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Workshop Report

Bioavailability and Bioequivalence: Focus on Physiological Factors and Variability

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Abstract. This is a summary report of the EUFEPS & COST B25 conference on Bioavailability and Bioequivalence which focused on physiological factors and variability. This conference was held at The Royal Olympic Hotel in the centre of Athens (Greece) during the 1–2 of October in 2007. The issues discussed in the conference involved physiological factors affecting drug absorption, the role of pre-systemic effects on bioavailability (BA), the impact of variability in bioequivalence (BE) studies, and a final closing panel session on unresolved issues in BA/BE regulations. Several important aspects of drug absorption were highlighted. It was presented how the complexity of gastrointestinal (GI) physiology and the site dependent absorption can impact on drug BA. Similarly, the effects of food and formulation were also studied. The second session focused on integrating the complexities of GI into modeling the inter-individual variability of absorption and the prediction of first-pass metabolism from *in-vitro* data. The necessity to measure metabolites, the value of Biopharmaceutical Classification System (BCS), and the more recently proposed Biopharmaceutical Drug Disposition Classification System (BDDCS) were assessed as well. This session closed with presentations of pharmacokinetic software delegates. In the second day of the conference, the problem of high intra-subject variability in BE studies was analyzed. Study design considerations, the use of multiple-dose studies and the role of statistics in BE were also highlighted. Finally, the current thinking of regulatory authorities (EMEA and US-FDA) was presented. The conference closed with a last session on unresolved issues in the regulatory level.

KEY WORDS: bioavailability and bioequivalence; highly variable drugs; impact of variability on BE studies; physiological factors affecting drug absorption.

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INTRODUCTION

Drug transit through the body is a composite procedure arising from the complexity and the diversity of interactions between the drug, physiological mechanisms and various exogenous factors. Accordingly, the relationship between drug intake and clinical response is considered highly complex and is potentially affected by intrinsic and extrinsic variables. A major cause for deviations of drugs' responses can be ascribed to product bioavailability (BA), namely the rate and extent of drug absorption.

Various sporadic *in vivo* observations in the 1950s and 1960s raised the first intimations of bioequivalence (BE) with multi-source drug products (1–7). It was realized that product efficacy depends on the proportion of the drug which is ultimately absorbed (extent of absorption) from its formulation and how rapidly the drug absorption is being held (rate of absorption). Thus, the two key terms, extent and rate of absorption, formed the basis of BA and BE testing. Since then BE assessment relies on the assumption that the therapeutic effect of a drug product is a function of concentration of the active moiety in the systemic circulation.

Both BA and BE exert a critical role in drug development and regulatory context. Aim of this conference was: (a) to provide an insight into the physiological factors that can influence drug absorption, (b) to address the role of pre-systemic effects on BA, and (c) to assess the impact of variability in BE studies.

The conference consisted of four sessions. The three first sessions were in accordance with the objectives quoted above, while there was also a final discussion session on unresolved issues in BA/BE regulations.

PHYSIOLOGICAL FACTORS AFFECTING DRUG ABSORPTION

Drug absorption is influenced by the physicochemical properties of the drug itself, the formulation effects, and several physiological factors. These physiological factors include gastric emptying, intestinal motility, blood flow rate, gastrointestinal pH, and first pass metabolism. In addition, regional differences in GI physiology amplify the potential to affect drug absorption processes. Changes to splanchnic blood flow and bile secretion may lead to different first-pass metabolism of the drugs and bile-salt solubilisation of lipophilic drugs, respectively (8).

Physiological factors are subject to species variation and hence differences in oral BA may be the result of differences in the physiological factors mentioned above. The existence of transporter proteins can potentially contribute to the observed variability.

In addition, coadministration of drugs with meals or other drugs may alter GI physiological conditions and influence drug absorption. Aging and GI disease states may lead to alterations in GI physiology and physiological response, resulting in further changes in the extent and rate of drug absorption.

Complexity of GI Physiology and Impact on Drug Absorption

The first session started with the presentation of Dr. C. Wilson (Strathclyde Institute, UK) who underlined the complexity of the GI physiology and its impact on drug absorption. He discussed the impact of changing posture or meal intake on the pulses of gastric emptying which in turn leads to high variability in drug exposure. It was shown that stomach is not homogenous and that the swollen objects or dosage forms can alter gastric emptying

Special emphasis was placed on the application of investigational tools such as triggered capsules or imaging techniques (e.g. MRI) which assist in mapping the absorption of a drug and the disintegration of formulations. Accordingly, this can lead to the development of appropriate *in vitro–in vivo* correlations. It was shown that factors such as circadian rhythm, time of dosing and meal intake control entry of drugs into the colon and the colonic residence. The relative colon transit of a pellet and a tablet given together was also studied using radio-labelling techniques. Administered to a fasted volunteer, the two preparations empty at around the same time but in the colon the tablet is treated as a consolidated mass and is propulsed ahead of the pellets.

In addition, the use of "steady state" conditions allowed the study of drug residence in various parts of the gut. A marked difference between the contents of right and left sides was present for steady-state studies. Besides, this difference is exaggerated in case of pathological conditions like active left-sided colitis. Overall, it was concluded that understanding of gastrointestinal physiology as well as knowledge of disease processes are necessary factors to explain variability in drug absorption.

Site Dependent Drug Absorption and Impact on Drug Absorption

Dr. T. Gramatté (SocraTec, DE) described how site dependent drug absorption can impact on drug absorption. It was demonstrated that the use of various methods to investigate regional intestinal drug absorption was shown. In indirect approaches, absorption from the intestinal lumen is estimated indirectly by following the appearance of the drug within the systemic circulation. Besides, the direct approach relies on the measurement of drug from the intestinal lumen and can also be combined with the simultaneous measurement of plasma concentrations of drug. Intestinal perfusion studies with several drugs (e.g. ranitidine) demonstrated that absorption can vary depending on the site of perfusion.

It was concluded that for small hydrophilic drugs (like paracetamol) and substances with sufficient lipophilicity there was a uniform and efficient mucosal permeation along the entire small intestine. However, a reduction of drug absorption from distal parts is prominent for high hydrophilic drugs. In these cases a sharp decrease, along quite relatively short distances of the small intestine, in AUC values was present. According to the intestinal perfusion studies it was noted that day-to-day variations of segmental intestinal transit times and regional fluid fluxes can have a significant impact on net drug absorption and contribute to high intra-subject variability.

Impact of Food on Drug Absorption

The presence of food in the gastrointestinal (GI) tract can significantly alter the oral BA of drugs due to changes in the rate and/or extent of absorption (9–11). These changes can further lead to variations in efficacy and toxicity profiles because medications are often taken under conditions of varying food and fluid intake.

Notwithstanding the physical and chemical interactions that may occur between drugs and specific food components, altered postprandial absorption is generally a function the changes associated with conversion from the fasted to the fed state (12,13). Changes due to (a) secretion of gastric acid and bile and pancreatic fluids, (b) modification of gastric and intestinal motility patterns, and (c) alterations in visceral blood and lymph flow have the most significant impact on absorption.

The impact of food on drug absorption was addressed by Dr. W. Weitschies (University of Greifswald). Several examples of drugs were used to demonstrate how food intake influences GI-physiology. Using, magnetic marker monitoring very impressive images of *in vivo* results were presented. For example, it was illustrated the different position of extended release amoxicillin-clavulanic acid tablets in the stomach depending on whether the tablet was administered fasting, after a first bite of a breakfast, and after the breakfast. Other results included monitoring of drug serum concentration after ingestion of a meal, recording of the frequency pattern of tablet movements in the stomach and the distribution of the capsules in the GI-tract. These techniques led to some findings such that the storage function of the stomach, the retention of large objects in the stomach (gastric-sieving), and the gastro-ileocecal reflex (i.e., the existence of jet propulsion into the colon).

Formulation Effects on Drug Absorption

The formulation factors that may impact on BA and BE can be classified into two categories (14): (a) In the first group belong factors that can affect drug dissolution or release which is considered as a prerequisite to the drug absorption process. (b) The second category comprises factors related to excipients or inactive ingredients which can influence drug stability, absorption, and metabolism.

A presentation about formulation effects on drug absorption was given by Dr. S. Stockbroeckx (Johnson & Johnson). A pharmaceutically stable formulation is required in order to avoid downstream issues such as polymorphic conversions. The beneficial properties of a drug product include enhanced solubility, stability and BA. Other issues comprise the ability to reduce odors or tastes, stabilize flavors, reduce stomach injury, and to minimize evaporation.

The current trend in drug development is summarized in the findings that drug candidates are obtaining higher molecular weight, become less soluble (more lipophilic), and require more sophisticated drug formulation technologies. These technologies can be divided into two categories. The first incorporates the "classical" (or Noyes–Whitney based) strategies such as: use surfactants to alter the wetability/dispersability, increase the surface area of the drug by reducing (micronizing) particle size and change the salt form to increase the saturation solubility of the drug. In the second category belong methods such as the preparation of solid dispersions, the use of complexation and nanosizing.

Of special interest is particle size reduction which increases the surface area and consequently dissolution rate. Cavitation is believed to be the main cause of size reduction. Common methods for reducing particle size include micronization and nanonization. Micronization can be completed by using traditional milling techniques (such as dry, wet and air milling) as well as with other particle sizing approaches like supercritical fluid processing. The nanosizing method usually requires special techniques to reduce aggregation and includes wet milling and high pressure homogenization.

ROLE OF PRE-SYSTEMIC EFFECTS ON BIOAVAILABILITY

The second session of the conference involved presentations which described the role of pre-systemic effects on BA. Drug absorption from the gastrointestinal tract is a very complex and often not well characterized procedure. Both extent and rate of drug absorption may be affected by many factors which can be divided into three categories: The first group comprises physicochemical factors such as pK_a, aqueous solubility, stability, diffusivity, lipophilicity, salts, surface area, particle size and crystal form. The second category includes physiological factors like intestinal blood flow, gastrointestinal pH, gastric emptying, intestinal transit time, and absorption mechanisms. The last category contains formulation factors, namely, tablet, capsule, suspension, etc (15,16).

In order to face-off this complexity several models have been proposed (14,17–19). The first approach was pH-partition hypothesis which was later used as a guideline for the prediction of drug absorption. According to this hypothesis, ionizable compounds diffuse through biological membranes primarily in their un-ionized forms. Hence, the degree of ionization limits the extent of absorption of drug compounds across lipid membranes. However, the pH-partition hypothesis is an oversimplification which does not consider drug solubility. To face this problem, the absorption potential (AP) concept was proposed. The AP is a predictor of the extent of absorption and can be calculated from a simple equation which comprises the partition coefficient, solubility, and the dose of the drug. Some years later, the more complex dispersion models have appeared. However, due to their complexity these model did not exhibit a wide spread and were followed by the much simpler mixing tank model. More recently, the mass-balance approach was introduced which correlates membrane permeability with the extent of drug absorption. Due to the fact that the underlying assumption of this approach is steady-state conditions, the rate of drug absorption cannot be estimated. In order to face-off this demerit the Compartmental Absorption and Transit (CAT) model has been proposed which allows the prediction of both the extent and rate of absorption. The Advanced Dissolution, Absorption and Metabolism (ADAM) model represents a mechanistic representation incorporating the main factors that may impact on the rate and extent of drug absorption such as: dissolution, region-specific gut wall permeability and metabolism, transport effects, physiological factors (e.g., gastric emptying, intestinal transit times, distribution of P450 enzymes in gut wall etc), effect food, and algorithms to incorporate variability in drug absorption processes (20).

Integrating the complexities of physiology and biology of GI tract into modeling the inter-individual variability of oral drug absorption

The session regarding the role of pre-systemic effects on BA started with the presentation of Dr. A. Rostami-Hodjegan (University of Sheffield, UK). He focused on the integration of physiology and biology encountered in GI tract into modeling the inter-individual variability of drug absorption. Several examples were presented which clearly demonstrated both the progress achieved so far and the limitations that are still present. Knowledge of the variability of the biological systems is necessary to develop useful models. Thus, it is of crucial importance to identify the variables that contribute significantly in the process of drug absorption which will allow reliable predictions of drug absorption. Therefore integrated gastrointestinal physiological and pharmacokinetic mechanistic models take into account variables pertinent to physicochemical/pharmaceutical issues (e.g. disintegration, dissolution, solubility etc), physiology/pathophysiology (e.g., gastric emptying, intestinal mobility etc), fluid dynamics in the GI-tract, fluid secretion rate and transit time in stomach and small intestine/colon etc.

However, there are still limitations to the models developed so far. For example, a reliable prediction of the extent of intestinal first-pass drug metabolism from *in vitro* data is still challenging as the current models do not yet fully accommodate the additional complexities from gradients of enzymes and drug transporters in the gut.

As a conclusion, it was underlined that the development of such models should be in accordance with the general principle that everything should be made as simple as possible, but not simpler.

Assessment of intestinal and hepatic first-pass: Necessity to measure metabolites? Pre-systemic elimination may occur when orally administered drugs are subject to metabolism during their

passage from the gut lumen to the systemic circulation. Intestine and liver are the organs that may be potentially involved. In the case of the gut and the liver, the phenomenon is a result from the anatomical arrangement of the splanchnic circulation which allows these organs to act as a barrier. The role of metabolites in BE assessment has been a controversial issue for many years (21–25). One of the major concerns raised for metabolites is the relative variability between the measured plasma concentrations of parent drug and metabolite.

There are several situations where metabolite data are considered in BE studies. Firstly, metabolite data may be used when parent drug is an inactive compound whereas the metabolite exerts significant pharmacological activity. Another situation for the use of metabolites arises when the serum concentration of the parent drug is relatively low to allow a reliable measurement. Additionally, in case of highly variable drugs the use of metabolite data is suggested to be a possible alternative.

The presentation of Dr. H. Blume (SocraTec, DE) focused on the problem of assessing metabolites in BE studies. It was highlighted the long debate which results in different regulatory requirements in FDA and EMEA. Though, it is generally approved that metabolite levels should be measured in BA studies, there is no general consensus in the use of metabolites for the assessment of BE. For bioequivalence studies determination of drug metabolites usually applies in case of non-linear pharmacokinetics or when the metabolites are formed pre-systemically and contribute significantly to efficacy or safety issues.

Prediction of Intestinal First-pass Drug Metabolism From *In-vitro* Data

Intestinal first-pass effect is generally not regarded to contribute significantly to drug metabolism. However, the importance of first pass biotransformation can be clearly considered since gut is the most important extrahepatic site of drug metabolism and comprises both phase I and II enzymes (including many CYP enzymes).

In the presentation of Dr. G. Tucker (University of Sheffield, UK), the objective was the prediction of intestinal first-pass drug metabolism from *in vitro* data by applying "minimal" models, namely models which consider the intestine as a single compartment. The models which incorporate intestinal drug absorption can be classified into two categories: (a) the full physiologically based pharmacokinetic models, and (b) the "minimal" models. The first category comprises the segmented segregated flow model (26), the ACAT model (27) and the ADAM model used in Simcyp (20). The focus of was on the "minimal" models. Two models were compared for a set of several CYP substrates; the "well-stirred" gut model and the "Q_{Gut}" model. The simple "well-stirred" gut model was unable to reflect the metabolism–permeability interplay and led to poor predictions of intestinal first-pass metabolism. However, incorporating the feature of permeability interplay, the "Q_{Gut}" showed an improvement in predictability, although several sources were used to estimate permeability clearance. Overall, a more reliable prediction requires the application of more sophisticated models such as ACAT and ADAM.

BCS and BDDCS

The presentation of Dr. L. Benet (UCSF, USA) highlighted the value of having simple models such as the Biopharmaceutical Classification System (BCS) and the more recent Biopharmaceutical Drug Disposition Classification System (BDDS) (14,28).

BCS was outlined to optimize the development of oral dosage forms based on rate-limiting factors for absorption such as aqueous solubility and membrane permeability. According to BCS drugs are classified into four categories; Class I includes drugs with high aqueous solubility and high membrane permeability, Class II comprises drugs with poor aqueous solubility and high permeability, in Class III belong drugs with high aqueous solubility and poor membrane permeability, while Class IV includes drugs with poor aqueous solubility and poor membrane permeability. The main goal of BCS was to predict *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility and may help decisions to obtain regulatory waivers for BE studies.

In depth examination of BCS classes revealed that compounds belonging either to Class 1 or Class 2 of BCS are eliminated primarily via metabolism, whereas the major routes of elimination for Class 3 and Class 4 compounds are urine and bile. The Biopharmaceutics Drug Disposition Classification System (BDDCS) was proposed to early address issues related to drug disposition, drug interaction and transporter–enzyme interplay. Hence, BDDCS provides a road map for the design of preclinical and Phase 1 clinical studies without the need of running expensive and time consuming permeability studies in humans. In addition, it was recommended that for the definition of Class I compounds, the regulatory agencies may use the extent of drug metabolism (i.e., greater than ≥90% metabolized) instead of the extent of drug absorption (i.e., more than 90% absorbed). A criterion about the definition of 90% of metabolism was also proposed which is based on the mass balance of the phase I and phase II metabolites present in urine and feces.

IMPACT OF VARIABILITY IN BIOEQUIVALENCE STUDIES

The third session, on the second day of the Conference, focused on variability in BE studies. Highly variable drugs and drug products are those exhibiting within-subject (intra-subject) variability greater than 30% in BA parameters (29,30). This high variability can be ascribed either to the drug substance itself or it can be secondary to the drug product formulation. The underlying causes of high variability include physiological, pathological and the physicochemical properties of the drug product. In the physiological factors belong regional pH, pancreatic or bile secretions, gastric emptying, intestinal motility, luminal/mucosal enzymes, circadian rhythm which can significantly vary between different subjects but also within the same subject. Other factors that can influence absorption are age, gender, drug interactions and food intake. Of special importance, for the determination of BA and BE, is the role of first-pass effect since the activity of cytochrome enzymes show large variability along the gastrointestinal tract. However, variability can arise due to formulation factors of the drug product. Hence, it is essential to keep batch-to-batch homogeneity which can be assessed through several *in vitro* tests. Regardless the cause,

high variability constitutes a major difficulty for the establishment of BE between the drug products.

Within-subject Variability: Design, Determination & Demonstration

The first speaker of the second day of the conference was Dr. K. Midha (University of Saskatchewan, Canada) who defined within-subject variability as a measure of variability in response within the same subject, when the subject is administered two doses of a solution on two different occasions. This variability may be intrinsic to the drug substance and/or the formulation. Even though, large between-subject variability may exist for many drugs and drug products, BE is concerned with interchangeability within a subject. It was shown that estimation of the within-subject variability following a solution can act as the most pure measure of variability which will further help to understand whether the drug or the formulation is highly variable. The use of replicate designs to measure within-subject variability of Test and Reference formulations allows the determination of pharmaceutical quality for each of the products.

The concept of "exposure" was also highlighted (31). According to "exposure", the key parameters are C_{max}, AUC and AUC_E which correspond to peak exposure, total exposure and "early" exposure. AUC_E refers to partial AUC namely the AUC estimate truncated at the median T_{max} of the reference formulation. All these exposure measures are considered to have clinical relevance in terms of efficacy and safety for orally administered drug products for systemic availability. The worthiness of AUC_E for the comparison of the variability in BE studies during the absorption phase was demonstrated using examples where the reference formulation exhibited higher variability than the generic product.

BE Design Considerations: Multiple Dose *Versus* Single Dose Studies

Dr. A. Van Peer's (Johnson & Johnson, Belgium) presentation focused on the use of multiple *versus* single dosing studies for the determination of BE. Published theoretical considerations and several examples were presented. According to existing European guidelines (32) multiple dose (steady-state) studies would be required in the existence of non-linear kinetics (dose- or time-dependence) and for some modified release drug products. Other situations include high intra-subject variability or when sensitivity problems preclude the precise estimation of plasma concentrations after single dose. Similarly, the US authorities (33,34) propose the use of steady-state studies when there is a difference in absorption rate but not in the extent of absorption. Other conditions involve extended release dosage forms, very low concentrations after a single administration of the drug and excessive variability.

A key issue in BE testing is the assessment of intra-subject variability which can only be addressed through replicate designs (35–37). Other advantages of replicate designs include the enrollment of a reduced number of volunteers and that information can be retrieved about intrinsic factors which influence the formulation performance.

The presentation highlighted literature examples of BE trials upon which the 90% confidence intervals were narrower after multiple-dose studies than single-dose studies (38). Another example demonstrated that in steady-state conditions of a modified release formulation lower intra- and inter-subject variability were achieved. Even though, diminished variability at steady-state is favorable in terms of human exposure in clinical trials (i.e., the required number of subjects in the study is reduced), regulatory authorities do not support the use of multiple dose BE studies since they may hide differences in absorption profiles between formulations.

It was noted that at steady-state pre-dose concentrations reflect intra-subject variability. This finding is of great importance during drug development since it offers a method to assess the variability of a drug from early multiple-dose pharmacokinetic data.

Can Statistics Address the BE for Highly Variable Drugs?

Dr. P. Macheras (National and Kapodistrian University of Athens, Greece) analyzed the question whether statistics can address the BE of highly variable drugs. His presentation started by reviewing the historical evolution of the statistical concepts adopted in BE testing. It was highlighted the fact that the first intimations of BE problems originated from multi-source drug products and also inspired by vitamins, aspirin, and tolbutamide (1–7). The measures of BE were initially defined regarding rate and extent of absorption. Afterwards, the aim changed to the concept of "exposure" which included the total, peak, and early exposure (31). Besides, several approaches have been proposed for the establishment of BE (33): Average bioequivalence focuses on the comparison of means of the product under study and the originator's. In order to incorporate variability, population bioequivalence has been proposed which assesses the total variability of the measure in the population. Individual bioequivalence was introduced as an effort to assess within-subject variability for test and reference formulation, as well as the subject-by-formulation interaction. The concept of average BE dominated along with some typical assessment criteria such as the 80–125% acceptance range, the log-transformation of AUC and C_{max} estimates, and the construction of the 90% confidence interval around the geometric mean ratio of the two products (32–34).

Reference was also made to the "75/75 rule" which, at the time it was proposed, considered as a backup to the classical statistical tests and applied in circumstances where the power to detect a difference was low (39–41). However, due to the scientific criticism, this rule was finally discontinued (FDA 1988).

Special emphasis was given to the solutions proposed to resolve the problem of high variability in BE assessment. Such methods include: steady-state studies, widening of BE to pre-fixed constant values (e.g., 0.75–1.33, 0.70–1.43 etc.), scaled limits, GMR-dependent scaled BE limits, and the leveling-off scaled limits. Scaling of BE limits was initially proposed in 1995 by Boddy and his co-workers (42). Several modifications have been proposed (43–45). According to the "mixed model", unscaled limits are applied up to a switching variability value while scaled limits are used afterwards. In another approach, an additional regulatory criterion is imposed concomitantly with the classic 90% CI in BE limits. This secondary criterion suggests that the estimated ratio of geometric means should be constrained in the range 0.80–1.25. Finally, the more recent concepts of GMR-dependent scaled limits and the leveling-off scaled BE limits have been discussed (46–48). These concepts

resolve the problem of discontinuity of the previous scaled limits and offer a new alternative for reducing producer risk in cases for highly variable drugs.

Current Thinking of EMEA Position on BE of Highly Variable Drugs

This session of the impact of variability in BE studies closed with the presentations of EMEA and FDA representatives which presented the current thinking of their authorities regarding BE of highly variable drugs.

Dr. T. Salmonson (Medicinal Products Agency, Uppsala, Sweden) shared his personal view on the current situation since EMEA has not yet come to a formal position from the 27 European member states, although there have been several attempts to develop a European position on this issue. The presentation was divided into three main sections: background, ongoing scientific debate, and what future may bring.

The first part focused on the cases in which it is now permitted to use a wider acceptance range for the ratio of C_{max}. The answer comes from the EMEA guideline which defines the cases in which the reference product is highly variable and the safety/efficacy issues are justified based on PK/PD relationships or at least on clinical data.

On the ongoing scientific debate section, the questioning of using clinical data to justify a wider acceptance range was raised. However, it was concluded that in reality this is quite difficult since member states disagree on level of evidence. The methodological/philosophical aspects of scaling have also been described. The usefulness of scaling can be justified on the basis that is unethical to perform studies larger than necessary and the injustice that BE may not be possible between reference *versus* reference comparisons. According to Dr. Salmonson, it is possible to define regulatory acceptance criteria based on scaling.

Finally, it was presumed that the future will bring an update of the current guideline along with the widening of C_{max} limits relying upon high variability and clinical justification.

Current Thinking of US-FDA on BE of Highly Variable Drugs

The final presentation of the conference was from Dr. Barbara Davit (Food and Drug Administration, USA) who showed the current thinking of US-FDA on BE assessment of highly variable drugs. Her presentation had two objectives: the first was to discuss issues of BE submissions for new generic drug products, while the second aim was to present a novel approach for BE assessment.

Based on surveyed BE studies during the years 2003–2005, the drugs were categorized in respect of the encountered intra-subject variability. About 20% of the drugs with acceptable *in vivo* BE studies were consistently characterized as highly variable. On average, the acceptable BE studies of highly variable drugs enrolled 50% more subjects than studies of lower variable drugs.

The proposed approach for the assessment of BE is based on simulation results. According to this approach, the appropriate sample size (in case of highly variable drugs) can be reduced by scaling BE limits with the intra-subject variability of the reference product. A partially replicated cross-over design is proposed in which the reference product is administered twice and test product once to allow estimation of the intra-subject variability of the reference product. In addition, this method requires imposing a GMR point estimate constraint in order to avoid the potential for declaring BE (49).

UNRESOLVED ISSUES IN BA/BE REGULATIONS

The conference closed with a open session regarding unresolved issues in BA/BE regulations. Several short presentations on a variety of BE issues took place. Also, an interaction with the conference participants was offered through a panel of three delegates of European regulatory agencies who provided their opinions to the questions raised.

CONCLUSIONS

The variability of "Highly variable drugs" is due to the drug substance pharmacokinetic characteristics, influencing the rate and extent of drug absorption. HVDs generally exhibit within-subject variability of >30%. The variability due to the product or formulation factors can be easily handled through using better formulations.

Current acceptance criteria for BE studies are that the 90% CI of the test/reference geometric mean ratio for AUC and C_{max} should fall within the limits of 0.8 to 1.25. HVD generally do not meet these acceptance criteria unless large number of subjects are employed in BE study. Simulation results suggest that the number of subjects needed for BE study of HVD can be reduced when average BE limits are adjusted by scaling to the within-subject variability of the reference product. Use of reference scaling is based on the general concept that reference variability should be used as an index for setting the standard. Reference scaling effectively decreases the sample size needed for demonstrating BE. The within-subject variability of the reference product is determined using partially replicated crossover study (reference product administered twice and test product administered once) design.

An additional constraint on point estimate of test/reference geometric mean ratio (0.8–1.25) will eliminate the potential of a test product with a large mean difference from the corresponding reference product. A minimum of 24 subjects should be involved in the BE study. The scaling factor needs to be used only when the within-subject variability is found to be more than 30%.

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