Bioequivalence studies: relevance for veterinary medicine

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Abstract

Bioequivalence (BE) studies are scientific methods that allow comparison of different medicinal products containing the same active substance, or different batches of the same medicinal products or, in a broad sense, different routes of administration of the same product. Actually, legislation on generic drugs and bioequivalence only exist in Brazil for drugs intended for human purposes. In the field of Veterinary Medicine, BE is being used in many countries as part of the necessary requirements for registration of animal health products, i.e., to provide efficacy and safety animal data and to allow consumers safety; indeed, they also assure the quality of the food derived from treated animals. The present manuscript was designed to review and discuss BE; for that, it was divided into three major parts: 1- understanding bioequivalence: importance of BE studies for animal and human health; 2- type of BE studies included; 3- general consideration on experimental design involved o BE studies.

Key words:
Pharmacokinetic.
Pharmacodynamic.
Bioavailability.
Bioequivalency.
Reference drugs.
Generic drugs.

Introduction

Bioequivalence (BE) studies are scientific methods designed to compare different health products containing the same active constituent or different batches of the same veterinary medical products, based on their formulation, pharmacokinetic and pharmacodynamic properties and residual profiles in the tissues of treated animals.[1,2,3] BE studies are used in a variety of situations, most often when a sponsor proposes manufacturing a generic version of an approved off-patent product. A BE study may also be part of a new animal drug application (NADA) or supplemental NADA for approval of an alternative dosage form, new route of administration, or a significant manufacturing change which may affect drug bioavailability. In veterinary medicine, BE studies are necessary and relevant because they allow the establishment of the necessary requirements for registration of generic animal health products that ensure animal and food safety for human being.

Actually, legislation on generic drugs in Brazil exists only for those to be used in humans; as a consequence, specific rules for bioequivalence (BE) studies on veterinary products are still lacking. In this regard, legislation on both generic drugs and BE in veterinary medicine are being employed and might be found in many countries.[1,2,3] Consideration on such maters seems appropriate and necessary; indeed, it may become a useful instrument for governmental authorities, pharmaceutical industries and veterinary professionals' managements in this field. The present manuscript was them designed, to discuss BE; it was divided into three major parts: 1- understanding bioequivalence: importance of BE studies for animal and human health; 2- type of BE studies included; 3- general consideration on

experimental design involved o BE studies.

Understanding BE: importance for animal and human health

Veterinary pharmaceutical product is a finished dosage form that contains the pharmacologically active substance(s) with or without excipient(s). Changes in active substance purity or in excipients in a veterinary medical product as well as in the manufacturing process may have significant effects on bioavailability.

It is scientifically valid to assume that, if an active substance or therapeutic moiety of a test animal health product reaches the systemic circulation with the same rate and extent as the active substance or therapeutic moiety of a reference animal health product, the local availability (concentration in tissue) of the active substance or therapeutic moiety will be similar for the test and reference products. The similarity of availability at the site(s) of action is the basis of concluding therapeutic equivalence of the products. However and importantly, it is usually accepted that tissue residue depletion of the generic product is not adequately addressed through BE studies. For that, sponsors of drug products for food-producing animals are generally requested to include BE and tissue residue studies in their data package.[2,3]

The *in vivo* BE of an animal health product is demonstrated if the rate and extent of absorption, as determined by comparison of measured parameters derived from relevant data (e.g. concentration of the active substance in the blood or pharmacological/clinical effects) do not indicate a biologically relevant difference in the rate and extent of absorption from the reference product. In other words, two products are considered to be bioequivalent when they are equally bioavailable; that is, equal in the rate and extent to which the active ingredient(s) or therapeutic ingredient(s) is (are) absorbed and become(s) available at the site(s) of drug action.[1,2,3]

As a general rule, the proposed generic product should be tested for BE against the original pioneer product. However, if the original pioneer product is no longer marketed, but remains eligible to be copied, then the first approved and available generic copy of the pioneer should be used as the reference product for BE testing against the proposed new generic product.[2] If several approved NADA's exist for the same drug product, and each approved product is labeled differently (i.e., different species and/or claims), then the generic sponsor is asked to clearly identify which product label is the intended pioneer[2]. BE testing should be conduct against the single approved product which bears the labeling that the generic sponsor intends to copy.

Thus, a generic animal health drug may be defined as a product that contain the same active ingredient, in the same dose range, in the same pharmaceutical formulation and that is administered to the same animal species, for the same route and for the same therapeutic/prophylactic/feed addictive purposes. For that, they present the same efficacy, same security for animal and human health and, the same quality of the reference standard, as statically proven by BE and by tissue depletion residue studies.

According to the Australian Pesticides & Veterinary Medicines Authority - APVMA[1]- a proposed product is considered to be generic to a reference product if: 1- ingredients are of equivalent pharmaceutical compendial standard, and 2- the active constituents are the same substances and are within ± 5% of that in the reference product or the dose range to the animal is within ± 5% of the reference product, and 3- the non-active constituents are the same substances and are within ± 5% of that in the reference product, except for those constituents defined in the Australian Guidelines on Minor Formulation Changes, where they can vary more than ± 5%, and 4- the product is in the same dosage form, and finally 5- the product has the same physico-chemical properties as the reference product (including pH, particle size, crystal form, and dissolution profile where applicable).

Type of BE studies included

BE can be demonstrated *in vivo* or, under specific conditions, *in vitro*. Figure 1 depicts the more relevant studies related to BE determination. The best approach would be that one that uses all methods available to display BE, i.e., that employs both *in vitro* and *in vivo* methods. However and in particular terms, the blood level study is generically preferred above all others being considered, since it is considered the most sensitive measure of BE. For that, the sponsor should provide justification for choosing either a pharmacologic or clinical end-point study over a blood-level (or other biological fluids or tissues) study. It should be noted, however, that tissue residue depletion studies were also included in this figure; this was done because, as already stated, it is usually accepted that tissue residue depletion of a generic product is not adequately addressed through BE studies.

In vivo BE studies must be conducted using the most accurate, sensitive and reproducible approach. It may be determined by one of several direct or indirect methods. Selection of the method depends upon the purpose of the study, the analytical method available, and the nature of the drug product. BE testing should be conducted using the most appropriate method available for the specific use of the product. A classification of such approaches proposed by the EMEA Committee for Veterinary Medical Products in descending order of accuracy, sensitivity and reproducibility[2] is listed bellow:

a) In vivo testing in target species in which the concentration of the active substance or therapeutic moiety or its representative metabolite(s) in blood, plasma, serum or if justified, other biological fluid or appropriate tissues is measured as a function of time. Reliance on *in vivo* BE data relies upon an assumption that the measured concentrations of active substance have meaning with respect to the objective of the trial and the intended label claim. It also necessitates that adequate drug concentrations are achieved to allow for the determination of product concentration *vs* time profile in the blood or other biological fluid.

b) In vivo testing in target species in which an appropriate acute pharmacological/clinical effect of the active substance or therapeutic moiety or its metabolite(s) is measured as a

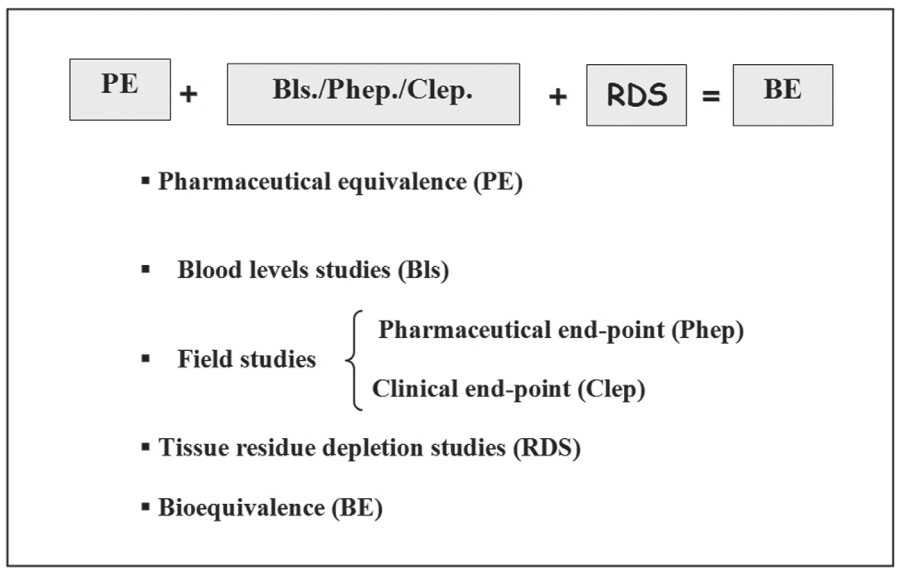

Figure 1 - Studies implicated in bioequivalence determination. Note that equivalence between a generic and a pioneer product should be obtained using *in vitro* pharmaceutical equivalence and/or *in vivo* tests such as the blood level, the pharmacological and clinical end-points studies. Note also that residue depletion studies are also necessary

function of time. This approach can be used when analytical methods are not available. Its use requires demonstration of dose-related response. For veterinary medical products intended for local effect, pharmacological/clinical end-points can be the most relevant approach for the demonstration of BE.

According to FDA BE Guidance for Industry[3], when absorption of the drug is sufficient to measure drug concentration directly in the blood (or other appropriate biological fluid or tissues) and systemic absorption is relevant to the drug action, then a blood (or other biological fluid or tissue) level BE study should be conducted. However, when the measurement of the rate and extent of absorption of the drug in biological fluids can not be achieved or is unrelated to drug action, a pharmacologic end-point (i.e., drug induced physiologic change which is related to the approved indications of use) study may be conducted. Lastly, and in order of preference, if drug concentration in blood (or fluids or tissues) are not measurable or are inappropriate, and there are no appropriate pharmacologic effects that can be monitored, then a clinical end-point study may be conducted, comparing the test (generic) product to the reference (pioneer) product and a placebo (or negative) control.

Finally, *In vitro* equivalence studies could support *in vivo* BE studies in the following case[2]:

a) In vivo BE has been demonstrated for the highest dosage strength and *in vitro* dissolution data is used to support the BE of the lower dosage strengths for that generic formulation. In these cases, the use of *in vitro* methods requires that the following conditions are all met: 1- the dosage strengths differ only by the mass of the active substance; 2- the drug is known to be associated with linear pharmacokinetics; 3- the composition of all formulations are qualitatively identical; 4- the ratios between active substances of the test- and reference products are identical.

b) In vitro comparability might be adequate to confirm the comparability of the reference product and its generic product to be administered orally. This applies particularly to immediate release oral dosage forms that are rapidly dissolving and contain drug substances that are both highly soluble and high permeable.

c) There is a very minor formulation change to an approved product (or a minor pre-approval change to a product that has undergone extensive clinical trials), and has been determined that the change requires only *in vitro* confirmation of comparability to the formulation that underwent the clinical trials.

d) Ensuring batch to batch consistency within a product.

It should be noted, however, that the Australian Guidelines on BE[1] states that for certain dosage forms (intravenous solutions, oral solubilized forms, topical dose forms, non-absorbed antacids and radio opaque media and oral tablets), an applicant may wish to address BE by providing *in vitro* data demonstrating pharmaceutical equivalence of a ´product to a reference product. In this case, such data should include: 1- nature of dosage form; 2- solubility of active constituent(s) in water; 3- relevant pharmaceutical characteristics including particle size, crystal form, and dissolution profile where applicable; 4- rate limiting steps in absorption of the active constituent(s) e.g. disintegration, dissolution, gut absorption where applicable or in access to the site of effect; and 5- relevant scientific argument regarding clinical consequences of inequivalence.

General consideration on experimental design involved on BE determination

As depicted in Figure 1, BE can be demonstrated *in vivo* or, under specific conditions, *in vitro*.

In vivo BE studies

BE may be demonstrated *in vivo* by one of several direct or indirect methods. In descending order of sensitivity: blood levels

studies, pharmacological end-point studies and clinical end-point studies. As stated before, the selection of the method is linked to the purpose of the study, the analytical method available and, among many other variables, the nature of the product analyzed.

Basic principles

In vivo BE studies usually involve an experimental part conducted in the field, where the active substance or therapeutic moiety are given to the target animal species, an analytical part where the active substance(s) or its representative metabolite(s) are analyzed in the blood, plasma, serum or other biological fluids or appropriate tissues and a statistical part. Figure 2 depicts these parts, the main steps they involve and the quality compliance test protocols that should be employed in each of them.

Veterinary Good Clinical Practice (GCP) should be used in all blood level, pharmacological end-point, clinical end-point and residue depletion studies if the products intendment is food-producing animals use. The global requirements referred to as VICH-GCP guidelines[4] are usually employed in the field experiments, because they were specially prepared to ensure the accuracy, integrity and correctness of the obtained data. VICH is a tripartite (EU, Japan and USA) programme aimed at harmonizing technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products.

Some relevant extracts from the VICH- GCP guidelines[4] are: 1- the investigator responsible for the study should have sufficient knowledge, scientific training and experience, as evidenced by a current *curriculum vitae* and other credentials, to conduct clinical studies to investigate the effectiveness and in-use safety of investigational veterinary products in the target species; 2- the investigator should delegate authority and work only to individuals qualified by training and experience to perform the assigned duties; 3- the field experiment should comply with the study protocol, being conducted according to GCP and applicable regulatory requirements; 4- the equipment and facilities used to conduct the study should be adequate and well-maintained; 5- the housing, feeding, and care of all animals at the study site should be carefully supervised; 6- all data should be collected and recorded, including unanticipated observations, in accordance with the study protocol and applicable regulatory requirements in an unbiased manner that accurately and completely reflects the observations of the study; 7- the investigator should ensure that

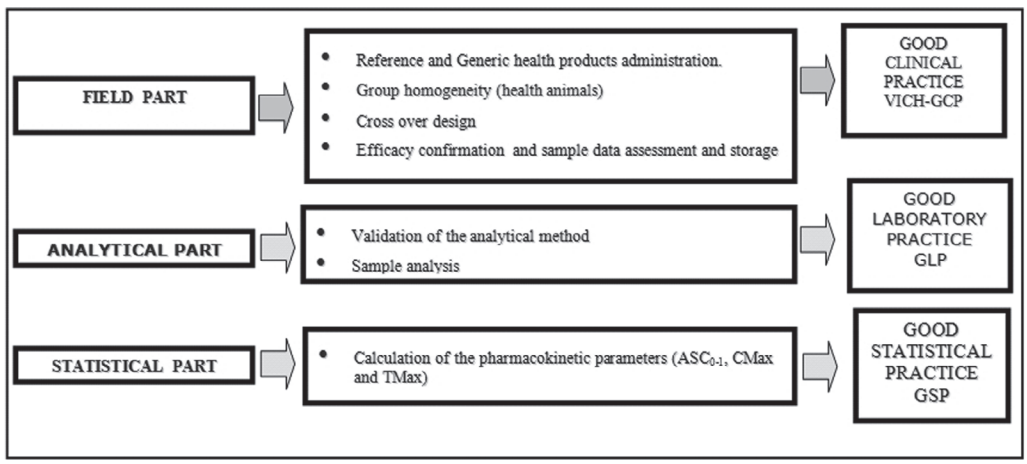

Figure 2 - The three different steps of bioequivalence studies. Note the requirements on quality compliance applied to each of them

all specimens required to be retained by the study protocol and any applicable regulatory requirements are identified in a manner that is complete, accurate, legible and precludes loss of identification from the specimen; 8- the special analysis and/or tests to be performed including the time of sampling and interval between sampling, storage of samples, and the analysis of testing should be carefully described; and, among others, 9- all study documentation should be securely stored for the period of time required by the relevant regulatory authorities, being protected from deterioration.

To ensure VICH-GCP, selected animals for BE studies must be from the target population for which the product is intended. Ordinarily, BE studies should be conducted with health animals representative of the species, class, gender, breed, age, weight, hormonal, nutritional and physiological maturity for which the drug was approved. Where possible, it is advisable to restrict the studies to the gender for which the pioneer product is approved, unless there is known interaction of formulation with gender. An attempt should be made to restrict the weight of the test animals to a narrow range and animals should not receive any medication prior to testing for a period of two weeks or more, depending upon the biological half-life of the ancillary drug.

Feeding may either enhance or interfere with drug absorption, depending upon the characteristics of the drug and the formulation. Feeding may also increase the inter- and intrasubject variability in the rate and extent of drug absorption. The rationale for conducting each bioequivalence study under fasting or fed conditions should be provided in the protocol. In this respect, the protocol should describe the diet and feeding regime which will be used in the study. However, and importantly, if a pioneer product label indicates that the product is limited to administration either in the fed or fasted state, then the BE study should be conducted accordingly.

Concerning the analytical part of the BE studies, it should be conducted and reported in conformity with the international principles of GLP and quality compliance methods. Specifically, it should be conducted in accordance with requirements stated in the 32nd, 47th and 52nd meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[5] or with the European Community legislation reported in Directives 87/18/EEC and 88/320/EEC. The FDA considerations included on Assay methods specified in the BE Guidance to Industry document[3] and the VICH guideline GL1 Validation of analytical procedures (CVMP/VIVH/97/076) might also be useful.

In this respect, it's undisputable that a properly validated assay method is pivotal to the acceptability of any pharmacokinetic study. For that, the submission should contain adequate information necessary for the reviewer to determine the validity of the analytical method used to quantitate the level of the drug under study in the biological matrix (e.g., blood or tissues). Thus, the following aspects should be addressed in assessing method performance: 1- concentration range and linearity, defined by at least five concentrations; 2- limit of detection (LOD), estimated as the response value calculated by adding three times the standard deviation of the background response to the average background response; 3- limit of quantification (LOQ), at least ten times the standard deviation of the background response to the average background response; 4- specificity, demonstrated by the analysis of six independent sources of control matrix; 5- accuracy (recovery), based upon the mean value of six replicate injections, at three dose levels (high, mid-range and LOQ); 6- precision, evaluated using at least three known concentration of analyte freshly spiked in control matrix; 7- analyte stability, determined with incurred analyte in the matrix of dosed animals in addition to control matrix spiked with pure analyte; 8- analytical system stability, to assure that the system used remains stable over the time course of the assay; 9- quality control (QC) samples, to assure that the complete analytical

method, sample, preparation, extraction, clean-up, and instrumental analysis was performed according to acceptable criteria; 10- replicate and repeat analysis, determined prior to running the study and recorded in the method protocol.

Finally, Good Statistical Practice (GSP) is necessary to analyze the obtained data and to allow their interpretation.[6]

Blood level studies

These studies are the most sensitive measures of BE. They are performed when absorption of the drug is sufficient to measure drug concentration directly in the blood and systemic absorption is relevant to the drug action.[1,2,3] These studies compare a test (generic) product to a reference (pioneer) product using parameters derived from the concentrations of the drug moiety and/or its metabolites, as a function of time, in whole blood, plasma, serum (or in other appropriate biological fluids or tissues). Generally, the study should encompass the absorption, distribution, and depletion (elimination) phases of the drug concentrations *vs* time profiles.

The potency of the pioneer and generic products should be assayed prior to conducting the BE study to ensure compendial specifications are met. FDA authorities recommend that the potency of the pioneer and generic lots should differ by no more than ± 5% for dosage products[3]. The animals should be dosed according to the labeled concentration or strength of the product, rather than the assayed potency of the individual batch (i.e., the dose should not be corrected for the assayed potency of the product). The BE data or derived parameters should not be normalized to account for any potency differences between the pioneer and generic lots.

A single dose study at the highest approved dose will generally be adequate for the demonstration of BE. However, a multiple dose study may be appropriate when there are concerns regarding poorly predictable drug accumulation, (e.g., drug with nonlinear kinetics) or a drug with a narrow therapeutic window. A multiple dose study may also be needed when assay sensitivity is inadequate to permit drug quantification out to three terminal elimination half-lives beyond the time when maximum blood concentrations (CMAX) are achieved, or in cases where prolonged or delayed absorption exist.

Figure 3 exemplifies a time *vs.* plasma concentration curve. As depicted, the pivotal parameters for BE estimation on blood level studies are: CMAX, the maximal concentration or peak concentration achieved by the drugs under test in plasma; TMAX the corresponding time to reach CMAX and, area under the time *vs.* concentration curve $AUC_{(0\text{-}t)}$.

As presented in figure 3, the rate of absorption will be estimated by the maximum observed drug concentration (CMAX) and the corresponding time to reach this maximum concentration (TMAX). By another way, the extent of product bioavailability is estimated by the area under the blood or plasma concentration vs. time curve (AUC). AUC is most frequently estimated using the linear trapezoid rule. Other methods for AUC estimation may be proposed in the study protocol and should be accompanied by appropriate literature references. In the case a multiple dose BE study is used, the AUC should be calculated over one complete dosing interval $AUC_{(0\text{-}t)}$.

For a single dose BE study, AUC should be calculated from time 0 (predose) to the last sampling time associated with quantifiable drug concentration $AUC_{(0\text{- LOQ})}$. The comparison of the test and reference product value for this noninfinitive estimate provides the closest approximation of the measure of uncertainty (variance) and the relative bioavailability estimate associated with $AUC_{(0\text{- inf})}$, the full extent of product bioavailability[7]. However, because of the possibility of multifunctional absorption kinetics, AUC can not always be determined when the available drug has been completely absorbed. Therefore, it is generally accepted that the duration of sampling should be

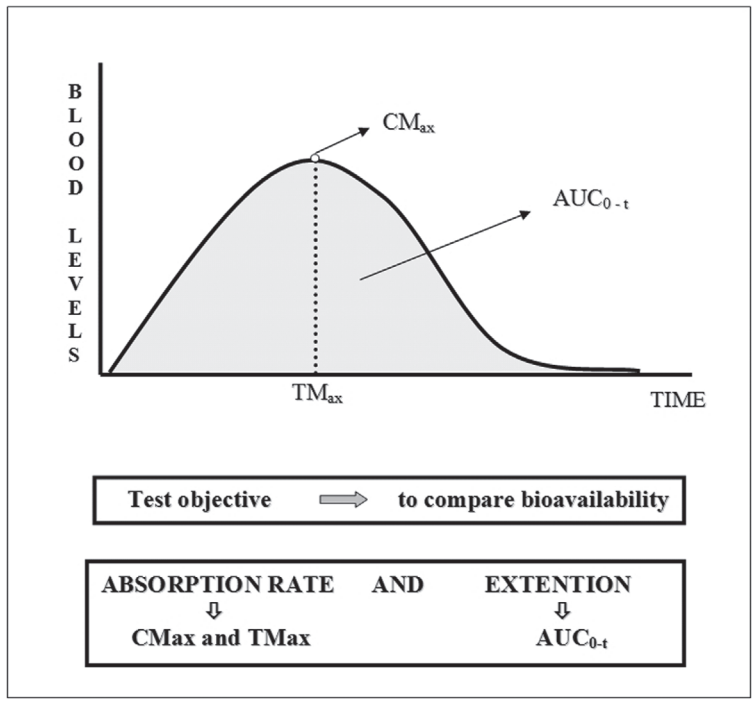

Figure 3 - Pivotal parameters analyzed in a Blood Level Study of Bioequivalence. CMax is the maximal concentration or peak concentration in the blood, TMax the corresponding time to reach CMax and $AUC_{(0-t)}$ the area under the time *vs*. concentration curve

extended until such a time that $AUC_{(0\text{-}LOQ)}/AUC_{(0\text{-}inf)} >= 0.80$. For that, the sampling times should extend to at least three multiple of the drug's apparent elimination half-life, beyond the time when CMAX are reached. Further detail on this subject might be found elsewhere.[8,9]

Other relevant pharmacokinetic measures that should also be part of BE studies (but preclude statistical comparison between the pioneer and the drug under test) are: apparent volume of distribution (*Vd*), elimination half-life ($t_{1/2}$) and depuration or clearance (D). *Vd* is a measure that links the quantity of drug within the organism (the dose administered) and its concentration in the plasma (C). The elimination half-life ($t_{1/2}$) of a drug is the time necessary to reach 50% of CMAX. Finally, the capacity of an organism to eliminate a drug is measured by its clearance that is obtained multiplying the apparent volume of distribution (*Vd*) by the drug's constant of elimination (K*e*). The following formulas might be used to calculate those values.[10]

$$Vd = \frac{\text{Dose administered}}{C} \; ; \; T_{1/2} = \frac{\log 2}{Ke} \text{ and } D = Vd \,.\, Ke$$

In general, product BE should be based upon total (free plus protein bound) concentrations of the parent drug (or metabolite, when applicable). However, if nonlinear protein binding is known to occur within the therapeutic dosing range (as determined from literature or pilot data), then both the free and total drug concentrations for the generic and pioneer products should be included. Similarly, if the drug is known to enter blood erythrocytes, the protocol should address the issue of potential nonlinearity in erythrocyte uptake of the drug administered within the labeled therapeutic dosing range.[3]

The total number of sampling times necessary to characterize the blood level profiles will depend upon the curvature of the profiles and the magnitude of variability associated with the bioavailability data (including pharmacokinetic variability, assay error and interproduct differences in absorption kinetics). Anyway, the sampling

times should adequately define peak concentration(s) and the extent of absorption. As stated, it is generally assumed that the sampling times should extend at least three terminal elimination half-lives beyond TMAX, as stated above.

Usually, maximum sampling time efficiency is achieved by conducting a pilot investigation. In this case, the pilot study should identify the general shapes of the test and reference curves, the magnitude of the difference in product profiles, and the noise associated with each blood sampling time (e.g. variability attributable to assay error and the variability between subjects, for parallel study designs, or within subjects, for crossover designs). This information should be applied to the determination of an optimum blood sampling schedule. Further information on this subject might be found elsewhere.[11]

Pilot studies are also recommended as a means of estimating the appropriate number of subjects to be used, i.e., the sample size for pivotal BE study. Furthermore, this number depends on several factors including variance of the response, differences in the two formulations and level of the rejection of the hypothesis. Estimated sample size will vary depending upon whether the data are analyzed on a log or linear scale. Useful references on sample size estimates might be found elsewhere.[6,12]

A two-period cross-over design is commonly used in blood level studies. The use of cross-over designs eliminates a major source of study variability: between subject differences in the rates of drug absorption, drug clearance, and the volume of drug distribution. Crossover design has advantages in terms of power and number of animals needed. In a typical two period cross-over design, subjects are randomly assigned to either sequence A or sequence B with the restriction that equal numbers of subjects are initially assigned to each sequence. The design should be as follows:

	Sequence A	Sequence B
Period 1	test	pioneer
Period 2	pioneer	test

However, a one-period parallel design may be preferable in the following conditions: 1- the drug induces physiological changes in the animal (e.g. liver microssomal enzyme induction); 2- the drug has a very long terminal elimination half-life; 3- the duration of the washout time for the two-crossover study is too long; and, 4- the drug follow a delayed or prolonged absorption. Anyway, when using a cross–over study, the washout period should be sufficiently long to allow the second period of the study to be applicable in the statistical analysis.

Concerning the statistical analysis, the use of 90% confidence intervals is usually taken as the best available method for evaluating BE study.[1,2,3,10,13] Thus, two products are considered to be equivalent when the 90% confidence intervals found for the reasons $AUC_{Generic}/AUC_{Pioneer}$ and $CMAX_{Generic}/CMX_{Pioneer}$ are within 80% and 120% or when generic and pioneer data are not different from each other at $P<0.05$. Further data on statistical analysis of blood level BE studies might be found elsewhere.[6,13,14]

Pharmacological end-point studies

Where the direct measurement of the rate and extent of absorption of the new animal drug in biological fluids is inappropriate or impractical, the evaluation of a pharmacologic end-point related to the labeled indications for use is normally acceptable. These studies may be conducted analyzing the physiological changes induced by the test drug and the reference or pioneer product.[2,3]

Typically the design of a pharmacologic end-point study should follow the sane general considerations as the blood level studies. However, specifics such as the number of subjects or sampling times will depend on the pharmacologic end-point monitored. The parameters to me measured will also depend upon the pharmacologic end-points and may differ from those used in blood level studies. As with blood level studies, when pharmacologic end-point studies are used to demonstrate BE, a tissue

residue study will also be required in food-producing animals.

For parameter which can be measured over time, a time *vs* effect profile is generated, and equivalence is statistically determined with a method of analysis that is essentially the same as for the blood level BE study. Thus, the use of 90% confidence intervals is usually taken as the best available method for evaluating the obtained data. For pharmacologic effects for which effects *vs* time curves can not be generated, then alternative procedures for statistical analysis should be discussed and justified in the experimental study protocol.

Clinical end-point studies

If measurement of the drug or its metabolites in blood, biological fluids or tissues is inappropriate or impractical, and there are no appropriate pharmacological end-points to monitor (e.g., most production drugs and some coccidiostats and antihelmintics), then well-controlled clinical end-point studies are acceptable for the demonstration of BE.[3] In this case, a parallel group design with three groups should be used. The groups should be a placebo (or negative control), a positive control (reference/pioneer product) and the test (generic) product. The purpose of the placebo (or negative control) is to confirm the sensitivity or validity of the study.

Some clinical end-point studies, however, may not include a placebo (or negative control) for ethical and/or practical considerations. If a placebo is omitted, then the response(s) to the test and reference products should each provide a statistically significant improvement over baseline.

Clinical end-point studies should be conducted using the target animal species, with consideration for the sex, class, body weight, age, health status, and feeding and husbandry conditions, as described on the pioneer product labeling. The dosage(s) approved for the pioneer product should be used in this study. Finally, the length of time that the study is conducted should also be consistent with the duration of use on the pioneer product labeling. As with other studies, when clinical end-point studies are used to demonstrate BE, a tissue residue depletion study will also be required in food-producing animals.

In general, the response(s) to be measured in a clinical end-point study should be based upon the labeling claims of the pioneer product. In this respect, it may not be necessary to collect data on some overlapping claims (e.g., for a feed additive which is added as the same amount per ton of feed for both growth rate and feed efficiency, data from only one of the two responses need to be collected).

Statistical analysis should be used in clinical end-point studies, to compare the test product and the reference product. However, a traditional hypothesis test should be performed before, comparing both the test and the reference products separately to the placebo (or negative control). The hypothesis test is conducted to ensure that the study has adequate sensitivity to detect differences when they actually occur. If no improvement ($a = 0.05$) is seen in parameter (i.e., the mean of the test and the mean of the reference products are each not significantly better than the mean of the placebo (or negative control), the study should be considered inadequate to evaluate BE.

Assuming that the test and reference products have been shown to be superior to the placebo (or negative control), the determination of BE is based upon the confidence interval of the difference between the two products. The use of 90% confidence intervals is usually taken as the best available method for evaluating clinical end-point data.

If the results are ordered categorical data (e.g., excellent, good, fair or poor), a non-parametric hypothesis test of no difference between test product and placebo (negative control) and between the reference product and the placebo (or negative control) should be performed. As above, if these tests result in significant differences between the test product and the negative

control and the reference product and negative control, then a non-parametric confidence interval on the difference between the test and the reference products is calculated. Another acceptable approach for categorical data is to calculate the confidence interval on the odds ratio between the test and reference products after showing that the test and the reference products are significantly better than the control. Further detail on statistical analysis of clinical end-point data might be found elsewhere.[13]

Tissue residue depletion studies

The panel on Human Food Safety at the 1993 Veterinary Drug BE Workshop addressed tissue residue depletion studies for generic animal drugs[13]. As stated many times before in the present document, the center has concluded that in addition to BE study, a tissue residue depletion study should be conducted for approval of a generic animal drug product in food producing species. Indeed, two drug products may have the same plasma disposition profile at the concentrations used to asses product BE, but may have different tissue disposition kinetics when followed out to the withdrawal time for the pioneer product. Therefore, to show the withdrawal period at which residues of the generic product will be consistent with the tolerance for the pioneer product, a tissue residue depletion study is necessary.[1,2,3] However, and importantly, for purposes of calculating a withdrawal period for a generic animal drug, only the generic product would be tested (i.e., not the pioneer product), and only the marker residue in the target tissue would be analyzed.[3]

In this respect, it should not be forgotten, that the results of a BE study or tissue depletion study in one animal species can not generally be extrapolated to another species. Possible species differences in drug partitioning or binding in tissues could magnify a small difference in the rate or extent of drug absorbed into a large difference in marker residue concentration in the target tissue. Furthermore, differences in drug metabolism and excretion are also known to exist among different food-producing animals. Therefore, for a pioneer product labeled for more than one food-producing species, a BE study and a tissue residue depletion study will generally be requested for each major food-producing species on the label.[1,2,3]

Traditional withdrawal studies, as those described in FAO/WHO *Codex alimentarius* guidelines[15] are considered the best design for collecting data for the calculation of withdrawal periods for drugs used in food-producing animals. In this respect, it was not until the 42nd JECFA meeting that specific requirement for residue data were presented.[5] The committee requested detailed reports, including individual animal data, on the followings: 1) the chemical identity and properties of the drug; 2) the use and (recommended) doses; 3) pharmacokinetic metabolic and pharmacodynamic studies in experimental and food producing animals, and in humans, where available; 4) residue depletion studies with radiolabelled drug in target animals from zero withdrawal time to periods extending beyond the recommended withdrawal time; 5) information on total residues, including free and bound residues, and major residue components to permit selection of a marker residue and a target tissue; 6) residue depletion studies with unlabeled drug for the analysis of marker residue in target animals and in eggs and milk; 7) a review of routine analytical methods that may be used by regulatory authorities for the detection of residues in target tissues; and 8) a description of the analytical procedure that was chosen by the sponsor for the detection and determination of residues in target tissues (with sensitivity equal to or less than MRL, ideally $\leq$0.5 MRL).

Usually, in these withdrawal studies, animals are divided into different groups being slaughtered at carefully preselected time points following the last (or single) administration of the test product and different matrix or target tissue samples are collected for residue analysis. A statistical

tolerance limit approach is used to determine when, with 95% confidence limits, 99% of treated animals would have tissue residues bellow the codified limits.

Figure 4 shows an example of this study; it depicts the depletion of a marker residue in a given tissue of a food-producing animal. It is relevant to note that in this curve, the Maximum Limit of Residue (MLR) is a point on the curve describing the upper one-sided 95% confidence limit while the median is the corresponding point in the regression line. In other words, the withdrawal time is correctly defined by the upper one sided 95% confidence limit.

According to international legislation[1,2,3], the generic animal drug will be assigned the withdrawal time supported by the residue depletion data, or the withdrawal time currently assigned to the pioneer product, whichever is the longer. If the generic sponsor wants to request a shorter withdrawal period for one specific product, he should provide all data necessary to support his request, including those that show no other food safety concerns for the drug are evident.

Finally, the analytical methodology used to determine the withdrawal period for the generic product does not have to be more rigorous than the approved methodology used to determine the existing withdrawal period for the pioneer product. However, it has to be fully validated and performed according to GLP and GSP standards, as stated above. Thus, if an analytical method other than the approved method is used, the generic sponsor should provide data comparing the alternate method to the approved method.

In vitro pharmaceutical equivalence

A product is considered to have pharmaceutical equivalence to a reference product if the formulation ingredients are the same and package in a size container made from identical material.[2,3] Specifically, 1- ingredients in both products should be of equivalence pharmaceutical compendial standard; 2- the active constituents should be the same substance and should be within ± 5% of that in the reference product or the dose range to the animal should be within ± 5% of the reference product; 3- the non-active constituents should be the same substances and should be within ± 5% of that in the reference product; and 4- the product should have the same physicochemical properties as the reference product.

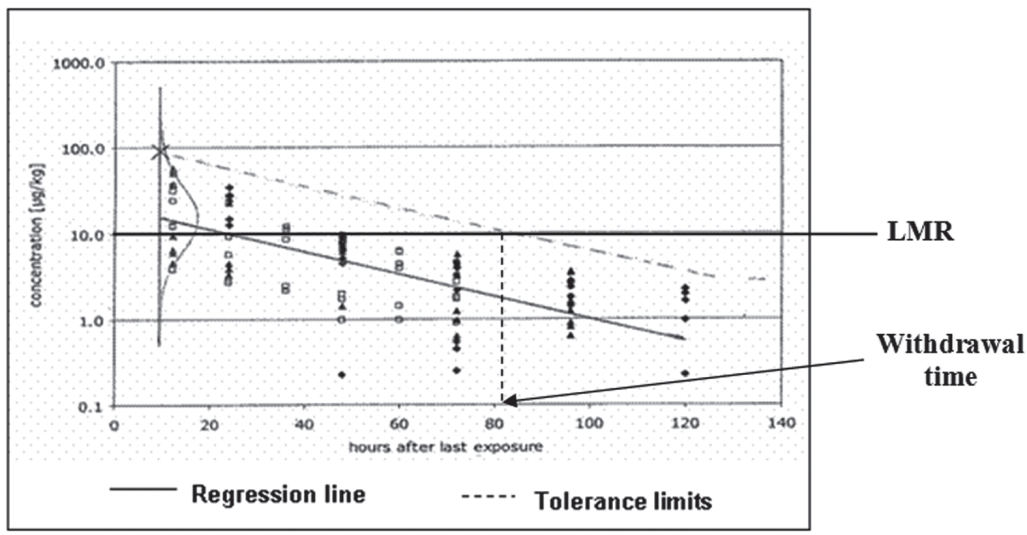

Figure 4 - Depletion of a marker residue in a given tissue of a food producing animal. Note that in curves like this one, the Maximum Limit of Residue (MRL) is a point on the curve describing the upper one-sided 95% confidence limit while the median is the corresponding point in the regression line. Note also that the withdrawal time is correctly defined by the upper one-sided 95% confidence limit

According to the EMEA guidelines for the conduct of BE studies for Veterinary Medical products[2] *in vitro* BE studies could support *in vivo* BE in the following cases: 1- *in vivo* BE has been demonstrated for the highest dosage strength and *in vitro* dissolution data is used to support the BE of the lower dosage strengths for that generic formulation; 2- *in vitro* comparability might be adequate to confirm the comparability of the reference product and its generic product to be administered orally; this applies particularly to immediate release oral dosage forms that are rapidly dissolving and contain drug substances that are highly soluble and highly permeable; 3- there is a very minor formulation change to an approved product, and it has been determined that the change requires only *in vitro* confirmation of comparability to the formulation that underwent the original trials; and, 4- ensuring batch to batch consistency within a product.

According to EMEA guidelines[2], data for *in vitro* pharmaceutical equivalence should include: 1- the nature of the dosage form, the solubility of the active constituent(s) in water; 2- the pharmaceutical characteristics of the product, including particle size, crystal forms, and dissolution profile where applicable; 3- information on rate limiting steps in absorption of the active constituent(s) e.g. disintegration, dissolution, gut absorption where applicable or in access to the site of effect; and, 4- relevant scientific argument regarding clinical consequences of inequivalence.

It should be noted, however, that the *in vitro* pharmaceutical equivalence test must be a validated predictor of the *in vivo* dissolution of the product, i.e., the *in vitro* test conditions has to have been previously related to *in vivo* conditions. An *in vitro* test cannot be used when the mean dissolution time is higher than the mean absorption time. Moreover, the longer is the dissolution time, the more difficult will be the extrapolation between the *in vitro* and *in vivo* conditions. It is therefore not recommended to perform *in vitro* test when the dissolution time is too long. On the contrary, when the process of dissolution is not the rate limiting step with respect to the rate and extent of absorption, the equivalence between the dissolution profiles of the generic and the reference products would not be required.

The experimental units collected for *in vitro* test are tablets, defined quantities of a paste, or a powder in a specified packing. These units should be collected using a sampling plan, based on a randomization procedure. This sampling procedure should be the same for the pioneer formulation and for the generic formulation, and should be representative of the entire product population. Finally, the number of batches from which experimental units are sampled for the *in vitro* test should be related to the expected variability between batches.

In vitro pharmaceutical equivalence should take into account the main sources of variation, which are likely to influence the final result: product batch, time of conservation and apparatus used in the test. Precautions to avoid bias must be taken, such as an equal repartition of units of each formulation in each analytical run.. When relevant, replicates of measures should be made, in order to take into account the variation inherent to the analytical method.

In the case of a study design comparable to the *in vivo* BE study design is relevant, the sample size should be determined to provide sufficient power in the demonstration of equivalence. The residual error (coefficient of variation) used in the calculation of the sample size should be obtained from pilot studies, or estimated from the variance reproducibility of the analytical method. These points must be documented in the experimental protocol.

In vitro pharmaceutical data to be statistically analyzed must be selected *a priori* and must be justified with regard to the correlation with the pharmacokinetic. It could be sufficient to discuss the relation between the dissolution time and the absorption rate for the products compared[2]. *In vitro* BE could be then demonstrated by comparison of dissolution profiles after

fitting to a mathematical model or by comparison of parameters like 50% dissolution time and 90% dissolution time and total amount dissolved and AUC. Statistical analysis could be comparable to the analysis used in the case of an *in vivo* BE study. However, GSP should be employed and the predetermined equivalence interval should be carefully justified. It should be kept in mind that exemptions of *in vivo* studies are only possible when results of *in vitro* studies could lead to the deduction of similar pharmacokinetic behavior between the two products compared.

Conclusion

It is indisputable. High efficiency in the field of Veterinary Medicine, particularly in animal production is achieved when new technologies are used, quality assurance methods are always an objective and, animal health products, such as veterinary medicines and feed additives, are employed. The use of animal health products in animal production however, should be done following the standards on Good Clinical Practice (GCP) on Veterinary Drugs Use. Thus, only products approved for food-producing animals use and their generic formulation (that have statistically proven BE) should be employed. However, the requirements for *in vivo* BE study may be waived for certain products. According to FDA Guidance to Industry (FDA, 2002) categories of products which may be eligible for waivers include, but are not limited to, the followings: 1- parenteral solutions intended for injection by intravenous, subcutaneous, or intramuscular routes of administration; 2- topically applied solutions intended for local therapeutic effects. 3- other topically applied dosage forms intended for therapeutic effects for non-food animals only; 4- inhalant volatile anesthetic solutions. For those waivers, *in vitro* pharmaceutical equivalence and residue depletion studies would be enough to guarantee their registration. Finally, it is felt relevant to point out again that in spite of the relevance and the common use in other countries, BE studies are still not requested for veterinary products registration in Brazil.

Estudos de bioequivalência: importância para Medicina Veterinária

Resumo

Os estudos de Bioequivalência (BE) são utilizados para a comparação de diferentes produtos farmacêuticos que contêm o mesmo princípio ativo, de diferentes lotes de um mesmo produto ou, ainda e de uma maneira ampla, de diferentes vias de administração de um mesmo medicamento. No Brasil dos dias de hoje, encontramos legislações sobre medicamentos genéricos e bioequivalência apenas na área de Medicina Humana. No campo da Medicina Veterinária, os testes de BE têm sido considerados, em muitos países, como requerimentos necessários para o registro de produtos destinados aos animais visto que eles asseguram, ao mesmo tempo, a eficácia do produto, a saúde dos animais tratados e a qualidade dos alimentos provenientes desses animais. O presente trabalho faz uma revisão crítica sobre BE. Para tanto, o assunto foi dividido em três grandes partes: 1- Entendendo a bioequivalência: importância de estudos de BE para a saúde animal e humana; 2- tipos de estudos de BE; 3- considerações gerais sobre delineamentos experimentais que envolvam estudos de bioequivalência.

Palavras-chave:
Farmacocinética.
Farmacodinâmica.
Biodisponibilidade.
Bioequivalência.
Medicamento de referência.
Medicamento genérico.

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