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AAPS/FDA Workshop Report

Bioequivalence of Topical Dermatological Dosage Forms—Methods of Evaluation of Bioequivalence

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The three-day AAPS/FDA workshop on "Bioequivalence of Topical Dermatological Dosage Forms—Methods for Evaluating Bioequivalence," held on September 4–6, 1996 in Bethesda MD was attended by 260 scientists from industry, academia and regulatory authorities. The goals and objectives of the workshop were to:

1. Discuss scientific issues and approaches for bioequivalence (BE) evaluation of topical drug products;
2. Explore principles of dermatopharmacokinetics (DPK) in BE evaluation;
3. Discuss DPK and statistical evaluation for BE of dermatological products; and
4. Review other methodologies applicable to BE demonstrations for topical drug products.

INTRODUCTION

With the exception of topical corticosteroids, the only means an US generic company has to demonstrate bioequivalence of a topical dermatological product to an innovator's product is through comparative clinical trials with a bioequivalence endpoint. An innovator company wishing to replace an already approved post-1962 topical dermatological product with a new formulation exhibiting appreciable compositional changes is also faced with the need to demonstrate bioequivalence using clinical studies, again with the exception of topical corticosteroids. In the specific instance of topical corticosteroids, the demonstrations of BE of two physically alike (e.g., cream versus cream) formulations may now be done using a vasoconstriction protocol, as outlined in FDA Guidance (*Topical Dermatologic Corticosteroids: In Vivo Bioequivalence, June 2, 1995*), irrespective of whether the product is for an Abbreviated New Drug Application or for updating an existing New Drug Application.

Clinical efficacy trials aimed at showing the bioequivalence of topical dermatological products are relatively insensitive, time-consuming, and costly. To gain adequate statistical power required to make a clear BE determination, they may require as many as 300 patients. A problem in the topical dermatological area is that no recognized surrogate measures are currently available that might be used in replace of clinical efficacy studies. For drugs where effect is related to concentration in the systemic circulation, the concentrations of a drug and/or active metabolite in blood and/or urine have been viewed as surrogate measures of clinical safety and efficacy. For many years, FDA has thus relied on blood and/or urine concentration time curves as a measure of BE. A key assumption in this approach is that concentrations of a drug in blood are also in equilibrium with concentrations in the target organ/tissue. This workshop explored the possibility that a dermatopharmacokinetic characterization might provide an alternative approach to clinical trials for the determination of BE of topical dermatological products, analogously to the use of concentration-time curves for systemically administered drugs. If accepted, this approach might allow dermatopharmacokinetic studies to

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replace comparative clinical trials as a means of documenting bioequivalence of selected topical drug products.

The DPK approach includes any measure of drug concentration in the skin, whether directly or indirectly related to the drug's therapeutic action, which can be determined continuously or at least intermittently for a period of time. This may include measurement of either drug concentrations in stratum corneum (SC) over time and/or drug concentrations in serial biopsy samples. To be a useful DPK measure, the time-integrated DPK response must reflect both local safety and efficacy of the topical drug product. One assumption in the DPK approach is that excipients are pharmacologically inactive. In some instances, however, an excipient may exert a direct or an indirect effect, by enhancing or inhibiting drug penetration into the skin. Such effect should be accounted for by DPK methodology through implementation of proper experimental controls (i.e., placebo formulations).

DPK methods should be validated and verifiable. Validation should include all aspects of sampling, e.g., SC stripping and measurement of drug concentration in the SC, or any other analysis. At every critical step in the method development, accuracy, precision, sensitivity, specificity, and other standard aspects of validating an assay methodology should be established. Beyond these obvious checks and balances, all measurements must stand up to rigorous scientific scrutiny.

Before a DPK method is adopted as a basis for BE, it must be shown that differences in DPK capture or reflect significant clinical important differences in formulations. Delivery of a drug into the stratum corneum may not be the only factor in therapeutic efficacy. Other formulation factors may contribute to a topical product's therapeutic efficacy. Therefore, a multi-tiered approach to BE assessment may be a prudent strategy. For instance, one might determine that DPK, e.g., SC concentration-time profiles, are the same in the test and reference product which have qualitatively same composition (Q1), similar physicochemical properties such as pH, viscosity, consistency, residues upon drying, and comparable in vitro release rates.

The most promising DPK method involves assessment of drug concentrations in SC through skin stripping (SS). The SC is the rate limiting barrier for most topically applied drug products. The SC also lies in a direct path to the viable tissues of the skin where many diseases of the skin manifest themselves, making either the SC and/or the viable tissues below the site where most drugs must be delivered. Therefore, the concentration of a topically applied drug in the SC for therapeutic efficacy may theoretically be expected to be related to its concentrations in viable tissues such as the epidermis and dermis. Because dermatological products deliver the drug locally and close to the intended site of action in the skin, DPK measurement may provide a means of assessing BE of two dermatological drug products. Two formulations that produce comparable SC drug concentration-time curves may be bioequivalent just as two oral formulations are judged bioequivalent if they produce comparable plasma concentration-time curves. The successful application of DPK thus rests on the assumption that SC concentration-time curves are directly related to concentration-time curves of the active drug substance in the epidermis and dermis.

The results of preliminary investigations indicate that SS allows assessments of both drug uptake into and clearance from the SC. Assessments based on common pharmacokinetic metrics, such as area under the curve (AUC), maximum concentration (Cmax), and time to maximum concentration (Tmax) in SC, have been demonstrated. It should be pointed out that although the DPK metrics are similar to those obtained from plasma based traditional BE studies, (AUC, Cmax, Tmax), the interpretation of DPK is different. SC parameters reflect the driving concentrations that deliver the drug to the epidermis or dermis (site of action). Although these results are useful, actual methodological details for a DPK study involving SS would necessarily be product specific. Because the formulation is removed prior to determining a drug's concentration from the SC, the Cmax obtained by this procedure is not functionally equivalent to Cmax of a drug following oral administration. Subjects employed in a DPK study would ordinarily be individuals exhibiting normal skin, similar to the use of normal healthy subjects in BE determination of oral drug products. Employing patients with diseased skin may introduce additional variability in drug penetration into SC, although it might suggest a subject by formulation interaction. Neither in vitro diffusion cell studies with human skin sections nor in vivo work performed on animals would be acceptable as the sole criteria for BE assessment of topical products. Both cadaver skin and animal skin are known to differ significantly in their physiological properties from normal human skin, and thus both are inappropriate for BE assessment. For this reason, DPK measurements obtained by harvesting SC from cadaver, animal, or ex-vivo human skin (the latter by surgical harvesting) will deviate in important ways from those obtained from live human skin. An important asset of the stripping DPK procedure is that the test and reference formulations can usually be applied to a given subject at the same time, allowing each subject to become his or her own control. Adequate sampling from a sufficient number of stripping sites would be required to characterize drug uptake into and clearance from the SC. Based on preliminary investigations, all the conditions important in the application of the DPK approach in assessing BE seemed manageable.

SPECIFIC CONSIDERATIONS/CONCERNS WITH THE SKIN STRIPPING METHOD

Skin is known to be a highly variable organ in its chemical and physical properties. It exhibits appreciable site-specific inter-intra subject permeability differences in its barrier function properties. Therefore, considerable thought and attention must be given to validation of the SS method and experimental design when conducting a BE study based on measurements of drug concentration in SC. These considerations are discussed in the following paragraphs.

Skin stripping is a technique sensitive operation. Each technician's ability to remove, reproducibly and carefully, the SC should be demonstrated. Appropriate tape or tape discs used for the purpose of SS should be demonstrated to have uniform adhesive properties and to have reproducible properties relative to SC removal. Validation in this regard can be achieved in terms of reproducible amount of skin (weights) or protein contents recovered from test sites. Within subject variability in SS recoveries may be minimized during the experiment through randomization of the product applications to specific sites.

A pilot study should be performed to optimize the sampling scheme for each investigation using a reference product. In the experimental design, both test and reference formulations should be applied simultaneously to each subject at separate, randomized sites for each paired treatment duration. As the next best alternative, crossover comparisons could be made at the selected times for sampling within a fixed study group. Two to five mg of a formulation should be applied to each square centimeter of designated area. As currently practiced and as envisioned for the future, sites on the volar surface of both forearms should be designated for the applications. Care should be taken to avoid positions too close to the wrists or the elbows due to differences in vasculature along the forearm.

Both drug uptake into and elimination from SC should be measured. The elimination phase is characterized after removing all residual formulation from the skin site by swab or other treatment and after taking one or two SS collections to further assure that no formulation (drug) residue remains on the surface. The swabbing of the treated skin site or other removal procedure(s) requires validation, since swabbing with solvents may affect the percutaneous penetration of the drug. A minimum of three time points for drug uptake and three to four time points for drug elimination from SC should be charted in terms of concentration for each phase.

Other experimental concerns with the procedure that needs to be addressed in the course of developing and implementing a DPK stripping study include the method of preparation of the skin site (e.g., a volar forearm) prior to applying any formulation. If the formulations are applied too close to one another, cross contamination between neighboring sites of application may occur and may affect the results. Preliminary work shows that a template or equivalent device should be used to block out each application area and assure consistent removal of stratum corneum from the exact treatment area. Dose consistency in terms of the amount applied per each designated area (square cm) should also be validated. To assure usefulness of the SS methodology, a dose proportionality study should be considered to assure that linearity exists between the amount of drug applied to the SC and the amount of drug collected via SS. Early studies suggest that dose proportionality is linear for standard concentrations of selected topical drugs.

Validating the surrogacy of DPK might be achieved either by developing suitable correlations with pharmacodynamic measurements. Existing data with corticosteroids suggest a relationship between the pharmacodynamic vasoconstrictor response and amounts of these drugs recovered through stripping. In vitro antiviral and antifungal bioactivity has also been shown to correlate with skin stripping data. Based on these preliminary findings, the DPK approach seems to offer a valid means by which to determine the BE of topical dermatological products. Although a need exists to unequivocally establish the linkage between clinical efficacy and the DPK measurements, correlations to establish this linkage will be difficult, if not impossible, because of the variability in clinical response to topical products. The simplicity of the DPK experimental design and procedure needs to be balanced against the need to avoid biases in the comparison of the test and reference formulations. Properly deployed, the stripping method promises to be less expensive than clinical studies, yet definitive and conclusive.

PROTOCOL OUTLINE FOR A SKIN STRIPPING BE STUDY

The following outlines an example of procedural steps involved in the SS methodology. In general, two studies (1) pilot study and (2) pivotal BE study should be carried out. The pilot study should be used to validate the methodology and to optimize the sampling scheme. The BE study should be used to demonstrate the BE between the test and reference products.

- Apply the test and/or reference drug products concurrently at multiple sites.
- After an appropriate interval, remove the excess drug (one site) by wiping three times lightly with a tissue or cotton swab. Appropriate time duration should be determined in the pilot study. For example, it can be 0.25, 0.50, 1.0, 3.0 hours.
- Apply the adhesive tape (e.g., Transpore tape from 3M Company, St. Paul, Minnesota or D-Squame tape from Cuderm Corporation, Dallas, Texas) with uniform pressure, remove and discard the first stripping, as this represents unabsorbed drug on the skin surface.

Repeat the procedure if one tape strip is not sufficient to remove all excess/unabsorbed drug from the skin surface.

- Apply (at the same site), remove and collect nine successive tape strips (from the same site).

Use more than nine skin strippings, if necessary to collect majority of the drug in SC.

- Repeat the procedure of removing excess drug and SS for each site at other designated time points.
- Extract the drug from combined nine SS (2–10 in this example) and determine the concentration using an appropriate validated analytical method.
- Express the results as amount of drug per square cm area of the adhesive tape (e.g., ng/sq cm).
- The above procedure will provide information about the drug uptake in SC.
- To determine a drug elimination phase from SC, apply the drug product (test and/or reference) concurrently at multiple sites (e.g., four sites), allow sufficient exposure period until it reaches apparent steady-state level (in this example, it is three hours); remove excess drug from the skin surface as described above, including the first SS. After predetermined time intervals, collect skin samples using nine (in this example) successive tape strips, and analyze them for drug content. The intervals in this case can be 1, 3, 5 and 21 hours after drug removal.

OTHER TECHNIQUES FOR SAMPLING SKIN

Other methods to determine the drug concentration profile in the local tissues of the skin following its topical application include surface biopsy, surface scraping, sebum collection, sampling of hair and/or nail, collecting fluid from suction blisters, or excising the epidermal roofs of such blisters, shave biopsy, and punch biopsy. Assessing the concentration of systemic deposition of drug substances in hair shafts and/or nail clippings might have its purpose in forensic medicine, but appears to have little utility in comparative drug delivery investigations. Of the other techniques, carefully sectioned horizontal punch biopsies have provided useful information concerning the gradients of drugs which are established across the skin's various strata. Work has

also been performed in terms of charting drug delivery using suction blisters. However, because of scarring, pain and other drawbacks, neither of these techniques appears to offer the same possibilities as SS. Like SS, biopsies and the other mentioned procedures also have to be carefully validated for the specific application.

ADDITIONAL PROMISING APPROACHES TO DETERMINING BE

Certain other procedures may prove useful for specific drugs. For example, pharmacodynamic approaches have already proved useful to document BE of selected topical corticosteroid drug products. This approach is based on the well-known skin blanching effects of corticosteroids. Another pharmacodynamic endpoint that may prove useful is the increase in Trans Epidermal Water Loss (TEWL) and desquamation rate of the SC following the application of retinoic acid. Preliminary data demonstrate TEWL and SC desquamation increase in proportion to a topical retinoic acid dose. This happens over the course of several days, and the phenomenon is readily followed with respect to time.

Another tool which may prove useful is in vitro permeation assessment. Available evidence suggests that rate of permeation of drugs from their formulations and the temporal profiles of such permeation may be similar as long as the formulations themselves are the same. Where differences in clinical endpoints have been shown to exist, permeation rates have been shown to vary in kind. These findings, however, should be regarded now as investigational in nature. The methodology takes considerable skill and experience to work. All comparisons must be performed with skin membranes cut from the same section of unblemished excised skin. The skin sections must also be checked for leaks prior to applying the formulations. Applications of formulations to excised skin should approximate clinical application in the in vivo setting. Considerable work also shows that while in vitro permeation technique is not now suitable for BE assessment of two products, it might be useful for drug developmental purposes.

Confocal laser scanning microscopy appears to have future promise for DPK assessments. This tool allows an investigator to focus a beam to a given depth within a tissue and to take a reading of the concentration of an agent at the level of focus. Since the individual measurement is near instantaneous, a concentration profile can be generated following topical application of a drug product. To date the work done have been based on sectioned buccal mucosa and on fluorescence markers, but the method may possibly be extended to cornified epithelia and UV-absorbers in time. Elimination of interference in UV-absorption by endogenous substances may be difficult.

Another promising technique currently under investigation is microdialysis. In this methodology, a drug or other agent applied to the skin is detected and its concentration measured via an invasive probe placed at the dermis level. Because only trace amounts of compounds are collected, a high degree of analytical sensitivity is required. Exact positioning of the probe is difficult to accomplish, and reading from one placement to the next must therefore be taken at different depths. Nevertheless, since such probes can be left in place for multiple days, the possibility exists that a single probe can be used to study the delivery of drug from the test and reference formulations by applying these sequentially. Much work is needed on this method to establish its value in documenting BE.

SUPAC-SS

The FDA guidance for "Nonsterile Semisolid Dosage Forms, Scale-Up and Post Approval Changes: Chemistry, Manufacturing and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation" (SUPAC-SS) is intended to lower the regulatory burden while assuring the safety and effectiveness of these products under certain post-approval changes. It defines three levels of changes, (1,2,3), tests and filing documentation associated with each level of change. Level 1 changes are those that are unlikely to have any detectable impact on formulation quality and performance; level 2 changes are those that could have a significant impact on formulation quality and performance; and level 3 changes are those that are likely to have a significant impact on formulation quality and performance. The SUPAC-SS guidance allows certain changes in the category of components and composition, manufacturing site, manufacturing process and equipment and scale of manufacturing. Changes in approved formulations graded as level 1 will require reporting in the annual report only. The manufacturer will have to show that in vitro release rates of pre-change and post-change formulations are the same for changes designated as level 2 changes. For changes designated as level 3 change in component and composition, bioequivalence between the pre-change formulation and the post-change formulation or between the post-change test and reference product has to be demonstrated. If the product is a corticosteroid, vasoconstriction comparability is all that needs to be established. In a recently held workshop on September 8–10, 1997 on *Assessment of value and application of in vitro testing of topical dermatological drug products*, it was concluded that in vitro release test is an appropriate tool to assess product sameness under SUPAC related changes for semi-solid dosage forms.

CONCLUSIONS

Skin stripping is a specific dermatopharmacokinetic method that assesses drug concentration in stratum corneum as a function of time. The method involves application of test and reference product to multiple sites on the forearm with each site yielding a single drug concentration. Both drug uptake and elimination phases of dermatopharmacokinetic (DPK) profile should be evaluated to determine traditional metrics, i.e., AUC, Cmax, and Tmax.

Two general views were expressed at the Workshop on the potential universality of skin stripping technique across different therapeutic classes. Some expressed the opinion that because only SC concentrations are assessed, then only diseases in which the SC is the site of action are amenable, i.e., antifungal class of topical dermatological drugs. Others noted that regardless of how far through the skin layers, stratum corneum—epidermis—dermis, the drug needs to penetrate, it needs to pass through the SC first before reaching deeper skin layers. Because the SC is the rate limiting barrier for drug penetration into the skin, concentration in the SC may provide

meaningful information for comparative evaluation of topical dosage forms.

With proper validation, DPK is expected to be a viable method for BE evaluation of topical dermatological drug products. In addition to DPK data for BE, qualitatively same formulation of test and reference product, an in vitro drug release rate data, and, in certain instances, a comparative pharmacodynamic evaluation may be helpful in establishing the BE of the test to the reference product. A combination of these techniques may provide sufficient information for use of DPK in BE assessment in lieu of clinical trials. This will allow industry to pursue the development of safe and effective generic topical products in a scientifically and regulatorily sound manner.