Bioequivalence studies: main methodologies for design

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Regulatory approach

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Abstract
The objective of bioequivalence studies is to demonstrate that two formulations with the same active ingredient have such a similar pharmacokinetic profile that it can be assumed that they will behave in the same way as the reference product, ensuring their pharmacological and therapeutic equivalence, even allowing them to be exchanged. This regulatory focus article seeks to provide general bases on the types of design of bioequivalence studies, as well as their advantages and limitations. In El Salvador, the entity in charge of clinical trials oversight and authorization, and the establishments where they are carried out is the National Directorate of Medicines.

Keywords: bioequivalence, design, healthy subjects, test drug and reference drug, generic drug.

Introduction

A drug availability proportion in its site of action is known as bioavailability (Villar, 2012).

Bioequivalence is the absence of significant differences on a drug bioavailability post-dosification, comparing its reference product, this justifies the decision of not carrying out clinical trials, costly and complex, under the principle that, if one generic specialty and its reference are administered on a same subject, containing the same active ingredient, its absorption kinetics and blood distribution must be such that the site of action concentration resembles it; hence, its therapeutic activity resembles it as well. It is tested measuring the reagent's blood concentration at determined intervals, in different subjects, to determine the pharmacokinetics behavior's resemblance and to establish the bioequivalence between both formulations. In general, a product is considered bioequivalent when the confidence interval of 90% for the generic Cmax and AUC against the logarithmically transformed data reference is at 80 %-125 % (Laosa et al, 2009).

This process' social contribution is based on its potential to facilitate the access to quality products and the health system resources streamlining.

Background

The World Health Organization (WHO), promotes the use of international standards for quality drug assessment making expert committees such as The Working Group on Bioequivalence, which develops consultation guidelines regarding updated bioequivalence at the pace of technological and pharmaceutical advances. The member states either adapt them according to particular circumstances or have been stakeholders in regional harmonization groups. This organization has also established norms for quality assessment, raw materials, labeling, toxicological profiles, pharmacological products, and the interchangeability of generic drugs, with bioequivalence studies being a requirement. Given the

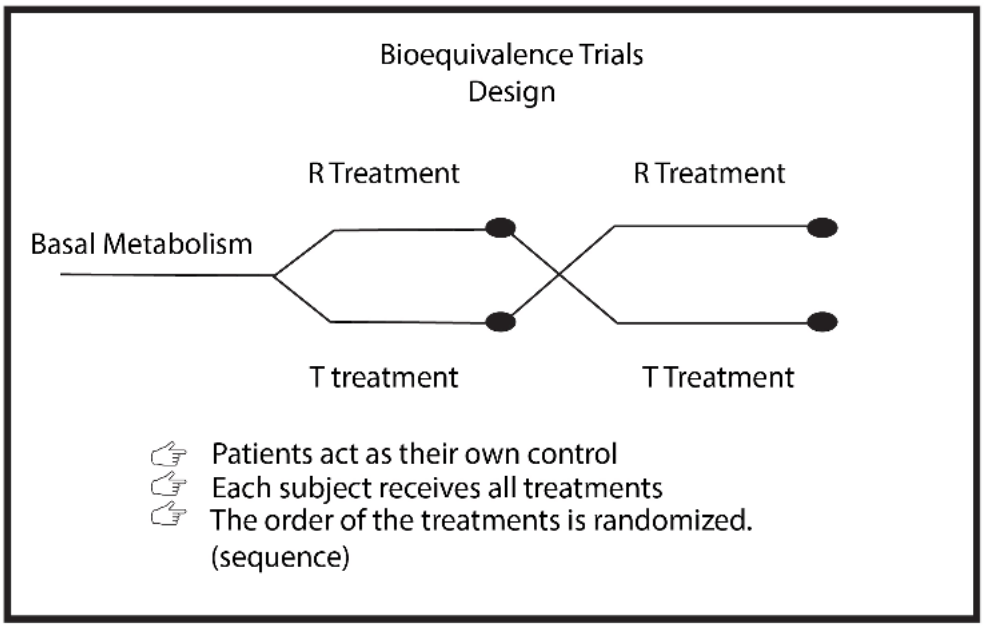
ethical and methodological challenges this involves, as well as the necessity of qualified personnel, it is costlier; consequently, its progressive application is commendable, according to the capabilities of each country, as well as the prioritization under the evaluation of risk to health (Giarcovich, 1997). Likewise, the regulatory agencies must ensure the fulfillment of international ethical codes and the prevalence of individual rights over science's interests, besides the compliance with Good Clinical Practice and Good Laboratory Practice standards to ensure the reliability and truthfulness of the results (Montpart & Martín, 2002).

Methodological Designs: According to the 5040/2006 disposition from the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, Argentina, (ANMAT, 2006), the bioequivalence studies require the multidisciplinary contribution from experts in regulatory matters, pharmacokinetics and statistics. During the study, two products are administered: T (test product) and R (reference product), hence common bioequivalence study designs include parallel and crossover.

In a bioequivalence study once the objective has been set, the methodology and study protocol must be planned, subject profile, dosage, sampling times, parameters, analysis methods for biological samples and results analysis and interpretations. Among the designs worth mentioning are:

Crossover design: The participants are randomly assigned to the treatment or control formulation to be compared, following the sequence for the established dosage. In this type of comparative design each individual acts as their own control diminishing the interindividual variability and given the randomisation in the administered doses it is possible to better detect the bioavailability differences or similarities. Following the first administration according to the randomized sequence, a proper washout period is allowed to ensure that the administered drug and its metabolites have been completely eliminated. This washout period is 5 or more half-lives of the drug. To control the variables that affect the absorption and availability the design also requires the standardization of the subject habits and lifestyles such as liquid and food intake, rest, timing between a blood sample and another (See Figure 1)

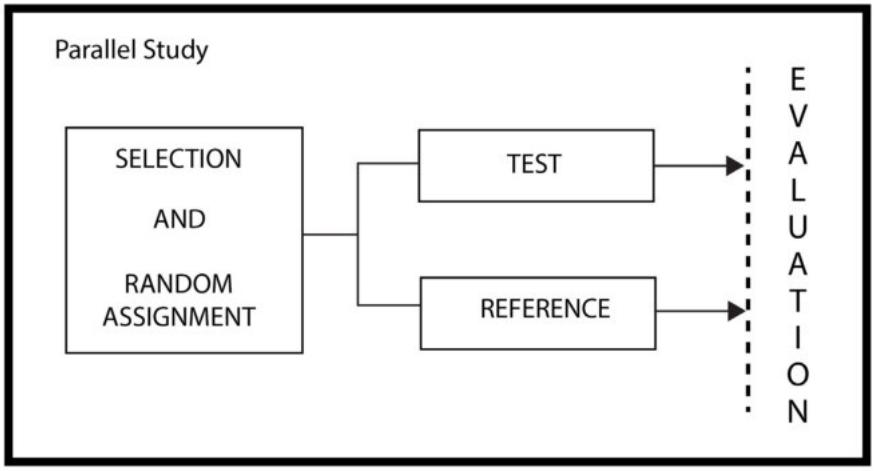
Figure 1. Crossover design diagram



Source: Laosa, et al; 2009

Alternative designs: Some drugs can provoke adverse or unacceptable effects for the participants or require the usage of elevated doses. In these cases, a parallel design can be useful (see Figure 2). Such designs must be justified by the sponsor who will subscribe the volunteers with stable conditions. Similarly if products with prolonged half-life must be evaluated (>24 hours), where a crossover design becomes eventful, a parallel group study design can be used. In these designs the sample collection time must be appropriate to allow a full gastrointestinal transit (between 2 and 3 days). The blood sample collection will extend up to 72 hours. Thus, in these studies a larger number of volunteers are required and occasionally according to the active ingredient (safety reasons) must be performed on patients.

Figure 2. Parallel design diagram



Source: Disposición 5040/2006; (ANMAT, 2006)

For other drugs with "high pharmacokinetic variability", those which Coefficient of Intraindividual Variations (%) is equal or greater than 30%, it is recommended to apply a replicated two-sequence crossover design (TR/RT) and four periods (e.g: Sequence 1: RTTR; Sequence 2 TRRT), see Figure 3.

Figure 3. Replicated crossover design

		PERIOD			
		1	2	3	4
SEQUENCE	1	E	R	E	R
	2	R	E	R	E

Source: GUÍA TÉCNICA G-BIOF 01, Instituto de Salud Pública de Chile, (ISP; 2007)

In certain situations crossover studies with multiple doses are more appropriate e.g. the evaluated active ingredient is too strong or toxic to be administered to healthy volunteers even in single doses. Such studies could base their evaluations on pharmacokinetic or pharmacodynamic parameters although the latter requires more patients. The employed posology must follow the regular posology recommendations.

Single dose designs are preferred for modified release formulation due to their sensitivity to detect the main ingredient's release from its condition as a drug to the systemic circulation.

A critical aspect for the evaluation of drugs is food since it may alter their absorption and their impact must be studied, however, the bioequivalence criteria for its acceptance are essentially the same as for simple release products.

Participants

The objective of the subject's selection criteria in the bioequivalence studies is to reduce the variability contributed by the subject's demographic and anthropometric characteristics or by pathological situations, so that if relevant differences emerge in the drugs under study pharmacokinetics behavior, these can not be attributed to the subject's heterogeneity, but to the drugs behavior (Committe for proprietary Medicinal Products, 2001).

For this, healthy volunteers must be incorporated including both genders, normal weight or suitable body mass index (BMI), from 18 to 55 years old, neither alcohol/ tobacco consumers, nor other conditions that may increase the risk of bias that makes the interpretation of results difficult. Moreover, the volunteers must be able to understand and comply with the protocol, as well as sign the consent.

The international regulation advises participants to be part of one study at a time, every 3 months, for safety reasons and to the possible pharmacotherapy interactions, in addition, to the blood volume extracted for plasma samples, which may not be as important in a 2x2 study, but it may be in a different design in which a higher blood volume is required. Besides, the enrollment of an extra number of subjects above the required amounts according to the sample size calculation must be established by protocol, given the possible losses or withdrawals that may occur during the development of study, and that it is not always advisable to make replacements, as the model and statistical analysis may get complicated. Nevertheless, the reasons for subject withdrawal must be informed. Even if it is wanted to replace the losses during the study, this intention must be indicated in the protocol (ANMAT, 2006).

Dose selection

According to Laosa, et al. (2009), the dose selection must be evaluated taking into account three basic aspects simultaneously:

-Is the dose safe enough?
-Will the analytical method used for quantification have sufficient sensitivity to the chosen concentration of the drug?
-Will the selected dose be the most sensitive for detecting remarkable differences in pharmacokinetic features?
Doses within the range of the Dose-Related Reference Range authorized for marketing must be selected.

Effect of food on Bioequivalence

Regarding this aspect, according to Laosa, et al. (2009) and PAHO (2005), the subjects who participate in a bioequivalence study must receive the same diet whilst the trial is ongoing, since some foods and drinks may interfere in the metabolic process of drugs therefore modifying its pharmacokinetic behavior, as well as its bioavailability, so that the diet is standardized and alcoholic beverages, food containing xanthines and drugs of abuse are restricted, in some cases, participants in fasting conditions are desirable.
This bioavailability modification could be the consequence of different mechanisms (Center for Drug Evaluation and Research (CDER), 2002), such as:
- gastric emptying delay
- bile flow stimulation
- Gastrointestinal pH change
- Increased splenic blood flow
- Change in luminal drug metabolism
- Physical or chemical interaction with a pharmaceutical form or a pharmacotherapeutic substance.

According to studies, the influence of food on bioequivalence are usually greater when the drug is administered post-feeding , specially if a high-calorie and high- fat diet is consumed, since the gastrointestinal physiology is altered, consequently, the bioequivalence of pharmaceutical products under trials.

In class I active ingredients, according to the Biopharmaceutics Classification System (BCS), in fast-dissolving immediate-release formulations, effects on bioequivalence are less likely to occur, due to the substances being highly soluble and permeable, resulting in an independent absorption of pH and site.

Washout periods

The washout period must be enough to completely eliminate the previous dosage from the systemic circulation said timeframe must be the same for all subjects. The literature recommends more than five times the mean life of the active ingredient (ISP, 2007). According to the active ingredient's nature, the washout period could be longer. In cases where the active metabolites have a long half-life or if the drug elimination speed presents high interindividual variability (Agencia Nacional de Regulación, Control y Vigilancia Sanitaria (ARCSA), Ecuador, 2018).
It is necessary to consider a longer washout period when the drug presents high interindividual variability to allow the subjects with low elimination rates to eliminate the drug. It is important to verify the active ingredient or its metabolites concentration in blood before drug administration in the second period.
The shortest washout period must be seven days unless a shorter period is justified if the main ingredient has a short half-life (ARCSA, 2018).

Sampling time

When the biological samples correspond to blood, they must be collected with enough frequency to allow adequate Cmax evaluation (peak drug concentration), the area under the curve, and other pharmacokinetic parameters. A pre-dose sample must be included in the experimental sampling points at least 1-2 points before Cmax, 2 points around Cmax and 3-4 points during elimination phase (ISP, 2007). Considering at least 7 sampling points to measure the pharmacokinetic parameters. A larger number of samples are required for some pharmaceuticals to reduce possible differences within the absorption and elimination rates among the participants thus determining the peak drug concentration in blood and the constant of elimination speed in all subjects. It is advisable to carry out the sampling without exceeding 72 hours to ensure that the 80% of the area under the curve is quantified. The time depends on the nature of the administered drug.

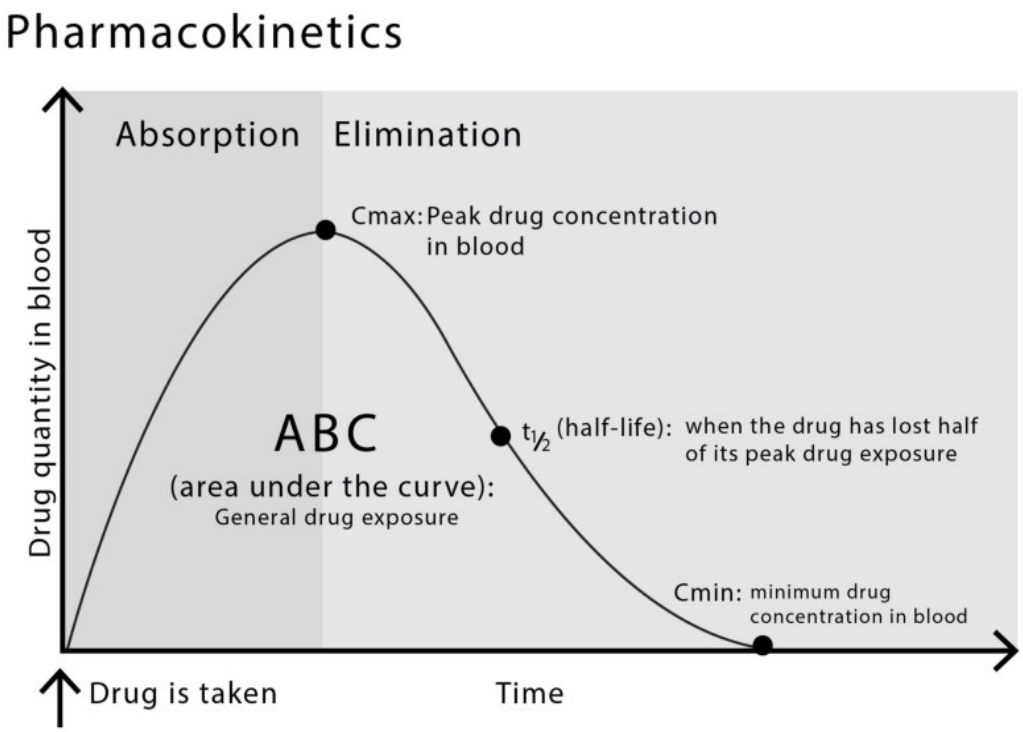
Sampling and collections fluid

In general, the drugs concentration is evaluated by carrying out biological fluid sampling, the blood is the biological fluid with a higher frequency of usage, since the metabolites or the drug are measured in the serum or plasma.
Also, sampling of different biological fluids such as urine can be performed, if the drug is eliminated through the renal route with no changes, however, the usage of such fluid presents the inconvenience that the Tmax (Transport maximum) and the Cmax (Maximum concentration) can not be calculated. (CECMED,n.d).
It is recommended to analyze the sample in the recollection center and, if possible, sampling must be enough to calculate every pharmacokinetic parameters. In the case of blood sampling, it is required to control the storage conditions in order to avoid the analyte degradation (ISP, 2007).

Pharmacokinetic analysis

After the reference and trial drug administration, the plasma concentration of each formulation and its changes at different time points over a defined period must be quantified. Blood samples will be extracted by venipuncture for this purpose. Nevertheless, the minimum necessary must be maintained to reduce the discomfort caused to participants. The validation of analytical methods is mandatory; these methods must be accurate, sensitive, specific, and replicable to ensure that the results are reliable and correspond to the analyte being measured.
Drug Regulatory Agencies suggest the following pharmacokinetic criteria for the studies on bioequivalence.
The Area Under the Curve (AUC) of concentration over time (see Figure 4) allows measurements from drug administration to the last quantifiable sample on the time axis, facilitating concentration extrapolation down to zero. The calculation extends along the elimination phase, ensuring that it does not

Figure 4. Area Under the Curve (AuC); Concentration vs. Time



Source: Clinical Info Departamento de Salud y Servicios Humanos Estados Unidos

exceed 20% of the total AUC. It is important to note that the AUC reflects the drug's bioavailability. Additionally, peak drug concentration and time parameters measure the rate at which the drug is utilized by the body (Laosa et al., 2009).

The elimination half-life is helpful for comparing kinetic profiles among formulations, testing the existence of concordance with what is described in the literature, and assessing whether the washout period has been sufficient. The AUC and Cmax are primary parameters for evaluating bioequivalence, while Tmax and half-life constitute secondary parameters (Laosa et al., 2009).

Statistical analysis

A common concern for the drug regulating agencies is the acceptance of a product as bioequivalent when it is not. Therefore, statistical procedures without exceeding the 5% alpha error (0.05) are recommended so that the probability to conclude that two formulations are bioequivalent when they are not would be just 5% (Laosa et al, 2009).

It is worth mentioning that the statistical evaluation used for the bioequivalence studies is proven with confidence intervals of relative bioavailability. Two pharmaceutical products will be considered bioequivalent in speed and strength if the "width of the differences among their respective variables is less or equal to 20%" (Sigler, 1999).

Bioequivalence Research Center

These are all the centers where bioequivalence studies are carried out, those can be private institutions, public or being inside hospital facilities, complying with the applicable good practices, as well as the infrastructure basic requirements, personnel with sufficient technical competence, equipment, instruments and materials, quality management system, storage and biological sampling transportation, storage of test and reference drug, among some other legal and regulatory dispositions which must be enforced for its correct functioning (Salvadoran Technical Regulation Agency, OSARTEC for its acronym in Spanish, 2023).

Bioequivalence studies are carried out in the Clinical Unit. All the necessary medical services must be available for the participants in case of any emergency, in this view an Intensive Care Unit (ICU) must ideally exist in the Clinical Unit or at least specialized transportation for the transfer of subjects to hospital centers (ANMAT, 2006), in cases where it is warranted.
In El Salvador, hospitals and clinics, both public and private, that offer services to conduct studies on bioequivalence must be registered with the National Directorate of Medicines (DNM, by its acronym in Spanish) as a 'Research Center.' They are obligated to comply with the requirements outlined in the "Guía para la apertura de establecimientos regulados por la DNM" (Guide for the opening of establishments regulated by the DNM).

Conclusion

Bioequivalence studies serve as important tools for public health systems. These clinical trials are utilized to demonstrate that a pharmaceutical product is safe, effective, and of high quality, aiming to establish its equivalence to the innovative product. Therefore, it is crucial that these studies are conducted in strict compliance with national and international regulations, as well as Good Clinical Practice (GCP) guidelines. The selection of a method for conducting bioequivalence trials depends on the drug's properties and the sampled population.
Nowadays, the National Directorate of Medicines (DNM, by its acronym in Spanish) is the regulatory agency responsible for authorizing research centers. It verifies compliance with international and current national regulations, ensuring the safety of subjects and the reliability of data. This ensures that the population has access to safe, effective, and quality drugs at an affordable price.

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