Pharm Res (2010) 27:2018–2029
DOI 10.1007/s11095-010-0220-9

MEETING REPORT

From Drug Delivery Systems to Drug Release, Dissolution, IVIVC, BCS, BDDCS, Bioequivalence and Biowaivers

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Received: 7 July 2010 / Accepted: 9 July 2010 / Published online: 16 July 2010
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ABSTRACT This is a summary report of the conference on drug absorption and bioequivalence issues held in Titania Hotel in Athens (Greece) from the 28th to the 30th of May 2009. The conference included presentations which were mainly divided into three sections. The first section focused on modern drug delivery systems such as polymer nanotechnology, cell immobilization techniques to deliver drugs into the brain, nanosized liposomes used in drug eluting stents, encapsulation of drug implants in biocompatible polymers, and application of differential scanning calorimetry as a tool to study liposomal stability. The importance of drug release and dissolution were also discussed by placing special emphasis on camptothecins and oral prolonged release formulations. The complexity of the luminal environment and the value of dissolution in lyophilized products were also highlighted. The second session of the conference included presentations on the Biopharmaceutics Classification Scheme (BCS), the Biopharmaceutics Drug Disposition Classification System (BDDCS), and the role of transporters in the classification of drugs. The current status of biowaivers and a modern view on non-linear *in vitro–in vivo* (IVIVC) correlations were also addressed. Finally, this section ended with a special topic on biorelevant dissolution media and methods. The third day of the conference was dedicated to bioequivalence. Emphasis was placed on high within-subject variability and its impact on study design. Two unresolved issues of bioequivalence were also discussed: the use of generic antiepileptic drugs and the role of metabolites in bioequivalence assessment. Finally, the conference closed with a presentation of the current regulatory status of WHO and EMEA.

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INTRODUCTION

During the last few years, a plethora of multi-source drug products has reached the market. Most of these drug products are complex compounds which create the need for new ways of drug delivery in order to transport them to the desired site in the body. This is very important, since drug product's efficacy actually depends on the amount of the active moiety that is ultimately absorbed from its formula-

tion and how rapidly the absorption process takes place. The extent and rate of absorption constitute the basis of bioequivalence (BE) testing (1,2). According to the regulatory authorities, two drug products of the same active moiety and at the same molar dose are considered bioequivalent if their rate and extent of absorption are similar enough to ensure comparable *in vivo* performance (1,3). Assessment of bioequivalence is generally achieved by performing clinical studies which intend to prove the similarity between a product under evaluation (test product, T) and a product already approved by the regulatory authorities (reference product, R). However, there are situations in which BE studies can be substituted with an other type of evidence, like *in vitro* data. The term *biowaivers* refers to these exceptions to the requirement to perform clinical studies (2). The most common types of biowaivers adopted by the regulatory authorities include *in vitro–in vivo* correlations (IVIVC) and the application of the Biopharmaceutics Classification Scheme (BCS) (4–6).

The aim of the conference was to highlight some important aspects of drug delivery systems, drug release and dissolution. Special emphasis was given to biowaivers and the utility of BCS and the newly proposed Biopharmaceutics Drug Disposition Classification System (BDDCS) (7). The current status of the WHO and EMEA guidelines was also addressed. Finally, the conference ended with a presentation of some special topics of bioequivalence testing, such as the impact of high within-subject variability on study design, the necessity of bringing generic antiepileptic drug products to the market, and the role of metabolites in BE testing.

DRUG DELIVERY SYSTEMS, DRUG RELEASE, DRUG DISSOLUTION

The first section of the conference involved presentations which focused on special types of drug delivery systems (DDSs) and on the role of drug release/dissolution on drug absorption. The term *drug delivery* refers to methods of drug administration used to supply drugs in the body. Except for the common methods of drug administration, like the immediate-release oral dosage forms which have been used in therapeutics for several years, many other formulation technologies have appeared more recently. These new drug delivery technologies include methods which either modify the drug release profile or ways to deliver certain types of medications, such as peptides, proteins, gene-based drugs, *etc*.

Nanotechnology holds a significant potential as an effective drug delivery system. Nanoparticles which are used as drug delivery vehicles are generally lower than 100 nm and may consist of several types of biodegradable materials, such as natural or synthetic polymers, lipids, and metals. These nanosystems have been extensively investigated for drug and gene delivery applications since they exhibit several advantages, such as targeted drug delivery at the site of the disease, improved uptake of poorly soluble drugs, *etc*.

Drug Delivery, Polymer Nanotechnology and Molecular Imaging

Recent advances in nanotechnology offer the potential to revolutionize current clinical diagnostic and therapeutic techniques (8,9). Dr. I.C. Kwon (Korea Institute of Science and Technology, Korea) addressed the issue of utilizing polymeric nanoparticles for molecular imaging purposes.

Nanomaterials are used in the pharmaceutical field for various applications. Among them, molecular imaging is of special interest (10,11). It aims to visualize the cellular function and the follow-up of molecular processes without perturbing them. A common modality that can be used for noninvasive molecular imaging is magnetic resonance imaging (MRI). However, a common drawback of the MRI technique is the limited sensitivity compared to other imaging techniques. To this point, the use of nanoparticles allows information on biological processes at the molecular and cellular levels to be obtained and greater knowledge of disease processes and the effects of therapy to be gained. Nanoparticles may interact chemically with their surroundings and therefore alter the image according to molecular changes occurring within the area of interest. One such process that was presented is the angiogenesis in cancer, from the stage of signaling to the stage of migration of the endothelial cells towards tumor.

Cell Immobilization to Deliver Therapeutic Compounds into the Brain

Cell immobilization represents an alternative approach for the sustained delivery of therapeutic agents (12,13). According to this technique, different types of cells are immobilized within a polymeric matrix, which is further enclosed in a semipermeable membrane. This membrane protects transplanted cells from immune rejection without the need for immunosuppression. Therefore, the therapeutic agents can be released with a controlled rate from the immobilized cells, while these cells are kept isolated from the host immune system.

Dr. G. Orive (University of the Basque Country, Spain) highlighted the versatility of microencapsulation technique and its use in the treatment of numerous medical diseases, including Parkinson's and Alzheimer's diseases (14). Statistical data from several pre-clinical *in vivo* studies were provided in order to confirm the usefulness of the abovementioned technique. However, advances in biology and genetic engineering will offer the opportunity to further improve drug delivery of therapeutic compounds.

Nanosized Liposomes for Construction of Drug Eluting Stents: Improving Blood Compatibility

Nanotechnology can also be applied in the field of drug-eluting stents (DES). DES refers to coronary stents which are placed into the narrowed arterial vessels and slowly release a therapeutic agent to inhibit cell proliferation (15). Issues regarding the use of nanosized liposomes for the construction of DES were analyzed by Dr. S. Antimisiaris (University of Patras, Greece).

After analyzing the structure and the advantages of all types of liposomes used in stents, Dr. Antimisiaris proceeded to her team's approach on this matter. The goal is to coat polymer-covered (PC) stents with drug-encapsulating liposomes and examine whether sustained release of the drug can be achieved (16–18). The blood compatibility of stents can also be improved due to PC stents and plain metallic surfaces. Regarding PET-covered stents, it was demonstrated that after coating with heparin-eluting liposomes, blood compatibility is evaluated in the case of reference and liposome-coated stents, by measuring plasma re-calcification time. In terms of plain metallic surfaces, the scope of these surfaces is to allow the binding of drug-encapsulating nanosized liposomes on functionalized metallic surfaces.

Encapsulation of Drug Implants in Biocompatible Polymers

In the same vein, Dr. D. Hatziavramidis's (National Technical University of Athens, Greece) presentation focused on the encapsulation of drug implants in biocompatible polymers. These drug delivery devices represent a new era in pharmaceutics development. The main objective of encapsulation is the localized and controlled-release delivery of drugs to tissues, so the drug can act beneficially on the target tissue (14). These controlled-release systems are in direct and sustained contact with the target tissues, and some of them degrade *in situ*. Their intended use is for diseases which lack efficient treatments.

Two examples illustrated the special features of this technique. The first one focused on the delivery of anti-VEGF agent for posterior eye diseases (19). A model for gel swelling/deswelling was described with a system of partial differential equations, while a pharmacokinetic model was applied to simulate the disposition of the anti-VEGF agent in the eye. The second example referred to the encapsulation of pancreatic islet cells for diabetes type 1 treatment (20). This technique is achieved through encapsulation of biocompatible and cross-linked polymer semi-permeable membranes. The aim is to build an encapsulation apparatus for pancreatic islets for animal model studies and then to scale it up in order to meet the demands of humans. Finally, a generally accepted ascertainment is that all these experiments are still on-going, and further planning of the processes must be conducted.

Differential Scanning Calorimetry (DSC): A Tool to Study the Thermal Behaviour of Lipid Bilayers and Liposomal Stability

Thermal analysis refers to a group of methods used to characterize the physical and chemical changes derived from the temperature changes of specimens. Thermal analysis includes methods such as thermomechanical analysis, thermogravimetric analysis, differential thermal analysis, and differential scanning calorimetry (DSC). Among these methods, DSC is the most frequently used technique (21). The latter technique is based on the principle to measure the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material.

The presentation by Dr. C. Demetzos (University of Athens, Greece) focused on the application of DSC and its use as a tool to study the thermal behavior of lipid bilayers and liposomal activity (22,23). After an initial reference to basic definitions, such as drug, DDSs, lipid bilayers and liposomes, the DSC technique and its advantages and applications were described. The DSC technique can serve as a tool to study the stability of nanoparticles and liposomes used as drug delivery systems. For nanoparticles, important factors for their stability include size, vesicle shape, fluidity, elasticity, and ζ-potential. In the case of liposomes, the composition of the liposomal bilayers, the storage conditions, and the preparation method represent factors that may significantly affect their physical stability. Results from the DSC experiments, based on thermodynamic parameters (such as composition of liposomal bilayer, storage conditions and formulation process), are useful to choose the right phospholipids for formulating liposomes used as drug carriers in therapeutics.

Camptothecins: From Thermodynamics to Application

Dr. M. Savva (Arnold & Marie Schwartz College of Pharmacy and Health Science, Long Island, NY, USA) moved the discussion on the camptothecins (CPT) class of anticancer agents. Camptothecins were first isolated in 1966, and after elucidation of their mechanism of action, several analogs were synthesized (24). Among them, 10-Hydroxy Camptothecin (10-HC) demonstrated promising activity against a range of tumors. This CPT derivative exhibits cell toxicity by stabilizing a ternary complex between the nuclear enzyme topoisomerase I and double-stranded DNA, thus leading to single and double strand breaks. However, the open ring form of CPT is inactive and must be closed to inhibit topoisomerase I. Besides, the CPT molecule is highly susceptible to hydrolysis and poorly

soluble in water, a finding which restricts the clinical application of CPTs (25,26). It should be mentioned that solubility increases as the environment becomes more acidic, as it exists in many cancer cells' microenvironment (27). In addition, the formulation task of CPTs becomes more difficult since X-ray and thermal studies have demonstrated that the CPT compounds exist in polymorphic forms. Therefore, the challenges in the development of CPTs aim at increasing their stability and solubility in aqueous media and developing the appropriate DDSs for them. A number of different delivery strategies have been investigated and have been used to modulate the systemic delivery of this class of agents (28).

Regarding stability properties of camptothecins, real-time monitoring data reveal that the hydrolysis rate increases exponentially with temperature, whereas camptothecins' use is also limited by the instability of the active lactone form. Camptothecins exist in a pH-dependent equilibrium between active lactone and inactive carboxy forms, which can be altered by binding to human serum albumin (HSA). Results have shown that stability of CPT becomes worse in the presence of HSA. On the contrary, stability of another CPT analog, 7-Ethyl-10-Hydroxycamptothecin (SN-38), is improved in the presence of HSA.

Oral Prolonged Release Formulations: The Industry View

It is widely known that the rate at which a drug is released from its formulation depends on many factors. Among them, formulation properties exert a predominant role on drug release. During the past years, and in association with the progress in the pharmaceutical technology field, the number of modified release formulations was enormously increased. In these formulations, the release of the active substance is modified for some therapeutic purpose, such as to maintain activity for an extended time, reduce toxic effects, *etc*.

In this vein, Dr. E. Karavas (Pharmathen SA, Greece) presented the industry view on oral modified-release formulations. Currently, trends in the pharmaceutical industry are toward the development of improved DDSs instead of discovering new chemical entities which cost much more. The new drug delivery products are modified-release formulations which can program the release of the drug at the right time and site of action. Special emphasis was placed on prolonged release formulations which exert several advantages, such as reduced dosing frequency, less fluctuating plasma level, improved safety/efficacy ratio, and more uniform drug effect. The challenges in oral drug delivery were also pointed out. These include gastric retention platforms, colon-targeted drug delivery systems, and the development of formulations which offer improved intestinal absorption for poorly soluble drugs (29–31). Some of the recently developed chronotherapeutic drug delivery systems include diffucaps/surecaps, compression-coated systems, and layered systems. The improvement of DDSs allows the pharmaceutical industry to provide more safe and efficient drugs with a lower price.

The Luminal Environment and the Performance of Orally Administered Drugs

Drug absorption is a composite, often not well-characterized, process which requires the concurrent consideration of many variables. Both extent and rate of absorption are influenced by several factors, such as the physicochemical properties of the drug itself (pKa, aqueous solubility, lipophilicity, particle size, surface area, *etc*.), formulation factors (*e.g.*, the pharmaceutical form), possible food effects, and physiological factors of the gastrointestinal (GI) tract (*e.g.*, intestinal blood flow, pH, GI motility, mucus, bacteria, *etc*.) (32). It becomes evident that prediction of drug absorption is a very difficult task.

Dr. C. Reppas (University of Athens, Greece) addressed the issue of the complexity of the lumen environment and the performance of orally administered drugs. It was underlined that the luminal composition and hydrodynamics can affect drug dissolution properties as well as the stability of drugs in the GI tract and their absorption via the intestinal mucosa. In the fed state, lipids are a part of the administered food and perhaps of the drug formulation. However, the lipids' digestion in the fed stomach may alter drug absorption, by affecting drug solubilization, drug release, and dissolution kinetics (33). The issue of intra-intestinal composition and its effect on the stability of scavengers of reactive oxygen species was also underlined. The pH buffering species in the intestinal lumen affect the intralumenal fate of these scavengers (34,35). Finally, it was demonstrated how the contents (fasted or fed state) of the colon can affect drug solubility and performance in general (36).

The Importance of Dissolution in Lyophilized Products Used in Emergency Situations

Lyophilization (freeze drying) is a method often used in the pharmaceutical industry to preserve vaccines, proteins, and some chemical compounds (*e.g.*, amphotericin-B). According to this method, water and any other solvents are removed by sublimation and desorption. Due to the fact that the lyophilization process removes most of the water from the sample, lyophilized products become highly absorbent. Also, the materials can be easily stored and reconstituted to their original form for injection.

The presentation by Dr. G. Digenis (University of Kentucky, Lexington, USA) focused on the importance of dissolution in lyophilized products used in emergency situations, such as the case of dantrolene-sodium, which is used to treat malignant hyperthermia (37). In conditions like this, the dissolution of the

active ingredient at the reconstitution stage determines the onset of drug's action. Therefore, a product with a shortened reconstitution time offers a significant therapeutic advantage in treatment of patients in such conditions.

Special reference was made of the "manufacturing" process of a lyophilized product, by presenting all steps: formulation at elevated temperature, cooling, filtration sterilization, filling of the vials, and, finally, lyophilization cycle (*i.e.*, stoppering and packaging). The critical step in the production chain is lyophilization, since it determines the rate of reconstitution of the product. Finally, the presentation underlined the value of co-solvents (both aqueous and non-aqueous) and the way these solvents affect, beneficially or not, the morphology of ice crystals, the surface of the dried cake, the drying rate of the lyophilizate and the reconstitution time (38).

IVIVC, BCS, BDDCS, BIOWAIVERS

The second day of the conference involved presentations which focused on the use of *in vitro–in vivo* correlations (IVIVC), the Biopharmaceutics Classification Scheme (BCS), the more recently proposed Biopharmaceutical Drug Disposition Classification System (BDDCS), and the role of biowaivers in the drug approval process.

In general, BE studies are required for the initial approval of a generic drug and for some post-approval changes of a drug product in order to ensure equivalence with a dosage form already proven to be effective and safe. However, BE studies can be substituted with other type of evidence, such as *in vitro* data, to save time and reduce cost. The term *biowaivers* refers to waivers of the requirement to perform clinical bioequivalence studies (5). The most common type of biowaivers, adopted by the regulatory agencies (*e.g.*, FDA, EMEA) or the WHO, used in place of clinical BE studies include application of IVIVC, the BCS, and the BDDCS (3–7).

BCS: Current Status

This session started with the presentation by Dr. V. Shah (Scientific Secretary of FIP), who focused on the current status of BCS. The biopharmaceutics classification scheme was introduced by Amidon and his co-workers in 1995 as a framework to classify drug substances according to their solubility and permeability properties (6). The aim of BCS is to optimize the development of oral dosage forms relying only on rate-limiting factors for absorption, like aqueous solubility and membrane permeability. According to BCS, drugs are classified into four categories. Compounds with high solubility and permeability values belong to Class I, whereas highly permeable and low-solubility drugs fit in Class II. Class III comprises moieties with high solubility and low permeability values, while drugs with poor aqueous solubility and poor membrane permeability are classified into Class IV. The main objective of BCS is to predict *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility.

The BCS classification of drugs is now adopted by many regulatory authorities (3,5,39) and is used as a tool to allow regulatory waivers for immediate-release, but non-narrow therapeutic, drug products. A drug substance belonging to BCS Class I can claim biowaiver if it dissolves very rapidly (namely, more than 85% dissolution in less than 15 min) in media with pH 1.2, 4.5, 6.8. Similarly, if dissolution occurs just rapidly (*i.e.*, more than 85% in 30 min or less), bioequivalence studies can be waived if the similarity factor (f_2) value is greater than the value of 50 (40,41). BCS application for Class II and III is challenging and provides opportunities to lower the regulatory burden with a scientific rationale. According to WHO guidelines (39), a biowaiver can be claimed for BCS Class II drugs if dissolution occurs rapidly and both the test and reference products exhibit similar dissolution profiles. A biowaiver for Class III drugs (weak acids) can be claimed in cases where T and R products are rapidly dissolving and the formulation does not consist of any excipients that could possibly alter GI motility. In general, the role of excipients is very critical for a BCS-based biowaiver, since excipients may modify gastrointestinal and/or absorption kinetics. Finally, it should not be disregarded that BCS also serves as a precursor classification tool for the BDDCS, which in turn is used to predict drug transport, absorption, and disposition (7).

Predicting Drug Disposition via Application of BDDCS

The session continued with Dr. L. Benet (University of California, San Francisco), who showed how the application of biopharmaceutical drug disposition classification system can lead to early predictions of drug disposition, drug interaction, and elucidation of the transporter-enzyme interplay (42,43,47). BDDCS originated from BCS, after it was discovered that drug moieties belonging either to Class I or II of BCS are eliminated primarily via metabolism, whereas drugs classified in Classes III and IV are not metabolized and are eliminated unchanged via bile or urine. This implies that for drugs already in market, permeability estimates can be obtained from the extent of metabolism, whereas for a new molecular entity the major route of elimination can be predicted from estimates of permeability (*e.g.*, from Caco-2 cells).

Dr. Benet also highlighted the fact that the use of BCS prompted regulatory authorities to redefine permeability in terms of the extent of absorption *i.e.*, in terms of a

thermodynamic measure. On the other hand, application of BDDCS allows for the use of the extent of drug metabolism (namely, if it is greater than 90%) instead of the extent of drug absorption (44). Even though the extent of metabolism is also a thermodynamic parameter, its use (instead of permeability) seems to be advantageous for oral drugs currently in the market. This finding can be ascribed to the fact that orally administered drugs do not reach market unless they exhibit acceptable absorption.

Application of BDCCS also allows the prediction of transporter-enzyme interactions during drug absorption (42). BDDCS Class I compounds are not substrates for GI or liver transporters, but they are transporter substrates in the kidneys and the blood–brain barrier. For BDDCS Class II drugs, efflux transporter effects are important for both intestine and liver, whereas uptake transporters can be important in the liver but not in the intestine. In the case of Class III drugs of BDDCS, the absorptive transporter effects are more potent, but they can be modulated by efflux interactions. Finally, both absorptive and efflux effects were suggested to be important for Class IV drugs.

The Role of Transporters in the Biopharmaceutic Classification of Drugs

The abovementioned presentation made clear that drug transporters exhibit an important role in the disposition of drugs through the body (43). Particularly, for orally administered drugs, both uptake and efflux transporters located at the gut and the liver may influence bioavailability.

The role of transporters, in terms of the biopharmaceutic classification of drugs, was also discussed in the subsequent presentation of Dr. Benet in the conference. Special emphasis was placed on Class II drugs, since these drugs represent the predominant class of new molecular entities, while in the meantime transporter-enzyme interaction in the liver is different from that in the intestine. It was recalled that uptake transporters are important for the liver but not for the GI absorption. On the contrary, the efflux transporter-enzyme effects in the liver counteract with those dominating in the intestine. The different interplay between the transporters in the intestine and the liver was highlighted by presenting specific examples of drugs (45–48).

The effect of high-fat meals on drug absorption was also addressed in light of BDDCS classification (42). In the case of Class I drugs, no significant effect was described for the extent of absorption (F), while an increase in peak time (T_{max}) might be expected. For Class II compounds, an increase in F can be observed. It is anticipated that high-fat meals cause a decrease in F and an increase in Tmax values for Class III drugs. For Class IV drugs, a prediction cannot be made.

Biopharmaceutic Classification of Drugs Viewed in Terms of the Fraction of Dose Absorbed: The Critical Role of Supersaturated Dissolution Phenomena

Dr. P. Macheras (University of Athens, Greece) offered with his presentation an alternative insight on the biopharmaceutics classification scheme by placing emphasis on the fraction of dose absorbed and underlining the critical role of supersaturated dissolution phenomena.

The value of BCS for claiming a regulatory waiver is doubtless. However, some concerns have appeared in literature regarding the solubility dissolution and permeability criteria of BCS. For example, the BDDCS system suggests that permeability, for defining Class I biowaivers, can be substituted by the extent of metabolism (44). In addition, the recently proposed quantitative biopharmaceutics classification scheme (QBCS) highlighted the critical role of dose on absorption (49). QBCS explicitly classifies drug compounds into four categories using the dose-solubility ratio instead of solubility itself. The importance of dose and solubility-dose ratio for identifying biowaivers among Class II drugs was also highlighted with a dynamic model which describes drug intestinal phenomena (50). This intestinal tube model considers the dynamics of two consecutive processes: dissolution and wall permeation. It was shown that the underlying reason for full absorption originates from the dynamic character of the dissolution-uptake processes occurring simultaneously in the GI environment.

The critical role of supersaturated dissolution phenomena was also addressed. A reaction-limited model of dissolution, which is not based on diffusion principles, was described as a useful alternative for explaining supersaturated dissolution data (51). This model is not based on classic diffusion principles, but incorporates time-dependent coefficients. It was concluded that supersaturated dissolution data tend to be more physiologically relevant for biopharmaceutic classification purposes (52). Examples of supersaturated formulations demonstrated that biowaivers can also be claimed for this type of formulation.

Biowaivers: Current Status

The current status of biowaivers accepted by the regulatory authorities was addressed by Dr. Shah's second presentation in the workshop. The primary mission of regulatory authorities is to assure safety and efficacy of the marketed drugs, and, for this reason, clinical data are required. However, in the case of the drug approval processes, adoption of a biowaiver allows the use of evidence other than *in vivo* data (3,5). In other words, it is the objective of biowaivers to lower the regulatory burden without any particular loss of drug product quality. For oral dosage forms, biowaivers are based on dissolution data.

Based on the specific drug product properties, several different types of biowaivers can be identified. Biopharmaceutics classification scheme represents a common tool for biowaiver. It is now accepted that for BCS Class I drugs, no bioequivalence studies are required if the two drug products under comparison are rapidly or very rapidly dissolving in aqueous media at pH values 1.2, 4.5, and 6.8 (3,5,39). Under certain conditions, a biowaiver can be claimed for BCS II and III classes. Another type of biowaiver refers to the cases where a BE study is conducted at one strength and the applicant claims equivalence at other strengths (2,53). This biowaiver is applicable to conventional-release tablets (or capsules), extended-release tablets, and beaded capsules.

Dr. Shah also addressed the issue of quality by design (QbD) process as a method to ensure product quality (54,55). In case of generic products, the term QbD simply refers to scientifically designing a drug product so as to meet specific objectives and to be equivalent to another product already approved by the regulatory authorities. Special emphasis was placed on the design of equivalence for generic topical antifungal products. The terms Q1, Q2, and Q3 were introduced to classify product similarity. Depending on the extent of similarity, application of QbD allows the identification of the appropriate *in vivo* bioequivalence study. In other words, QbD represents an essential part of modern approaches to ensure pharmaceutical quality as well as to reduce the need for testing and expand the design space beyond past experience (55).

The Development of Biorelevant Dissolution Methods

Oral drug administration represents the most convenient and preferred way to deliver drugs. However, drug absorption is influenced by the physicochemical and physiological properties of the environment in the GI tract. When a drug substance moves from the stomach into the small intestine, it meets a rapidly changing environment, such as the different enzymes, bile components, and rising pH values from acidic to neutral.

Dissolution testing has an important role throughout the drug product development process. In general, dissolution methods represent the most often used tools to predict the *in vivo* behaviour of a drug formulation from the *in vitro* data. Even though simple dissolution media can be used, for quality control reasons, more complex dissolution media should be applied when the prediction of *in vivo* drug performance is intended. The composition of the luminal environment differs significantly from that of the simple aqueous solutions and depends on the location of the GI tract, the dosing conditions, and the inter- and intra-variability, necessitat the use of biorelevant dissolution media which mimic gastric and GI conditions.

In this context, Dr. M. Vertzoni (University of Athens, Greece) described the development of biorelevant dissolution methods with special emphasis on biorelevant media and biorelevant hydrodynamics. It is worth mentioning that biorelevant media have been successfully applied to the prediction of the *in vivo* performance for drugs with dissolution-dependent or solubility-dependent absorption kinetics, and, without dispute, they have facilitated the assessment of intralumenal dissolution (56,57). Biorelevant media can either simulate the fasted or the fed state conditions of the gastrointestinal tract. Fasted media are used to mimic either the gastric (*e.g.*, SGF_{SLS}, SGF_{Triton}) or the small intestinal conditions (HIF, CIF, SIF, *etc*) (58,59). The media used to simulate the fed state conditions are also divided into those simulating the gastric and those simulating the small intestinal conditions. In both cases, *in vitro* simulation in the fed state can be described by the gradual digestion approach (60,61) or the snap-shot media approach (62,63). The presentation ended with a description of the apparatus used to simulate the luminal hydrodynamics. However, the predictive value of such apparatus for drug release requires further validation (64).

Non Linear IVIV Correlations: An Exception to the Rule?

The second day of the workshop closed with the second presentation by Dr. Macheras, who introduced a novel insight into the non-linear *in vitro–in vivo* correlations. IVIVC refers to the establishment of a relationship between an *in vitro* property of a drug product (*e.g.*, extent and/or rate of dissolution) and a relevant *in vivo* response (*e.g.*, extent of absorption) (2,65). Usually, successful IVIVCs are considered those in which a linear relationship is established between the *in vitro* and the *in vivo* parameters, whereas any non-linear IVIVCs are considered failed and remain unpublished. It is proposed that the latter should be referred to as *in vitro–in vivo* relationships (IVIVR) in order to distinguish them from the linear cases (66). The reasons for non-linearity can be attributed to the complexity of absorption process, such as the heterogeneity in the topology of GI, the intestinal motility, the interplay between drug and food. Non-linear IVIVC are described with models assuming first-order kinetics (66), proportional odds model (67), and fractal kinetics (68). Monte Carlo simulations have also successfully been applied to study drug release for Euclidean and fractal geometries (69–71). An alternative approach is to use fractional kinetics (72,73). In this case, differential equations of fractional order, which are related to the geometry of the reaction space, are used to describe absorption kinetics. Methods based on fractional calculus have been successfully applied to account for kinetics in constrained topologies (74).

BIOEQUIVALENCE

The key topic of discussion during the third day of the workshop was bioequivalence (BE). Special emphasis was placed on the role of within-subject variability (WSV) encountered in BE studies and the impact of WSV on the study design. During this session, two specific topics on BE were also discussed: the necessity of bringing generic antiepileptic drug products to the market and the role of metabolites in BE assessment. Finally, the session ended with a presentation of the current status of the WHO and EMEA guidelines for bioequivalence.

Within-Subject Variability: Design, Determination, Demonstration

The first presentation of the day was given by Dr. K. Midha (President of FIP), who addressed the issues of within-subject variability, its determination using simple cross-over or replicate designs, and the importance of WSV in bioequivalence assessment (75–77). Within-subject variability was defined as a measure of variability in response within the same subject, *i.e.*, when two doses of a solution are administered to the same subject on two different occasions.

Within-subject variability can be intrinsic due to the drug substance itself or can also be ascribed to the formulation. Using the standard cross-over 2×2 design, the estimated residual variability is considered to be a measure of WSV. In fact, residual variability comprises four components: the true WSV, subject by formulation interaction, within-formulation variability, and unexplained random variability. However, the most honest estimate of WSV of a drug moiety can be obtained after oral administration of a solution of the drug in a replicate design. In this case, formulation variability is excluded, and the estimated WSV comprises only the true WSV and the unexplained error. Estimation of true WSV of test and reference allows one to infer the pharmaceutical quality of a drug product. The more variable a formulation appears to be, it is considered to be of more poor pharmaceutical quality. Another important issue concerned with WSV is the fact that high WSV is often observed at early time points during absorption, a finding which might lead to difficulties in establishing BE using early time exposure concepts.

Bioequivalence: Variability and Impact on Study Design

The presentation by Dr. A. Van Peer (Johnson and Johnson, Belgium) focused on WSV and its impact on the clinical study design. He referred to both simple cross-over designs, which are the most preferable among the regulators, and replicate designs in which the T and R formulations are given twice.

In cases of high WSV, there is an increased rejection rate of BE for truly equivalent drugs (77–79). In such situations, several methods have been proposed, like the widening of BE limits to predefined constant values (*e.g.*, 0.75–1.33) (1,78), increase of sample size, application of steady-state studies (78) or replicate designs (78,79), and use of scaled BE limits (80–85). More recently, scientists working for the FDA have proposed the application of a three-period semi-replicate design according to which the test formulation is administered once, while the reference product twice (86). This design allows the estimation of the true WSV of the reference product, which is then used to estimate scaled BE criteria.

During this presentation, an algorithm for the design of average BE studies was also described. It was proposed that for low to moderate WSV values the ordinary 2×2 design suffices. However, for highly variable drugs, replicate designs might be preferred. Group-sequential design should be applied when resources are limited to achieve the desired statistical power or in cases when there is uncertainty about the magnitude of WSV (87).

Generic Products of Antiepileptic Drugs: Is It an Issue?

The issue of generic antiepileptic drugs (AEDs) is rather old; generic carbamazepine and valproic acid products have been marketed for a long time, and, currently, generic formulations are available for several AEDs. However, a controversy still persists regarding whether generic AEDs can be used interchangeably with the brand name drug with respect to safety and efficacy issues (88). This issue was discussed by Dr. M. Bialer (The Hebrew University, Israel), who further highlighted the special features of antiepileptic drugs when subjected to bioequivalence analysis.

When a physician prescribes a drug, he faces the dilemma of drug interchangeability, which in turn is decomposed into drug prescribability or drug switchability (89,90). *Prescribability* refers to the physician's first choice for a drug-naïve patient, while *switchability* indicates the situation when a patient taking one product is switched to another formulation of the same active substance. The current BE criteria, using AUC, Cmax and the relevant statistical procedures, can sufficiently justify the prescribability of generic products; however, these criteria do not ensure the switchability between different formulations. In addition, empirical evidence from physicians implies that in the case of AEDs, not all generics are equal to the brand-name for all patients. Presumably, shifts between different AEDs are more risky than shifts between a brand and a generic product (88,91). Moreover, the primary end-point

in treating epilepsy is seizure control without side effects, and this condition should not be sacrificed on the basis of any cost. Actually, switchability of AEDs can safely be addressed only if individual bioequivalence is considered, an opinion also justified by several national guidelines for generic prescription of AEDs (92). Therefore, it was concluded that a switch from one antiepileptic drug to another is not recommended unless sufficient evidence, such as individual BE data, is available.

Towards the Elucidation of the Role of Metabolites in Bioequivalence Assessment

The role of drug metabolites in the determination of BE represents another unresolved issue in the field of bioequivalence. In most of cases, BE studies are carried out focusing only on the measurement of the parent drug (P). Even though the role of metabolites (M) in bioequivalence assessment has been the subject of many discussions, it still remains a controversial issue (75,93,94). The basic argument in favor of the use of the parent drug for BE assessment relies on the fact that the concentration (C)-time (t) profile of the parent drug is more sensitive to detecting differences in formulation performance (95). However, there are situations where metabolite data are preferred (2,3,53). Such situations arise from the relative efficacy/safety profile of parent drug *versus* metabolite, the variability of the pharmacokinetic parameters of P and M, the type of kinetics (linear or non-linear), and the concentration levels of P and M.

Dr. V. Karalis (University of Athens, Greece) addressed the issue of metabolite assessment in bioequivalence studies and presented the results of simulated BE trials. A basic prerequisite of his presentation was to set the criteria for the definition of the preferred analyte standing only on bioequivalence terms. Since the bioequivalence decision depends on the ability of the measured moiety to identify differences in the responses between the test and reference formulation, the analyte of choice would be the one which would reflect better the differences in the extent and the rate of absorption. The term *better* simply implies that the analyte of choice would be the one which carries the information of bioequivalence with higher sensitivity and lower variability. In bioequivalence terms, the BE decision depends on the sensitivity of the measure to reflect the changes of the GMR for the measure under study (*e.g.*, AUC or Cmax) and the variability of this measure.

This task was implemented by generating data for a variety of pharmacokinetic models, scenarios and conditions. Regarding AUC, the performance of metabolite was found to be very similar to that of parent drug for all scenarios and models examined. However, a more complex behaviour was found for Cmax. In all cases, M data showed higher permissiveness in the percentage of acceptances. It is widely accepted that the analyte of choice is the parent drug; however, there are situations where the use of metabolite data might be advantageous. Primarily, this was found to be true when P undergoes high formation rate and/or is eliminated rapidly. Also, metabolite data may be preferable when parent drug is absorbed slowly, metabolite is eliminated slowly, and first pass effect is followed by concurrent formation of the metabolite.

WHO Guidelines on Registration to Establish Interchangeability

The session closed with two presentations focused on the guidelines of bioequivalence. The first speaker, Dr. Midha, highlighted the major advances of the new WHO guideline regarding interchangeability of multi-source drug products (39).

In the case of very potent or toxic drugs, the WHO guideline suggests that the bioequivalence study should be conducted either at the lowest strength or in patients. For long half-life drugs, the use of truncated AUC in BE studies is recommended, *i.e.*, a sample collection for a time adequate to ensure completion of GI transit (usually up to 72 h after drug administration) (96). In the case of truncated AUCs, there is no need for greater assay sensitivity to define the disposition phase. In addition, more blood samples are clustered around Cmax, which leads to greater precision of the estimated Cmax and Tmax values.

It is generally proposed that only the parent drug should be measured in a BE study. However, there are situations where metabolite data can be used instead (see section "Towards the Elucidation of the Role of Metabolites in Bioequivalence Assessment" of this manuscript for a more detailed description). Usually, a non-stereoselective analytical assay should be applied. Only in cases of different metabolic and safety/efficacy profiles of the enantiomers is a stereoselective assay necessary. The BE limits should usually lie within the 0.80–1.25 range. For Cmax, a wider acceptance range (0.75–1.33) can be adopted, but even in these cases the point geometric mean ratio (GMR) estimate should lie between the 0.80–1.25 interval.

The vast majority of BE studies are conducted in the fasted state. Nevertheless, fed studies are preferred when there are labeling restrictions for administering the drug in the fasting state or when it is known that the drug might cause GI disturbances. Especially for modified release products, it is suggested that the appropriate study design is the one conducted in the fed state using the highest marketed dose. The WHO proposes that multiple-dose studies should be considered for drugs with non-linear kinetics, for extended release formulations with an accumulation tendency, and in cases where the assay lacks of sensitivity.

In addition, the WHO guideline refers to some special considerations, such as fixed dose combination products, application of truncated AUCs in BE determination, and highly variable drugs. The latter corresponds to drugs with WSV greater than 30% in their bioavailability parameters (77,97). Finally, genetic phenotyping is required for drugs showing phenotyping-linked metabolism. In all cases, a prior justification of the methods of analysis is required.

The New EMEA Guideline for Bioequivalence

The workshop closed with the presentation of Dr. J. Morais (University of Lisbon, Portugal), who outlined the revolution of the regulatory status in Europe since 2001 (1,53). He also referred to the new features added to the new EMEA draft guideline issued in July 2008 (3).

Due to the rising number of procedures for generics' approval (mutual recognition procedure and decentralized procedure), many difficulties arise for the interpretation of certain aspects of the current guidance. The aim of the new EMEA guideline is to define the conditions when BE studies are necessary to set the requirements for their design, conduct, and evaluation. This new guidance provides a clearer view on specific BE issues, such as fed/fasting conditions, stereoselective analytical methods for enantiomers, metabolite data, and strength, to be tested in the bioequivalence study. In addition, special emphasis is given to study designs; the study designs are covered more explicitly, and, apart from the classic 2×2 cross-over design, several other designs, like the parallel group, the replicate, and the two-stage designs, are analyzed. According to the new EMEA guideline, multiple-dose studies can also be conducted in case of dose- or time-dependent pharmacokinetics and for drug tolerability reasons. Also, the possibility for a BCS-based biowaiver is incorporated regarding Class I drugs and under certain conditions for Class III compounds. Finally, the new EMEA draft guideline refers to the narrowing of acceptance limits in case of narrow therapeutic index drugs.

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