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WORLD HEALTH ORGANIZATION'S GUIDELINES FOR BIOEQUIVALENCE STUDIES USING PHARMACOKINETIC MEASUREMENTS

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ABSTRACT
The purpose of this study is to understand World Health Organization's guidelines for Bioequivalence studies in humans. It is important for anyone preparing a trial of a medicinal product in humans that the specific aims, problems and risks or benefits of the proposed human study be thoroughly considered and that the chosen design be scientifically sound and ethically justified. All research involving human subjects should be conducted in accordance with the ethical principles, including respect for persons, beneficence (maximize benefits and minimize harms and wrongs) and non- maleficence (do no harm).

KEYWORDS: Bioequivalence, Multisource pharmaceutical products, Pharmacokinetic parameters, Area under the Curve.

INTRODUCTION

Multisource pharmaceutical products must be shown, either directly or indirectly, to be therapeutically equivalent to the comparator product if they are to be considered interchangeable. Suitable test methods to assess equivalence are:

- Comparative Pharmacokinetic Studies in humans, in which the active pharmaceutical ingredient (API) and/or its metabolite(s) are measured as a function of time in an accessible biological fluid such as blood, plasma, serum or urine to obtain pharmacokinetic measures, such as AUC and C_{max} that are reflective of the systemic exposure;
- Comparative Pharmacodynamic Studies in humans;
- Comparative Clinical Trials; and
- Comparative *In- vitro* tests.

The present study describes the equivalence studies using pharmacokinetic measurements. Acceptance of any test procedure in the documentation of equivalence between two pharmaceutical products by a drug regulatory authority depends on many factors, including the characteristics of the API and the pharmaceutical product. Where an API produces measurable concentrations in an accessible biological fluid such as plasma, comparative pharmacokinetic studies can be performed. In vivo documentation of equivalence is needed when there is a risk that possible differences in bioavailability may result in therapeutic non equivalence[1].

MATERIALS AND METHODS

The secondary data used in the study was obtained from various official reports published by World Health Organization and internet. The study is of descriptive type and method used is the description.

Study Protocol

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol and its attachments and/or appendices should state the aim of the study and the procedures to be used, the reasons for proposing the study to be undertaken in humans, the nature and degree of any known risks, assessment methodology, criteria for acceptance of bioequivalence, the groups from which it is proposed that trial subjects be selected and the means for ensuring that they are adequately informed before they give their consent. A signed and dated study protocol together with the study report should be presented to the authorities in order to obtain the marketing authorization for the multisource product[1-3].

Study Design

Bioequivalence studies are designed to compare the in vivo performance of a multisource product with that of a comparator product. Pharmacokinetic bioequivalence studies on products designed to deliver the API for systemic exposure serve two purposes:

• As a surrogate for clinical proof of equivalence; and
• They provide an in vivo measure of pharmaceutical quality.

The design of the study should minimize the variability that is not caused by formulation effects and eliminate bias as far as possible. Test conditions should reduce variability within and between subjects. In general, for a pharmacokinetic bioequivalence study involving a multisource and a comparator product, a two- period, single- dose, cross- over study in healthy volunteers will suffice. However, in certain circumstances, an alternative, well-established and statistically appropriate study design may be adopted.

A two-period, two-sequence, single-dose, cross-over, randomized design is the first choice for pharmacokinetic bioequivalence studies. Each subject is given the multisource and the comparator product in randomized order. An adequate wash-out period should follow the administration of each product. The interval (wash-out period) between doses of each formulation should be long enough to permit the elimination of essentially all the previous dose from the body. The wash-out period should be the same for all subjects and should normally be more than five times the terminal half-life of the API. Consideration will need to be given to extending this period if active metabolites with longer half-lives are produced and under some other circumstances. For example, if the elimination rate of the product has high variability between subjects, the wash-out period may be longer to allow for the slower elimination in subjects with lower elimination rates. Just prior to administration of treatment during the second study period, blood samples are collected and assayed to determine the concentration of the API or metabolites. The minimum wash-out period should be at least seven days. The adequacy of the wash-out period can be estimated from the pre-dose concentration of the API and should be less than 5% of C_{max}[5].

SUBJECTS

Number of Subjects: The number of subjects required for a sound pharmacokinetic bioequivalence study is determined by:

- The error variance (coefficient of variation) associated with the primary parameters to be studied, as estimated from a pilot experiment, from previous studies or from published data;

- The significance level desired (5%);
- The statistical power desired;
- The mean deviation from the reference product compatible with bioequivalence and with safety and efficacy;
- The need for the 90% confidence interval around the geometric mean ratio to be within 80–125% bioequivalence limits for log transformed data.

The number of subjects to be recruited for the study should be estimated by considering the standards that must be met. It should be calculated by appropriate methods. The number of subjects recruited should always be justified by the sample-size calculation provided in the study protocol. A minimum of 12 subjects is required.

Selection of Subjects: Pharmacokinetic bioequivalence studies should generally be performed with healthy volunteers. Clear criteria for inclusion and exclusion should be stated in the study protocol. If the pharmaceutical product is intended for use in both sexes, the sponsor may wish to include both males and females in the study. The risk to women will need to be considered on an individual basis, and if necessary, they should be warned of any possible dangers to the fetus if they should become pregnant. The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study. Confirmation should be obtained by urine tests just before administration of the first and last doses of the product under study. Generally subjects should be between the ages of 18 and 55 years, and their weight should be within the normal range according to accepted life tables. The subjects should have no history of alcohol or drug abuse problems and should preferably be non-smokers. The volunteers should be screened for their suitability using standard laboratory tests, a medical history, and a physical examination. If necessary, special medical investigations may be carried out before and during studies depending on the pharmacology of the individual API being investigated, e.g. an electrocardiogram if the API has a cardiac effect. The ability of the volunteers to understand and comply with the study protocol has to be assessed. Subjects who are being or have previously been treated for any gastrointestinal problems, or convulsive, depressive or hepatic disorders, and in whom there is a risk of a recurrence during the study period, should be excluded. If the aim of the bioequivalence study is to address specific questions (e.g. bioequivalence in a special population) the selection criteria should be adjusted accordingly.

Monitoring the Health of Subjects during the Study: During the study, the health of volunteers should be monitored, so that onset of side-effects, toxicity, or any disease may be recorded and appropriate measures taken. The incidence, severity, and duration of any adverse reactions and side-effects observed during the study must be reported. The probability that an adverse effect is drug-induced is to be judged by the investigator. Health monitoring before, during and after the study must be carried out under the supervision of a qualified medical practitioner licensed in the jurisdiction in which the study is conducted[9].

Study Standardization

Standardization of study conditions is important to minimize the magnitude of variability other than in the pharmaceutical products. Standardization should cover exercise; diet; fluid intake; posture; and the restriction of the intake of alcohol, caffeine, certain fruit juices and concomitant medicines for a specified time period before and during the study[8].

Study Conduct

Selection of Dose: In bioequivalence studies the molar equivalent dose of multisource and comparator product must be used. Generally the marketed strength with the greatest sensitivity to bioequivalence assessment should be administered as a single unit. This will usually be the highest marketed strength. A higher dose (i.e. more than one dosage unit) may be employed when analytical difficulties exist. In this case the total single dose should not exceed the maximum daily dose of the dosage regimen. Alternatively, the application of area under the curve (AUC) truncated to 3 × median t_{max} of the comparator formulation would avoid problems of lack of assay sensitivity in many cases. In certain cases a study performed with a lower strength can be considered acceptable if this lower strength is chosen for reasons of safety.

Sampling Times: Blood samples should be taken at a frequency sufficient for assessing C_{max}, AUC and other parameters. Sampling points should include a pre-dose sample, at least 1– 2 points before C_{max}, 2 points around C_{max} and 3– 4 points during the elimination phase. Consequently at least seven sampling points will be necessary for estimation of the required pharmacokinetic parameters. For most medicines the number of samples necessary will be higher to compensate for between-subject differences in absorption and elimination rate and thus enable accurate determination of the maximum concentration of the API in the blood (C_{max}) and terminal elimination rate constant in all subjects. Generally, sampling should continue for long enough to ensure that 80% of the AUC (0→ infinity) can be accrued, but it is not necessary to sample for more than 72 hours. The exact duration of sample collection depends on the nature of the API and the input function from the administered dosage form.

Sample Fluids and Their Collection: Under normal circumstances blood should be the biological fluid sampled to measure the concentrations of the API. In most cases the API or its metabolites are measured in serum or plasma. If the API is excreted predominantly unchanged in the urine, urine can be sampled. The volume of each sample must be measured at the study centre, where possible immediately after collection, and included in the report. The number of samples should be sufficient to allow the estimation of pharmacokinetic parameters. However, in most cases the exclusive use of urine excretion data should be avoided as this does not allow estimation of the t_{max} and the maximum concentration. Blood samples should be processed and stored under conditions that have been shown not to cause degradation of the analytes. This can be proven by analysing duplicate quality control samples during the analytical period. Quality control samples must be prepared in the fluid of interest (e.g. plasma), including concentrations at least at the low, middle and high segments of the calibration range. The quality control samples must be stored with the study samples and analysed with each set of study samples for each analytical run. The sample collection methodology must be specified in the study protocol[3,9].

Parameters to be assessed

In bioavailability studies, the shape of and the area under the plasma concentration versus time curves are mostly used to assess rate (C_{max}, t_{max}) and extent (AUC) of absorption. Sampling points or periods should be chosen such that the concentration versus time profile is adequately defined to allow calculation of relevant parameters. For single-dose studies, the following parameters should be measured or calculated:

• Area under the plasma/serum/blood concentration–time curve from time zero to time t (AUC_{0-t}), where t is the last sampling time point with a measurable concentration of the API in the individual formulation tested. The method of calculating AUC-values should be specified. In general AUC should be calculated using the linear/log trapezoidal integration method. The exclusive use of compartmental-based parameters is not recommended.

• C_{max} is the maximum or peak concentration observed representing peak exposure of API (or metabolite) in plasma, serum or whole blood. AUC_{0-t} and C_{max} are considered to be the most relevant parameters for assessment of bioequivalence.

In addition it is recommended that the following parameters be estimated:

• Area under the plasma/serum/blood concentration–time curve from time zero to time infinity ($AUC_{0-\infty}$) representing total exposure, where $AUC_{0-\infty} = AUC_{0-t} + C_{last}/ß$; C_{last} is the last measurable drug concentration and ß is the terminal or elimination rate constant calculated according to an appropriate method;

• t_{max} is the time after administration of the drug at which C_{max} is observed.

For additional information the elimination parameters can be calculated:

• $T_{1/2}$ is the plasma (serum, whole blood) half-life.

For steady-state studies the following parameters can be calculated:

• AUC_{Γ} is AUC over one dosing interval ($_{\Gamma}$) at steady-state;

• C_{max};

• C_{min} is concentration at the end of a dosing interval;

• Peak trough fluctuation is percentage difference between C_{max} and C_{min}.

When urine samples are used, cumulative urinary recovery (Ae) and maximum urinary excretion rate are employed instead of AUC and C_{max}[5,8].

Studies of Metabolites

Generally, evaluation of pharmacokinetic bioequivalence will be based upon the measured concentrations of the parent drug released from the dosage form rather than the metabolite. The concentration–time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution and elimination. It is important to state *a priori* in the study protocol which chemical entities (pro-drug, drug (API) or metabolite) will be analysed in the samples.

In some situations it may be necessary to measure metabolite concentrations rather than those of the parent drug:

• The measurement of concentrations of therapeutically active metabolite is acceptable if the substance studied is a pro-drug.

• Measurement of a metabolite may be preferred when concentrations of the parent drug are too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time, or when the parent compound is unstable in the biological matrix.

It is important to note that measurement of one analyte, API or metabolite, carries the risk of making a type-I error (the consumer risk) to remain at the 5% level. However, if more than one of several analytes is selected retrospectively as the bioequivalence determinant, then both the consumer and producer risks change. When measuring the active metabolites wash-out period and sampling times may need to be adjusted to enable adequate characterization of the pharmacokinetic profile of the metabolite[2-4].

Statistical Analysis

The primary concern in bioequivalence assessment is to limit the risk of a false declaration of equivalence. Statistical analysis of the bioequivalence trial should demonstrate that a clinically significant difference in bioavailability between the multisource product and the comparator product is unlikely. The statistical procedures should be specified in the protocol before the data collection starts. The statistical method for testing pharmacokinetic bioequivalence is based upon the determination of the 90% confidence interval around the ratio of the log-transformed population means (multisource/comparator) for the pharmacokinetic parameters under consideration and by carrying out two one-sided tests at the 5% level of significance. To establish pharmacokinetic bioequivalence, the calculated confidence interval should fall within a preset bioequivalence limit. The procedures should lead to a decision scheme which is symmetrical with respect to the two formulations (i.e. leading to the same decision whether the multisource formulation is compared to the comparator product or the comparator product to the multisource formulation). All concentration-dependent pharmacokinetic parameters (e.g. AUC and C_{max}) should be log-transformed using either common logarithms to the base 10 or natural logarithms. The choice of common or natural logs should be consistent and should be stated in the study report. Logarithmically transformed, concentration-dependent pharmacokinetic parameters should be analysed using analysis of variance (ANOVA). Usually the ANOVA model includes the formulation, period, sequence or carry-over and subject factors.

Parametric methods, i.e. those based on normal distribution theory, are recommended for the analysis of log-transformed bioequivalence measures. The general approach is to construct a 90% confidence interval for the quantity $\mu T - \mu R$ and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits. The nature of parametric confidence intervals means that this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance. The anti- logs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the multisource and comparator products. The same procedure should be used for analysing parameters from steady state trials or cumulative urinary recovery, if required.

For t_{max} descriptive statistics should be given. If t_{max} is to be subjected to a statistical analysis this should be based on non-parametric methods and should be applied to untransformed data. A sufficient number of samples around predicted maximal concentrations should have been taken to improve the accuracy of the t_{max} estimate.

For parameters describing the elimination phase ($T_{1/2}$) only descriptive statistics should be given. Methods for identifying and handling of possible outlier data should be specified in the protocol. Medical or pharmacokinetic explanations for such observations should be sought and discussed. As outliers may be indicative of product failure, post hoc deletion of outlier values is generally discouraged. An approach to dealing with data containing outliers is to apply distribution-free (non-parametric), statistical methods. If the distribution of log-transformed data is not normal, non-parametric statistical methods can be considered. The justification of the intent to use nonparametric statistical methods should be included a priori in the protocol[6].

Acceptance Ranges

Area under the Curve - Ratio: The 90% confidence interval for this measure of relative bioavailability should lie within a bioequivalence range of 0.80– 1.25. If the therapeutic range is particularly narrow, the acceptance range may need to be reduced based on clinical justification. A larger acceptance range may be acceptable in exceptional cases if justified clinically.

C_{max} - Ratio: In general the acceptance limit 0.80– 1.25 should be applied to the C_{max} - ratio. However, this measure of relative bioavailability is inherently more variable than, for example, the AUC-ratio, and in certain cases a wider acceptance range (e.g. 0.75–1.33) may be acceptable. The range used must be defined prospectively and should be justified, taking into account safety and efficacy considerations. In exceptional cases, a simple requirement for the point estimate to fall within bioequivalence limits of 0.80–1.25 may be acceptable with appropriate justification in terms of safety and efficacy.

t_{max} - Difference*:* Statistical evaluation of t_{max} makes sense only if there is a clinically relevant claim for rapid onset of action or concerns about adverse effects. The nonparametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically relevant range. For other pharmacokinetic parameters the same considerations as outlined above will apply[10].

Reporting of Results

The report of a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with good clinical practice rules. The relevant ICH guideline can be used in the preparation of the study report. The responsible investigator(s) should sign their respective sections of the report. Names and affiliations of the responsible investigator(s), site of the study and period of its execution should be stated.

The names and batch numbers of the pharmaceutical products used in the study as well as the composition(s) of the tests product(s) should be given. Results of *in vitro* dissolution tests should be provided. In addition the applicant should submit a signed statement confirming that the test product is identical to the pharmaceutical product which is submitted for registration. The bio- analytical validation report should be attached. The bio- analytical report should include the data on calibrations and quality control samples. A representative number of chromatograms or other raw data should be included covering the whole calibration range, quality control samples and specimens from the clinical trial. All results should be presented clearly. All concentrations measured in each subject and the sampling time should be tabulated for each formulation. Tabulated results showing API concentration analyses according to analytical run (including runs excluded from further calculations, including all calibration standards and quality control samples from the respective run) should also be presented. The tabulated results should present the date of run, subject, study period, product administered (multisource or comparator) and time elapsed between drug application and blood sampling in a clear format. The procedure for calculating the parameters used (e.g. AUC) from the raw data should be stated. Any deletion of data should be justified. If results are calculated using pharmacokinetic models, the model and the computing procedure used should be justified. Individual blood concentration/ time curves should be plotted on a linear/linear and log/linear scale. All individual data and results should be given, including information on those subjects who dropped out. The drop-outs and/or withdrawn subjects should be reported and accounted for. Results of all measured and calculated pharmacokinetic parameters should be tabulated for each subject–formulation combination together with descriptive statistics. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated if necessary. If the statistical methods applied deviate from those specified in the trial protocol, the reasons for the deviations should be stated[7].

CONCLUSION

The present study describes the bioequivalence studies in humans using pharmacokinetic measurements to establish the interchangeablity of multisource pharmaceutical products. They must be shown, either directly or indirectly, to be therapeutically equivalent to the comparator product through suitable test methods. Comparative pharmacokinetic studies in humans is one of such method, in which the active pharmaceutical ingredient (API) and/or its metabolite(s) are measured as a function of time in an accessible biological fluid such as blood, plasma, serum or urine to obtain pharmacokinetic measures, such as AUC and C_{max} that are reflective of the systemic exposure. Equivalence studies using pharmacokinetic measurements are most often used to document equivalence for most orally administered pharmaceutical products for systemic exposure. Pharmacokinetic studies should be carried out in accordance with the provisions and prerequisites for a clinical trial i.e. in accordance with the ethical principles, including respect for persons, beneficence (maximize benefits and minimize harms and wrongs) and non- maleficence (do no harm).

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