A Comprehensive Review on Bioequivalence Studies in Human Subjects

Muhammad Zaman¹*, Sherjeel Adnan¹, Muhammad Farooq¹, Ali Aun¹, Muhammad Uzair Yousaf¹, Ayesha Naseer¹ and Maryam Shareef¹

¹Faculty of Pharmacy, The University of Lahore, Pakistan.

Authors’ contributions

This work was carried out in collaboration between all authors. Author MZ supervised and designed the study. Authors AA and MUY wrote the first draft of manuscript. Authors AN and MS managed the literature search. Authors SA and MF has done the proof reading and final editing of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: Purpose of the current review was to provide information about the bioequivalence and the bioequivalence study steps in human subjects to conduct bioequivalence studies easily as well as to increase the use of alternative generic products and to decrease the healthcare cost.

Methodology: For this review different research and review articles were studied. The steps of bioequivalence studies on human subjects were closely understood. Bioequivalence gained increasing attention during the last 3 decades after it became clear that marketed products having the same amounts of the drug may exhibit marked differences in their therapeutic responses, which is making difficulties for physicians and pharmacists for choosing therapeutically equivalent drug for patient. Generally, these differences were caused mainly by impaired absorption. Now a considerable body of evidence has accumulated indicating that drug response is better correlated with the plasma concentration or with the amount of drug in the body than with the dose administered. Consequently, on the basis of simple pharmacokinetic concepts and parameters, bioavailability and bioequivalence studies have been established as acceptable surrogates for expensive, complicated and lengthy clinical trials and are used extensively.

*Corresponding author: E-mail: muhammad.zaman@pharm.uol.edu.pk;
worldwide to establish and ensure consistent quality and a reliable, therapeutically effective performance of marketed dosage forms.

**Conclusion:** This study provides the basic information and specifications which are required to fulfill while performing the bioequivalence studies.

**Keywords:** Bioequivalence; human subjects; active drug substance; study designs; experimental circumstances.

**1. INTRODUCTION**

Bioequivalence is a term in pharmacokinetics used to evaluate the predictable in vivo biological equivalence of two proprietary preparations of a drug. If two drug products are said to be bioequivalent it means that they would be predictable to be, for all objectives and purposes, the same [1].

Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent and their bio-availabilities after administration in the same dose are similar to such a degree that their effects, with respect to both efficacy and safety, can be expected to be for all intents and purposes the same. Pharmaceutical equivalence infers the same amount of active substances, in the same dosage form, for the same route of administration and meeting the same or comparable standards [2].

The two drugs; test drug and reference supposed to be bioequivalent if:

- The rate and extent of absorption of the test drug do not show an important difference from the rate and extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental circumstances in either a single dose or multiple doses,
- The extent of absorption of the test drug does not show a major difference from the extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses [3].

In a few cases, a drug product that differs from the reference listed drug in its frequency of absorption, but not in its extent of absorption, may be measured bioequivalent if the difference in the rate of absorption is calculated and the rate of absorption is not disadvantageous to the safety and effectiveness of the drug product. Bioequivalent drug products may contain different inactive ingredients, provided the manufacturer identifies the differences and provides information that the differences do not affect the safety or efficacy of the product [3].

Bioequivalence studies should be showed the comparison of two medicinal products holding the same active substances [4]. Two products sold by different names, containing same active ingredients, must be presented to be therapeutically equivalent to one another in order to be measured interchangeable [4]. Several test methods are existing to measure equivalence, including:

- Comparative bioequivalence studies, in which the active drug substance is measured in an accessible biological fluid such as plasma, blood.
- Comparative clinical trials.
- Comparative pharmacodynamic studies in human.

It should be remember generic drug applications are termed “abbreviated” because they are generally not required to include preclinical and clinical data to establish safety and effectiveness [5]. Many guidelines and regulations covering the licensing of generic products have been announced to ensure that the medicinal products reaching the market have well established efficacy and safety profile [6].

**2. POSSIBLE PROBLEMS**

Absence of bioequivalence may be supposed when the proof from the well-controlled clinical trials in patients of different marketed drug products do not give comparable therapeutic effects. These drug products want to be designed either in vitro (e.g., drug dissolution) or in vivo (e.g., bioequivalence study) to determine if the drug product has bioavailability problem [3].

Moreover, during the progress of a drug substance, certain biopharmaceutical properties of the active drug substance or the design of the drug product may show that the drug may have
variable bioavailability or bioequivalence problem [3,7]. Some of these problems include:

- The active drug material has fewer than 5 mg/ml solubility in aqueous medium.
- The dissolution rate is slow (i.e., less than 50% in half an hour).
- The particle size or external area of the active drug ingredient is critical in determining the bioavailability.
- Certain structural forms of the active drug ingredient (e.g., polymorphic forms) dissolve poorly, thus disturbing absorption.
- The active drug ingredient or beneficial moiety is absorbed in large portion in a particular part of the GI tract or is absorbed from a specific place only.
- Preparation may have a high proportion of excipients to active ingredients (i.e., greater than 5:1).
- The amount of permeability of the active drug constituent is less than 50% when compared to an intravenous dose.
- The active drug substance is quickly metabolized or excreted.
- The active drug constituent is unstable in different surroundings of gastrointestinal tract.

3. DESIGN AND EVALUATION OF BIOEQUIVALENCE STUDIES

Bioequivalence studies are done to relate the bioavailability of the generic drug product to the brand-name product. Numerical methods should be needed to detect change in rate and extent of absorption that are not attributable to substance variability. Once the bioequivalence is well-known, it is likely that both the generic and brand-name dosage forms will produce the same therapeutic effect [8].

The study design and evaluation of well-controlled bioequivalence studies need cooperative response from pharmacokinetics, statisticians, clinicians, bio analytical chemists, and others. The basic scheme for a bioequivalence study is determined by:

- The scientific questions to be answered
- The nature of the reference material and the dosage form to be tested
- The accessibility of analytical methods
- Benefit-risk and proper attention should be paid with regard to testing in humans

For bioequivalence studies, the test and reference drug products must contain the pharmaceutical equivalent drug in the same dose strength, in similar dosage forms (e.g., immediate release or controlled release), and be given by the same route of administration. Both a single-dose and a multiple-dose study may be required. Before start the study, the ‘Institutional Review Board (IRB)’ of the clinical facility in which the study is to be performed must approve the study. The IRB is responsible for protection the rights and safety of human subjects [3].

When several studies have been performed the complete form of evidence must be well-thought-out. It is not satisfactory to ignore failed studies simply because another one has passed. The details for the failure should be discussed. A combined analysis of all studies can be provided if relevant, however, it is not acceptable to combine failed studies to show Bioequivalence [7].

The study should be designed in such a manner that the formulation effect can easily be distinguished from other effects of drug product. If two formulations are to be studied in comparison, a two period, two sequences cross over design may be the design of choice with the two phases of treatment separated by an adequate washout period which should ideally be equal to or more than five half-life’s of the moieties to be measured [6].

4. CRITERIA FOR HUMAN SUBJECTS

The basic management value in performing studies is ‘do not do unwanted human tests’. Generally, the study is done in normal, healthy male and female volunteers who have given educated authorization to be in the study. Critically ill patients are not involved in an in-vivo bioavailability study unless the concerning physician defines that there is a potential benefit to the patient [9]. The number of subjects in the study will depend on the standard inter subject and intra subject variability. The subjects are generally fasted for 10 to 12 hours (overnight) prior to drug administration and may continue so fast for a 2 to 4 hours period after dosing [3,10].

5. ANALYTICAL METHODS

The analytical method used in an in-vivo bioequivalence study to calculate the concentration of the active drug ingredient or therapeutic moiety, or active metabolite(s) of
active drug ingredient, in body fluids or excretory products, or the method used to measure an severe pharmacological effect, must be established perfectly and have sufficient sensitivity to measure, with proper precision, the actual concentration of the active drug ingredient or therapeutic moiety, or active metabolite(s) of active drug ingredient, achieved in the body. For bioavailability studies, both the parent drug and its active metabolites are generally measured [11].

For bioequivalence studies, the parent drug is measured. The active metabolite might be measured for some very high hepatic clearance (first-pass metabolism) drugs when the parent drug concentrations are too low to be reliable. Any amendment of an analytical method would require revalidation of the procedures. Analytical method validations should be performed to support pharmacokinetics, bioequivalence, related studies in a new drug application or an abbreviated new drug application [3,11].

6. REFERENCE STANDARD

For bioequivalence studies, one formulation of the drug is selected as a reference standard against which all other formulations of the drug are equated. The reference drug product should be administered by the same route of administration as the comparison formulations unless an alternative route or additional route is needed to answer specific pharmacokinetic questions. For example, if an active drug is poorly bioavailable after oral administration, the drug may be compared to an oral solution or an intravenous injection [3,12]. For bioequivalence studies on a proposed generic drug product the reference standard is the reference listed drug (RLD), which is listed in Approved Drug Products with Therapeutic Equivalence Evaluations” the Orange Book, and the proposed generic drug product is often referred to as the “Test” drug product. The RLD is generally a formulation currently marketed with a fully approved NDA for which there are suitable scientific safety and efficacy data. The RLD is usually the innovator’s or original manufacturer’s brand-name product and is administered according to the dosage references in the labeling. Before beginning an in-vivo bioequivalence study, the total content of the active drug substance in the test product (generally the generic product) must be within 5% of that of the reference product. Furthermore, in vitro comparative dissolution or drug-release studies under different specified conditions are usually performed for both test and reference products before performing the in-vivo bioequivalence study [3].

7. EXTENDED-RELEASE FORMULATIONS

The tenacity of an in-vivo bioavailability study involving an extended-release drug product is to find out that if (1) the drug product meets the controlled-release claims made for it by the manufacturing company, (2) the bioavailability profile established for the drug product rules out the occurrence of any dose dumping (release of all the drug from the formulation at once), (3) the drug product's plasma steady-state performance is comparable to that of a currently marketed non-extended-release formulation, and (4) the drug product's formulation offers consistent pharmacokinetic performance between individual dosage units. A comparative bioavailability study is used for the development of a new extended release drug product in which the reference drug product may be either a solution or suspension of the active ingredient or a currently promoted non-controlled release drug product such as a tablet or capsule. For example, the bioavailability of a non-controlled-release (immediate-release) drug product given at a dose of 25 mg every 8 hours is compared to an extended-release product containing 75 mg of the same drug given once daily. For a bioequivalence study of a new generic extended release drug product, the reference drug product is the currently marketed extended release drug product listed as the RLD in the Orange Book and is administered according to the dosage recommendations in the approved labeling [3].

Immediate release solid dosage form are routinely subjected to tests such as content uniformity, weight, hardness, friability, and disintegration, the test that is most often associated with the assessment of in vivo performance is the dissolution test [11].

8. COMBINATION DRUG PRODUCTS

Generally, the tenacity of an in-vivo bioavailability study relating a combination drug product containing more than one active drug substance is to determine if the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product is equivalent to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in
separate single ingredient preparations. The reference material in such a bioavailability study should be two or more currently marketed, single-ingredient drug products, each of which contains one of the active drug ingredients in the combination of drug product. The FDA may, for valid scientific reasons, specify that the reference material be a combination drug product that is the subject of an approved NDA [3].

9. STUDY DESIGN

Intended at many drug products, the FDA, Division of Bioequivalence, Office of Generic Drugs, gives guidelines for the performing in-vitro dissolution and in-vivo bioequivalence studies. Related guidelines appear in the United States Pharmacopeia NF.

Subjects included in the study should be healthy male or female volunteers and who signed consent. Sampling times should be selected on the basis of the onset of the drug to be tested for bioequivalence. Criteria for bioequivalence depend on the dose frequency and the presence of food in the body of the subject. The release rate and extent of absorption should be same for the drugs under consideration.

Now days, three different studies may be essential for solid oral dosage forms, including

- A fasting study,
- A food intervention study, and/or
- A multiple-dose (steady-state) study
- Crossover study design

9.1 Fasting Study

Bioequivalence studies are mostly determined by a single-dose, two-period, two-treatment, two-sequence, and open-label, randomized crossover design comparing equal doses of the test and reference products in fasted, adult, healthy subjects. Such study is essential for all immediate-release and modified-release oral dosage forms [6]. Both male and female might be used in the study. Bioequivalence studies are usually resolute by a single-dose, two-period, two-treatment, two-sequence, and open-label, randomized crossover design comparing equal doses of the test and reference products in fasted, adult, healthy subjects. This study is necessary to all immediate-release and modified-release oral dosage forms [6].

9.2 Food Intervention Study (Fed Study)

Administration of food with an oral drug product may intrude the bioavailability of the drug. Food intervention or food effect studies are commonly carried out using meal conditions that greatly affects GI physiology such that systemic drug availability is maximally affected. That’s why alcohol and any over the counter drug was discontinued for at least three days before the start of the experiment and throughout the experiment period [10]. The test meal is a highly-fat and high-calorie meal. A typical test meal may consist of two eggs fried in butter, two strips of bacon, and two pieces of toast with butter, four ounces of brown potatoes, and eight ounces of milk. These are approximately 150, 250, and 500 - 600 calories from protein, carbohydrate, and fat [3].

9.3 Multiple-dose Study

In selected cases, a multiple-dose, steady-state, randomized, two-treatment, two-way crossover studied comparing equal doses of the test and reference products may be carried out in healthy non-smoker adults [10]. For such studies, three successive trough concentrations (C_{min}) on three continuous days should be resolute to ascertain that the subjects are at steady state. The last morning dose is given to the subject after a one night fast, with persistent fasting for at least 2 hours following dose administration. Blood sampling is performed likewise to the single-dose study [3].

9.4 Crossover Study Design

Many researchers use crossover study design for their clinical trials. There are certain considerations that are related to the crossover design, but play no role in regular parallel-group trials, must receive sufficient attention in trial planning and data analysis for the results to be of scientific value [13]. The design resembles a retrospective non-randomized crossover study but differs in having only a sample of the base population-time [14].

In the simplest and general situation, a crossover trial involves two treatments (test drug and reference drug) which are successively administered in each subject selected in the study. Subjects who meet the selection criteria are given informed consent [3].
Table 1. Latin-square crossover design for a bioequivalence study of three drug products in six subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study period 1</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
</tr>
</tbody>
</table>

Where: A is reference and B and C are the test products.

In the crossover design, each subject is his own control, and subject-to-subject deviation is reduced. Furthermore, deviation due to sequence, period, and treatment (formulation) are reduced, so that all the subjects do not receive the same drug product twice on the same day and in the same order. The intra-subject variation is usually lesser as compared to inter-subjects changeability [15].

9.5 Parallel Study Design

The crossover design may not be applied for drugs with long half-lives (longer than 24 hours). In such cases, researchers can use parallel design in which each treatment is administered to a separate group of subjects [16].

9.6 Replicated Crossover Study Design

A replicate crossover study may be a suitable substitute to the parallel, and can be conducted as either a three-period or four-period replication of treatment. In this design, one or both treatments could be administered to the same subject on two separate occasions [16]. Replicated crossover designs can also be used for the determination of bioequivalence individually, to estimate within subject diversity for both the Test and Reference drug products, and to provide an estimation of the subject-by-drug product interaction variance. Generally, a four-period, two-sequence, two-product design is suggested by the FDA [3,16].

Table 3. Replicated crossover study design

<table>
<thead>
<tr>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1</td>
<td>T</td>
<td>R</td>
<td>T</td>
</tr>
<tr>
<td>Sequence 2</td>
<td>R</td>
<td>T</td>
<td>R</td>
</tr>
</tbody>
</table>

Where: R = Reference; T = Treatment

10. EVALUATION OF DATA

10.1 Analytical Method

The analytical method for the evaluation of bioequivalence studies must be validated for accuracy, precision, sensitivity, and specificity. The exercise of more than one analytical method during a bioequivalence study is not recommended, because different methods may give in different values. Data should be presented in both tabulated and detailed graphical form for evaluation. The plasma drug concentration time curve for each drug product and each subject should be presented [3].

10.2 Pharmacokinetic Evaluation of the Data

Mostly bioequivalence studies are performed by using cross-over study design, in which all the treatments are applied simultaneously to the subjects [12]. For single dose studies, including a
fasting study or a fed state study, the pharmacokinetic evaluation of data, include calculation for the AUC_0-t, that is area under the plasma concentration - time curve, from 0 hr to the last quantifiable concentration and AUC_0-∞, which is the area under the plasma concentration - time curve, from zero to infinity to be calculated as the sum of AUC_0-t in addition the ratio of the last measurable concentration to the elimination rate constant, T_max (time required to achieve maximum drug concentration in blood), and C_max (maximum drug concentration achieved in blood after the drug administration) [15,17]. Moreover, the elimination rate constant, k, the elimination half-life, t_1/2, and other parameters also are estimated [3]. For multiple-dose studies, pharmacokinetic analysis includes calculation for each subject of the AUC for steady state concentration, (AUC_0-t), T_max, C_min, C_max, and the percent fluctuation [100 x (C_max – C_min)/C_min]. Proper statistical evaluation should be performed on the projected pharmacokinetic parameters [3].

10.3 Statistical Evaluation of the Data

This retrospective analysis compares the two drug products (reference and test) for bioequivalence measures from single-dose and multiple doses clinically. Bioequivalence measures evaluated were drug peak plasma concentration (C_max) and area under the plasma drug concentration versus time curve (AUC), which represents the drug rate and extent of absorption, respectively. To establish bioequivalence, the calculated data should fall inside the prescribed limit, usually, 80 – 125% for the ratio of the product averages. Normal crossover design studies are usually used to obtain the data [3]. Another approach proposed by the FDA is termed individual bioequivalence [8]. Individual bioequivalence needs a replicate crossover design, and estimates within-subject variability for the Test and Reference drug products, as well as subject-by product interaction. Currently, only average estimates are used to set up bioequivalence of the selected drug products. To establish bioequivalence, there must be no statistical difference between the bioavailability of the reference and test drug product [3].

10.4 Analysis of Variance (ANOVA)

An analysis of Variance (ANOVA) is one of the statistical procedures, used to present data as well as test the data for the differences between the subject groups [3]. The various pharmacokinetic parameters (e.g. AUC, C_max) resulting from the plasma concentration-time curve are subjected to ANOVA in which the variance is partitioned into mechanism due to subjects, periods and treatments [18]. A bioequivalent product should not produce any significant difference in all pharmacokinetic parameters tested [3].

11. CONCLUSION

At present, many pharmaceutical manufacturing companies evolving alternative generic drug products for many drug products. Bioequivalence study is vital for generic drug approval process. It is hoped that, this review will provide an easy and quick overview for Regulatory consideration required for bioequivalence studies. This review covers major aspect of requirement of bioequivalence study along with the regulatory specifications.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

5. Tamboli AM, Todkar P, Zope P, Sayyad F. An overview on bioequivalence: Regulatory consideration for generic drug


17. Qayyum A. Bioequivalence studies: INTECH Open Access Publisher; 2012.


© 2016 Zaman et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14036