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Bioequivalence: Issues and perspectives
Shubha Rani

ABSTRACT
The design, performance and evaluation of bioavailability and bioequivalence have evolved over the last two decades. Bioequivalence (BE) means the absence of a greater-than-allowable difference between the systemic bioavailability of a test product and that of a reference product. Due to efforts by academia, the pharmaceutical industry and health authorities, there is a far-reaching consensus on essential questions of bioequivalence trials which have been reflected in the many regulatory guidelines. Despite the introduction and wide acceptance of stringent requirements for bioequivalence studies, there is, however, increasing awareness that some fundamentals of bioequivalence assessment need to be reconsidered in principal. This includes several debatable issues such as practical strategies for bioequivalence of highly variable drugs, drugs with long half life, drugs under genetic polymorphic metabolism, biopharmaceuticals and endogenous substances; single bioequivalence range for all drugs; inclusion of female volunteers; individual versus average bioequivalence; metrics of absorption; etc. This article is an attempt to highlight current thinking on issues in bioequivalence.

KEY WORDS: Bioequivalence, current thinking on bioequivalence studies, issues in bioequivalence

The design, performance and evaluation of bioavailability and bioequivalence (BE) studies have received significant attention from academia, the pharmaceutical industry, and health authorities[1]-[5] over the last decade, and there has been an attempt to achieve international harmonization of regulatory requirements. According to the FDA Orange Book, test and reference products are said to be bioequivalent ‘if the rate and extent of absorption of the test drug do not show a significant difference from the rate and extent of absorption of the reference drug, when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either single dose or multiple doses.’ The International Consensus statement is: ‘two pharmaceutical products are considered to be equivalent when their bioavailabilities, from the same molar dose, are so similar that they are unlikely to produce clinically relevant differences in therapeutic and / or adverse effects.’

Approaches for testing the BE of drug formulations have been evolving over the past quarter century. Consequently, several resolved and unresolved issues pertaining to practical strategies for highly variable drug BE studies, individual vs average BE, metrics of absorption, shape analysis in single and multiple dose BE studies, statistical comparison of intra-subject variability of test and reference products in BE studies, special BE problems with biotechnology-derived drugs, problems in BE of endogenous substance and long half-life drugs, pharmacokinetic and clinical pharmacodynamic considerations in BE, and problems in BE of drugs under genetic polymorphic metabolism have been the focus of discussion and debate.

Scientific discussions between academia, the pharmaceutical industry, and health authorities were encouraged during workshops and conferences organized by the various organizations to resolve these issues.[6]-[10] Due to these efforts, there is a far-reaching consensus on the essential questions of BE trials. All these aspects have been reflected in the many regulatory guidelines. A glimpse of the statistical evolution in BE trials has been given in our earlier paper.[11] This article is an attempt to highlight the current thinking on some of the above mentioned issues.

Highly Variable Drugs
The definition of high intra-subject variability of pharmacokinetic data, proposed at the Bio-International Conference 1989 and confirmed at the one in 1992[12] is as follows: ‘Drugs that exhibit intra-subject variability of >30% (CV_{ANOVA}) are to be classified as highly variable.’ The assessment of BE for highly variable drugs has been very difficult and problematic.[12,13] One of the consequences of high intra-subject variability is that an unwieldy number of subjects may be required to provide an adequate statistical power, even when both the formulations are bioequivalent. With the common sample size of 20 in a BE study, with \( \alpha = 0.05 \) and intra-subject CV = 15%, the possibility of a true ratio of 100% (AUC or \( G_{\text{max}} \)) meeting the 90% confidence interval criterion is about 100%.
However, when intra-subject CV increases to 30% (or higher), the possibility of a true ratio of 100% (AUC or C_max) meeting the 90% confidence interval criterion (with the sample size of 20), reduces to 45% (or less); to attain only a 90% chance of passing the BE criteria for intra-subject variability of 30%, 40 subjects have to be recruited. The sample sizes become very large as the %CV increases (e.g., if the %CV is 45%, the required number of subjects to show BE is 88).[12]

At the Bio-International '92 Conference,[13] to overcome this problem, it was suggested to investigate the highly variable drug products in steady-state situations. This suggestion was the outcome of experience gained from several BE studies; e.g., verapamil was examined in a single dose vs multiple dose (replicate design) study and it was observed that coefficients of variation were 31% for AUC and 32% for C_max for the single dose study; however, it was 19% for AUC and 23% for C_max in the multiple dose study. In another study, a reduction from 26% to 18% in the coefficient of variation of AUC was observed from the multiple dose study. In another study, a reduction from 26% to 18% in the coefficient of variation of AUC was observed from the single to the multiple dose study for nifedipine.[13]

The AAPS/FDA Workshop’s (Crystal City, Arlington, VA, March 6–8, 1995) report stated that, ‘For some highly variable drugs and drug products, the bioequivalence standard should be modified by changing the BE limits while maintaining the current confidence interval at 90%.’ The intention was to scale the BE limits based on the intra-subject variance associated with the reference formulation, while maintaining the consumer risk at 5%.[16] This requires the estimation of intra-subject variation. In a test vs reference, two-period cross-over design, the residual error term includes several components:

- Intra-subject variation in ADME (with a component of assay variability)
- Intra-formulation variation (tablet-to-tablet variation)
- A subject-by-formulation interaction term
- Other random variations, not explained by the ANOVA model

If a solution of the drug is given on two occasions in a two-period cross-over design, the residual error contains no intra-formulation variations and no subject-by-formulation interaction term. Consequently, the residual error gives the intra-subject variation. Thus, a pilot study would be needed to provide estimates of intra-subject variability to scale the BE limits. The drawback of this method is that the scaled BE criterion relaxes the BE requirements for highly variable drugs but imposes more stringent BE requirements on low variability drugs with a wide therapeutic window.[17,18] The limited sampling method[19] (LSM) and metabolite assessment[20] were also suggested to determine the BE of highly variable drugs. However, severely limited utility of these methods[19,20] have been shown by researchers. Hence, there is a need for new concepts in the design and analysis of BE experiments for drugs with high intra-subject variability.

**Average vs Individual Bioequivalence**

It is well known now that two formulations are considered bioequivalent if the confidence interval of the ‘test/reference’ ratio of the geometric means (in the population) of the bioavailability characteristics under investigation lie in the BE range of 80-125%, i.e., the BE of two drug formulations is determined by the drug regulatory authorities in terms of the mean responses following administration of test and reference formulations. This definition of the BE has been termed ‘average BE.’

Anderson and Hauck[21] and Sheiner[22] defined two situations. In the first, the subject is naive to the drug and has not taken any of its formulations. Here the overall efficacy and safety of the drug is important, while it has already been established for the reference formulation. Hence the question is whether the new, test formulation can be prescribed instead of the reference formulation. This should be done only if the test formulation’s properties, on the average, are similar to those of the reference drug product. Consequently, the average BE of the two formulations is important in this instance.

In the second situation, the subject has already been taking the reference drug product. The question is whether the reference product can be substituted by the test formulation. In this situation, determination of the intra-subject variation in response to the drug is important. Individual BE is thus evaluated with this in mind.

The importance of assessing not only the average but also individual BE has recently been gaining increasing recognition. The fact that the average bioavailability of test and reference formulations is similar does not indicate that the bioavailability metrics of the test and reference products are similar in all or in most of the individuals. It has repeatedly been noted that the suitability of drug products in individuals is not assured even when the requirements of average BE are met.[21,23–26]

Indeed Anderson and Hauck,[21] as well as Endrenyi and Schulz,[27] demonstrated that a distressingly large proportion of subjects can exhibit very different responses following drug substitution. Moreover, individual BE based on replicate designs also assesses the comparability of formulations in terms of pharmaceutical quality, such that the test formulation is ‘penalized’ if the intra-subject variability of the test is more than the intra-subject variability of the reference or ‘rewarded’ if the intra-subject variability of the test is less than the intra-subject variability of the reference.[28]

Hence, assessment of individual BE seems to be an appealing and exciting alternative to the current practice of evaluating average BE. In contrast to the current average BE procedure, the proposed individual BE approach offers flexible equivalence criteria based on the individual therapeutic window and the variability of the reference drug product. The proposed criteria rewards manufacture of less variable drug products, allows scaling criteria for highly variable/ narrow therapeutic range (NTR) drugs, and promotes the use of subjects from the general population in BE studies. However, the criteria for individual BE are stricter and, therefore, more subjects would be required.[28] Currently, there is a lot of emphasis on developing a practical approach for individual BE to substitute or complement average BE.

The recent US FDA guidelines[28] state that although average BE is recommended for a comparison of BA measure in most BE studies, the individual BE may be useful in some instances. The average BE approach focuses only on the comparison of population averages of a BE measure of interest and not on the variances of the measure for the test and reference products. In contrast, the individual BE approach includes comparisons of both averages and variances of the measure.
Even if guidelines have come for individual guidelines, transition from average to individual BE requires consideration of a number of factors involving the benefit-cost to manufacturers and the benefit-risk to public health. Also, the regulatory authorities have to explore and clarify several issues before implementing the regulation of individual BE.

**Metrics for Absorption for Immediate-release Formulations**

BE is demonstrated when the rate and extent of absorption of the drug are sufficiently similar for the two formulations (test and reference) when administered under similar experimental conditions. The underlying principle is that the products should be therapeutically equivalent if the products show BE with respect to each other. The rate and extent measures thus become surrogate indicators of therapeutic outcome. The area under the plasma concentration curve is a widely used estimate of the extent of drug absorption. \(C_{\text{max}}\) and \(T_{\text{max}}\) are measures for rate of absorption. While there is consensus concerning AUC as the appropriate metric for the extent of bioavailability, there has been intensive discussion regarding \(C_{\text{max}}\) and \(T_{\text{max}}\) as measures of rate of absorption. It is well known that \(C_{\text{max}}\) has serious shortcomings as an indirect measure of rate of drug absorption.\[31-35\] Ideally, a metric should reflect specifically the investigated kinetic feature/parameter (e.g., extent/rate of absorption), be linearly related to it, and should exhibit kinetic sensitivity and, generally, statistical insensitivity. AUC reflects the extent of absorption in an ideal manner for most drugs. In contrast, \(C_{\text{max}}\) is related nonlinearly to the rate of absorption, is nonspecific to it (reflecting also the extent of absorption as well as the rates of disposition processes), and lacks kinetic sensitivity. Consequently, there are many other metrics that have been suggested for assessing the rate of absorption of the drug, such as: \(C_{\text{max}}/\text{AUC}_{0-\infty}\), \(\text{AUC}_{\text{upto } T_{\text{max}}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference or test, whichever occurs first}}\), \(\text{AUC}_{\text{upto } T_{\text{max}}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference}}\), \(\text{AUC}_{\text{upto } T_{\text{max}}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference}}\), \(\text{AUC}_{\text{upto } T_{\text{max}}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference}}\), \(C_{\text{max}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference or test, whichever occurs first}}\), and \(C_{\text{max}}/\text{AUC}_{\text{upto } T_{\text{max}}-\text{of reference}}\). The intercept parameter\[36\] as a measure of absorption rate, and early exposure\[37\] as an alternative to assessment of absorption rate by \(C_{\text{max}}\) and \(T_{\text{max}}\) was also proposed. None of the various measures of rate listed above showed consistently high sensitivity to changes in rate. Furthermore, the simulation studies showed that each rate measure has advantages and limitations that depend on the kinetic characteristic of the drug, its formulation, and its assay sensitivity. Therefore, for those drugs that offer particular problems with BE testing, simulation of BE trials could be particularly helpful to assess the applicability of various measures in the context of the specific situation. Indeed, it is of less practical importance.

Considerable time and effort have been spent to find a universal measure of rate of absorption. In general, there is no universal measure of rate of absorption. Moreover, there is no agreement on the decision criterion for \(C_{\text{max}}\). The US FDA requires the same level as for AUC (90% confidence interval between 80.00 and 125.00%). The European Union and Canada have more flexible criteria, according to the characteristic of the drug (narrow therapeutic range, etc.). In Canada, generally, there is no confidence interval on \(C_{\text{max}}\) but the ratio of log-transformed \(C_{\text{max}}\) must be in the range of 80.00 to 125.00% and, for certain complicated drugs, confidence intervals are applied.

**Long Half-life Drugs**

When conducting BE trials of drugs having long half-lives, there are two major problems: (i) unavailability of subjects for long periods as well as higher chances of dropouts with routine cross-over designs; and (ii) intra-subject variation with time. The proposed solutions for these issues are the use of parallel designs and the use of truncated areas for AUC. However, parallel group design requires more number of subjects to show BE compared to a cross-over design for the same power.\[31-35\] The use of truncated areas has been shown to be beneficial.\[32-35\] However, more research is required in this direction. Recently, the US FDA has accepted the use of AUC from 0 to 72 h in place of \(\text{AUC}_{0-\infty}\), \(\text{AUC}_{0-\infty}\), for drugs that show low intra-subject variability in distribution and clearance. The use of the LSM to determine the BE of highly variable drugs with long half-lives was also suggested.\[44\] However, Jackson showed severely limited utility of the LSM.\[19\]

**Single Bioequivalence Range and Narrow Therapeutic Range Drugs**

A single BE range for all types of drugs has been agreed upon by the regulatory authorities.\[5,8,10\] For drugs having a narrow therapeutic range, such as digoxin, dimercaprol, theophylline, and carbamazepine, this may result in a conclusion of bioequivalence when the drugs are actually bioinequivalent.\[15\] Conversely, drugs having a wide therapeutic window, such as oral antibiotics, antacids, antihistamines, vitamins, and certain analgesics, may be declared bioinequivalent based on the current single acceptance range; however, variations in pharmacokinetics may not have crucial effects on the clinical outcomes.\[46\] Even though both the situations are inappropriate, the first one is very risky.

There are many NTR drugs which may have significantly different clinical effects even if there is a small change in the dose. The FDA defines a drug as having a narrow therapeutic range if: [47]

- There is less than a two-fold difference between median lethal dose and median effective dose values
- There is less than a two-fold difference between minimum toxic concentrations and minimum effective concentrations in the blood

With NTR drugs, the therapeutic drug levels are not much different from the drug levels that produce no effect or toxic effects. Therefore, considerably small changes in the drug levels can lead to marked change in pharmacodynamic effects. Consequently, careful titration and patient monitoring is required for safe and effective use of the drug products. Hence, current FDA BE evaluation guidelines may not be appropriate for the assessment of NTR drugs because a 20-25% acceptable difference in bioavailability would alter the therapeutic effects of these drugs.\[48\] A number of studies to examine whether the various levothyroxine (an antithyroid drug) formulations are in fact bioequivalent or therapeutically equivalent ended in controversy, with some investigators concluding that the formulations they tested were therapeutically equivalent and others pointing out the high rates of abnormal TSH values in
patients who were switched from one formulation to another.\textsuperscript{[45-46]}
Even the major thyroid associations, the American Thyroid Association, the Endocrine Society, and the American Association of Clinical Endocrinologists, state that patients could be harmed medically if they were forced to switch from one brand to another without appropriate monitoring and, when needed, dose adjustment; they also warned that even small differences between doses...can have major clinical implications for thyroid patients, for example, causing atrial fibrillation, osteoporosis, or uncontrolled hypercholesterolemia.\textsuperscript{[55,56]}

Therefore, many researchers feel that BE criteria should be drug specific. Current guidelines for establishing BE were viewed as possibly being not appropriate for NTR drugs. The US FDA\textsuperscript{[1]} guidance recommends that sponsors and/or applicants should contact the appropriate review division to determine whether a drug should or should not be considered to have a narrow therapeutic range, and sponsors should consider additional testing and/or controls to ensure the quality of such drug products. However, this guidance recommends that the traditional BE limit of 80-125\% remain unchanged for the bioavailability measures (AUC and $C_{\text{max}}$) of NTR drugs, unless otherwise indicated by a specific guidance. Recently, the European regulatory authorities issued a document entitled "Questions and Answers on the Bioavailability and Bioequivalence Guideline,"\textsuperscript{[7]} which says that in specific cases, e.g., when there is a narrow therapeutic range, the acceptance interval may need to be tightened.

**Endogenous Substances**

A range of problems usually arises in pharmacokinetic studies on endogenous substances. These possess specific rhythms in baselines, fluctuation in baseline values, and important gender differences in baseline values.\textsuperscript{[58]} Hence a bioavailability study performed for endogenous substances requires baseline subtraction and, consequently, baseline evaluation before dosing, which should cover a similar period during which blood is withdrawn after dosing.\textsuperscript{[59]} There are also many other issues; for example:
- Increased data dispersion is encountered.
- A period effect can be encountered, even in the baseline, which is not attributable to periods but to subjects.
- Extrapolation of AUC to infinity is not possible as the end point is not zero but the baseline, together with its fluctuations.

Besides this, there are other problems. In certain cases endogenous synthesis may be inhibited by exogenous doses, which further complicates the assessment of bioavailability. When net AUC is less than 20\% of baseline values, the recommended statistical procedures fail to demonstrate BE. Marzo\textsuperscript{[59]} suggested that clinical trials be conducted or the appropriate statistical methods be used in such situations. Also, it is recommended that in these cases protocols must be tailored on a case-by-case basis.

**Genetic Polymorphism**

It is well known now that several drugs are subject to genetic polymorphic metabolism.\textsuperscript{[60]} With drugs under genetic polymorphic metabolism, different half-life values in poor metabolizers (PMs), extensive metabolizers (EMs), and ultra-rapid metabolizers (UMs) are produced. Higher values of plasma concentrations and half-life are encountered when a PM is investigated, which, in the extrapolation procedure of AUC to infinity, could lead to adding more than the 20\% accepted by guidelines.\textsuperscript{[61]} To overcome these problems, some solutions are suggested: First, prior screening of subjects could identify PMs and UMs, who should not be used in the trial. However, this approach, albeit mandatory for steady-state studies, is time-consuming.\textsuperscript{[59]} Second, we can investigate drugs that undergo polymorphic metabolism using a larger number of subjects so that a few enrolled PMs or UM could be dropped when the statistical methods are applied.\textsuperscript{[59]} Third, a common situation is that an investigation is performed in a given number of subjects, for instance in 24 subjects. Data results in bioequivalence and it has been statistically proven that not fewer than 30 subjects are required to assess BE. In this case a separate design on an additional 12 subjects can be conducted with the same protocol and the results of the two trials could be combined, which would solve the problem. A prior pilot study on 6-12 subjects to evaluate the number of subjects statistically required is another solution. Whatever the case may be, these procedures involve high costs. The existing guidelines do not provide any specific solutions to these possible problems.

**Use of Healthy Subjects**

Homogeneous groups of healthy subjects are usually enrolled in BE studies to avoid the inter-subject variability. Actually, the use of normal healthy subjects minimizes the chances of bioinequivalence due to changes in the disease process over time rather than differences in the formulations.\textsuperscript{[62]} These healthy subjects are adult males in the age group of 18–55 years, with height and weight in the normal range, nonsmokers, and without any concomitant medication. The information obtained from studies on such subjects is extrapolated for the patients. Pharmacokinetic behavior of a drug in healthy subjects may not, however, predict the pharmacokinetics in patients. The pharmacokinetics in patients may be influenced by many factors, such as disease condition, differences in first-pass metabolism, diet, the influence of fed vs fasted state conditions, and gastrointestinal factors such as gastric pH, blood flow, and bacterial flora. Formulations that are bioequivalent in healthy subjects, may be bioinequivalent in patients.\textsuperscript{[63]} Therefore, caution is recommended when considering the generic substitution of drugs with high inter-subject variability or with differing pharmacokinetic properties in different patient groups.\textsuperscript{[84]}

There is an ethical issue also against investigations using healthy subjects for drugs that cause serious adverse events.\textsuperscript{[60]} A common case is the dose-repeated regimen with extended-release formulations which is ethically debatable with several drugs, e.g., those acting on the central nervous system and antiarrhythmics. The suggested option in these cases is to use the target population, but even here other major problems arise, such as the use of concomitant medications by the subjects, high incidence of dropouts, and difficulty in completing the study in a reasonable time period, etc. This will end up in high cost and longer time period. Consequently, we do not perceive any alternative to this issue.
Single vs Multiple Dose Studies

In general, BE evaluation is done for single dose studies only. At present, regulatory authorities recommend multiple dose studies in some specific instances, including controlled/modified release formulations, when the drug’s pharmacokinetics is dose dependent or when the drug has large intra-subject or inter-subject variability in pharmacokinetics.[66]

Based on the results of single dose studies only, we presume the BE of the formulations after multiple doses. However, there are many concerns over this practice. First, a single dose study does not give any idea of the drug/metabolite accumulation after multiple doses, which may result in more adverse events.[67] Second, Elkoshi et al.[68] found that two formulations of omeprazole sodium were bioinequivalent because of a difference in the quality of their enteric coating. This observed difference was more prominent after multiple dosing. This implies that multiple dose studies are more sensitive in BE testing of enteric-coated formulations. Third, most of the drugs are administered for long periods, and maintenance of the steady-state concentration is necessary for the desired therapeutic effects. Moreover, the steady-state drug concentration is often comparatively higher than the concentration after a single dose. It has been indicated that apparently inert compounds used in the preparation of formulations may influence the absorption, distribution, and metabolism of the drug at steady-state, whereas the difference may not be noticeable following a single dose.[69]

On the other hand, multiple dose studies have their own drawbacks. Drugs having long half-lives and drugs producing autoinduction require longer period to achieve steady-state. Antiarrhythmic agents and drugs acting on the central nervous system cannot ethically be given to normal healthy subjects in a multiple dose regimen because of safety concerns.[70]

Measurement of Metabolite in Bioequivalence

Metabolites play an important role in drug development. According to the US Code of Federal Regulations (section 320.26, 1982), guidelines on the design of a single dose bioavailability study state that, 'The peak concentration and total area under the curve for the active drug ingredient or its metabolite in the blood should be measured to establish bioavailability.' By the late 1980s, questions were being raised whether the metabolites should be measured in BE studies as is being determined in bioavailability studies.[69] At the Bio-International (I) scientific meeting (1989), there was disagreement among the scientific community on the issue of measuring the metabolites in BE studies. Chen and Jackson[69] concluded that metabolites should not be used to determine BE as they are not sensitive to changes in drug absorption. The conclusion from the Bio-International (II) (1992) conference was that the decision to include metabolite data should be made on a case-by-case basis.[70] It was also suggested that regulatory agencies be consulted at the time of protocol preparation. As the parent compound is most sensitive to differences in the formulations, it is now accepted by the researchers that the BE studies should be solely based on the parent drug if the following conditions are met: (i) The active metabolite(s) concentration time profile is, at all time points, less than, say, 10% of the comparable value of the parent drug; (ii) The type of pharmacologic or toxic responses produced by the parent drug and active metabolite are substantially similar; (iii) It has been clearly established that all the pharmacokinetic processes that govern plasma concentration of the parent drug and active metabolite are linear at all plasma concentrations likely to occur when the drug is used clinically; and (iv) The drug delivery system is not designed to be an extended-release product. In the following situations, it is preferred to measure the metabolite: (i) when a prodrug is administered; (ii) if the concentration of the parent drug in blood is too small to quantitate; (iii) if the parent compound is unstable; (iv) if the half-life of the parent compound is very short.[70]

In the 3rd Bio-International Conference, it was still not possible to come to a conclusion regarding metabolite measurement as there was not enough data to give general guidelines on the inclusion of metabolite data for BE.[71]

Despite the varying opinions on metabolite measurement, the regulatory authorities— Health Protection Branch, Canada,[10] European Agency for Evaluation of Medicinal Products,[72] and the US FDA[5]— agree that in most cases the BE assessment can be based solely on the parent compound, though in some cases the metabolite measurement could be required.

Inclusion of Female Subjects in Bioequivalence Studies

Gender-related differences in pharmacokinetics are apparent in many drugs studied. Theoretically, the sex-related differences in pH, motility, transit time or differential metabolism/degradation between formulations in the gastrointestinal tract may affect the pharmacokinetics of the formulations for orally administered drugs.[73–76] Through an exploratory analysis, Chen et al.[77] showed that substantial and significant sex-by-formulation interactions can occur in BE studies. Although the FDA (in 1998)[78] instructed that new drug applications must provide data on safety and efficacy by sex, an investigation by the US General Accounting Office in 2001 revealed that more than one-third of the FDA approved drugs did not include the required information. Despite the fact that women are involved in the clinical trials of new drugs, failure to analyze sex-related differences in pharmacokinetics limits the generalizability of such data. The question is whether women should be included in BE trials? The US FDA has already recommended the use of female subjects in the BE trials whenever there are chances of sex-related pharmacokinetics.[79]

Bioequivalence of Biopharmaceuticals

Biotechnology pharmaceuticals are typically manufactured using biological processes, e.g., using living cells to produce proteins from inserted DNA. In the past two decades, an increasing number of biopharmaceuticals produced by genetically modified cells have entered the market. During biopharmaceuticals development, they have been re-engineered at the DNA level to meet the needs of high-level production in foreign hosts. As a result, the biochemical and biophysical characteristics of these molecules is inextricably intertwined with the processes of production and purification. Consequently, the properties of biotechnology products are highly dependent on the production process and their biological behavior remains partly unpredictable. This is the classic ‘product, process,
specifications’ paradigm, often shortened to ‘process = product’. An example is the recombinant EPO, which was prescribed to millions of patients without major problems. Later, an epidemic of pure red-cell aplasia (PRCA) was identified, which was linked to antibodies that are induced by a specific recombinant EPO product. Affected patients survive only by frequent blood transfusions and, although antibody levels decrease when the treatment is stopped, about 50% of the patients remain transfusion dependent. The epidemiological data indicate that there may be many factors responsible for the development of PRCA, such as use of ex-US formulation of EPO-α, storage and handling account of EPO-α, and subcutaneous administration of EPO.

The available analytical methods do not allow a full prediction of the biological and clinical properties of a biopharmaceutical. We lack the technology to establish whether the structures of two biopharmaceuticals are completely identical. However, advances in analytical methodology are at a pace to meet the biotechnology research and regulatory requirements. The example of EPO cited above shows that a full clinical evaluation is essential to show equivalence. In the near future, the patients of some biopharmaceuticals will expire and consequently, new generics will come into the market. It is inconceivable at present to manufacture a biopharmaceutical that can be shown to be therapeutically equivalent to another product, other than by extensive clinical comparisons. In addition, a commitment to post-marketing surveillance should be mandatory before marketing authorization is granted.

The question for regulatory agencies is how to establish pharmaceutical and therapeutic equivalence of biologics. Assurance of pharmaceutical equivalence and BE need not necessarily be assurance of efficacy of biologics. Thus, other measures of efficacy such as biomarkers and/or surrogate markers may have to be included in the final equation concerning the regulatory approval of generic biologics. The FDA had held workshops to discuss the scientific and technical issues surrounding follow-on proteins in 2004 and 2005. A background white paper and draft guidance were to follow but have not so far been seen. The guideline on similar biological medicinal products was discussed by the Committee for Proprietary Medicinal Product (CPMP) in June 2004 and released for consultation in November 2004, before coming into effect on 30th October, 2005. Later, several of the supporting guidelines came into effect on 1st June, 2006.

Conclusions

Despite the introduction and wide acceptance of the stringent requirements for BE studies, there is, nevertheless, an increasing awareness that some of the fundamentals of BE assessment need to be reconsidered in principal. In general, there is concordance for the proposed BE approach; however, the use of the recommended procedure for BE may require further examination of the issues discussed above. For example, equivalence of average bioavailability may not be sufficient to provide reasonable assurance that an individual patient could be switched from a therapeutically successful reference formulation to a generic substitute. According to the present guidelines, BE is achieved if only $C_{\text{max}}$ and AUC values for both products are in agreement, i.e., the shape of the plasma concentration–time curve does not have to be similar. For certain drugs, e.g., some cardiovascular agents, even under the steady-state conditions, the rate of release is important and from the therapeutic viewpoint, the specific timing of the peak concentration may influence the therapeutic outcome. In addition, very specific and sensitive surrogate markers for rate of absorption are not always available. In addition to the total AUC and $C_{\text{max}}$, a comparison of the plasma concentration–time profile during the absorption phase may be useful in these cases. For drugs with longer $t_{1/2}$, the requirement to follow plasma concentration for more than 3.3 of $t_{1/2}$ is impractical. It has been shown that data generated from a certain segment of the area under the plasma concentration–time curve may provide the necessary information with accuracy and certainty. A single BE criterion, such as the 0.80-1.25 equivalence range, which at present is applicable to all drugs, does not necessarily reflect the therapeutic range or the intra-subject coefficient of variation of the investigated drug. BE criteria are not very clear for endogenous substances. The current BE criteria may conclude bioinequivalence even if the products are bioequivalent for highly variable drugs.

In addition, clinicians are increasingly encouraged by health care decision makers to help control health care expenses by prescribing generic medications. Also, consumers have the habit of switching from one brand to another, especially if it is cheaper. According to a US Congressional Budget Office estimate, in 1994 alone, consumers saved US$ 8-10 billion on prescription drugs by buying generic drugs instead of their branded counterparts. Therefore, it is the responsibility of researchers and regulatory authorities to ensure that the issues in BE evaluation should be resolved on a priority basis.

There is a need to implement an improved method for BE, so that clinicians are more confident about the BE when substituting one drug for another. Finally, it is important that regulators, the industry, and professionals harmonize in their acceptance of BE, especially in light of the challenges to be faced with the rising understanding of the problems associated with the current criteria for BE. As discussed in our earlier paper, the concepts for design and interpretation of BE studies have evolved during the last 25 years and there is no reason to believe that this process of progress is yet complete. We may expect further modification of our procedures.

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