Wld Hlth Org. techn. Rep. Ser., 1974, No. 536

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES No. 536

Bioavailability of Drugs: Principles and Problems

Report of a WHO Scientific Group

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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WHO SCIENTIFIC GROUP ON THE BIOAVAILABILITY OF DRUGS: PRINCIPLES AND PROBLEMS

Geneva, 25–30 June 1973

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BIOAVAILABILITY OF DRUGS: PRINCIPLES AND PROBLEMS

Report of a WHO Scientific Group

A WHO Scientific Group on the Bioavailability of Drugs met in Geneva from 25 to 29 June 1973. The meeting was opened on behalf of the Director-General by Dr. V. Fattorusso, Director, Division of Prophylactic and Therapeutic Substances, who outlined the terms of reference for the present meeting in relation to the Organization's programme for the promotion of drug quality, efficacy, and safety.

1. INTRODUCTION

A resolution of the Seventeenth World Health Assembly requested the Director-General to "undertake, with the assistance of the Advisory Committee for Medical Research, the formulation of generally acceptable principles and requirements for the evaluation of the safety and efficacy of drugs". In compliance with this request, WHO has already convened five Scientific Groups to establish principles for the pre-clinical testing of drug safety,[1] the clinical evaluation of drugs,[2] and the testing and evaluation of drugs for teratogenicity,[3] carcinogenicity,[4] and mutagenicity.[5] The present Group was convened to discuss the bioavailability of drugs. Its aim was to review methodological progress in this field and to reach agreement on principles to guide the evaluation and testing of drug products for the biological availability of their medicinal ingredients.

The Group considered the biopharmaceutical and pharmacokinetic factors influencing the plasma concentration of drugs and their excretion in the urine and established priorities for determining the order in which

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 341.
[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 364.
[3] *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 403.
[4] *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 426.
[5] *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

various types of drug products should be tested for bioavailability. Examples of products requiring the earliest attention were suggested. Due attention was given to various types of experimental design in the review of methods of testing. Although it was felt that every drug required an individual approach and that it was "inadvisable to establish and prescribe rigidly formulated regulations specifying in detail the tests to be performed",[1] the Group outlined basic principles that should be applied to the testing of drugs for bioavailability. The correlation of bioavailability data in man with data from other sources was discussed. In this field even more than in others, the coordinated efforts of multidisciplinary teams of professionals were considered to be essential.

2. GENERAL CONSIDERATIONS

Patients vary greatly in their response to a drug. This variability depends upon factors such as dose, severity of disease, rate of drug metabolism and excretion, and other pharmacokinetic factors. However, an important and often unrecognized source of variability is the bioavailability of the drug from the dosage form [2] being used, i.e., its time course and extent of absorption.

The time course and the extent of entry into the general circulation of drugs administered by a route other than the intravenous one may be affected appreciably by the release characteristics of the dosage form used. Unrecognized variability in drug absorption can have serious clinical implications. Drugs that are not absorbed may leave a patient without medication when it is urgently required. Differences in the absorption of the medicinal ingredients of drug products [3] from different sources or production batches, or in different dosage forms, can also cause patients to be undermedicated or overmedicated. The result may be therapeutic failure or serious adverse effects, particularly in the case of drugs with a good correlation between intensity of effect and plasma [4] concentration and a small therapeutic index.

The entry of a drug into the general circulation after the administration of a drug product usually involves two consecutive processes: (*a*) release of the drug from the dosage form into solution, and (*b*) transfer of the

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[1] *Symposium on the toxicology of drugs*, Copenhagen, WHO Regional Office for Europe, 1964 (unpublished document). A limited number of copies of this document are available to officially or professionally interested persons on request to the WHO Regional Office for Europe, Copenhagen, Denmark.

[2] *Dosage form:* the form in which a drug is administered to a patient, e.g., tablet, oral solution, or injection.

[3] *Drug product:* the commercial dosage form offered for sale by a manufacturer.

[4] The term "plasma" is used throughout to refer to blood, serum, or plasma.

dissolved drug across biological membranes and through organs, such as the liver, into the general circulation. The drug release process is frequently the limiting factor in the rate of absorption when drugs are administered in solid form. Drug release from a tablet dosage form involves both disintegration and dissolution. The rate of dissolution will be affected by certain characteristics of the drug formulation.[1] These include particle size and shape, crystal form, and additives such as colouring agents, lubricants, disintegrants, and suspending agents, as well as manufacturing variables such as the compression pressure and the moisture content of tablets. If the release rate of a drug from a dosage form is unduly decreased, absorption may be incomplete. This is particularly true of drugs that are administered orally or rectally because of their limited sojourn in the gastrointestinal tract. An incompletely absorbed drug is usually variably absorbed because changes in factors such as gastrointestinal motility and the transit rate can cause pronounced differences in the extent of absorption.

Bioavailability is determined relative to a reference formulation, which may be an intravenous injection (a dosage form that is known to be completely available to the general circulation) or another dosage form known to be well absorbed, such as an oral solution. The sampling site for bioavailability determinations depends upon the therapeutic use of the drug but is usually the general circulation (i.e., venous or arterial blood) or the urine. There are, however, agents whose bioavailability should be determined at other sites, for example, topical preparations and agents intended to act in the gastrointestinal lumen.

The primary purpose of bioavailability studies is to determine how well a particular product is absorbed by patients who require the drug for the prevention or treatment of disease. Such studies are designed to develop and maintain standards that ensure, as far as possible, that supposedly equivalent products and production batches of the same drug have similar bioavailability characteristics. For practical reasons and because of limited knowledge of the effects of disease upon bioavailability, most studies are at present carried out on healthy human subjects. An essential goal is to develop *in vitro* tests (e.g., dissolution rate tests) and animal models that could serve as production and stability controls for individual products, since it is obviously not feasible to conduct bioavailability studies on every production batch of every product. The results of such *in vitro* and animal studies must, however, be correlated with the results of studies in man and can only be considered meaningful when such correlation is adequately demonstrated.

In bioavailability studies on patients or healthy subjects, scrupulous attention must be given to maintaining ethical standards consistent with

[1] *Drug formulation:* the composition of a dosage form, including the characteristics of its raw materials and the operations required to process it.

the Helsinki Declaration. It is desirable to have an institutional review committee confirm that the protocol complies with accepted ethical standards for research on human subjects. Informed consent should be obtained and all safeguards instituted to assure the wellbeing of the subjects involved in the studies.

Bioavailability is most frequently determined on the basis of drug plasma concentration or excretion in urine, and the following sections are concerned with the factors that affect these concentrations.

3. BIOPHARMACEUTICAL AND OTHER FACTORS INFLUENCING THE PLASMA CONCENTRATION OF DRUGS AND THEIR EXCRETION IN URINE

3.1 Biopharmaceutical factors influencing bioavailability

3.1.1 *Dosage form and site of administration*

Among the factors influencing the plasma levels of a drug are the dose and dosage form used. Solutions injected intravenously achieve immediate high plasma levels. Drugs are also often injected by the intramuscular or subcutaneous route, and in special circumstances they may be given sublingually, by suppository, or by inhalation. However, since most drugs are administered orally, either in solid or in liquid form, the following discussion may be assumed to apply to the oral dosage forms unless otherwise specified.

3.1.2 *Drug formulation and manufacturing variables*

A dosage form has to meet several criteria, including reproducibility, stability, and patient acceptability. To be suitable for commercial distribution the formulation must also possess adequate physical characteristics. In these respects the solid oral dosage form has proved to be very satisfactory. It has not, however, been without problems. These have occasionally resulted in therapeutic aberrations stemming from the method of manufacture of the particular product and the nature and amount of excipient used.

Particle size. After the process of disintegration has taken place, the dissolution rate of a drug is often determined by its particle size. For some poorly soluble drugs, small particle size (micronization) is desirable. In the absence of an adequate dissolution rate, drug particles may be carried past the optimal site of absorption.

Polymorphism. Many chemicals may exist in different polymorphic forms or crystalline habits, and on occasion these may have very different dissolution rates. When polymorphic forms occur they can usually be distinguished by X-ray diffraction techniques.

Disintegrants. Tablet disintegration is often critically affected by the amount and nature of the disintegrant incorporated. It has been suggested that a delay in disintegration of a dosage form may reduce the bioavailability of a drug. Tablet hardness may likewise affect the disintegration time of the tablet and its subsequent dissolution.

Lubricants. The compression of tablets or the filling of capsules is often facilitated by the use of lubricants. Care must be taken that they do not affect adversely the dissolution rate of the active ingredient.

Surface-active agents. For some dosage forms that disintegrate well, the dissolution step may be inhibited by poor dispersibility of the individual drug or excipient particles. Deaggregation may be effected by the use of surface-active agents or other substances having an influence on electrostatic properties. Similar considerations apply to suspensions, emulsions, etc.

Granulation. The techniques used in the process of granulation of pharmaceutical powders may influence the release characteristics of the dosage form.

Coatings. Special tablet or capsule coatings may be desirable for some purposes, but their use has frequently been associated with inadequate drug release or erratic bioavailability.

3.2 Other factors influencing drug plasma concentration and excretion in urine

As mentioned above, bioavailability studies take the form of comparative experiments in which a drug is compared with a reference formulation of the same drug. The reference formulation may be an intravenous solution (in determinations of "absolute bioavailability") or a non-intravenous formulation (in studies of "relative bioavailability"). Since it is reasonable to assume that any difference in bioavailability of the compared products will be reflected in the plasma concentration curve and/or in the excretion pattern of the drug in urine, determination of bioavailability is usually based on drug concentration measurements in the plasma and urine. However, comparison is complicated by the fact that both the plasma concentration curve and the excretion pattern of a drug in urine are affected by factors other than the biopharmaceutical properties of the drug product itself. Insofar as possible, these effects must be controlled.

The influence of factors such as body weight, sex, disease states, genetic differences in drug metabolism, and age of the test subjects can be to a large extent controlled by the use of crossover techniques.

On the other hand, the effects of factors that can be controlled directly by the investigator should be minimized by rigidly standardizing the experimental conditions. There are many such factors. For example, food intake and concomitant administration of other drugs may influence the absorption of the test drug. The same is true of factors such as stress, anxiety, and water intake, which influence gastrointestinal motility. The time of administration is important in the case of acidic and basic drugs because their rate of excretion by the kidney may vary over the course of the day. In addition, the possible influence of the pH of the urine on the elimination kinetics of these drugs should be considered. Drug kinetics may also be influenced by physical activity and body position. Administration or exposure to drugs and other substances, either prior to or concomitant with testing, may result in the induction or inhibition of drug-metabolizing enzymes that can influence both the bioavailability and the elimination of the test drug.

4. METHODS OF TESTING

4.1 General considerations

Bioavailability studies are carried out (*a*) during the development of a new drug product; (*b*) after its development, for continued quality control; and (*c*) in comparative evaluations of different products of the same drug from different manufacturers.

There are two basic methods available:

(1) the measurement of the concentration–time course or rate of excretion of the drug in body fluids after single or repeated doses; and

(2) repeated measurements of pharmacodynamic or biochemical responses to the drug and its active metabolites.

As the second method is usually much more complex, most work has been done with the first, on the assumption that the onset, intensity, and duration of the drug response bear a definable relationship to the concentration–time patterns of the drug and/or its metabolites in body fluids.

The detailed protocol of bioavailability studies depends on the physical and biological characteristics of the individual drug. It is usually necessary to characterize the pharmacokinetics of the drug before studies of bioavailability of the dosage form can properly be undertaken. There is no set of rules or guidelines that can be rigorously applied to every drug. However, a crossover design will usually diminish variance and consequently reduce the number of subjects required.

4.2 Preliminary procedures

4.2.1 *Sampling the product*

The method of obtaining the sample of the product to be used in the study must be given careful consideration and be stated explicitly. Batch numbers, date of manufacture, and storage conditions should be recorded.

Part of each sample should be tested to ensure that the product meets the appropriate pharmacopoeial or other quality specifications before a bioavailability study is begun. A further part of the sample should be kept for other *in vitro* studies if these are required.

Comparative studies should be done without modifying the dosage form available commercially.

4.2.2 *Selecting human test subjects*

Bioavailability studies may be performed in healthy human subjects or in patients requiring the drug. In the selection of healthy adults, consideration must be given to factors such as age, sex, and weight. Individuals with a history of significant gastrointestinal, liver, kidney, heart, thyroid, or other organ damage should be excluded. Before a volunteer is accepted for a study, an appropriate physical examination and clinical laboratory tests should be carried out. The types and numbers of tests to be performed will vary with the drug being studied but they should be chosen for their ability to indicate whether the volunteer has damage to any of the above-mentioned organs. Subjects taking other medication may be included provided the intake remains constant throughout the study period and consideration is given to its possible effects on the study. The use of agents such as alcohol that influence drug metabolism or gastrointestinal physiology should be kept to a minimum.

The same general evaluation should be carried out on patients who are prospective subjects. However, the criteria for inclusion may have to be less rigid; the disease process may produce abnormal laboratory test values and concomitant drug therapy may be essential to the patient's well-being. When complicating factors exist, every effort should be made to keep them constant throughout the study.

4.3 Experimental procedure

4.3.1 *General methods*

Bioavailability may be assessed by measuring the total area under the plasma concentration–time curve or by determining the total excretion of drug and/or its metabolites in urine. The extent of bioavailability must be determined by comparison with a reference formulation, such as an intravenous injection, an oral solution, or another well specified and well

absorbed oral dosage form. From the scientific standpoint the last form is least desirable and should be used primarily when the preparation of a solution is not feasible (e.g., when the drug is insoluble or unstable in solution). Data obtained after an intravenous injection or infusion yield the least ambiguous results because the entire dose reaches the systemic circulation. Comparison with an intravenously administered formulation is usually the only means of determining absolute bioavailability. However, extreme care is needed when studying drugs for which the safety of this route of administration has been incompletely evaluated. Nor is the measurement of absolute bioavailability always most desirable. Under special circumstances (e.g., studies of topical products) bioavailability might be more relevant if measured at the drug's actual site of action rather than in the systemic circulation.

4.3.2 *Single dose* versus *repetitive dosing schedules*

Single and repetitive dosing schedules each have advantages and disadvantages for bioavailability studies.

Single doses involve less drug exposure for healthy subjects but require measurements over 3 drug half-lives or more. This method may be unsuitable if the drug has complex pharmacokinetic properties, for example, if it enters the enterohepatic circulation.

With repetitive dosing, blood sampling can be completed within one dosing interval and thus fewer blood samples are required, although each product must be administered for about 5 half-lives before the samples are obtained. Analytical methods usually need not be so specific as in single-dose studies and detailed knowledge of the pharmacokinetic characteristics of the drug is less important. An important advantage of repetitive dosing studies is that they can be carried out in patients who are receiving the drug regularly as part of their therapy.

4.3.3 *Pharmacokinetic measurements*

Especially for single-dose studies, specific and sensitive analytical methods are necessary since the drug concentration must be followed in the plasma for at least 3 half-lives. Although specific chemical methods are preferable, the level of certain drugs (e.g., antibiotics) may be satisfactorily determined by nonspecific but sensitive bioassays. If an adequate analytical method is not available, bioavailability may be evaluated from pharmacodynamic measurements. For example, this approach has been used for atropine derivatives. Drugs with radioactive labels are also used for bioavailability testing but have the disadvantage of exposing the subject to radiation. Another drawback is that the physicochemical characteristics and formulation of the labelled drug may differ from those of the unlabelled drug. If radioactive labels are used, detailed *in vitro* dissolution studies

must demonstrate that the labelled dosage form has the same release characteristics as the commercial product. It should be emphasized that measurement of the excretion of total radioactivity in the urine does not always provide an accurate assessment of bioavailability, especially if the drug is significantly metabolized when it first passes through the intestine and liver.

Measurements of the area under the plasma concentration–time curve can be made with an appropriate numerical integration procedure such as the trapezoidal rule. Sufficient samples should be obtained so that the area under the curve is adequately quantified. Area measurements after repetitive dosing are necessary during only one dosing interval and should be done after a steady state has been attained.

Equal doses of the reference formulation and the product being tested should be used. This is particularly important with drugs that have appreciable nonlinear pharmacokinetic characteristics.

5. CORRELATION OF BIOAVAILABILITY DATA IN MAN WITH DATA FROM OTHER SOURCES

It would be advantageous if there were a good correlation between the bioavailability of a drug product in man and its bioavailability in animal models or as measured by various *in vitro* methods, such as the disintegration or dissolution test.

5.1 Therapeutic efficacy and adverse effects

There have been cases where differences in the bioavailability of a drug product have been detected because of changes in therapeutic efficacy or adverse reactions in man. Often, hower, such sporadic observation appears to be too insensitive to detect even clinically significant changes in bioavailability.

5.2 Animal models

Research is being carried out on the testing of bioavailability in intact animals and animals with everted sacs and gut pouches. The predictive value of such models for bioavailability in man has not yet been established.

5.3 *In vitro* tests

The two most common *in vitro* tests are the disintegration test and the dissolution test. The former attempts to measure the first stage of drug release by specifying a maximum time for disintegration of the dosage form into particles of a certain size. The latter measures the amount of