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Bioavailability- A New Era of New Drug Design and Development

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Bioavailability is used to describe the fraction of an administered dose of medication that reaches the systemic circulation, one of the principal properties of the drugs. By definition, when the drug is administered intravenously, its bioavailability is 100%. However when a medication is administered via other routes (such as oral), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration. "The rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For the drug products which are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

Keyword: Bioavailability, Bloodstream, Clinical Trial, New Drug Entity.

1. Introduction

The bioavailability of drugs in recent years has become interesting subject in drug development and also in the early stages of drug discovery. This is a tool to finding that most of the candidate drugs that failed in clinical trials because of problems with toxicology and absorption, distribution, metabolism, excretion i.e. ADME, rather than through lack of efficacy. The very hard efforts are being made in the pharmaceutical industry to improve success rates by taking into account the toxicology and ADME aspects in drug discovery. Oral bioavailability is the fraction of an oral administered drug that reaches systemic circulation. After intravenous administration, a drug is directly and fully available in the bloodstream and can be distributed via systemic circulation to the point where a pharmacological effect takes place. If a drug is administered orally, it has to cross further barriers to reach the systemic circulation, which can significantly reduce the final extent of a drug in the bloodstream. Oral bioavailability is one of the most important properties in drug design and development. A high oral bioavailability reduces the amount of an administered drug necessary to achieve a desired pharmacological effect and therefore could reduce the risk of side-effects and toxicity. A poor oral bioavailability can result in low efficacy and higher inter-individual variability and therefore can lead to unpredictable response to a drug.

2. Objective of Bioavailiability Study

1. Primary stages of development of a suitable dosage form for a new drug entity.

2. Determination of influence of excipient, Patient related factors and possible reaction with other drug on efficient of absorption.
3. Development of new formulation of existing drug.
4. Control of quality of drug product during early marketing in order to determine the influence of processing factors, storage and stability on drug absorption.

3. Factor Affecting Bioavaility

If the size of the dose to be administered is same, then bioavailability of a drug from its dosage form depends upon three major factors,

1. Pharmaceutical factors related to physiochemical properties of the drug and characteristics of dosage form.
2. Patient related factors
3. Route of administration.

If the goal is to compare the two formulations of same drug then the experimental design should maintain the remaining factors constant. The resultant bioavailability may differ with respect to the amount absorbed, the rate of absorption or both.

Bioavailability reflects the extent of the systemic availability of the active therapeutic moiety and is generally assessed by measuring the 'area under the concentration time curve' (AUC), the peak plasma concentration (Cmax) and the time to reach C_{max} (Tmax). The extent of the systemic availability is determined by the extent of drug absorbed from the site of administration. For a drug that obeys linear pharmacokinetics, the AUC and Cmax values increase proportionately with the dose. Consequently, if two formulations/dosage form of the same drug exhibit comparative AUC values, they are considered to have similar systemic availability. The bioavailability of an oral dosage form or a drug is generally compared with an intravenous solution (100% standard), to determine the absolute bioavailability.

Bioequivalence studies compare both the rate and extent of absorption of various multisource drug formulations with the innovator (reference) product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects.

"The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study."

4. Types of Bioavailability And Bioequivalence Studies:

4.1. Pilot Studies:

If the sponsor choose ,a pilot study it can be carried out in a small number of subjects before proceeding with a full bioequivalence study .The study can be used to validate analytical methodology ,asses variability optimize sample collection time interval and provided other information .For example for

conventional immediate release product careful timing of initial sample may avoid a subsequent finding in a full scale study that the first sample collection occurs after the plasma concentration peak .For modified release product, a pilot study can help determine the sampling schedule to asses lag time and dos dumping. A pilot study that document bioequivalence may be appropriate, provide its design and execution is suitable and a sufficient number of subjects have completed the study.

4.2. Pivotal Studies

General recommendations for a standard bioequivalence based on a pharmacokinetics measurement are provided in attachment. The study should be design in such a manner that the formulation effect can be distinguished from other effect. Typically, if two formulations are to be compared, a single dose, two period, two sequence, cross over design is design of choice with the two phase of treatment separated by an adequate wash out period which should ideally be equal to or more than five half lives of moiety to be measured. Non replicable study designs are recommended for bioequivalence of immediate release and modified release dosage form

.Alternative design include the parallel design for very long half life substance or replicate design for substance with highly variable disposition.

5. General Conduct of Studies
In the following sections, requirements for the design and conduct of comparative bioavailability studies are formulated. Investigator(s) should have appropriate expertise, qualifications and competence to undertake a proposed study and is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance in question. For the pharmacokinetic basis of these studies reference is made to the recommendation "Pharmacokinetic studies in man". The design and conduct of the study should follow Malaysian Guidelines for Good-Clinical Practice including reference to an Ethics Committee.

5.1. Design
The study should be designed in such a way that the treatment effect (formulation effect) can be distinguished from other effects. In order to reduce variability a cross over design usually is the first choice. Other designs or methods may be chosen in specific situations, but should be fully justified in the protocol and final study report.

The subjects should be allocated to treatment sequences in a randomized order. In general, single dose studies will suffice, but there are situations in which steady-state studies may be required:
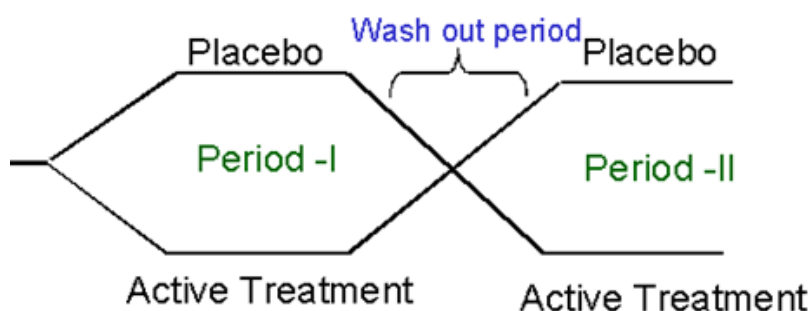
a) If problems of sensitivity preclude sufficiently precise plasma concentration measurement after single dose;
b) If the intra-individual variability in the plasma concentrations or disposition rate is inherently large;
c) In the case of dose- or time-dependent pharmacokinetics;
d) In the case of extended release products (in addition to single dose studies).

In such steady-state studies, the administration scheme should follow the usual dosage recommendations.

More commonly used replicated crossover designs to compare two formulations are:
▪ Four-sequence and two-period design (Balaam's design):
▪ Two-sequence and four-period design:
▪ Four-sequence and four-period design:
▪ Two-sequence and three-period design
▪ Crossover design for three medications (Williams' design).

Crossover design for four medications (Williams' design).

Fig 1: Crossover Design

Treatment Group	Treatment Period I	Wash-out Period	Treatment Period II	Wash-out Period	Treatment Period III	Wash-out Period	Treatment Period IV
1	A		B		C		D
2	B		C		D		A
3	C		D		A		B
4	D		A		B		C

Fig 2: Latin Crossover Design

6. Subjects
6.1. Number of subjects
The number of subjects required is determined by the error variance associated with each of the pharmacokinetic parameters of primary interest (e.g. AUC, C max) as estimated from a pilot experiment, from previous studies or from published data, by the coverage probability of the confidence interval from relative bioavailability, by the expected deviation from the reference product compatible with bioequivalence and by the required power. The clinical and analytical standards imposed may also influence the statistical determined

10 number of subjects. However, the minimum number of subjects should not be smaller than 12 and the number of recruited subjects should always be justified.

6.2. Selection of Subjects
The subject population for bioequivalence studies should be selected with the aim to minimize variability and permit detection of differences between pharmaceutical products.
Therefore, the studies should be performed with healthy volunteers. The inclusion/ exclusion criteria should be clearly stated in the protocol.
Subjects could belong to both sexes; however, the risk to women of childbearing potential should be considered on an individual basis.
In general, subjects should be between 18 – 55 years old, capable of giving informed consent and of weight within the normal range according to accepted life tables or Body Mass Index (BMI) or LIC chart. They should be screened for suitability by means of clinical laboratory tests, an extensive review of medical history, and a comprehensive medical examination. Subjects should preferably be nonsmokers and without a history of alcohol or drug abuse. If moderate smokers are included (less than 10 cigarettes per day) they should be identified as such and the consequences for the study results should be discussed.

If the purpose of the bioequivalence study is to address specific questions such as investigation of differences in bioavailability in different subsets of the population or drug-drug interactions the selection criteria and the statistical analysis should be adjusted accordingly.

6.3. Inclusion of Patients
If the investigated active substance is known to have adverse effects and the pharmacological effects or risks are considered unacceptable for healthy volunteers, it may be necessary to use patients instead under suitable precautions and supervision. In this case the applicant should justify the alternative.

6.4. Genetic Phenotyping
Phenotyping and/or genotyping of subjects may be considered for safety or pharmacokinetic reasons.

7. Standardization of the study
The test conditions should be standardized in order to minimize the variability of all factors involved except that of the products being tested. Therefore, standardization of the diet, fluid intake, exercise and posture is recommended.

Subjects should preferably be fasting at least during the night prior to administration of the products. If the reference product is administered with food, subjects should take a standard meal at a specified time before the treatment. The time of day for ingestion should be specified and as fluid intake may profoundly influence gastric passage, the volume of fluid which varies according to different guidelines should be constant. All meals and fluids taken after the treatment should also be standardized in regard to composition and time of administration.

The subjects should not take other medication during a suitable period before and during the study and should abstain from food and drinks, which may interact with circulatory, gastro-intestinal, liver or renal function (e.g. alcoholic or xanthine-containing beverages or 11 certain fruit juices). As the bioavailability of an active substance from a dosage form could be dependent upon gastrointestinal transit times and regional blood flows, posture and physical activity may need to be standardized. All the above criteria should be clearly stated in the protocol.

8. Sampling
Blood samples in principle should be used. Urine samples also can be used. Blood samples should be taken at a frequency sufficient for assessing Cmax, AUC, Tmax and other parameters. The sampling schedule should be planned to provide an adequate estimation of Cmax and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of absorption; this is generally achieved if the AUC derived from measurements is at least 80 % of the individual AUC extrapolated to infinity.

In a steady-state bioequivalence study, when the circadian rhythm is known to have an influence on bioavailability, sampling schedule should be carried out over a full 24 hours cycle.

9. Washout Period
Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (wash-out period). In steady-state studies washout of the last dose of the previous treatment can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least three (3) times the dominating half-life).

10. Characteristics to be Investigated
In most cases evaluation of bioequivalence will be based upon the measured concentrations of the parent compound. This may be impossible if (1) the concentration of the parent compound is too low to accurately measure in the biological matrix (e.g. major difficulty in analytical method, product unstable in the biological matrix) thus giving rise to significant variability or if (2) the half-life of the parent compound is too short to derive any meaningful pharmacokinetic parameters. In these situations, a major biotransformation product should be used provided it reflects the bioavailability of the active substance. Measurement of the concentrations of an active biotransformation product is also essential if the parent compound is a prodrug. Where the biotransformation product is formed predominantly by a saturable first pass metabolism, concentration-time curves of the metabolite cannot be used to assess bioavailability. If urinary excretion (rate) is measured the product determined should represent a major fraction of the dose and the excretion rate should be considered to parallel plasma concentrations of the active substance. In bioavailability studies, the shape of, and the area under the plasma concentration curve or the cumulative renal excretion and excretion rate are mostly used to assess extent and rate of absorption. Sampling points or periods should be chosen, such that time concentration profile is adequately defined so as to allow the estimation of relevant parameters. From the primary results the bioavailability characteristics desired are estimated, namely AUCo-t, AUCo-∞, Cmax, Tmax or any other justifiable characteristics. The method of calculating AUC-values should be specified. For additional information, t½ and MRT can be estimated. For studies in steady state AUCo-t and fluctuation should be provided. The exclusive use of modeled characteristics is not recommended unless the pharmacokinetic model has been validated for the active substance and

the products. If pharmacodynamic effects are used as characteristics the measurements should provide a sufficiently detailed time course and the initial values in each period should be comparable and the complete effect curve should remain below the maximum physiological response. Specificity, accuracy and reproducibility of the measurements should be sufficient. The non-linear character of the dose/response relationship should be taken into account and base line measurements should be subtracted before data analysis.

10.1 Chemical Analysis

The bioanalytical methods used to determine the active principle and/or its biotransformation products in plasma, serum, blood or urine or any other suitable matrix must be well characterized, fully validated and documented to yield reliable results that can be satisfactorily interpreted. The main objective of method validation is to demonstrate the reliability of a particular method for the quantitative determination of an analyte(s) concentration in a specific biological matrix. The characteristics of a bioanalytical method essential to ensure the acceptability of the performance and reliability of analytical results are:

1. Stability of the analyte(s) in the biological matrix under processing conditions and during the entire period of storage.
2. Specificity
3. Accuracy
4. Sensitivity
5. Precision and
6. Response function.

The validation of a bioanalytical method should comprise two distinct phases:

1. The prestudy phase in which the assay is developed to comply with the six characteristics listed above and
2. The study phase itself in which the validated bioanalytical method is applied to the actual analysis of samples from the biostudy mainly evaluating stability, accuracy and reproducibility.

In addition, it is necessary to validate the method of processing and handling the biological samples. All procedures should be performed according to pre-established Standard Operating Procedures (SOPs). All relevant procedures and formulae used to validate the bioanalytical method should be submitted and discussed, Any modification of the bioanalytical method before and during analysis of study specimens requires adequate revalidation; all modifications should be reported and the scope of revalidation justified.

For the validation of analytical methods reference can be made to the relevant ICH guidelines "Validation of analytical procedures: Definition and Terminology" and "Validation of Analytical Procedures: Methodology" Attention should be given to the requirements of the note for guidance on the "Investigation of Chiral Active Substances" as far as relevant for bioavailability and bioequivalence studies.

11. Reference and Test Product

Generic products, being pharmaceutical equivalents or alternatives are normally compared with the corresponding form of a well established "Innovator" medicinal product (reference product). The choice of reference product should be justified by the applicant and agreed upon by the regulatory authority.

All investigated products must have been prepared in accordance and conformed with Good Manufacturing Practice. Batch control results of the test product should be reported. The test product must originate from a batch of at least 100,000 units or 1/10 of a full production batch whichever is larger. This should be prepared by a manufacturing process which meaningfully simulates that which will be used in production; in case of production batch smaller than 100,000 units, a full production batch will be required.

If the product is subjected to further scale-up, samples of the product from the production batches should be compared with those of the test batch, and should show similar *in-vitro* dissolution profiles when employing suitably discriminatory dissolution test conditions. The study sponsor will have to retain a sufficient

number of product samples for one year in excess of the accepted shelf-life to allow retesting, if requested by the regulatory authority.

12. Data Analysis

The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant.

The primary concern in bioequivalence assessment is to limit the risk of erroneously accepting bioequivalence which should not exceed the nominal risk of 5%, and to try to minimize the risk of erroneously rejecting bioequivalence.

12.1. Statistical analysis

The statistical method for testing bioequivalence is based upon the 90% confidence interval for the ratio of the population means (Test/Reference) for the parameters under consideration. This method is equivalent to the corresponding two one-sided test procedure with the null hypothesis of bioinequivalence at the 5% significance level. The statistical analysis (e.g. analysis of variance [ANOVA]) should take into account sources of variation that can be reasonably assumed to have an effect on the response.

The validity of the assumptions underlying the statistical analysis (e.g. additivity, normality) may often be improved by transforming the raw data prior to analysis, preferably using a logarithmic transformation. This is suggested for the pharmacokinetic parameters that derived from measures of concentration e.g. AUC, Cmax, etc.

The statistical methods for Tmax should be non-parametric. For all pharmacokinetic parameters of interest in addition to the appropriate 90% confidence intervals for the comparison of the two formulations, summary statistics such as median, minimum, maximum should be given.

12.2. Handling Deviations from the Study Plan

The method of analysis should be planned in the protocol. The protocol should also specify methods for handling drop-outs and for identifying biologically implausible outliers. *Post hoc* exclusion of outliers is not generally accepted. If modeling assumptions made in the protocol (e.g. for extrapolating AUC to infinity) turn out to be invalid, a revised analysis in addition to the planned analysis (if this is feasible) should be presented and discussed.

12.3. A Remark on Individual and Population Bioequivalence

To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter. However, studies with replicate design may be helpful for substance with highly variable absorption.

12.4 *In vitro* Dissolution Complementary to a Bioequivalence Study

The results of *in vitro* dissolution tests, obtained with the batches of test and reference products that were used in the bioavailability or bioequivalence study should be reported. These results should be reported as profiles of amount dissolved versus time for individual dosage units. The specifications for the *in vitro* dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioequivalent to the reference product and would be expected to be similar to those of the reference product.

For immediate release products, if the dissolution profile of the test product is dissimilar compared to that of the reference product and the in vivo data remain acceptable, the dissolution test method should be re-evaluated and optimized. In case that no discrimatory test method can be developed this reflects in vivo bioequivalence a different dissolution specification for the test product could be set.

12.5 Reporting of Results

The report of a bioavailability or bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules. This implies that the authenticity of the whole of the report is attested by the signature of the study monitor. The responsible

investigator(s) should sign for their respective sections of the report.

Names and affiliations of the responsible investigator (s), site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s) of the test product(s) should be given. In addition the applicant may submit a signed statement, confirming the test product is the same as the one which is submitted for marketing authorization.

All results should be clearly presented and should include data from subjects who eventually drop-out. Drop-out subjects and withdrawals should be fully documented and accounted for. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The data used to estimate AUC should be reported. If pharmacokinetic models are used to evaluate the parameters the model and computing procedure used should be justified. Deletion of data should be justified. All individual subject data should be given and individual plasma concentration/time curves presented on linear/linear, and log/linear scale. The analytical report should include the results for all standard and quality control samples as well. A representative number of chromatograms or other raw data should be included covering the whole concentration range for all, standard and quality control samples as well as the specimens analyzed. The analytical validation report should be submitted as well. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated.

13. Testing Competative (Generic) Products Under Fed Conditions

The fed study is to be designed in such a way that the effects of formulation can be distinguished from other factors. If two formulations are being compared, a randomized two-period, two-sequence crossover study is commonly considered the design of choice. An adequate washout period between periods is needed to avoid drug carryover effects. Replicate studies, although not mandated, offer the advantage of providing a comparison of intra-subject variances for the test and reference products. All facets of the study are to be tightly controlled. The full characteristics, including lot numbers and expiry dates, of the test and reference products shall be known. Normally, subjects fast for 10 hours prior to ingesting a standardized meal. The meal is to provide the greatest changes from the gastrointestinal physiology of a fasting state. A meal with high-fat and high-calorie content is recommended (e.g. 150, 250 and 500-600 calories from protein, carbohydrate, and fat, respectively). The meal shall be ingested over a period of 30 minutes or less. The product dose shall be ingested 30 minutes after start of the meal. Generally, the highest safe strength/dose of the test or reference product will be administered with about 8 ounces (240 mL) of water. Further fluid shall be withheld for about 2 hours; standardized meals will be permitted beginning at four hours after drug administration. All subsequent meals will be carefully standardized. For most drugs, subjects shall not be allowed to recline until at least two hours after product ingestion. Physical activity and posture shall be standardized to limit effects on gastrointestinal blood flow and motility. Blood samples (about 12 to 18, including a pre-dose sample) are to be drawn at appropriate, specified, and carefully recorded times (to capture increasing and decreasing concentrations during the absorption, distribution and elimination phases). The collections shall continue for about three terminal drug half-lives in order to capture at least 80% of the total area. At least three to four samples shall be obtained from the terminal log-linear phase to derive an acceptable estimate of the terminal constant (λz) from linear regression. For long half-life drugs, a truncated AUC (e.g. up to 72 hours) is generally considered adequate. Blood samples or the harvested plasma/serum are to be analyzed for the administered drug or metabolites by means of a validated analytical method.

14. Conclusion

Bioavailability for oral dosage forms is defined as the percentage of an administered dose that enters the systemic circulation. The bioavailability of drugs injected into the bloodstream is 100%. Drugs with poor oral bioavailability present

challenges at every stage of development, as well as post-marketing. In the clinic, these compounds show widely variable inter- and intra-patient responses, which may add months – and potentially avoidable costs – to development programs. Once approved, a poorly-bioavailability drug can potentially negatively affect cost of goods per dose as a consequence of the API loading required to achieve effective systemic levels.

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