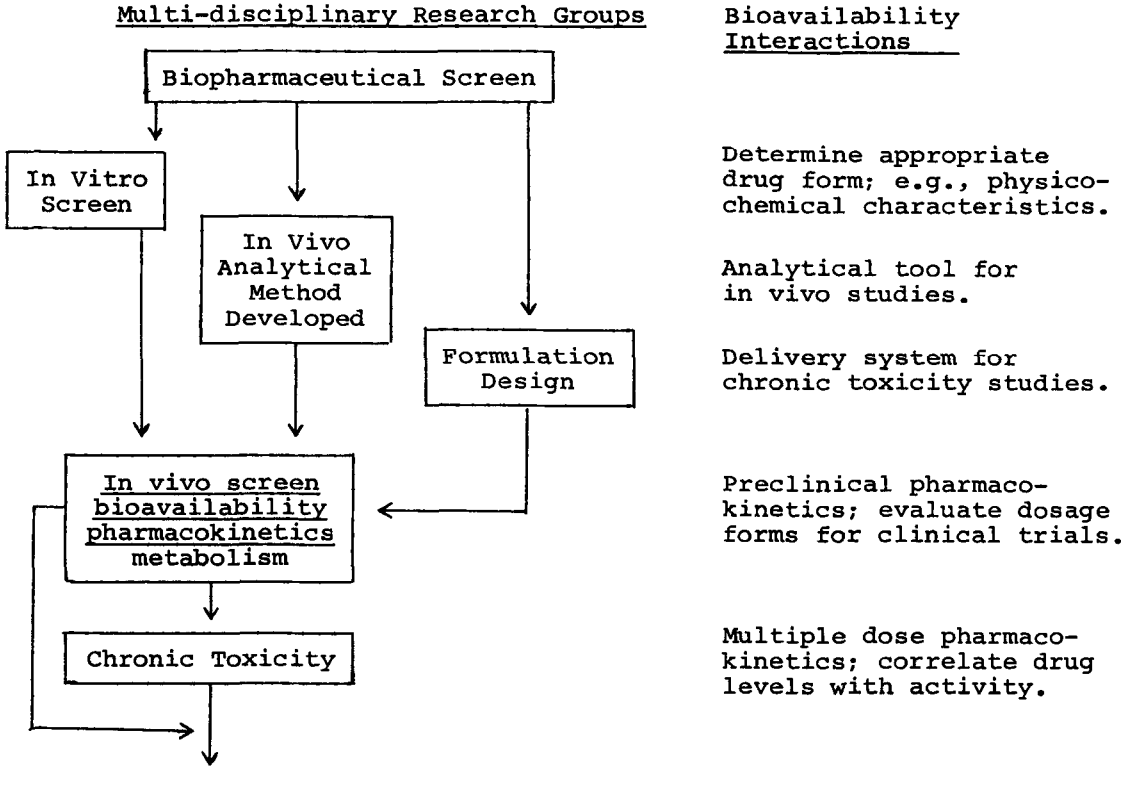
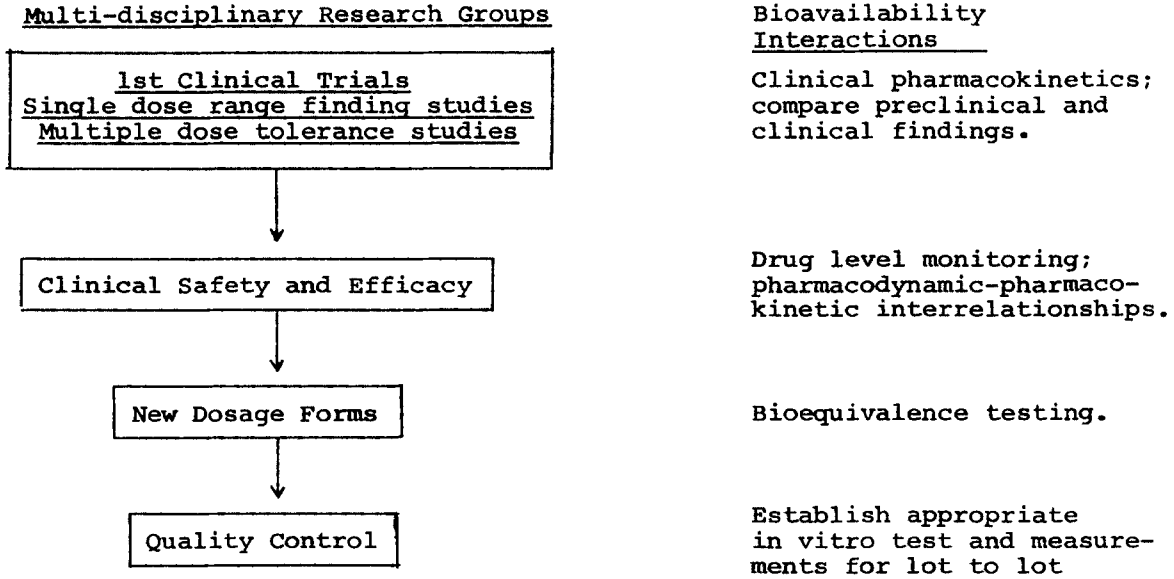


Figure 3c (cont.). Organization of a bioavailability oriented multi-disciplinary drug research program.



crease in pH to allow for more complete absorption of a relatively insoluble substance. One might consider a derivative more stable over the physiological pH range of 1 to 8, or an ester or analog that exhibits a pK_a which overcomes a permeability limited absorption problem. In addition to absorption, other pharmacokinetic parameters might be altered by chemical manipulation to suit a specific clinical need. Examples would include a more highly bound drug that would decrease the elimination rate of the drug from the body; a more lipophilic compound or a compound with a suitable pK_a to provide higher tissue levels or drug, or conversely a more hydrophilic derivative that would probably be metabolized to a lesser extent. Dialogues between the chemist and pharmacokineticist may provide a new dimension to a drug development program.

Bioavailability aspects of drug development rely heavily on the Quality Control Department, even while a drug is still in the early stages of development. A major goal of the Quality Control Department is to establish the testing procedures and specifications which will be used to monitor the future marketed drug product. However, Quality Control also performs extensive tests at the various stages of chemical synthesis of compounds and manufacturing of dosage forms.

Variability in the physicochemical measurements of a drug per se, e.g., dissolution rate, particle size, crystalline form and stability may indicate a potential for altered bioavailability of the drug in vivo. Therefore, Quality Control must also be aware of potential bioavailability problems for a drug. The advantage is that when testing procedures are designed for a drug, Quality Control will also monitor the specific physicochemical properties which may affect the bioavailability of the drug.

Bioavailability studies are routinely performed in the early stages of drug development, but not on every lot of the marketed product. Although the bioavailability of each marketed lot of an established dosage form is not routinely evaluated, the bioavailability of these lots is inferred since the Quality Control testing guarantees that every lot of drug substance and drug product released for use is physically and chemically identical to a standard lot of drug compound whose bioavailability was previously established. For example, distinguishing between polymorphic forms of a drug may not be a routine Quality Control test. However, for certain drugs the bioavailability of the substance is dependent upon the polymorphic form used. Chloramphenicol is a classical example where blood levels are significantly influ-

enced by the polymorphic form (2). The polymorphic forms of chloramphenicol must be closely controlled in order to guarantee the bioavailability of the drug. Nitrofurantoin is another example where the physicochemical properties of the drug affect its bioavailability (3). The crystal size of nitrofurantoin was found to relate directly to its bioavailability as monitored by its urinary excretion profile. Although crystal size may not be a parameter routinely monitored by Quality Control, for some drugs this may be necessary to ensure bioavailability. Therefore, interaction of Quality Control with bioavailability investigators during drug development will allow for the establishment of appropriate specifications to ensure reproducible bioavailability and therefore efficacious drugs.

Such involvement with Quality Control scientists will become more evident as pharmaceutical investigators realize that the properties of the drug substance per se, as well as the delivery system must be standardized to ensure efficiency in drug development and a successful drug product. Prior to marketing a drug product, Quality Control should establish standards which are sensitive to potential changes in drug bioavailability, as well as purity. Such criteria will make the Quality Control program more relevant.

Before even considering the implementation of bioavailability studies per se, it is of the utmost importance that scientists involved in the initial biological screening programs of new drug substances develop uniformity standards for the drug substance and delivery systems used to administer these drugs. Bioavailability can influence the onset and intensity of a pharmacological response. Uniformity of drug delivery is essential in order to accurately evaluate the pharmacologic activity and toxicity of drugs. Since the physicochemical properties of the drug substance per se, as well as the drug delivery system, can affect the bioavailability and potency assessment of drug, one should strive for reproducible and optimal bioavailability to achieve an optimum potency evaluation. Uniform drug delivery is also important for the correlation of data from laboratory to laboratory. Such uniformity standards will require control and release of the drug substance by a Quality Control group and the design and use of a delivery system for administering drug during the screening programs. Although a delivery system may vary for different drugs, all investigators studying an individual drug should agree upon a common delivery system. The absolute bioavailability of a drug from its delivery system may not be defined at the time of early

pharmacological screening. Therefore, initial potency and toxicity data may be a reflection of dose absorbed and not dose administered. However, when the bioavailability of drug from the uniform delivery system is established, the initial potency data can be reevaluated in terms of its absolute bioavailability. It should be emphasized that an LD_{50} value is not an absolute value but can vary as a function of the form of the compound and the delivery system used to administer a compound. Consider the two examples presented below:

Drug A:

Compound Form	LD_{50} mg/kg, p.o.	ED_{50} mg/kg, p.o.
Non-micronized	>15,000	630
Micronized	7,900	520
Sodium Salt	3,250	394

Drug B:

Particle size	LD_{50} mg/kg p.o.
Coarse	3340
Regular	2461
Fine	1796

In the first case, the sodium salt exhibited a much lower LD_{50} than micronized or non-micronized free acid. In the second case, the LD_{50} was shown to be sensitive to changes in particle size of the magnitude which might not usually be controlled during the early stages of drug development.

Uniformity of administration of a drug with an undefined bioavailabiaity profile also provides better precision in defining a therapeutic index for a drug, e.g., the ratio of LD_{50} to ED_{50}. A low therapeutic index may result in terminating a drug candidate. If poor bioavailability is suspected, a comparison of oral and intravenous LD_{50} values provides a rough index of the magnitude of the absorption problem. Such comparisons can also be used as the basis for choosing a uniform delivery system that provides optimal absorbability. If one is unable to obtain toxicological manifestations as the oral dose administered is increased, it is likely that drug absorption has become a limiting factor. In such cases, one may not have a "non-toxic" drug, but merely an inconclusive toxicity study.

The pharmacological activity and intended therapeutic use of the drug must also be considered in evaluating bioavailability data. A drug which must exhibit a rapid onset of activity such as a hypnotic

or analgesic agent must be absorbed rapidly. Therefore, one would be more critical in choosing a more rapidly absorbed form of a compound for these indications as compared to an agent where efficacy is based upon chronic administration of the drug.

Finally, a bioavailability and pharmacokinetics program in conjunction with acute and chronic toxicity studies, can provide useful information relative to drug and/or metabolite level profiles in various body fluids, tissues, and excreta. Such information may correlate drug response profiles with toxic manifestations in various animal species. The data may also suggest a potential for drug accumulation and/or enzyme induction. If such correlations could be made, then drug level profiles might be used as a meaningful indicator of drug efficacy and safety.

Most of the Quality Control and in vitro studies can be performed using relatively simple analytical procedures since drug concentrations are usually high. However, bioavailability studies require sensitive and specific assay procedures to quantitate drugs and/or metabolites in biological fluids, tissues, or excreta (4). Such procedures may be developed using either chemical techniques or with radioactive drug. The procedures,require extraction and clean-up steps to separate intact drug from the sample matrix and other

drug related components. The use of a suitable thin layer chromatographic step may be sufficient to achieve specificity, especially when using a radio labeled drug.

Since the success of a bioavailability-oriented drug development program depends upon the successful development of analytical procedures, these investigators must be an integral part of the overall organization of a drug development program. Ideally, an assay procedure will be developed sufficiently early to allow for bioavailability assessment of a formulation designated for chronic toxicity studies as well as to monitor drug levels during such studies.

Much of the drug screening program will precede the defining of the biopharmaceutical properties of a drug substance. This results from the large number of compounds screened, which precludes full evaluation of each candidate. However, potential candidates for chronic toxicity studies should first be submitted for biopharmaceutical evaluation, so that the drug form and delivery system which ensures optimal and reproducible bioavailability will be used for all subsequent preclinical and clinical studies. Since these studies are both time consuming and costly, properly designed biopharmaceutical studies can negate much

wasteful repetition required with changes in a drug form or delivery system.

An orally administered solid drug must first dissolve in the gastrointestinal tract prior to absorption across the gastrointestinal mucosa into the systemic circulation. Therefore, the two biopharmaceutical parameters associated with absorption can be evaluated in vitro to determine whether dissolution and/or permeability may be a rate limiting step in the oral absorption of a drug. The utility of the data from the evaluation of the intrinsic dissolution rate as a function of pH (5), and the permeability of the drug across the everted rat intestinal sac preparation (6) in defining these two absorbability parameters has been discussed previously. The confirmation of these in vitro predictions is based upon the bioavailability data generated in animals prior to investigation in man.

An example of the effect of drug solubility and dissolution rate on the oral absorption of a short-acting hypnotic in beagle dogs has been reported (7). The oral administration of this drug in polyethylene glycol 400 solution resulted in a marked increase in rate and extent of bioavailability over the soybean oil suspension when administered at a dose of 100 mg/kg. Drug absorption was erratic from the suspen-

sion with only 10 percent of the dose absorbed when compared to the solution.

The projected therapeutic dose, based on the pharmacological data, is useful in assessing the clinical relevance of such bioavailability studies. If the projected dose is high, the solubility and dissolution characteristics would be much more critical than if the projected dose were low. A high dosed drug exhibiting slow dissolution characteristics may be both slowly and incompletely absorbed, whereas with a low dosed drug the intrinsic slow dissolution problem may be overcome solely by virtue of the fact that less drug is required to go into solution. If aqueous solubility decreases with an increase in pH, there may be more reason for concern since the potential for incomplete absorption is greater, especially if dissolution is not attained rapidly in the more acidic environment of the stomach.

It is important to emphasize that the evaluation of bioavailability data is based on the pharmacokinetic treatment of such data. Although the mathematical sophistication used in a pharmacokinetic analysis may vary with the expertise of the investigator and the quality of the available data, the more sophisticated the pharmacokinetic treatment the more

useful the information one is able to obtain from the data.

Pharmacokinetics is the study of the rates of the transfer processes associated with the absorption, distribution, metabolism, and excretion of a drug in the intact animal. The primary standard of a pharmacokinetic study would be based on the data obtained following intravenous administration. Since the entire dose administered is placed directly in the bloodstream, the pharmacokinetic parameters obtained are unaffected by all the potential complications associated with drug absorption. Subsequent to obtaining the primary pharmacokinetic parameters, the parameters of absorption can be evaluated and defined in relation to the intravenous standard. This absorption evaluation is an important aspect of the early studies since in many cases it is the success of developing the orally administered dosage form that motivates its marketing.

In pharmacokinetics one attempts to describe biological events in mathematical terms usually by designing mathematical models. In order to validate a model many in vivo experiments and computer simulations are required. However, in the early preclinical stages of drug development, one should attempt to utilize model independent curve fitting techniques or

the simplist model which is compatible with the observed data.

The preclinical in vivo parameters of absorption, when isolated from those of the physiological disposition of the drug, allow for meaningful predictions of absorption of a drug in man in most cases, even when the parameters of physiological disposition are different in the animal and man. Comparison of areas under the blood level curves following oral and intravenous administration provides an index of the extent of bioavailability. The shape of the oral blood level curve in comparison to the intravenous curve and/or absorption rate calculations can provide an index of the rate of bioavailability. Such analyses of bioavailability have been presented elsewhere in greater detail (8,9).

Additional preclinical parameters such as volume of distribution, clearance, elimination rate, and extent of recovery of the intact drug in the urine are useful in evaluating in vivo pharmacological and toxicological studies as well as in planning for the first clinical studies. However, until one is able to obtain and compare clinical with preclinical parameters, such pharmacokinetic evaluations should be kept simple.

Preliminary drug metabolism studies are valuable at this stage of drug development since the bioavailability of a drug may be impaired due to "first pass" metabolism of the drug in the gastrointestinal tract and/or liver prior to reaching the systemic circulation. When "first pass" metabolism is found to occur, there may be species variability where some species may not exhibit "first pass" metabolism. In addition, one may observe intra and inter subject variability in the extent of the "first pass" metabolism. Levodopa was shown to undergo gastrointestinal "first pass" metabolism in the dog (10); whereas an experimental benzodiazepine compound was shown to undergo liver "first pass" metabolism (5). Since the metabolites formed during this "first pass" will probably be absorbed, the identity and pharmacological activity of these substances should be defined. Since several animal species may be used in the preclinical stages of drug development, the potential for "first pass" metabolism and species variability may influence the interpretation of the pharmacological and toxicological data per se, and their relevance for the subsequent clinical studies.

Throughout the entire drug development program, drug delivery systems for both preclinical and clinical studies are required. Since most investiga-

tors are not trained to translate the biopharmaceutical characteristics of a drug substance into a dosage form or delivery system, the formulation experts in a pharmaceutical formulations research department must also be an integral part of the drug development program. The literature is replete with examples of failures in bioavailability due to formulation factors. These reported failures occur in man usually with commercially available drugs. There is a paucity of literature pertaining to preclinical bioavailability problems. However, with negligible input from pharmaceutical scientists during drug development, the potential for such bioavailability failures is great.

An in vivo preclinical bioavailability evaluation should be run on dosage forms intended for the first clinical trials so that the problems discussed herein vis-a-vis preclinical testing are not manifested during the first clinical trials.

Preclinical pharmacokinetic data will be useful in the design of the first clinical studies. Ideally, if the protocols for the initial clinical tolerance studies were designed to include blood and urine specimens for analysis, the resulting clinical pharmacokinetic data can be of value in designing a dosing regimen for the clinical efficacy studies. The

clinical pharmacokinetic data when compared with the preclinical findings may also suggest a suitable animal model for extended pharmacological and toxicological studies.

In conclusion, therefore, bioavailability might be considered the thread that weaves together the various multi-disciplinary aspects of a drug development program. Since the purpose of drug development is to obtain an efficacious compound with a reproducible onset and duration of activity, bioavailability measurement provides the basis for the assessment and correlation of such information across various scientific disciplines. The inclusion of bioavailability considerations at each stage of drug development aids in defining potential problems and suggests the means of overcoming such problems.

Those investigators that apply bioavailability testing solely as an endpoint in dosage form evaluation and not throughout drug development may find it necessary to repeat many costly and time consuming studies to correct these problems. Such problems are not easily corrected retrospectively since they may have resulted from a multiplicity of factors. Overall, a sequentially designed and integrated drug development program considering bioavailability in relation to the many disciplines involved will have the greatest potential for success.

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