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REVIEW ARTICLE

Bioavailability: A Pharmaceutical Importance in New Drug Development

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ABSTRACT

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. Therefore, it is not surprising to see that the number of publications on bioavailability of drug has been highly increasing steadily for some time. In this review, attention is focused to briefly discuss some terms of bioavailability, absolute bioavailability, and relative bioavailability, measurement of bioavailability and in-vitro dissolution and bioavailability, in-vitro in-vivo correlation, biopharmaceutical classification system, bioequivalence.

Keywords: Bioavailability, in-vitro and in-vivo correlation, bioequivalence, Biopharmaceutical classification system.

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INTRODUCTION

Bioavailability

It is defined as the rate and extent absorption unchanged drug from a drug product and becomes available at the site of action. Absorption is the process of movement of unchanged drug from the site of administration to systemic circulation or site of measurement i.e. plasma. The amount of intestinal absorption is dependent on lipophilicity, drug stability, aqueous solubility and intestinal permeability. The drug bioavailability is affects its pharmacological effect due to any alteration in drugs. The movement of drug between one compartment and the other (extra vascular tissues and intravascular i.e. systemic circulation) is referred to as drug distribution. The elimination is the process to remove the drug from the body and terminate its action [1]

Objective

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. [2]

Considerations in Bioavailability Study Design

Absolute bioavailability:

Absolute bioavailability compares the bioavailability of the active drug in systemic circulation administration of drug non-intravenously i.e., after oral, rectal, transdermal, subcutaneous, or sublingual administration, and comprising the bioavailability of the same drug administered intravenously. The absolute bioavailability is denoted by symbol (**F**).

The determination of absolute bioavailability of a drug, a pharmacokinetic study must be done to obtain a plasma drug concentration vs time plot for the drug after both intravenous (IV) and non-intravenous administration(oral, rectal, transdermal, subcutaneous, or sublingual). The formula for calculating *F* for a oral route administered drug is given below.

$$F = \frac{[AUC]_{\text{oral}} D_{\text{iv}}}{[AUC]_{\text{iv}} D_{\text{oral}}}$$

Therefore, a drug given by the intravenous route (direct administered i.v. shows 100% bioavailability) will have an absolute bioavailability of 1 ($F=1$) while drugs given by other routes usually have an absolute bioavailability of less than 1 ($F < 1$). If we compare the two different dosage forms having same active ingredients and compare the two drug bioavailability is called comparative bioavailability. [3-4]

Relative Bioavailability

When the systemic bioavailability of a drug after administered orally is compared with that of an oral standard of the same drug (aqueous or non aqueous solutions). It is denoted by symbol (**Fr**).

$$F_r = \frac{[AUC]_{\text{test}} D_{\text{std}}}{[AUC]_{\text{std}} D_{\text{test}}}$$

It is used to characterize absorption of a drug from its formulation. F and F_r are generally expressed in percentage (%)

Measurement of Bioavailability

The quantitative evaluation of bioavailability divided in to two categories

1) **Pharmacokinetics Method:** (Indirect Method)

These are widely used and base on assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of drug. It has two methods:

a) **Plasma level-time studies:**

It is most reliable method and method of choice in comparison to urinary data. The method is base on the assumption that two dosage forms that exhibit superimposable plasma level-time profile in a group of subjects should result in identical therapeutic activity.

If single dose study, the method requires collection of serial blood sample for 2 to 3 biological half lives after drug administration, their analysis for drug conc. and making a plot of plasma level-time profile. With i.v. dose, sampling should start within 5 minute of drug administration and subsequent sample taken at 15 min intervals. To adequately describe the disposition phase, at least 3 sample point should be taken if the drug follows one compartment kinetics and 5 to 6 points if it fits two compartment models. In case oral dose, at least 3 point should be taken on the ascending part of curve for accurate determination of K_a .

The 3 parameters of plasma level-time studies which are considered important for determining bioavailability are

(i). C_{max} : The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.

(ii). t_{max} : The peak time that gives an indication of the rate of absorption and

(iii). **AUC:** The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by following equation:

$$F = \frac{[AUC]_{\text{oral}} D_{\text{iv}}}{[AUC]_{\text{iv}} D_{\text{oral}}}$$

$$F_r = \frac{[AUC]_{\text{test}} D_{\text{std}}}{[AUC]_{\text{std}} D_{\text{test}}}$$

If multiple dose study, in this method drug administration for at least 5 biological half lives with dosing interval equal to or greater than the biological half lives to reaches the steady state.

b) Urinary excretion study

It is base on the principle that the urinary excretion of unchanged drug is directly proportional to plasma concentration of drug.

Method:

Collection of urine at regular intervals for a time span equal to 7 biological half life. Analysis of unchanged drug in collected sample and determine the amount of drug excreted in each interval and cumulative amount excreted. At each sample collection, total emptying of the bladder is necessary to avoid errors resulting from addition of residual amount to the next urine sample.

The 3 major parameters examined in urinary excretion data obtained with single dose study are:

- i) $(dx_u / dt)_{\text{max}}$ The maximum urinary excretion rate
- ii) $(t_u)_{\text{max}}$ the time for maximum excretion rate
- iii) x_u cumulative amount of drug excreted in the urine.

2) Pharmacodynamics Method: (Direct method)

The two pharmacodynamics methods involve determination of bioavailability

a) Acute pharmacological response

When bioavailability measurement by pharmacokinetics method is difficult, inaccurate or non-predictable, an acute pharmacologic effect such as change in EEG or ECG reading, pupil diameter etc. is related to the time course of given drug Bioavailability can then be determine by constriction of pharmacologic effect time curve as well as dose responses graph. The



methods require measurement of response for at least 3 biological half live of the drug in order to obtain a good estimation of AUC.

Disadvantage of this method

- 1) The pharmacological response tends to be more variable and accurate correlation between measured response and drug available from the formulation is difficult.
- 2) The observed response may due to the active metabolite whose concentration is not proportional to the concentration of parent of parent drug responsible for the pharmacological response.

b) Therapeutic Response

Theoretically the most definite this method is based on observing the clinical response to the drug formulation given to patient suffering diseases for which it is intend to be used.

Disadvantage of this method

- 1) In that **Quantization** of observed response is too improper to allow for reasonable assessment of relative bioavailability between two dosage forms of the same drug.
- 2) Many patients receive more than one drug, and the result obtained from a bioavailability study could be compromised because of a Drug-Drug interaction [5]

In Vitro Dissolution and Bioavailability

The purpose of in vitro dissolution studies in drug development process is to assess the lot to-lot quality of a drug product, guide development of new formulations; and ensure continuing product quality and performance after certain changes, such as changes in the formulations, manufacturing process, site of manufacture, and manufacturing scale-up process. However, for the IVIVC perspective, dissolution is proposed to be a surrogate of drug bioavailability. Thus, a more rigorous dissolution standard may be necessary for the in-vivo waiver. Generally, a dissolution methodology, which is able to discriminate between the study formulations and which best, reflects the in vivo behavior would be selected. The in vitro dissolution release of a formulation can be modified to facilitate the correlation development. Changing dissolution testing conditions such as the stirring speed, choice of apparatus, pH of the medium, and temperature may alter the dissolution profile.

Four types of dissolution apparatus

- a) rotating basket,

- b) paddle method,
- c) reciprocating cylinder,
- d) flow through cell,

These above four types are specified by the USP and also recommended in the FDA guidance especially, for modified release dosage form. However, the first two are preferred (basket and paddle). And other dissolution methodologies may be used. It is also recommended to start with the basket or paddle method prior to using the others.

A common dissolution medium

- 1) aqueous (water),
- 2) simulated gastric fluid (pH 1.2) without enzyme,
- 3) intestinal fluid (pH 6.8 or 7.4) without enzyme,
- 4) Buffers with a pH range of 4.5 to 7.5

Most commonly for sparingly aqueous (water) soluble drugs, surfactants (e.g., 1% sodium lauryl Sulfate) use in the dissolution medium is recommended. In BCS Class I drug, a simple aqueous (water) dissolution media is recommended. This type of drug shows lack of influence of dissolution medium properties. For most of the Class I drugs, aqueous (water) and simulated gastric fluid (pH 1.2) are the default mediums. A typical medium volume is 500 to 1000 ml. [6-9]

The test duration for immediate release is 15 to 60 minutes. For example, (a) a single time may point required for the BCS class I recommend at 15 minutes. (b) The two time points may be required for the BCS class II recommended at 15 minutes and the other time at which 85% of the drug is dissolved. In contrast, in vitro dissolution tests for a modified release dosage form require at least three time points to characterize the drug release. Deaerated water, a buffered solution (pH 04 to 08) or a dilute acid (0.001 to 0.1 N) may be preferably used as dissolution medium for modified-release dosage forms. A suitable distribution of sampling points should be selected to define adequately the profiles. The first sampling time (1-2 hours or 20- 30% drug release) is select to check dose-dumping potential. The intermediate time point has to be around 50% drug release in order to define the in vitro release profile. The last time point is to define essentially complete drug release. The dissolution limit should be at least 80% drug release. Further justification as well as 24-hours test duration are required if the percent drug release is less than 80. A dissolution profile of percentage or fraction of drug dissolved versus time then can be determined. The similarity of the dissolution profiles in particular dissolution testing conditions is evaluated using the similarity factor (f_2 metric) defined by equation 1:

$$f_2 = 50 \log \left[1 + \frac{1}{n} \sum_{t=1}^n W_t (R_t - T_t)_t^2 \right] \times 100 \dots \dots \dots [1]$$

Where,

- 1) R_t and T_t are the cumulative percentage dissolved at time point t for reference and test products, respectively, and
- 2) n is the number of pool points.

The f_2 equation is a logarithmic transformation of the sum of squares of the difference between test and reference profiles. This equation is only applicable in comparing profiles in which the average difference between R and T is less than 100. If this average difference is greater than 100, the equation will yield a negative number. The results values are between the 0 and 100. [10-13]

In-Vitro—Vivo Correlation (IVIVC)

The in vitro dissolution rate data must correlate with the in-vivo bioavailability data for the drug. A simple in-vitro dissolution test on drug product will be insufficient to predict its therapeutic efficacy. Mathematically, the term correlation means interdependence between quantitative or qualitative data or relationship between measurable variables and ranks. Convincing correlation in-vitro dissolution of drug and its in-vivo bioavailability must be experimentally demonstrated to guarantee reproducibility of biological response. Two definitions of IVIVC have been proposed by the USP and by the FDA.

United State Pharmacopoeia (USP) definition

The establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form.

Food and Drug Administration (FDA) definition

IVIVC is a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response. Generally, the in vitro property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed.

Correlation Levels

Five correlation levels have been defined in the IVIVC FDA guidance. The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form.

i) Level A Correlation

The purpose of Level A correlation is to define a direct relationship between in vivo data such that measurement of in vitro dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form. This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form. Generally, percent of drug absorbed may be calculated by means of model dependent techniques such as Wagner-Nelson procedure or Loo-Riegelman method or by model-independent numerical deconvolution. These techniques show a major advance over the single-point approach in that these methodologies utilize all of the dissolution and plasma level data available to develop the correlations. It is an excellent quality control procedure since it is predictive of the dosage forms in vivo performance. The level A correlation, an in vitro dissolution curve can serve as a surrogate for in vivo performance.

ii) Level B Correlation

The level B correlation uses all of the in vitro and in vivo data; it is not considered to be a point-to-point correlation, since there are a number of different in vivo curves that will produce similar mean residence time values. A level B IVIVC utilizes the principles of statistical moment analysis. In this level of correlation, the mean in vitro dissolution time (MDT vitro) of the product is compared to either mean in vivo residence time (MRT) or the mean in vivo dissolution time (MDT vivo).

A level B correlation does not uniquely reflect the actual in vivo plasma level curves.

iii) Level C Correlation

This is the weakest level of correlation as partial relationship between absorption and dissolution is established. In this level of correlation, one dissolution time point ($t_{50\%}$, $t_{90\%}$) is compared to one mean pharmacokinetic parameter such as AUC, t_{max} or C_{max} . Therefore, it represents a single point correlation and does not reflect the entire shape of the plasma drug concentration curve, which is indeed a crucial factor that is a good indicative of the performance of modified-release products. The Level C correlation is limited usefulness in predicting in vivo drug performance due to its obvious limitations. Level C correlations can be useful tool in the early stages of formulation development when pilot plant formulations are being selected. While the information may be useful in formulation development, waiver of an in vivo bioequivalence study (biowaiver) is generally not possible.

iv) Multiple-level C correlation

A multiple point level C correlation may be used to justify a biowaiver, provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A multiple level C correlation relates one or several pharmacokinetic parameters of interest (C_{max} , AUC, or other suitable parameters) to the amount of drug dissolved at several time points of the dissolution profile. A multiple Level C

correlation should be based on at least three dissolution time points covering the early, middle, and late stages of the dissolution profile.

v) Level D correlation

Level D correlation is not considered useful for regulatory purposes it is a rank order and qualitative analysis. It is useful in the development of a formulation or processing procedure [14-18].

Biopharmaceutical Classification System (BCS) and In Vivo-In Vitro Correlation (IVIVC)

The Biopharmaceutics Classification System (BCS) is a drug development tool that allows estimation and contribution of three fundamental factors including dissolution, solubility and intestinal permeability, which govern the rate and extent of drug absorption from solid oral dosage forms.

The classification is associated with drug dissolution and absorption model, which identifies the key parameters controlling drug absorption as a set of dimensionless numbers:

- a) **Absorption number:** It is the ratio of the mean residence time to the absorption time.
- b) **Dissolution number:** It is a ratio of mean residence time to mean dissolution time.
- c) **Dose number:** It is the mass divided by an uptake volume of 250 ml and the drug's solubility.
- d) **Mean residence time** here is the average of the residence time in the stomach, small intestine and the colon.
- e) **Fraction of dose absorbed** then can be predicted based on these three parameters.

For example: Absorption number 10 means that the permeation across the intestinal membrane is 10 times faster than the transit through the small intestine indicating 100% drug absorbed. In the BCS, a drug is classified in one of four classes based solely on its solubility and intestinal permeability:

Class I HIGH solubility / High permeability,

Class II LOW solubility / High permeability,

Class III HIGH solubility / LOW permeability

Class IV LOW solubility / LOW permeability

Class I In this class of the drugs like Labetolol, Atropine, Bucspirone, Salicylic acid, Theophylline, metoprolol shows a high Dissolution number and a high Absorption number. Drug dissolution or gastric emptying rate (if dissolution is very rapid) is the rate-limiting step to drug absorption.

Class II In this class of the drugs like phenytoins exhibits a low Dissolution number and a high Absorption number. In-vivo drug dissolution is then a rate-limiting step for absorption except in very high dose. The absorption for Class I drugs are faster than the Class II drug and it occur over a longer period of time. IVIVC is usually expected for Class I and Class II drugs.

Class III In this class of the drugs like cimetidine have a high Dissolution number but a low Absorption number, permeability is the rate-controlling drug absorption. Since the dissolution is rapid, the variation is due to alteration of GI physiological properties and membrane permeation rather than dosage form factors.

Class IV In this class of the drugs like cimetidine and chlorothiazide are exhibits low solubility and low permeability drugs. Drugs that fall in this class exhibit a lot of problems for effective oral administration.

Table no: 1 contain Biopharmaceutics Drug Classification and Expected IVIVC for Immediate Release Drug Products.

Class	Solubility	Permeability	IVIVC
I	High	High	Correlation (if dissolution is rate limiting step)
II	Low	High	IVIVC expected
III	High	Low	Little or no IVIVC
IV	Low	Low	Little or no IVIVC

Table no: 2 contain Biopharmaceutics Drug Classification for Extended Release Drug Products

Class	Solubility	Permeability	IVIVC
IA	High & Site Independent	High & Site Independent	IVIVC Level A expected
IB	IB High & Site Independent	Dependent on site & Narrow Absorption Window	IVIVC Level C Expected
Ila	Low & Site Independent	High & Site Independent	IVIVC Level A Expected
Ilb	Low & Site Independent	Dependent on site & Narrow Absorption Window	Little or no IVIVC
Va Acidic	Variable	Variable	Little or no IVIVC
Vb basic	Variable	Variable	IVIVC Level A expected

Bioequivalence

Bioequivalence is defined as 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.

The three major pharmacokinetic parameters to assess bioequivalence are:

- a. AUC is the principal criterion to characterize the extent of absorption and to assess bioequivalence.
- b. C_{max} is the rate and extent of absorption (wider acceptance criteria)
- c. T_{max} is the rate of absorption (considered only when clinically relevant)

Pharmaceutical alternatives: if drug products contain the same active moieties but differ in chemical form of that moiety or in the dosage form or strength (salt, ester, complex, and dosage form)

Chemical Equivalence: it indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount

Pharmaceutical equivalence: This indicates that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients.

Therapeutic equivalence: This term indicates that two or more drug products are pharmaceutical equivalents whose bioavailability or dissolution profiles, after the same molar doses, are similar to such an extent that their safety and efficacy can be assumed to be substantially equal [19-20]

Types of Bioequivalence Studies

a) In vivo study:

This method includes:

- A) when one or more following criteria apply to oral immediate release drug formulation with systemic action
 - 1 Narrow therapeutic window/safety margin; steep dose response curve
 - 2 pharmacokinetics complicated by variable or absorption window, non linear pharmacokinetic, high first pass metabolism > 70%
 - 3 The most documented evidence for bioavailability problems related to the drug or drug of similar chemical structure or formulation.
 - 4 Unfavorable physic-chemical properties eg. Low solubility, instability, Meta stable modification, poor permeability.
 - 5 Where high ratio of excipient to active ingredient exist.
- B) Non oral and Non Parenteral drug formulation design to act by systemic absorption (such as transdermal patches, suppositories)
- C) Sustained or otherwise modified release drug formulation design to act by systemic absorption
- D) Fixed- dose combination product with systemic action
- E) Non- solution pharmaceutical products which are for non systemic use eg. Oral, nasal, ocular, dermal, rectal, vaginal, etc



b) In -Vitro Study

In this method following circumstances equivalence may be assessed by the use of in – vitro dissolution testing

- A) Drug for which the applicant provides data to substantiate all of the following.
 - 1 In 250 ml of aqueous media highest dose strength is soluble of an over the pH 1-7.5 at 37⁰C
 - 2 At least 90% of the oral dose administered is on a mass balance determination to an i.v. reference dose
 - 3 Speed of dissolution as demonstrated by more than 80 dissolution within 15 min at 37⁰C using USP apparatus.1, at 50 rpm or apparartus2, at 100 rpm in a volume of 500 or 900 ml with following media
 - a) 0.1 N HCL or artificial gastric juice (without enzyme)
 - b) pH 4.5 buffer
 - c) pH 6.8 buffer or artificial intestinal juice (without enzyme).
- B) Different strength of drug manufactured by the same manufacturer, where all of the criteria are fulfilled.
 - 1) The ratio of active ingredients and excipient between the strength is same.
 - 2) The qualitative composition between the strength is essentially the same.
 - 3) The method of manufacture is essentially the same.
 - 4) An appropriate equivalence study has been performed on at least one of the strength of the formulation (low strength is chosen for reasons of safety [21].

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