Cutaneous Pharmacokinetic Approaches to Compare Bioavailability and/or Bioequivalence for Topical Drug Products

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KEYWORDS

• Topical • Dermatologic • Pharmacokinetics • Bioequivalence • Generics

KEY POINTS

- Extraordinary price increases for topical products, associated with insufficient competition from generics, has raised concerns about patient access to important dermatological treatments.
- The high cost of comparative clinical endpoint bioequivalence studies in patients has been a barrier to the development of topical generic products.
- The efficiency of cutaneous pharmacokinetics based bioequivalence approaches is facilitating the development of topical generics and enhancing patient access to affordable, high quality treatments.

INTRODUCTION

In simple terms, bioequivalence (BE) refers to biopharmaceutically equivalent product performance. Unlike the full evaluation of clinical safety and effectiveness that must be established when a new drug product is initially approved, the evaluation of BE involves a comparison of the test product to its reference product in a study whose fundamental scientific principles allow the clinical performance of the products to be inferred. This kind of assessment is typically relevant in two situations: when comparing a generic version of a drug product to its reference listed drug (RLD) product, or when a drug product experiences a change following approval, necessitating a demonstration of equivalent product performance despite the change.

The concept of BE exists at the intersection of science, medicine, law, and regulation. Title 21 of the US Code of Federal Regulations, Part 320 (21 CFR § 320)[1] followed by the US Food and Drug Administration (FDA) discusses bioavailability (BA) and BE requirements for drug products seeking marketing approval in the United States. These regulations evolved from the recommendations of a drug product BE study panel that was formed by the FDA's Office of Technology

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Assessment (OTA) in the 1970s.[2] Soon thereafter, the US Congress passed legislation that provided the framework for being able to approve generic drug products based on the results of a BE study, rather than based on clinical studies demonstrating effectiveness and safety. This legislation, the Drug Price Competition and Patent Term Restoration Act of 1984[3] (commonly known as the Hatch-Waxman Amendments) established the Abbreviated New Drug Application (ANDA) approval pathway for generic drug products and was particularly relevant for solid oral dosage forms, rather than topical or other locally acting dosage forms. Almost two decades later, the US Congress passed legislation that revised the Hatch-Waxman Amendments with Title XI of the Medicare Prescription Drug, Improvement and Modernization Act of 2003.[4] Under Title XI, "for a drug that is not intended to be absorbed into the bloodstream," the act authorized the FDA to "establish alternative, scientifically valid methods to show bioequivalence if the alternative methods are expected to detect a significant difference between the drug and the listed drug in safety and therapeutic effect." More specifically, 21 CFR § 320.24 describes several types of evidence to measure BA or establish BE.[5] Indeed, in recent decades, several test methods have been discussed and developed to evaluate topical BA and BE.[6–23] Among these, the use of pharmacokinetics (PK)-based approaches to evaluate the BA for topically applied drugs is an ideal approach by which to characterize the rate and extent to which an active ingredient becomes available at or near its site of action in the skin, and thereby, to assess the BE of test and reference topical products.[21] This is reflected in 21 CFR § 320.24, which specifies that "[an] in vivo test in humans in which the concentration of the active ingredient … in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time…; or [an] in vitro test that has been correlated with and is predictive of human in vivo bioavailability data" is considered to be among the most accurate, sensitive, and reproducible approach for determining the BA or BE of a product. This work provides a critical assessment of these cutaneous pharmacokinetic approaches to compare BA and/or BE for topical drug products.

IN VITRO CUTANEOUS PHARMACOKINETICS

The rationale for using an in vitro permeation test (IVPT) to support the assessment of topical BA or BE is readily apparent since it provides a simple means by which to measure the rate and extent of absorption of topically applied drugs. Use of the model as a potential BE surrogate was discussed by regulatory and industrial scientists in 1986, and although a substantial body of literature already existed at that time on its important role in the development of topical formulations, particularly as a screening tool to evaluate the impact of vehicle modification on absorption, it was concluded that "…more experience with this application of the technology was needed."[7]

The situation is vastly different today, and IVPT is recognized by scientists worldwide as a valid technique to quantify the absorption of chemicals into and through the skin. From a regulatory perspective its application in Europe, particularly in toxicology, is relatively well established. As a result of the acceptance of Guideline OECD 428 and Guidance Document OECD 28,[22,23] which describe the procedures to be used in the conduct of IVPT, all members of the European Union (EU) accept human in vitro permeation data to assess the risk associated with dermal exposure to pesticides, biocides, cosmetic ingredients, and industrial chemicals. The European Medicines Agency (EMA) has drafted guidelines requiring the use of IVPT to characterize the performance of transdermal products as part of new marketing authorization applications, and this applies to generic applications as well.[24] In the U.S. the Environmental Protection Agency (EPA) accepts human IVPT data as part of the assessment of systemic risk for the registration of pesticides.[25–27] In 2014, the FDA first recommended an IVPT study to support a demonstration of BE in the product-specific guidance for acyclovir cream, 5%, and has since recommended IVPT studies for numerous other topical products.[28] Of all the surrogate tests available to establish topical BE, IVPT stands out as the one that has been the most studied, that appears to have the broadest application and acceptance within the scientific community, and for which the most validation data exist.

The in vitro measurement of percutaneous absorption is possible, because excised skin retains its barrier properties for several days following excision from the body. Additionally, barrier function is not damaged by freezing for many months, and then thawing, so long-term storage is possible. Conceptually, the purpose of an IVPT study and its conduct are straightforward. To quote OECD 28, "The test preparation is applied to the surface of excised skin, which is mounted in a diffusion cell. The receptor fluid, which must have an adequate capacity to solubilize the test substance, is maintained in contact with the underside of the skin from the time of application until the end of the collection of the receptor fluid. The

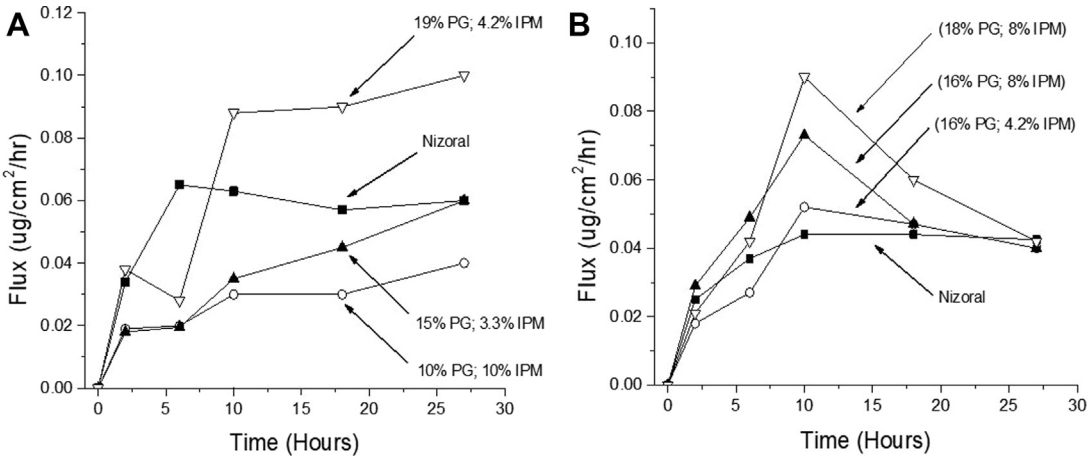

Fig. 1. Use of IVPT to match the absorption profile of a reference product (Nizoral). (*A*) Concentration of propylene glycol (PG) and isopropyl myristate (IPM) varied in 3 prototype formulations. Data suggest PG level should be between 15% and 19%. (*B*) Concentration of PG and IPM varied in 3 additional prototypes. Best match to reference product is PG = 16%, IPM = 4.2%. (*Data From* Franz TJ, Lehman PA, Raney SG. The cadaver skin absorption model and the drug development process. Pharmacopeial Forum 2008;34:1349-1356; with permission.).

test preparation remains on the skin for a specified period of time, relating to potential human exposure, and then the test preparation is removed by an appropriate cleansing procedure. The receptor fluid is sampled at time points throughout the experiment to ascertain the mass (and possibly rate) of the test substance (including any significant metabolite) passing through the skin. At the end of the study, the dislodgeable dose, the amount associated with the skin and the amount in the receptor fluid is determined. These data are necessary to calculate the total skin absorption, and allow for an estimate of the total recovery of the test substance."[23]

An example of the type of data obtained from IVPT, as well as an illustration of the sensitivity of the method, is presented in **Fig. 1**. During the development of a generic ketoconazole cream, reverse engineering failed to clearly identify the concentration of two cosolvents, propylene glycol and isopropyl myristate. Therefore, prototype formulation variants were prepared with different amounts of each of these two cosolvents, and the cutaneous PK of ketoconazole from each was compared relative to the RLD ketoconazole cream. Across 2 iterative stages of prototype formulation variation (**Fig. 1**), 6 prototype (test) formulations were evaluated, and IVPT studies were used to identify the one from which the rate and extent of ketoconazole BA best matched that of the RLD. Only 1 of the 6 was found to provide a rate and extent of absorption that closely matched that of the RLD product, and subsequent clinical evaluation confirmed its BE to the RLD product.[29]

The ultimate goal of the IVPT model system is to obtain data that are equivalent to those obtained in vivo. To this end, several expert groups have thoroughly examined all aspects of the in vitro methodology and, for the most part, are in agreement as to several critical elements in protocol design.[23,30–32] These can be summarized as:

- Use human skin (dermatomed to $\leq$500 μ or isolated epidermis).
- Either static or flow-through chambers are acceptable.
- Maintain skin surface temperature at 32° $\pm$ 1° C.
- Verify integrity of skin barrier through measurement of ^3H_2O flux, transepidermal water loss, or electrical resistance.
- Verify drug stability in receptor solution and sample processing procedures.
- Maintain adequate solubility conditions in receptor solution (ideally, a solubility 10 times greater than needed for experimental conditions).

The latter point is critically important since many topical drugs have limited water solubility. Erroneously low absorption values can be obtained solely on the basis of inadequate receptor solubility which acts to reduce the gradient for diffusion.[33–36] The recommendation to use dermatomed skin or epidermal membranes is directed at the same potential problem. The highly aqueous dermal compartment, normally approximately 1 to 2 mm in thickness, can serve as a potential barrier to the absorption of compounds with limited water solubility. Under in vivo conditions the diffusing drug can partition into the practically infinite sink of the systemic circulation in the uppermost region of the dermis. In the in vitro (IVPT) model the drug

typically traverses the epidermis and (when using dermatomed skin) part of the dermis before it can partition into the sink of a receptor solution with adequate solubility.

In Vitro/In Vivo Correlation: Percutaneous Absorption

Evidence to support use of the in vitro (IVPT) model to establish BE comes from studies of in vitro/in vivo correlation (IVIVC), and these fall into two main areas: studies that show good correlation between the amount of a compound absorbed in vitro with that absorbed in living humans, and studies that demonstrate the ability of the IVPT model to reach the same conclusion as in vivo human comparative clinical endpoint studies with respect to the BE of two drug products.

Lehman and colleagues reviewed the literature to collect data on compounds whose percutaneous absorption had been measured both in living humans and in the IVPT model.[37] Ninety-two data sets encompassing 30 compounds were collected from 30 published studies. Two analyses were performed: a comparison of the data from all studies irrespective of whether the conditions under which the in vitro study was conducted fully matched those of the in vivo study, and comparison of the data from only those studies in which full harmonization of the experimental conditions existed between the in vitro and in vivo studies. In vitro to in vivo (IVIV) correlation was examined by calculating the ratio of total absorption in vitro/total absorption in vivo, where total absorption was reported as a percent of the applied dose.

Examination of the IVIV ratios for all 92 data sets showed a definite trend for the observed values to follow the line of perfect 1:1 correlation (**Fig. 2**). The average IVIV BA ratio for all 92 data sets was 1.6; however, variability was relatively large, and IVIV BA ratios ranged from 0.18 to 19.7. A substantial improvement in correlation was found in the subset of data from in vitro studies in which the experimental conditions matched those used in vivo in all critical aspects. Eleven data-sets were identified in which the in vitro protocol was fully harmonized with the in vivo protocol (**Fig. 3**). The average IVIV BA ratio for the group now approached 1 (0.96), and the ratio for any individual data set differed from exact correlation (ie, a ratio of 1.0) by less than twofold (the ratios ranged from 0.58 to 1.28).

This analysis effectively demonstrated that absorption data obtained from the excised human skin IVPT model can closely match those obtained in living humans if the experimental conditions match those found in vivo. The two factors leading to exclusion of most of the original 92 data sets were the use of skin from different body sites and different formulations of the compound under study. The latter factor (discrimination of the BA from different formulations) is of special significance, as it is most relevant to the use of the model for BE testing.

In Vitro/In Vivo Correlation: Clinical Studies

Studies that support the use of the excised human skin IVPT model specifically for establishing the BE of topical drug products have been presented by

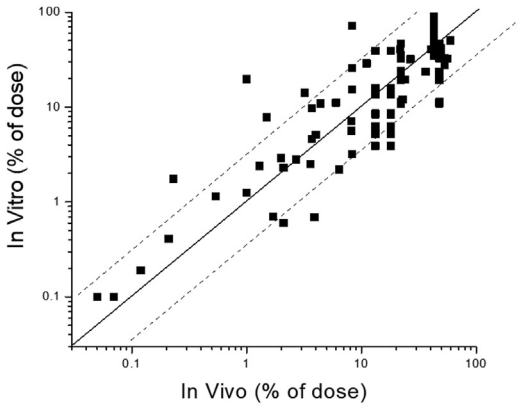

Fig. 2. IVIV ratios of total absorption for 92 data sets plotted on a log-log scale. Ratios ranged from 0.18 to 19.7, with an overall mean of 1.6. Solid line denotes ideal 1:1 correlation, and dashed lines denote ± threefold difference from ideal. (*From* Lehman PA, Raney SG, Franz TJ. Percutaneous absorption in man: in vitro-in vivo correlation. Skin Pharmacol Physiol 2011;24(4):224-30; with permission.)

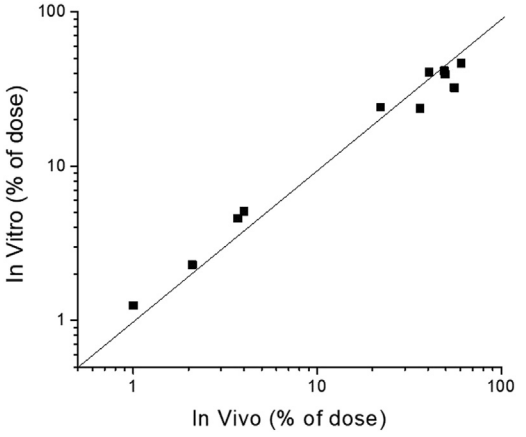

Fig. 3. IVIV ratios of total absorption for 11 fully harmonized data sets plotted on a log-log scale. IVIV ratios ranged from 0.58 to 1.28, with an overall mean of 0.96. Solid line denotes ideal 1:1 correlation. (*From* Lehman PA, Raney SG, Franz TJ. Percutaneous absorption in man: in vitro-in vivo correlation. Skin Pharmacol Physiol 2011;24(4):224-30; with permission.)

Franz and colleagues.[38] Seven prospective generic topical drug products (5 glucocorticoid creams and ointments and 2 tretinoin gels) were evaluated during their preclinical development. Absorption of the active pharmaceutical ingredient (API) was compared side by side to the reference products in the excised human skin IVPT model. All of the test products were later evaluated clinically (by a comparative clinical endpoint study or an in vivo vasoconstrictor assay) and shown to be BE to their respective reference products, thus, affording a unique opportunity to test and demonstrate the validity of the IVPT model to assess BE for topically applied drugs.

In agreement with the clinical data, the IVPT results showed that the BA of the test products were a remarkably close match to that of the reference products. Both tretinoin absorption studies were run as simulated BE studies and a sufficient number of replicate skin sections were included to calculate confidence intervals (**Table 1**). All parameters fell within the traditional BE limits (0.80–1.25) except for the maximum absorption rate of the 0.025% gel product, which fell slightly outside the upper bound of 1.25.

The 5 glucocorticoid studies were not designed as simulated BE studies but instead as screening studies in which only nine skin sections per product were evaluated. Several test formulations of each drug were initially compared to several lots of the reference products with the objective of selecting the best match (targeting a test/reference approximately 1.0) to move into a pivotal vasoconstrictor study. Of the 5 test formulations selected for clinical study, mometasone furoate was the only one in which the test/reference ratio (0.63) was not close to 1 (**Table 2**).

Yet, by vasoconstrictor assay, it and the other four glucocorticoids were found to be bioequivalent to the reference products and subsequently approved. This one instance of an apparent lack of agreement between IVPT and vasoconstrictor assay was determined not to be caused by a shortcoming in the IVPT model but potentially because of a greater discrimination sensitivity of the IVPT relative to the vasoconstrictor assay. An example of this is seen in **Table 2**, where alclometasone cream and ointment appear approximately equipotent by vasoconstrictor assay, yet differ by more than 15-fold in absorption when assessed by an IVPT.

In another study by Shin and colleagues, the effect of heat on nicotine BA was evaluated following topical application of nicotine transdermal delivery systems (TDS) both in vitro (using IVPT) and in vivo (human serum sampling). The study designs used for both IVPT and in vivo PK study were harmonized and included application of 1 hour transient heat after TDS application. An in vitro–in vivo correlation (IVIVC) was established for nicotine BA, and the result of this work showed that a well-designed and well-controlled IVPT study can be used to assess the relative heat effect on nicotine BA from the TDS products and can be predictive of the heat effect that was observed in vivo.[39]

In summary, IVPT is widely regarded as a valid method by which to quantify the absorption of chemicals into and through human skin, and this acceptance can be justified on the basis of good in vitro/in vivo correlation. Validation of IVPT data has also been specifically extended into the

Table 1
Comparison of primary in vitro endpoints for two strengths of generic tretinoin gels (test) versus the reference products.

	Test	Reference	Test/Reference	90% CI
0.01% gel				
Total absorbed	3.00	2.97	1.02	0.97–1.07
J_{max}	0.55	0.57	1.04	0.93–1.15
T_{max}	3.60	3.57	1.04	0.92–1.16
0.025% gel				
Total absorbed	3.49	3.47	1.03	0.95–1.10
J_{max}	0.91	0.88	1.11	0.95–1.28
T_{max}	3.66	3.72	0.98	0.97–1.00

Total absorbed = ng/cm^2/48 h; J_{max} = maximum rate of absorption, ng/cm^2/h; T_{max} = time of maximum rate of absorption, minutes.

90% confidence interval (CI) is calculated on the ratio of the means of natural log transformed data.

Test and reference values represent natural log transformed means.

Data from Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol 2009;22(5):276-86.

Table 2
Comparison of data obtained by the in vitro permeation test versus the in vivo vasoconstrictor assay on five generic glucocorticoid (test) products versus the corresponding reference (ref) products

	In Vitro Absorption (ng/cm^2/48 h)			In Vivo VC Assay (Negative AUEC$_{0\text{-}24\ h}$)		
	Test	Ref	Test/Ref	Test	Ref	Test/Ref
Alclometasone cream	4.52	4.39	1.03	18.5	16.8	1.10
Alclometasone ointment	66.95	70.0	0.96	16.0	17.4	0.92
Halobetasol cream	110.4	96.9	1.14	33.1	30.7	1.08
Halobetasol ointment	246.7	256.3	0.96	28.6	28.5	1.00
Mometasone ointment	213.4	338.7	0.63	13.7	12.3	1.11

Listed numbers are mean values.
Data from Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol 2009;22(5):276-86.

sphere of BE, where it has been shown that results obtained in the IVPT model are in agreement with those obtained from the clinical studies that were used as the basis for approval of 7 generic drug products. The IVPT model can provide relevant and accurate results with good IVIVC, and provides information regarding the variability in human skin permeation that is representative of the in vivo population of individuals from whom the skin was acquired. Also, it can be reproducible and discriminating. To facilitate its utility in the context of topical BE assessment, the procedures for the conduct of these studies have been increasingly standardized internationally by several expert groups. Commensurate with the power and utility of this method, validated IVPT studies are required to be performed and included within EMA regulatory submissions for all transdermal products.[24] Independently, in vitro (IVRT or IVPT) studies conducted for the purpose of demonstrating BE are required under 21 CFR Part 320 to be reported within ANDA submissions.[40] Also, the FDA recommends an IVPT study to support a demonstration of BE for numerous topical products.[28]

IN VIVO CUTANEOUS (EPIDERMAL) PHARMACOKINETICS

As previously noted, the BA or BE of systemically acting drug products such as an acetaminophen tablet is typically evaluated using PK studies.[41] For a dermal product, however, evaluating the cutaneous PK and quantifying the rate and extent to which drug becomes available in a solid tissue such as the skin has been challenging. Historically, there has been a lot of interest in developing techniques to evaluate the rate and extent of a drug's BA in the topmost layer of the skin, the epidermis. The stratum corneum (SC), which is composed of the keratinized remains of rapidly dividing epidermal cells bound together by a lipid matrix, is the outer most layer of the epidermis and is the area of the skin that is visible and accessible externally.

Historically, spectroscopy-based techniques such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) have been utilized for the quantification of drugs and to monitor changes in the structure of the SC barrier following the application of locally applied dermal products, either in vitro or following SC removal by tape stripping (TS).[42] Raman spectroscopy-based techniques were largely used for the evaluation of SC thickness.[43] However, recent advances in noninvasive imaging technology suggest that it may be feasible to use Raman based imaging techniques such as simulated Raman scattering (SRS) microscopy as a label-free, nondestructive tool to monitor the permeation of drugs across the different layers of the skin following topical application of dermal drug products.[44] Saar and colleagues illustrated that differences in the rate of permeation of a drug across the intercellular pathway in the SC (compared with the follicular pathway) could be directly observed using SRS, in addition to observing the metamorphosis of the formulation, including precipitation of the drug crystals and permeation of cosolvents.[45]

In 2013, Mateus and colleagues used Confocal Raman Spectroscopy (CRS) to evaluate drug disposition in the skin following topical application of ibuprofen solutions in vivo. Saturated solutions of ibuprofen in propylene glycol (PG) and PG: water (50:50, 75:25 v/v) solutions were simultaneously applied to 5 healthy subjects for 30 minutes. The semi-quantitative assessment

from the study indicated that the permeation profiles of ibuprofen across the skin were comparable to previously published TS data.[46] The observed differences in the permeation of ibuprofen from the 3 formulations suggest that it may be feasible to use Raman spectroscopy-based techniques to assess similarities and differences in drug distribution and to evaluate the BA of a drug from a topically applied drug product.

A practical matter impacting the utilization of such technology for dermal drug development is that a potential limitation to the quantification of drugs (or other compounds of interest) in the skin is the signal interference from the skin itself. Another potentially significant limitation is the increasing attenuation of the signal at deeper levels beneath the surface of the skin, potentially impacting the quantitative or semiquantitative measurement of a drug in the deeper epidermis. There are also technical challenges related to how rapidly measurements can be conducted, how compatible specific technologies may be with different APIs, how data analysis can be automated, and how the models can be validated to be sensitive and discriminatory so that they can be used to support product development and regulatory assessments.

Under the Generic Drug User Fee Amendments (GDUFA) science and research program orchestrated by the FDA's Office of Generic Drugs,[47] CRS-based methods are being developed for the noninvasive evaluation of epidermal PK (University of Bath (Grant# 1U01FD006533) and Massachusetts General Hospital/Harvard Medical School (Grant# 1U01FD006698)). Simultaneously, other research groups are developing automated image analysis tools to evaluate drug distribution in the skin; Jeong and colleagues evaluated the uptake of two drugs (minocycline and tazarotene) within human facial skin using a selective visualization method to monitor and quantify local drug distributions within the skin. Specifically, fluorescence lifetime imaging microscopy (FLIM) was used for the study since both molecules have fluorescence lifetimes that are distinct from the skin's autofluorescence. The publication suggests that the approach to data analysis can be generalized, and that integrating the analysis technique with real time or portable instruments will allow rapid assessment of drug distribution in vivo.[48]

In summary, techniques used to evaluate epidermal PK have advanced substantially over the last 25 years. Although TS-based methodologies have been used previously to quantify and compare the BA of topically applied drugs in the SC, more recent advances suggest that it is feasible to use noninvasive Raman spectroscopy-based techniques to monitor the cutaneous PK and drug distribution in the epidermis following topical application on the skin. Nonetheless, there are current challenges related to the use of these spectroscopic methods for monitoring many topical dermatologic drugs (because many of these drugs may not have an unique Raman spectra) and challenges related to the analysis of relatively large amounts of data with substantially greater spatiotemporal resolution compared to data generated with traditional approaches by which BA/BE are currently being evaluated.

IN VIVO CUTANEOUS (DERMAL) PHARMACOKINETICS

A comparison of in vivo dermal PK for topically applied drug products using dermal microdialysis (dMD) and dermal open flow microperfusion (dOFM) is being investigated to use these methodologies for a BE study as an alternative to a comparative clinical endpoint BE study. Both dMD and dOFM are similar in that both use a thin, hollow tube (or, in the case of dOFM, an open metal mesh[49]), referred to as probe that is inserted below the skin surface, into the dermis, and perfused with a physiologic solution so that drug can be collected in the perfusate from the surrounding tissue.[17,49,50]

In dMD, the probe has a polymeric, porous semipermeable membrane that is often made from material that is the same as or similar to kidney dialysis filters. The porous membrane allows the exchange of analytes (e.g., a drug) between the continuously perfused isotonic fluid and the dermal interstitial fluid (ISF) via passive diffusion across the dialysis membrane. Thus, only drugs that are unbound and soluble in the ISF can be measured using dMD probes, and the collected dialysate is free of proteins or other large molecules and can typically be analyzed without any sample preparation (cleaning).[51]

In dOFM, the membrane is a fenestrated metal mesh, and it uses a push/pull mechanism to collect diluted ISF containing the analyte (drug) of interest (including both bound and unbound drugs).[52] Thus, theoretically, drugs can be quantified using the dOFM technique irrespective of their protein binding characteristics or their lipophilicity; however, the samples collected using this technique often require processing to clean up the sample prior to quantitative analysis.[53]

In both dMD and dOFM, the insertion of the probe is associated with mild discomfort that can be reduced or eliminated by the use of local analgesia (eg, the application of ice packs). However, the probe insertion produces a localized trauma that

leads to histamine release and subsequent local hyperemia and edema. For this reason, a period of time (eg, 60–90 minutes or more) is typically needed for the tissue reaction to subside and for physiologic re-equilibration, prior to starting an experiment.[51]

A comprehensive discussion of the procedures related to dMD studies can be found in the article by Holmgaard and colleagues[51] and of the procedures related to dOFM in articles by Bodenlenz and colleagues[53,54] As discussed by Holmgaard and colleagues, the designs of dMD probes differ in size, shape, and material and are selected based on the intended site of implantation.[51] Linear probes are usually thinner and more flexible and cause less tissue disruption during insertion; therefore, they are the most widely used probes for dMD. In most cases, a guide cannula is used to insert the dialysis probe into the middermis (ideally a depth of 0.6–1.0 mm) horizontal to the skin surface, typically on the ventral forearm or the thigh. The precise depth within the skin can be determined by ultrasound, and, with practice, consistent placement at approximately the same depth is achievable. Once the cannula is withdrawn, the probe is fixed in place, and one end is connected to a fluid delivery pump and the other end to a collection system. Isotonic saline or another physiologic solution (such as Ringer solution) is pumped through the probe, generally at a low flow rate (0.9–1.5 μL/min) to allow sufficient time for the drug to diffuse from the ISF to the perfusion solution. Nevertheless, a total equilibrium between the 2 phases is not completely achieved, and, to determine the drug concentration in the surrounding tissue fluid, a recovery rate or extraction efficacy is routinely calculated, defined as the ratio of drug concentration in the dialysate to that in the surrounding tissue fluid. A recovery rate can be obtained from several different procedures and depends on the experimental conditions.[54,55] As an example, in a study by Kuzma and colleagues in Yucatan mini pigs, the BA of metronidazole from metronidazole topical gel and a cream product was evaluated.[56] In this study, deuterated (D3)-metronidazole was used as an internal standard to calibrate the dMD method, and it was added to the physiologic buffer that was perfused through dMD probes. The concentration of D3-metronidazole was measured in the dialysate samples, and a correction factor (defined as the relative loss of the D3-metronidazole compared with the concentration in the perfusate) was used to monitor probe performance through the duration of the study and to estimate the actual concentration of metronidazole in dermal ISF. That being said, although using a recovery rate or correction factor can be critically important in some research areas, its use for BE may not be essential, as the test/reference ratio in the dialysate (the relative amounts rather than the absolute amounts) can be the basis for a statistical comparison of data obtained for the test and reference products.

Because of the hydrophilic nature of the perfusion fluid, adequate recovery of lipophilic drugs can be challenging. The perfusate can be modified by the inclusion of serum albumin or other additives such as Intralipid, Encapsin, or cyclodextrins to improve recovery. However, in a study in which estradiol absorption from a commercial TDS was examined, detectable drug levels were found in only 8 of 10 in vitro experiments in spite of the addition of 7% serum albumin to the perfusion fluid.[57] Likewise, the measurement of glucocorticoid absorption in humans following topical application has only been reported with 4% clobetasol propionate in alcohol using Intralipid in the perfusate, but not with any commercial products at the most common 0.05% clobetasol propionate product strength.[58]

Clinical Correlation

Substantial work has been done in humans to demonstrate the feasibility of dMD and dOFM for measuring the cutaneous BA of drugs following topical application. Several of these studies have special relevance to BA and BE, because they involve a direct comparison of the rate and extent of topically applied drug absorption from different vehicles, and confirm the ability of dMD to accurately confirm a comparable dermal PK for products having equal BA and sensitively discriminate differences in the dermal PK between products with inequivalent BA. For example, Kreilgaard and colleagues used dMD to compare the absorption of 5% lidocaine from a commercial product to that of a laboratory-made microemulsion formulation and found greater absorption from the microemulsion vehicle.[59] Over the 4-hour collection period, the average areas under the curve (AUCs) were 2900 plus or minus 2690 versus 867 plus or minus 488 mg/L for the microemulsion and commercial product, respectively. The lag time was also found to be shorter for the microemulsion compared with the commercial product, 87 versus 110 minutes, respectively ($P<.02$). A second part of the study compared the PK results obtained by dMD with a pharmacodynamic (PD) response, pain reduction. Both products diminished the pain elicited by a standardized stimulus compared with a placebo microemulsion, but the PD test could not distinguish between the 2 active products, illustrating the greater sensitivity of dermal

Table 3
Comparison of lidocaine permeation from a cream and ointment formulation determined by dermal microdialysis

	AUC[a] (ng/mL/min)	C_{max}[a] (ng/mL)	Lag Time[b] (min)
Cream			
Mean	15,983	112	26.0
CV (%)	41	41	18
Ointment			
Mean	3309	27.5	45.6
CV (%)	42	41	27
t-test (*P* value)	0.018	0.03	0.06

[a] Geometric mean.
[b] Time at which drug level exceeded the lower limit of quantitation.
Adapted from Benfeldt E, Hansen SH, Volund A, Menne T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol 2007;127(1):170-8; with permission.

PK endpoints monitored by dMD relative to the PD endpoints.

Benfeldt and colleagues also evaluated the relative BA of lidocaine.[60] Two commercial 5% products (cream and ointment) were applied at different times to the ventral forearms of 8 subjects. Each was applied to 2 separate sites, and drug permeation was assessed using 2 dialysis probes per site. Analysis of the AUC over the 5-hour collection period showed an almost fivefold greater absorption from the cream product (**Table 3**). Of note, no statistically significant difference was seen between the results obtained from the 4 separate probes. In analyzing total variance, 19% was associated with differences between probes, 20% was caused by a difference between the 2 dosing sites, and 61% was caused by intersubject variability.

Tettey-Amlalo and colleagues examined the use of dMD for BE by measuring ketoprofen permeation in 18 subjects.[61] A single commercial 2.5% gel product was applied to 4 separate forearm sites and the dialysate collected over a 5-hour period from one probe per site. The experimental design allowed 2 sites each to be assigned as mock test and reference sites and, because 4 sites were available, 3 different randomization schemes could be used (TTRR/RRTT, TRTR/RTRT, TRRT/RTTR) to assess the BE of the test and reference products. Intrasubject variability for probes averaged approximately 10%, whereas intersubject variability for each probe averaged approximately 68%. Although the BE assessment for all 3 randomization schemes found the confidence interval for the test/reference ratio of log transformed data to fall within 0.80 and 1.25, 1 of the 3 sequences was found to lack the 90% power (**Table 4**). It was suggested that this may have been caused by regional variation within the forearm itself, which has been reported before,[62] because the aberrant sequence was the one comparing the most proximal and distal sites

Table 4
Comparison of ketoprofen permeation from a single 2.5% gel randomly assigned as both test and reference to 4 adjacent sites on the forearm

	AUC_{0-5} (ng/mL/h)		Statistical Analysis	
Sequence	Test[a]	Reference[a]	90% CI[b]	Power of ANOVA (%)
TTRR/RRTT	155.5 ± 98.9	150.0 ± 107.3	0.97–1.15	92.88
TRTR/RTRT	152.0 ± 99.2	153.5 ± 103.9	0.90–1.09	95.95
TRRT/RTTR	139.9 ± 87.3	165.6 ± 116.7	0.80–0.94	53.99

[a] mean ± SD.
[b] 90% confidence interval (CI) calculated on the ratio of the means (test/reference) using log transformed data.
Adapted from Tettey-Amlalo RN, Kanfer I, Skinner MF, Benfeldt E, Verbeeck RK. Application of dermal microdialysis for the evaluation of bioequivalence of a ketoprofen topical gel. Eur J Pharm Sci 2009;36(2–3):219-25; with permission.

(both having low AUCs) with the 2 middle sites (both having high AUCs).

Another study having a similar objective of specifically using dMD for evaluating the BE of various drug products was that of Garcia Ortiz and colleagues[63] Metronidazole permeation from 3 commercial products, approved as being BE in Europe, was measured concurrently in 14 subjects. Each product was randomly assigned to 1 of 3 adjacent sites on the ventral forearm, and 3 probes were inserted per site. Although no statistically significant differences in AUC (P>.05) were found following a 5-hour collection period, and there was high intersubject variability (116%–223%), and none of the products met traditional criteria for BE (the 90% CI calculated for the ratio of the means using log transformed data fell outside the bound of 0.80–1.25 for all comparisons of the 3 products to each other). It was estimated that 34 subjects would have been needed to attain sufficient statistical power for this analysis.

Although the aforementioned studies demonstrate the potential of dermal PK sampling techniques to assess the BA/BE of topical dermatologic products, 1 major limitation that existed in all of those studies had been the short duration of the study (eg, 4–6 hours), which may not be sufficient to adequately capture the dermal PK profile of topically applied drugs. Perhaps the most compelling evidence supporting the use of dermal PK to evaluate topical BE in human subjects comes from the work of Bodenlenz and colleagues, who compared the topical BA of acyclovir from test and reference products.[54] Among the notable advancements of their approach was the use of small portable pumps that allowed the subjects in the study sufficient mobility that the dermal PK could be monitored continuously for 34 hours. In addition, the investigators used dOFM probes and introduced several procedural controls into the study design, including the use of duplicate sets of probes and templates to stabilize anatomic flexion of the upper leg (thigh), where the probes were inserted, to enhance the precision of the results and the discrimination sensitivity of the methodology. Using traditional BE PK endpoints of C_{max} and AUC, and traditional BE limits of 80% to 125%, the investigators compared the reference product to itself as a positive control for BE, and compared the reference product to a test product as a negative control for BE. The positive control products were accurately shown to be bioequivalent, while the negative control products were discriminated as not being bioequivalent, both in the same population of the 20 subjects.

A noteworthy and unique advantage of dMD and dOFM over other cutaneous PK-based techniques is their ability to be used in diseased skin; this not only allows for measuring drug concentrations at or near the site(s) of action in the skin, but also for monitoring the intradermal biochemistry in patient populations to establish PK/PD relationships. In a pilot study by Quist and colleagues, 6 patients with chronic plaque psoriasis received methotrexate either orally or through subcutaneous injection and the drug concentration in dermal ISF was measured using dMD in psoriasis plaque and non-lesional skin, and in plasma using blood sampling for 10 hours.[64] Methotrexate levels and $AUC_{0–10\ h}$ were reported to be higher in lesional than nonlesional psoriatic skin and also much lower than those in the blood samples. In another study, 12 patients with moderate atopic dermatitis (AD) received topical treatment on either arm with tacrolimus topical ointment, 0.1% or a lotion containing 12% ω-6 fatty acids (polyunsaturated fatty acids; PUFA) twice daily for 5 consecutive days.[65] On day 6, dMD sampling was performed, and dialysate samples were collected at 30-minute intervals for 8 hours from 4 defined skin areas: lesional, nonlesional, and topically treated skin (treated with either tacrolimus or PUFA). Markers of oxidative stress (F2-isoprostanes; 5- and 8-prostaglandin F2α) and inflammation (9α,11α-prostaglandin F2α; and prostaglandin E2) were quantified. The results of this dMD study demonstrated that treatment with tacrolimus compared with PUFA appears to suppress eicosanoids more efficiently in AD skin, and the levels of eicosanoids were increased in clinically lesional skin compared with nonlesional AD skin. Bodenlenz and colleagues also investigated the PK of a highly lipophilic antipsoriatic drug using dOFM.[66] In that study, 12 patients received Dermovate (clobetasol propionate) topical cream, 0.05% once daily on small lesional and nonlesional skin test sites for 14 consecutive days. On days 1 and 14, dermal ISF was sampled by dOFM continuously from baseline to 24 hours after the dose while the cream remained on the skin and a nonocclusive dressing was used to protect the test sites. On day 1, quantifiable drug concentrations in the dermal ISF of nonlesional skin was obtained at approximately 10 hours after the dose, and maximum concentrations were observed at 18 hours after the dose (mean C_{max} 0.61 ng/mL). In lesional skin, the drug levels steadily increased on day 1 but did not reach the lower limit of quantitation (LLOQ) during the entire 24-hour sampling period in most subjects (mean C_{max} 0.19 ng/mL). On day 14, the C_{max} (mean C_{max} 1.00 ng/mL) was reached at 10 hours in nonlesional skin, while in lesional

skin, the clobetasol propionate levels were already quantifiable at baseline (before the dose), and moderately increased after dosing to reach C_{max} at 18 h (mean C_{max} 0.68 ng/mL). Overall, the authors concluded that the thickened psoriatic stratum corneum can decrease the skin permeation rate for lipophilic topical drugs like clobetasol propionate.

In summary, dermal PK techniques such as dMD and dOFM can directly monitor the dynamic concentrations of a drug in the dermis, which, for most dermatologic drugs, is at or near their site of action. Several studies have demonstrated the ability of these dermal PK techniques to monitor the permeation of several topically applied drugs with good sensitivity to distinguish differences in a drug's BA when applied in different vehicles. Based on the experimental variability observed in these studies, it is reasonable to expect that a well-controlled dMD or dOFM study would often have sufficient power to establish BE with a few dozen subjects. The results from 1 study on lidocaine absorption suggested that current traditional statistical requirements could be met with as few as 18 subjects, whereas another study with metronidazole estimated that 34 subjects would be needed. Similarly, the results from the dOFM clinical study with acyclovir demonstrated that 19 subjects would have been sufficient to have satisfied traditional BE criteria. Distinct limitations of dMD include potential difficulties in detecting drugs that are highly lipophilic or protein bound, and possibly the length of time that subjects can be comfortably immobilized with dialysis probes in place (if the dMD probes are not used with portable pumps). Further research with dOFM and dMD is certainly warranted, particularly to evaluate the utility of these techniques to monitor the dermal PK of drugs that are more lipophilic and protein bound than acyclovir.

SUMMARY

The practical assessment of BE for each drug product is not a one-dimensional issue, but rather, it routinely involves characterizing a multidimensional topography of product attributes and behavior that together define product performance in each case. As understanding of the physiochemical and structural complexity of semisolid drug products has evolved, it has become increasingly clear that the components, composition, and arrangement of matter in topical dermatologic products can be critical to their clinical performance.[67,68] Thus, it is important to consider such molecular and macromolecular qualities in the design of bioequivalent drug products, characterizing them as rigorously as possible. This might include matching characteristics like texture, rheology, specific gravity, phase state(s), particle size and distribution of the drug substance(s), globule size and distribution, polymorphic forms, pH, and other potentially critical physicochemical and structural characteristics, as relevant to a product. It may not always be possible to identify and perfectly match the arrangement of matter between a test and reference topical dermatologic drug product (or even between manufacturing batches of a test or reference product), and so appropriate in vitro and/or in vivo tests of product performance serve an important role as part of a multicomponent risk-based assessment of BE. As discussed in this work, it is now feasible for evidence from in vitro and/or in vivo cutaneous PK approaches to support a demonstration of BE.

A single approach alone may not always be sufficient to demonstrate BE, but the collective weight of evidence from orthogonal methods can be highly effective in affirming BE. The rational selection of such test methods, used in combination with rigorous physicochemical and structural characterization of the drug product, is particularly valuable for the evaluation of multidimensional aspects of product quality and performance that can collectively support a demonstration of BE. Such approaches, and in particular, cutaneous PK approaches to compare BA and/or BE for topical dermatologic drug products, will likely provide an efficient path forward for developers and regulators of topical semisolid generic drug products, where no viable path had previously existed, and to provide patients with access to generic topical medications whose qualities and performance have been evaluated and matched to those of the reference product more comprehensively than ever before.

CLINICS CARE POINTS

- Cutaneous pharmacokinetics based bioequivalence approaches ensure that topical generic products are safe and effective with the same rate and extent of drug bioavailability.
- Topical generic products approved using in vitro cutaneous pharmacokinetics based bioequivalence approaches additionally have the same look and feel as the brand name product.

DISCLOSURE

The information and opinions expressed in this article reflect the views of the authors and do not necessarily reflect the views or policies of the FDA or any other organizations with which the authors are affiliated.

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