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The global bioequivalence harmonisation initiative: Report of EUFEPS/AAPS third conference

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ABSTRACT

The European Federation of Pharmaceutical Sciences (EUFEPS) and American Association of Pharmaceutical Scientists (AAPS) have collaborated since 2015 to organize international conferences to support global harmonization of regulatory requirements for bioequivalence (BE) assessment. This collaboration has resulted in three Global Bioequivalence Harmonization Initiative (GBHI) workshops which provided a unique opportunity for scientists from academia, industry, and regulatory agencies to discuss current, complex BE issues. The 3rd GBHI workshop was held in April 2018 in Amsterdam/The Netherlands and covered the following topics: (a) the necessity of multiple-dose studies in BE testing; (b) BE of transdermal delivery systems, and (c) liposomal parenteral preparations. This report summarizes the extensive discussions that led to better understanding of the similarities and differences across the major regulatory agencies on these topics and paved the way for future international harmonization.

1. Introduction

The GBHI series of international conferences foster an open exchange of viewpoints amongst regulatory agencies, academia, and industry and have made significant contributions to the process of global harmonization of BE issues (Chen et al., 2018, 2019). The GBHI Scientific Planning Committee consists of expert scientists from academia, industry, and regulatory agencies, including the European Health Authorities and the U.S. Food and Drug Administration (FDA). The topics for all GBHI conferences are selected by this committee to identify differences in regulatory requirements and expectations where the current scientific evidence provides an opportunity for harmonization. An innovative feature of the third conference was the use of voting devices to allow the audience to convey their viewpoints on specific issues during the discussion period. All data were collected and incorporated into the session summaries.

2. BE topics

2.1. Necessity of multiple-dose studies in be testing

2.1.1. Current regulations

For extended-release (ER) drug products, there is no specific regulatory requirement from the FDA to demonstrate BE at steady-state. As such, steady-state studies are only recommended in patients when the safety and tolerability of a drug make it unethical or the need for washout between treatments make it impractical to conduct such studies in healthy volunteers. For example, the dosing regimen of Panobinostat Lactate Capsules does not allow for maintenance of the steady-state, but it does allow for an adequate washout period. Hence, for this drug product, a single-dose rather than a multiple-dose BE study is recommended in patients. If a steady-state study is conducted, it should be confirmed that the steady state has been reached (e.g., through the measurement of C_{min} (lowest concentration in a dosing interval) at several dosing intervals prior to pharmacokinetic (PK) sampling in each period). It was noted that whenever multiple-dose studies are

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conducted for US submission, the FDA does not consider C_{min} at steady state (C_{minSS}) as a primary parameter, and it remains controversial whether there is added value to consider C_{minSS} in multiple-dose studies if drug accumulation occurs. Steady state studies were also found to be less sensitive to detect clinically meaningful differences in lag time (T_{lag})

The EMA does not require a multiple-dose study for an immediate-release (IR) or delayed-release (DR) product but does require a multiple-dose study for prolonged-release products with accumulation, where the measured elimination phase is determined by the release from the drug product. In the latter situation, additional PK metrics are required to ensure a similar shape of the concentration-time profile. The concentration at the end of the posology interval at steady state should be compared as additional metric. Experience suggests that BE failure at the steady state might not be predicted in the single-dose study by the concentration at the end of the dosing interval, since the single-dose study may not anticipate non-linearity. Marked differences in the shape of the test and reference product have been noted in a multiple-dose study that were not anticipated by a single-dose study.

If there is no evidence of drug accumulation, the EMA can opt to waive the requirement for steady-state studies. There is a general waiver if the area under the curve (AUC) for the dosing interval after a single dose covers 90% of that extrapolated to infinity, but the shape of the profile should be further characterized in the single-dose study through partial AUC (pAUC) assessment. The pAUC approach was further clarified via the graph shown in Fig. 1.

2.1.2. Discussion

2.1.2.1. Should steady-state studies be required for MR products?. The FDA dropped the requirement for steady-state studies for MR products in the period 2000–2010, and Health Canada no longer required such studies after 2010. However, Europe still requires steady-state studies for MR products if the AUC to the end of the dosing interval in single-dose studies ($AUC_{0-\tau}$) is less than 90% of AUC_{0-inf}.

Simulations of multiple-dose pharmacokinetics for various types of ER drug products demonstrated that steady-state studies were associated with a decrease in statistical power, suggesting a lack of benefit in carrying out steady-state studies on MR products. The simulations were carried out assuming that there would be no excipient differences between products or that any differences in excipients would play no role in affecting the performance of the formulation.

On the other hand, the concentrations observed at the end of the dosing interval at steady state can be of clinical relevance for maintaining the desired therapeutic effect for certain products. An "end-of-dose-failure" phenomenon which has been demonstrated e.g. for the treatment with opioids or antiepileptic drugs and is caused by dropping of the C_{minSS} values below the minimum effective concentration needs to be avoided. For such cases BE at steady state was suggested.

Participants were almost equally split on the question of whether steady-state studies should generally be requested for MR products. However, achieving regulatory harmonization on this issue remained a topic of interest. The participants recommended that in jurisdictions where steady state studies continue to be required, the cut-off value for assessing drug accumulation (AUC within one dosing interval after a single dose administration /AUC_{0-inf}) from the single-dose studies should be lowered from 90% to 80%, since in cases of low accumulation, plasma profiles of multiple-dose studies do not differ significantly from those of single-dose studies. Further discussions and exchange of data between agencies were suggested to evaluate the costs and the benefits of requiring additional multiple-dose BE studies as a general practice to determine if a common position between agencies can be reached.

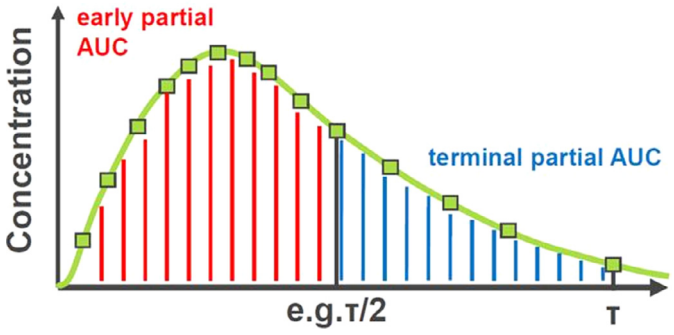

Fig. 1. pAUC assessment in a multiple-dose study.

2.1.2.2. What are the appropriate PK metrics for BE assessment?. It was noted that superposition principle could not be employed if the drug caused enzyme induction (e.g., carbamazepine). C_{max} and C_{min} are complex (composite) metrics which depend on the rate of absorption and the rate of elimination. For an MR product, C_{max} for use in a single-dose study is more sensitive to detect differences in the rate of absorption than C_{maxSS}. Other shape metrics that were discussed included plateau time $t_{75\%}$ or peak occupancy time POT-25 (which is mandatory in Russia) and half-value duration for single dose studies or percent peak to trough fluctuation at steady state. To assess the suitability of alternative PK metrics and their sensitivity, it was suggested that sponsors could submit studies with these additional metrics for further evaluation.

2.1.2.3. What are the considerations in using modelling and simulation as an alternative to steady-state studies to determine BE?. It was proposed that the superposition principle could be used, with the terminal elimination rate constant as predictor of drug concentrations beyond the last blood sampling time point in the single-dose study. However, this principle may not be applicable for drugs with nonlinear PK, and it is unclear if the prediction is dependent upon a one- or two-compartmental fit. When interpreting simulation results, the measurement of C_{minSS} in steady-state studies might not be reliable due to high variability and clinical utility for these measures compared to $AUC_{0-\tau}$ and C_{maxSS}. Therefore, applying the same BE criterion as that for $AUC_{0-\tau}$ and C_{maxSS} may be unnecessarily stringent. The need for additional PK parameters to ensure a similar shape of single dose profiles for BE assessment remains under discussion, and consensus was not reached regarding how to extrapolate the single-dose findings to the steady-state, particularly regarding C_{minSS}.

2.1.2.4. Is C_{minSS} an appropriate parameter in multiple-dose studies to establish BE?. Whether or not C_{minSS} is an appropriate BE parameter in multiple-dose studies remains controversial. Participants were equally split on the relevance of this parameter and the resulting need for conducting multiple-dose studies. The main justification for requiring multiple-dose studies in the EU is to evaluate C_{minSS} as a parameter to characterize the shape of the PK profile. However, if AUC and C_{max} are adequately characterized from single-dose studies, the steady-state PK parameters, $AUC_{0-\tau}$ and C_{maxSS} may add less value in characterizing the shape of the time-concentration profile. The participants proposed that if C_{minSS} is recommended, then perhaps a less stringent BE criterion should be applied for this parameter due to its inherently high variability and frequently lower level of clinical relevance compared to $AUC_{0-\tau}$ and C_{maxSS}. Less stringent criterion could include applying the non-inferiority approach (i.e., using only the lower limit of the 80–125% acceptance range as recommended by Health Canada) and/or considering only the ratio of the means of the test and reference products instead of the confidence interval.

2.1.2.5. How useful is pAUC in characterizing the shape of the single-dose PK profile?. There was extensive discussion around whether pAUC is a useful parameter to characterize the shape of the PK profile and whether its investigation in single-dose studies can be used to justify

waiving steady-state studies. BE of pAUCs with cut-off time at half-dosage interval should allow conclusion on similar shape after single dosing. However, the selection of an appropriate cut-off time for pAUC was challenged. In the absence of an established PK/Pharmacodynamic (PK/PD) relationship or dose-response data to justify the clinical relevance of pAUC, determining the pAUC based on a pre-set cut-off time (e.g., at half-dosage interval) should not be necessary as a general requirement for assuring BE. For MR products in general and for products with multiphasic release, pAUC should be requested only if supported by clinical evidence that the shape of the PK profile or drug-release features are critical for the drug's effect.

2.2. BE of transdermal delivery systems

2.2.1. Current regulations

The EMA and the FDA are the only two regulatory bodies with guidelines on this topic. The general EMA modified-release guideline (EMA 2014) which came into effect on 1 June 2015 covered transdermal drug delivery systems as well as intramuscular and subcutaneous depots. For Transdermal Therapeutic Systems (TTS), these guidelines summarize the requirements for in vitro testing, BE trials, investigations on patch adhesion as well as how to perform skin irritation and sensitization studies.

In contrast, the FDA has no general BE guidelines for TTS; instead, the FDA released product-specific guidances (PSGs) on these topics in 2016. Guidances for industry addressing skin irritation and sensitization date back to 1999 and are in principle applicable but are partly overruled by the PSGs. A draft guidance on adhesion assessment was issued in 2016. Following the conference, the 2016 PSGs issued by FDA were revised in October 2018. Also, fully revised draft guidances on adhesion, as well as on irritation and sensitization were published by FDA in October 2018 (FDA 2018a, 2018b).

Both the EMA and FDA commonly presume that the high-strength formulation is the most sensitive for BE testing; a waiver of in vivo BE testing for a lower strength is accepted if release is proportional to the effective surface area with similar release rates, and the qualitative composition is the same. For certain drugs, the FDA can allow assessment of the intermediate strength due to safety considerations (e.g., rivastigmine) as specified in the PSGs. Such assessments may differ between the two regions and have to be defined on a case-by-case basis with European authorities. The most relevant difference is that for the FDA, single-dose studies are generally sufficient; however, when high accumulation is a risk, the EMA requires multiple-dose studies, applying the same decision criterion ($AUC_{0\text{-}t}$ < 90% of $AUC_{0\text{-}inf}$) as for oral MR products. For these steady state comparisons, equivalence acceptance criteria are accordingly applied to C_{minSS} primarily to ensure avoidance of end-of-dose failure.

2.2.2. An industry perspective

An industry representative presented approval strategies for newly developed patches with dosing intervals divergent from the approved one. Such “generics with added value” are normally approved following identical or similar strategies as classical generic developments but considering slight modifications depending on the different product characteristics (EMEA 2008; FDA 1999).

Single-dose BE studies were considered not applicable to products with diverse dose regimes and that in such cases multiple-dose studies may be the only meaningful approach. PK-measures of relevance were discussed in such cases and the importance of C_{minSS} was specifically challenged. Arguments in favour of product-specific requirements were presented regarding relevance and acceptance criteria of C_{minSS}.

2.2.3. Discussion

2.2.3.1. What are the relevant PK measures for BE assessments of transdermal delivery systems?. PK measures to be used for BE assessments are defined on a product-specific basis in the US (e.g., for methylphenidate $pAUC_{2\text{-}9}$ is required). The requirements of the European Guideline on the pharmacokinetic and clinical evaluation of MR dosage forms (EMA 2014) are to be applied as specified in the general section. For drugs where no multiple-dose study is requested due to low accumulation risk, pAUC in addition to $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max} is requested. For multiple-dose studies $AUC_{0\text{-}t}$, C_{maxSS}, and C_{minSS} are requested.

2.2.3.2. What are the relevant principles and considerations for adhesion testing for BE assessment of transdermal delivery systems?. The assessment criteria for adhesion differ significantly between the EMA and the FDA. The EU asks that such studies are performed in the patient population. The EMA is in favour of a two-step approach which does not consider the reference product in the first step (90% CI of test should lie above 90%). Non-inferiority testing is accepted only if the test fails in the first step and if reference also shows poor adherence (<90%). The EU encourages discussion of the criterion for entering the second step of evaluation on a case-by-case basis and notes that deviations may be acceptable. The two-step approach of EMA also reflects the circumstance that better adhesion properties of the newly developed patch are acceptable. FDA favours non-inferiority testing with a predefined margin ($\delta = 0.15$) based on the 5-point score. Lastly the EMA focuses on adhesion at the end of the dosing interval for decision making and considers adhesion during the course of patch contact for descriptive evaluation only, whereas the FDA recommends covering the time course of adhesion over the entire dosing interval. Other considerations include which size patch to evaluate (e.g., should the biggest patch be characterised for adhesion properties as the worst-case scenario) and diversity in the nature of patches (e.g., very small patches impede exact measurements while for very big patches a grid might be difficult to be applied due to wrinkling). So far no adequate technical solution exists for adhesion assessments of opaque or stained patches, which do not allow differentiating adhered from non-adhered areas. Lastly, the development of alternative non-visual measurements was encouraged.

There are also significant differences in the scoring systems for adhesion testing. The EMA asks for a continuous variable based on the percentage of adhesion observed, whereas the FDA recommends an ordinal descriptive scale. The FDA's 5-point scale provides the necessary discrimination in adhesion of transdermal patches and when used as described in the guidance, provides an adequate assessment for comparison of adhesion, but may not estimate adhesion as precisely as the EMA percentage adhered approach. Discussion was encouraged regarding precision vs. adequacy for discrimination.

The relevance of adhesion testing was challenged for those cases when BE could unambiguously be proven and there is obviously no risk of patch detachment (i.e., the amount of absorbed drug depends solely on the surface area in contact with the skin). However, there is no current trend in either the EU or the US to weaken these acceptance criteria, even though from a practical perspective, precise adhesion assessment is extremely difficult – if not impossible – in the case of opaque or stained patches.

Statistical analyses highlight the pros and cons of the US and EU approaches to adhesion testing to determine BE of transdermal delivery systems. Two different adhesion studies were presented to illuminate the differences in outcomes between the two regulatory approaches. In both studies, mean adhesion of the Test products at the end of the dosing interval were similar and both below 90% (with Test in Study 1 being slightly above Test in Study 2), whereas in both trials mean adhesion of the Reference product at the end of the dosing interval was above 90%. However, in Study 2, the lower end of the 90% CI of Reference was slightly below 90%. Consequently, following the European approach, the Test failed in Study 1 in step 1 as non-inferiority testing was not possible because of the Reference data. In Study 2, the Test failed step 1 but was allowed to enter step 2 where non-inferiority could be demonstrated. These data show that minimal

differences between the Reference adhesion characteristics decide the yes/no decision in step 1 and call into question the meaningfulness of the 2-step approach in the European guideline. A Test product which is clearly non-inferior to Reference might fail if step 2 may only be applied in the case of a Reference product for which the 90% CI is below 90%. On the other hand, from a statistical point of view, the US scoring scale is too rough to be normally distributed which would result in lower statistical power as data transformation is not performed.

2.2.3.3. Is it necessary to investigate the influence of environmental factors on BE assessment of transdermal delivery systems?. The necessity of investigating the influence of environmental factors like heat and showers was discussed. Using an example of a fentanyl product, the FDA demonstrated their awareness of the relevance of heat on exposure and the potential importance of other environmental factors. However, examples of systematic investigation of such environmental factors on a confirmatory level have not been presented and discussed.

2.2.3.4. What are the best practices and considerations for assessing skin irritation for transdermal delivery systems?. Both the US and EU have compiled lists of topical exposure agents, and further collection of TTS relevant materials which could cause sensitisation was encouraged. The importance of an excipients list to support the estimation of sensitization potential for all regulatory bodies was emphasized.

An appraisal of the study design and the scoring used in skin sensitisation and irritation studies from a medical perspective was provided by an industry (CRO) speaker with specialization in dermatology. According to the normal requirements of the SmPC, e.g. for Rotigotine patches, the same application site shall not be used more than once within 14 days, whereas in the commonly requested irritation phase of the guideline-based irritation studies, each patch is to be applied repeatedly over 21 days to the same site. Adequately separated applications induce a temporary but reversible response whereas repeated applications to the same site overcome the threshold for appearance of dermatitis resulting in a cumulative irritation. In the currently requested study design, this normally comes along with a repeated tape stripping where the upper layer of the stratum corneum is repeatedly removed. This tape stripping-like procedure artificially increases irritant reactions but also increases the risk of allergic reactions as a consequence of the mechanical skin barrier damage. Also, in the subsequent discussion, the ethical justification of such a procedure was questioned as the subjects will carry this allergy life-long. It was pointed out that the 21-day daily application may cause false positive reactions and include a higher risk for ethically problematic iatrogenic sensitization. In the discussion, it was questioned whether an irritation study should consider waiving the sensitisation phase in those cases when excipients and active moiety are known not to have a skin sensitization potential.

In this presentation also the scoring system used by European and US regulatory bodies was challenged, as it was originally developed in the 1980s to characterise the effects of detergents and cosmetics and is not relevant to TTS products. Alternative scoring proposals that better reflect the irritation induced by a TTS are needed that can identify the leading symptom for irritation (i.e., increasing erythema) and the leading symptoms for allergy (i.e., papules and oedema).

There is a lack of data and clarity regarding the effects of age on irritation assessments. One FDA participant was doubtful whether there were large differences in irritation due to age but did notice the loss of subcutaneous fat in the 80+ group, leading to a lower torsional rigidity. He also agreed that discussion was needed as to whether sensitization studies are always needed.

Following the discussion of the conference, EMA updated its Question and Answer document (8.1) with a clarification on the requirements for sensitisation and irritation tests for transdermal products (EMA 2018a). The FDA also confirmed their interest in considerations for alternative scales for irritation scoring.

2.3. Liposomal parenteral preparations

2.3.1. Current regulations

Liposomes are phospholipid vesicles containing an aqueous interior within phospholipid bilayers. Liposomes have been developed as a drug delivery system to control and improve the PK, bio-distribution, safety, and efficacy profiles of therapeutic agents. It is important to appreciate that conventional plasma PK may not reflect access of drugs to the target sites and comparison of the total drug exposure alone may not be sufficient to demonstrate BE.

Currently there are over 10 liposome products approved by FDA, including those to treat cancer, age-related macular degeneration, and for pain management. In general, the FDA requires the generic parenteral liposome formulations and the reference product to be qualitatively (Q1) and quantitatively (Q2) the same, have equivalent physicochemical characteristics as well as equivalent systemic exposure of un-encapsulated drug and/or liposome associated drug. If these conditions are met, the FDA does not require non-clinical and clinical studies for the demonstration of safety and efficacy for generic liposome drugs. Given the diversity and complexity of liposomal drug products, the FDA is proactively developing PSGs for each approved liposomal new drug application (NDA) (that serves as the reference list drug) to aid generic drug development. Three case examples including doxorubicin HCl liposomes, verteporfin liposomes, and bupivacaine liposomes were presented. For verteporfin liposomes, the in vivo BE study can be waived since the liposome formulation is intended to have immediate and complete release upon administration. For doxorubicin HCl liposomes, equivalent systemic exposure of un-encapsulated and liposomal doxorubicin is recommended. These product-specific recommendations are based on careful analysis of liposome drug product properties, mechanisms of actions, and others relevant parameters.

The EMA perspectives on liposome drug products are generally based on the EMA reflection paper EMA/CHMP/806,058/2009. These complex formulations stand apart from conventional small molecule formulations as their formulation and manufacturing-specific characterization may not correlate with therapeutic performance, much like biologics. Since in vitro comparability is considered insufficient, the key question is how to ensure the similarity of in vivo biodistribution and release rates between different products to ensure similarity in efficacy and safety. The reflection paper provides general requirements regarding non-clinical PK studies while the issue of additional PD studies is less clear, as information is limited, and there is an argument for reducing the burden of PD testing for generic products. There may be grounds for waiving toxicology studies depending on pharmaceutical comparability and the nature of the toxicity produced by the product. The reflection paper also contains general recommendations on BE testing. It is accepted that clinical efficacy studies are not adequate to detect formulation variables.

Based on this document, the EMA is more cautious as compared to the FDA. However, the general nature of the reflection paper in contrast to the complexity of liposomal follow-on products highlighted the urgent need for product-specific guidelines as currently developed for liposomal doxorubicin. Provided that test and reference products have demonstrated qualitative and quantitative similarity, the proposal at the meeting was to request one crossover BE study at one dose level in patients, the evaluation of liposomal and unencapsulated doxorubicin within regular acceptance limits of 80 – 125%, while metabolite data might be dispensable. Of note, pAUCs are considered necessary in addition to overall AUC and C_{max}.

During the discussion, the necessity of determining the un-encapsulated active ingredient in addition to the encapsulated drug was challenged. Considering the extreme differences in half-lives between both forms, the overall pharmacokinetics seem to be controlled by the release of the active drug ingredient from the formulation and less by its own elimination rate constant. Moreover, this drug release can be directly determined as decline of the profile of the encapsulated drug. It

was, thus, suggested to limit the requirements to BE assessment based on the encapsulated drug only. However, there was no consensus whether to limit the requirements to BE assessment based on the encapsulated drug only, and more research is necessary on this topic.

EMA published its first product-specific BE guidance on liposome drug products in June 2018 (EMA, 2018b). The "Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific BE guidance" has very similar recommendations as the FDA PSG on doxorubicin HCl liposome injection in terms of single-dose crossover study design and the moieties to measure to determine BE. Both FDA and EMA still consider the measurement of the unencapsulated drug necessary to monitor the liposome stability in vivo. The only difference is that EMA recommends pAUC for encapsulated drugs while the FDA does not. Nevertheless, this is a great example how fruitful discussion at the GBHI meeting help narrow the gap between the EMA and FDA on BE recommendations for a specific liposome drug product. More research is necessary to fully harmonize the BE recommendation of liposome drug products.

2.3.2. A case study of the impact of liposome formulations on in vivo pharmacokinetics and pharmacodynamics of Doxil/Caelyx, a PEGylated stealth liposome containing doxorubicin

The development of PEGylated stealth liposomes containing doxorubicin facilitated the delivery of higher cumulative doses to cancer patients with reduced risk of cardiac toxicity and resulted in a product (Doxil/Caelyx and generic versions) approved for several important indications in cancer therapy. In addition to standard physicochemical characterization and appropriate release and stability assays, the methodology of high sensitivity differential scanning calorimetry and cryo-TEM have helped to characterize these formulations. The unique PK profile of pegylated liposomal doxorubicin (PLD) was described together with imaging studies showing the high localization of the drug payload and of liposomes in experimental tumours and humans via the enhanced permeability and retention (EPR) effect. PK-PD relationship and some of the adverse effects of PLD were discussed. Future directions in liposome-based therapies based on the PLD experience were also presented.

2.3.3. What methodologies are useful to determine the ratios and amounts of released and encapsulated drugs in liposomal parenteral formulations?

Using doxorubicin as an example, critical quality attributes of liposomes are listed in Table 1 below:

The encapsulated fraction is a function of the drug´s physicochemical characteristics, the lipid composition, and the process used for entrapment. The type of lipids, the drug-to-lipid ratio, and manufacturing process can have a large effect on the size and lamellarity as well as other physicochemical characteristics such as the zeta potential of the drug containing liposomes. These parameters can be determined by methodologies such as electron microscopy and dynamic light scattering techniques. More advanced techniques such as Small Angle X-Ray Scattering (SAXS) and Small Angle Neutron Scattering (SANS) allow not only a determination of the size distribution but also reveal the surface structure and sometimes internal organization of liposomal composites. Examples of using micro-positron emission tomography (PET) imaging to measure the in vivo release of a drug from preparations were presented and correlated with in vitro release rates. Nevertheless, such examples are still sparse today, making it difficult to establish experimental bio-predictive release conditions on a broader basis.

Pegylation of liposomal formulations decreases renal clearance, protects against enzymatic degradation and recognition as well as the uptake of liposomes from the reticuloendothelial system. The steric hindrance of pegylation in turn reduces immunotoxicity and prolongs circulation times of colloidal formulations in the systemic circulation by orders of magnitude. For example, the size and structure of doxorubicin formulations can be examined using electron microscopy techniques, where the formulations are found to have a coffee bean appearance. In four formulations of doxorubicin, there were subtle differences in appearance and size range. With regards to the plasma profiles, small quantities of unencapsulated doxorubicin in the product are much more rapidly cleared than the majority of encapsulated drug, and therefore the need for quantification of these amounts might be questioned. Lastly, a liposomal formulation can also be considered as a simple solubilizing system for a poorly water-soluble drug. Classifications can be constructed with regards to stable and leaky formulations where the encapsulated drug should be quantified with regards to equivalent efficacy and the unencapsulated drug concentrations be considered for safety reasons. The liposome classification system described in Hsu et. al. (Hsu and Huang, 2014) classifies four groups of preparations according to relative release rate and the extent of reticuloendothelial system uptake and may be useful for liposome preparations in general. Based on Monte Carlo simulations, Hsu et al. concluded that "the encapsulated form provides the most accurate assessment of BE for liposomal drug products with low reticuloendothelial system uptake" (such as doxorubicin). Of note, employing pAUC was supported by research results involving some formulations with rapid drug release.

2.3.4. Case studies demonstrating the relevance of non-dose proportional pharmacokinetics in BE assessment

With Daunoxome treatment in children, about 6% of unencapsulated drug is present in the liposome injection prepared according to instructions in the SmPC. The use of modelling to investigate the influence of dose on the fraction released indicates that a higher fraction is released with higher doses. Weight gain or losses occur frequently during treatment. According to simulations, a change in weight of 15% would lead to changes in the AUC of about 6.5% when dose adjustment according to body surface area (BSA) is applied. It was suggested that dose adjustment after weight loss should be done based

Table 1
Comparability of liposomal drug products and quality characterization.

Parameter	Aspects
• Lipidic components	• Source, characterization, assay, impurity profile, stability
• Other excipients	• Quality, purity, stability
• API-lipid ratio	• Acceptable range
• Morphology, size distribution	• Absence of aggregates, lamellarity
• Encapsulated fraction	• Dependent on API and process
• Stability of finished products (incl. In-use)	• API, lipids (Lyso-PC, Oxidation products)
• In vitro drug release / drug leakage test	• Method
• Batch-to batch variability	
• Need for reconstitution?	• Robustness, Training

on body surface area, although this is still a matter of debate. Effects of disease status and premedication (e.g., steroids and myelosuppressives), appear plausible, but were not detected in any popPK analyses thus far.

Amphotericin B liposome formulation has reduced nephrotoxicity and shows marked non-linear pharmacokinetics. The pharmacodynamics of different formulations, including emulsions, AmBisome (liposome formulation), and lipid complexes were compared with Amphotericin B deoxycholate (AMBd). It is important to look for follow-on products of AmBisome, but the carrier must have stability to avoid release before it reaches the fungus.

More product-specific guidelines for liposomal formulations such as those developed for pegylated liposomal doxorubicin are necessary.

2.3.5. What are the relevant criteria for assessing the therapeutic equivalence of generic liposomal products, including establishing both pharmaceutical equivalence and BE for these complex drug products?

There are many similarities in the challenges encountered in the development and approval process of biosimilars as compared to those for generic liposomal products. Outstanding questions are the requirements for pharmacokinetic testing, criticality of control over the manufacturing process of this complex product, and the choice of an RLD in similarity testing. In a recent FDA guidance document, attention was drawn to the assessment of critical quality attributes and to the exploration of design space. As little concrete information on these parameters is available for liposomes in the public domain, a link was made with accepted good practice for groups of other complex drugs, in particular monoclonal antibodies and antibody-drug conjugates. It was emphasized that a number of critical quality attributes have been suggested for comparability exercises of liposomal products, but there is no decision as yet regarding which are considered most important in terms of in vivo equivalence.

Understanding of liposomal formulations has greatly evolved in recent years, and follow-on products are greatly needed. Bioanalytical as well as quality-related methods are available, and attempts to approve such follow-on products are ongoing. However, the overall regulatory experience with follow-on liposomal formulations is still limited. Developing product-specific (e.g., liposomal doxorubicin) rather than general guidelines for these diverse liposomal products will enable harmonization of requirements for the approval of follow-on drugs.

3. Conclusions and perspective

The three topics selected for this workshop - necessity of multiple dose studies in BE testing, BE of transdermal systems, and liposomal parenteral preparations – were timely and of great interest to all the attendees. Even though a consensus could not be achieved on differences in regulatory requirements on any topic, there was open, extensive and informative exchange of views amongst regulators and attendees, providing greater clarity on the existing differences and the underlying scientific and regulatory rationale and paving the way for further harmonization. Subsequent to this workshop, updated or new BE guideline/guidance in the areas of transdermal products (FDA 2018a, 2018b) and liposomal products (EMA, 2018b) have been issued by the FDA and the EMA respectively. The success of this workshop highlights the importance of such engagements to furthering global consensus on BE topics and strengthens the commitment of the GBHI planning committee with the support of EUFEPS and AAPS to continuing this harmonization initiative.

Disclaimer

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