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CHALLENGES IN BIOEQUIVALENCE ASSESSMENT OF TOPICAL DERMATOLOGICAL DOSAGE FORMS

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ABSTRACT: The assessment of the bioequivalence of topical products not intended for absorption into the systemic circulation has presented a formidable challenge over the years. In particular, dermatological dosage forms such as creams, ointments, lotions and gels, apart from those containing topical corticosteroids, cannot readily be assessed for bioequivalence using "conventional" methodology and the only recourse to-date has been to undertake tedious, time consuming and expensive clinical end-point trials for such products. Although the human skin blanching assay (HSBA), also known as the vasoconstriction assay (VCA) has been successfully used for dermatological products containing topical corticosteroids but no surrogate methodology for the bioequivalence assessment of other topical dermatological products such as those containing nonsteroidal anti-infl ammatory drugs, anti-fungals, antibiotics and antivirals has been successfully accepted by regulatory agencies. The regulatory agencies and pharmaceutical industries are forging ahead to the development of new surrogate BE assessment approaches for other topical dermatological products. These promising approaches include dermatopharmacokinetic study (DPK), dermal microdialysis (DMD), in vitro studies, pharmacokinetic study (PK), near-infrared spectrometry (NIR), and confocal Raman spectroscopy (CRS). In addition, the expansion of biowaivers for topical dermatological products has been explored by pharmaceutical scientists.

KEYWORDS: Bioequivalence, Dermatopharmacokinetics, Microdialysis, Spectroscopy

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1.INTRODUCTION

Topical dosage forms are liquid or semisolid dosage forms, which are not intended for systemic absorption. These dosage forms comprise solutions, lotions, gels, ointments, patches, and foams; that are applied onto the skin either to elicit therapeutic effect within the skin or underlying subcutaneous tissue. Despite great advances in addressing the issues related to topical bioequivalence, many challenges remain due to the complexity of drug transport through the skin from different formulations and lack of harmonized guidance documents. It has been acknowledged that no single test procedure would be suitable for the development, biopharmaceutical characterization, and quality control of all semi-solid topical dosage forms[1]. Generally, when a new generic product becomes available in the market, the competition of generic pharmaceutical industry is felt immediately, and as a result, the prices of both reference list products (RLDs) and generic dosage forms reduce substantially. However, for topical dermatological products, the competition of generic industry remains limited despite the expiration of all exclusive protections of the RLD. This is mainly attributed to the limited number of acceptable bioequivalence(BE) assessment approaches to demonstrate the BE between generic topical dermatological products and RLDs[2]. This short review mainly focusses on the surrogate approches for bioequivalence assessment of topical semi-solid dosage forms and their recent advances other than the clinical trials as the new approches are less time consuming, require less number of subjects and have more sensitivity. Unlike the well-established approaches used for the determination of bioequivalence (BE) of oral dosage forms where the active ingredient(s) is/are intended to be absorbed into the systemic circulation, bioequivalence assessment of topical dosage forms not intended for absorption has proved to be quite difficult, daunting and extremely challenging. Currently, apart from undertaking clinical trials in patients to assess the bioequivalence of such products, the only surrogate method which has been found to be acceptable but which is constrained to topical corticosteroids only, is the human skin blanching assay (HSBA) also known as the vasoconstrictor assay (VCA)[3]. As with all types of generic products, regulatory agencies require the demonstration of therapeutic equivalence to its corresponding RLD, which includes the confirmation of pharmaceutical equivalence and bioequivalence. However, determination of topical BE for locally acting drugs in skin is more complicated than solid dosage forms. In contrast to orally administered products, most topical dermatological products are meant to be locally active, which provide limited systemic absorption, thus precluding the application of common procedures for BE determination of orally administered products (i.e., measuring the rate and extent of drug absorbed in plasma). The demonstration of BE between a generic topical dermatological products and its RLD is a long standing challenge for the lack of accepted bioequivalence methods[4]. Active pharmaceutical substances are usually applied to the skin in the form of semisolid formulations for topical treatment of dermatological diseases or for improvement of the skin condition. The skin may also be recognized as an alternative port of entry for systemically acting drugs. For the effectiveness of the formulations applied to the skin, the active

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications compounds incorporated into the semisolid base must reach the site of action. However, the skin acts as a barrier controlling the entry of molecules from the administered medications[5]. Transport of active substances through the skin may be described as series of consecutive steps, each of which can potentially be rate limiting. First, the drug needs to diffuse from the formulation to the skin surface. This process is characterized by the release rate. The release requires dissolution of the active substance and may be rate limiting process for skin delivery due to the fact that only small molecules can penetrate into the skin[6]. Active substances of highly lipophilic nature may be usually dissolved in hydrophobic bases whereas moderately lipophilic or hydrophilic substances form suspensions. However, the release of lipophilic active substances from hydrophobic bases is limited, even if they are dissolved in the base because of their strong affinity to the lipophilic components (low values of acceptor fluid/semisolid formulation partition coefficients)[7]. The physiochemical nature of the semisolid base influences the release rates of active substances in vitro. The type of the semisolid base determines the ability of the acceptor fluid to the penetration into the formulation. The release rate of hydrophilic and moderately hydrophilic active substances usually increases when more hydrophilic bases are used (hydrophobic<emulsion<hydrophilic). The high rate of the release from hydrophilic bases may be attributed to the readily dissolution of water-miscible components of the base in the acceptor fluid penetrating into the formulation. Hydrophilic components of the base may penetrate into the acceptor fluid and thus change the value of partition coefficient acceptor fluid/base of the active substance[8]. The stratum corneum is a selectively permeable barrier whose properties depend on many endogenous factors as well as are influenced by components of the topical formulations. The impact of semi-solid base components on the skin, in particular on the stratum corneum, includes: hydration and incorporation of some semisolid base components into the intercellular cement lipids leading to increased disordering of lamellar and lateral packing of lipids and/or increased solubility of the active substance within the stratum corneum lipids. These interactions may alter the stratum corneum permeability (influence on skin penetration and permeation rate) or change the value of skin/base partition coefficient (influence on the rate of the skin retention). The degree of the interaction between the base components and the skin can be assessed by a comparative analysis of the release rate and the skin permeation rate of the active substance. The stages of skin permeation once the active substance overcomes the stratum corneum are similar to the in vitro release through the artificial membrane whose properties resemble those of the deeper layers of the skin. These layers are more hydrophilic and permeable than the stratum corneum[9]. The proper selection of semisolid base type (hydrophobic, hydrophilic, emulsion) as well as its components are crucial for the effective skin and transdermal delivery of the active substance. Well characterized properties of the active compound, the semisolid base and the skin barrier (especially the stratum corneum) may help to predict the cutaneous and percutaneous absorption of the active substance. However, the difficulties in predictability of skin and transdermal delivery are usually seen due to the fact that characteristics of

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications the active substance, vehicle and the skin should be considered as a kind of multifactorial system, not separately. The base ingredients may interact with the active substance (solubilizing effect, complexes formation) as well as with the structure of the stratum corneum as percutaneous absorption promoters.

APPROACHES FOR BIOEQUIVALENCE ASSESSMENT OF TOPICAL DERMATOLOGI CAL PRODUCTS

CLINICAL END-POINT STUDIES

For most topical drug products, clinical endpoint trial is used to demonstrate BE between the generic product and its RLD. Even though it provides clinicians with a chance to directly evaluate the generic products, this method is the least sensitive and reproducible among all general approaches to demonstrate bioequivalence. Besides, clinical endpoint trial is often costly, time-consuming, difficult to conduct, and entails large patient population. The FDA has acknowledged the need to find more sensitive and more efficient surrogate approaches to demonstrate BE for topical dermatological products. Generally, for a specific new generic topical drug product, FDA will provide sponsors with product-specific clinical recommendations or consider whether a biowaiver is appropriate. If BE recommendation from FDA is not available or the sponsors prefer other rational alternative methods, the sponsors need to provide sufficient data to convince the FDA on using such method to demonstrate BE between the generic product and its RLD[10].

DERMATOPHARMACOKINETICS (TAPE STRIPPING)

The dermatopharmacokinetic (DPK) approach is comparable to a blood, plasma, urine pharmacokinetics approach applied to the stratum corneum. DPK encompasses drug concentration measurements with respect to time and provides information on drug uptake, apparent steady-state levels, and drug elimination from the stratum corneum based on a stratum corneum concentrationtime curve. When applied to diseased skin, topical drug products induce one or more therapeutic responses, where onset, duration, and magnitude depend on the relative efficiency of three sequential processes, namely, (1) the release of the drug from the dosage form, (2) penetration of the drug through the skin barrier, and (3) generation of the desired pharmacological effect. Because topical products deliver the drug directly to or near the intended site of action, measurement of the drug uptake into and drug elimination from the stratum corneum can provide a DPK means of assessing the bioequivalence of two topical drug products. The most important advantage of DPK approach is that both the generic product and the original one are evaluated in the same subject, thus reducing intersubject variability and the number of subjects[11]. Amongst the many variables that hamper the precision and reproducibility of this method is the fact that stratum corneum thickness differs between each individual - hence, normalization necessary. This can be accomplished by measuring the transepidermal water loss (TEWL) which is a noninvasive bioengineering technique that describes the outward diffusion of water through the skin. TEWL monitors the integrity of the SC water barrier function and is an indicator of skin water barrier alteration with increased readings often

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications indicating impairment of skin barrier function [12]. Even though the target site for topical dermatologic drug products in some instances may not be the stratum corneum, the topical drug must still pass through the stratum corneum, except in instances of damage, to reach deeper sites of action. In certain instances, the stratum corneum itself is the site of action. For example, in fungal infections of the skin, fungi reside in the stratum corneum and therefore DPK measurement of an antifungal drug in the stratum corneum represents direct measurement of drug concentration at the site of action. In instances where the stratum corneum is disrupted or damaged, in vitro drug release may provide additional information toward the BE assessment. In this context, the drug release rate may reflect drug delivery directly to the dermal skin site without passage through the stratum corneum. For antiacne drug products, target sites are the hair follicles and sebaceous glands. In this setting, the drug diffuses through the stratum corneum, epidermis, and dermis to reach the site of action. The drug may also follow follicular pathways to reach the sites of action. The extent of follicular penetration depends on the particle size of the active ingredient if it is in the form of a suspension[13]. Healthy volunteers with no history of previous skin disease or atopic dermatitis and with a healthy, homogeneous forearm (or other) skin areas sufficient to accommodate at least eight treatment and measurement sites (time points) should be recruited. The number of subjects to be entered may be obtained from power calculations using intra- and intersubject variability from the pilot study. The premarked sites are treated with predetermined amounts of the products (e.g., 5 mg/sq cm) and covered with a nonocclusive guard. Occlusion is used only if recommended in product labeling. Removal of the drug product is performed according to SOPs at the designated time points, using multiple cotton swabs or Q-tips with care to avoid stratum corneum damage[14]. Skin stripping proceeds first with the removal of the first 1-2 layers of stratum corneum with two adhesive tapes strip/disc applications, using a commercially available product (e.g., D-Squame, Transpore). These first two tape-strip(s) contain the generally unabsorbed, as opposed to penetrated or absorbed, drug and therefore should be analyzed separately from the rest of the tape-strips. The remaining stratum corneum layers from each site are stripped at the designated time intervals. This is achieved by stripping the site with an additional 10 adhesive tape-strips. All ten tape strips obtained from a given time point are combined and extracted, with drug content determined using a validated analytical method. The values are generally expressed as amounts/area (e.g., ng/cm2) to maintain uniformity in reported values. Data may be computed to obtain full drug concentration-time profiles, Cmax-ss, Tmax-ss, and AUCs for the test and reference products. A plot of stratum corneum drug concentration versus a time profile should be constructed to yield stratum corneum metrics of Cmax, Tmax and AUC. The two one-sided hypotheses at the α = 0.05 level of significance should be tested for AUC and Cmax by constructing the 90 percent confidence interval (CI) for the max ratio between the test and reference averages. Individual subject parameters, as well as summary statistics (average, standard deviation, coefficient of variation, 90% CI) should be reported. For the test product to be BE, the 90 percent CI for the ratio of means

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications (population geometric means based on log-transformed data) of test and reference treatments should fall within 80-125 percent for AUC and 70- 143 percent for Cmax[15].

PHARMACODYNAMIC APPROACHES

Sometimes topically applied dermatological drug products produce direct/indirect pharmacodynamic (PD) responses that may be useful to measure bioavailablity/bioequivalence (BA/BE). For example, topically applied corticosteroids produce a vasoconstrictor effect that results in skin blanching. This PD response has been correlated with corticosteroid potency and efficacy. Topically applied retinoid produces transepidermal water loss that may be used as a pharmacodynamic measure to assess BA/BE. Pharmacodynamic study is much simpler and involves less patient population than clinical endpoint trials. However, several issues have been identified previously. One of the most common issues is high intersubject variability, which requires a relatively larger number of subjects than dermatopharmacokinetic methods. Moreover, a pilot study must be performed to determine the dose duration and to select responders (subjects with confirmed adequate vasoconstriction). Moreover, the design of pilot test may influence the results of pivotal test[16]. The method involves application of the topical corticosteroid product to a number of skin sites and allowing the product to remain in contact with the skin for a fixed time. Excess product is then removed by gently washing and the degree of skin blanching or whitening of the skin is then assessed over a number of designated intervals of time. The visual assessment is based upon the utilization of an arbitrary intensity scale of 0 - 4where 0 indicates no blanching and numerical increase of numbers 1 - 4 are assigned to increasing degrees of blanching observed, respectively[17]. Chromameter assessment- An instrumental method involving a tristimulus colorimeter was subsequently introduced as an objective and thus preferred method. The Minolta chromameter, which is a portable instrument that uses tristimulus colorimetry involving reflectance spectroscopy, was adapted to measure skin blanching. This approach had subsequently been used for the objective measurement of skin color. The chromameter functions by emitting a white light (using a pulsed xenon arc lamp) onto the chosen area of assessment and measuring the intensity of reflected light through three particular wavelength filters (analyzed at wavelengths of 450, 560, and 600 nm) or using a photodiode array in more recent instruments. The detected signal is converted into three coordinates: L* (luminosity), a* (the amount of green or red), and b* (the amount of yellow or blue). The skin blanching response is measured relative to the color change in the skin. As the skin blanching response develops, the skin becomes lighter and its redness fades. As the skin becomes more pale the L* scale increases, a* scale decreases, and b* scale increases very slightly[18]. The FDA guidance suggests conducting two in vivo studies- a pilot dose durationresponse study and a pivotal in vivo bioequivalence study comparing test and reference products. The pilot study characterizes the dose-duration response relationship for the drug in terms of the Emax model and is conducted solely with the reference listed drug. The dose duration method as recommended in the guidance for documentation of bioequivalence is based on three dose durations:

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications ED₅₀, D₁ and D₂. The comparison of test and reference products in the pivotal study is conducted at a dose duration approximately equal to the population ED₅₀ determined in the pilot study.

MICRODIALYSIS

Microdialysis is a continuous sampling technique in which the molecule of interest is collected from the target tissue; thus providing insight into the time course of drug action or biochemical monitoring of the tissue. The technique can be imagined as an artificial capillary, in which a hollow semipermeable probe is carefully inserted into the site of interest: brain, muscle, eye, and skin. Therefore, it provides valuable information of unbound drug concentrations or biomarkers at the site closer to the pharmacological action compared to the conventional plasma/blood drug concentration versus time. Though it was developed for neurological research, it has gained acceptance in other areas of research. It was observed that probe insertion in the skin leads to inflammatory responses, both acute and chronic, and an immunological probe rejection response, all of which have the potential to affect experimental microdialysis in different ways. However, with respect to sampling of drug molecules from the skin, perturbation of blood flow to the local tissue is critical which would recover to normal in approximately two hours. The technique has been successfully adopted and demonstrated for dermatological research as well as for demonstrating the bioequivalence of topical dosage forms[19]. Dermal Microdialysis (DMD) is a relatively new application of microdialysis which allows continuous monitoring of endogenous and/or exogenous solutes in the interstitial fluid (ISF) of dermal tissue with minimal tissue trauma and involves the placement of small perfused membrane systems at given depths within the dermis. When a topical formulation is applied onto the skin and perfusate is pumped through the implanted membrane system, drug molecules from the topical formulation present in the dermal ISF diffuse (driven by the concentration gradient) into the lumen of the membrane, resulting in the presence of drug in the perfusion medium collected as dialysate. The dialysate is sampled at various intervals of time and the drug concentration in the dialysate can be determined quantitatively[20].

IN-VITRO STUDIES

In vitro study involves the measurement of drug release from base to receptor cell by using a vertical diffusion system separated by the excised skin or a synthetic membrane. The excised skin was used to model the lipid perturbation effects and study drug diffusion from transdermal products. The main limitation of excised skin is the high degree of variability, which must be standardized before use. Synthetic membrane has no lipid perturbation effects, but it is preferred by many researchers because it is easily resourced, less expensive, and structurally simple. In vitro drug release is sensitive to several physical and chemical parameters, such as drug solubility, particle size, and the arrangement and rheological property of semisolid dosage forms. In addition, in vitro release test is easier to be carried out than in vivo test, and also gives insights into drug permeation mechanism[21]. IVRT utilizes widely accepted Franz diffusion cells to estimate rate of drug release from drug products. It

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications involves the application of a drug product on to a membrane (synthetic membrane, excised animal skin, or excised human skin) that separates the donor and receiver chambers. The receiver chamber simulates sink conditions in vivo. The rate of delivery obtained from these studies is assumed to be similar to the in vivo situation. The method has been widely employed in discovery research for screening formulations and understanding mechanism of cutaneous drug transport. However, it is not recognized as a surrogate for in vivo BA/BE of new drug products. Nevertheless, in vitro study with a synthetic membrane is accepted as a valuable quality control tool to ensure product sameness under Scale-up and Post Approval changes (SUPAC), which include minor changes in composition, manufacturing process and equipment, site of manufacture, and scale-up and scale-down of manufacture[22]. For abbreviated new drug applications (ANDAs) bioequivalence has been documented for the highest strength, in vitro release may also be used to waive in vivo studies to assess bioequivalence between these lower strengths and the corresponding strengths of the RLD. If this approach suggests bioinequivalence, further studies may be important. To document BE of lower strengths in an ANDA, the following conditions are Important:

- Formulations of the two strengths should differ only in the concentration of the active ingredient and equivalent amount of the diluent.
- No differences should exist in manufacturing process and equipment between the two strengths.
- For an ANDA, the RLD should be marketed at both higher and lower strengths.
- For an ANDA, the higher strength of the test product should be BE to the higher strength of RLD[23].

SPECTROSCOPY STUDIES

Near-infrared spectrometry (NIR) and confocal Raman spectroscopy (CRS) are two major advanced noninvasive in vivo approaches for real-time monitoring of drug penetration across the skin. However, both NIR and CRS require that the molecule of interest should possess distinct spectral peaks with sufficient intensity. Near-infrared spectrometry represents a relatively novel in vivo approach for noninvasive assessment of chemicals' permeation across the skin. NIR wave is capable of penetrating the skin to a depth of several centimeters. Thus, by measuring IR spectrum and combining with linear multivariate statistics, the drug diffusion through skin can be quantified. NIR is superior to other methods due to its nondestructive, rapid, simple (without sample preparation), and quantitative properties, therefore the possibility of drug diffusion during scanning process can be eliminated, which is favorable for the analysis of volatile drugs. In addition, some NIR approaches are capable of real-time monitoring the rate and quantity of chemical penetration through the skin. The promising feature of NIR is relatively rapid data acquisition and in vivo applicability[24].

WAIVER OF BIOEQUIVALENCE STUDIES FOR TOPICAL DERMATOLOGICAL PRODUCTS

For topical dermatological solutions, the FDA will consider granting biowaivers, provided that the generic topical dermatological product fulfils the following requirements: (i) it is a solution; (ii) it

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications contains the same active ingredient in the same concentration and dosage form; (iii) and it does not contain other ingredients or change in formulation that may significantly affect drug availability[10].



In addition, in light of Biopharmaceutics Classification System (BCS) for oral solid dosage forms, which is based on sound scientific principles and has led to great success in promoting the development of generic oral solid products, the authors further proposed a Topical Drug Classification System (TCS) for topical products. The proposed TCS is based on qualitative (Q1) and quantitative (Q2) equivalence of composition, and the similarity of in vitro release rates (an estimator of microstructural consistency; Q3) between a generic topical product and its RLD. If the generic topical product has the same Q1 and Q2, and meets Q3 comparison requirements identified in SUPAC-SS [50], it will be classified as TCS class 1. If the generic topical product has the same Q1 and Q2, but is different in Q3, it will be classified as TCS class 2. If the product has different Q1 and Q2, but meets Q3, for example when the excipients are inert and have no significant impact on in vitro release rate, it can be classified as TCS class 3. Lastly, if Q1, Q2, and Q3 of the product are all different, the generic topical product will be classified as TCS class 4. Under the classification, only for TCS class 1 and TCS class 3 dosage forms, a biowaiver can be allowed. TCS class 2 and TCS class 4 are not eligible for biowaiver, for which the in vivo BE studies are required [4].

CONCLUSION

The intricacy of cutaneous drug delivery is very well addressed in the literature. Nevertheless, there is a knowledge gap between industry and regulatory agencies. It will not be possible to have a single step solution for demonstrating the BE of all Topical Dermatological Drug Products. However, FDA's Guidance documents on surrogate methods could not only reduce US healthcare costs by encouraging competition among companies, but also increase the emphasis on product quality, in particular Q3 equivalence. The future emphasis of BE assessment for topical dermatological products would be

Preet & Kaur RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications directed toward the refinement and standardization of the existing approaches, development of new alternative BE approaches, establishment of detail guideline for each method by FDA, and the exploration of expanding biowaivers of topical dermatological products.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

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