In Vivo Measurement of Human Skin Penetration: Alternatives for Measurement of Cutaneous Bioavailability

In the case of systemically delivered drugs, plasma or serum drug concentrations are traditionally used for assessment of pharmacokinetic (PK) parameters, and to establish bioavailability (BA). However, for topically administered drugs designed to exert a local effect in diseased skin, only a fraction of the administered drug usually reaches the systemic blood circulation, and active drug concentrations in the skin will be many times higher. Therefore, the evaluation of BA of these drugs must be done in the target tissue itself. The development and validation of appropriate methodologies to determine the rate and extent to which a topically applied drug reaches its site of action within the skin is one of the biggest challenges in dermatological research today.

Methodologies

Methods such as punch biopsies, suction blisters and shave biopsies can be used for the determination of in vivo percutaneous penetration, however these methods are invasive and not practical for obtaining kinetic data with drug concentration versus time profile. This article focuses on alternatives for determination of quantitative PK data in the skin, their usefulness and their limitations.

Dermatopharmacokinetics (DPK)

The DPK, or tape stripping, methodology allows determination of drug concentration, drug uptake and drug elimination in and from the stratum corneum (SC). The SC, the outermost 10-20µm of the epidermis, is not only the ultimate barrier to penetration of molecules through the skin, but also serves as a reservoir for substances applied to the skin. The SC is the rate-determining barrier to percutaneous absorption, and it is assumed that the SC concentration of the drug is directly related to the amount which diffuses into the underlying viable epidermis. Therefore, determination of a drug concentration versus time profile in this barrier layer may be a relevant strategy for determination of percutaneous penetration.

The method calls for standardised product application to test fields located on the volar aspect of the forearm. Residual product is removed after a defined treatment time, e.g. 30 minutes – 2 hours after application. Microscopic layers of the SC are then harvested by sequentially pressing adhesive tape strips or discs onto the skin and removing them by a sharp upward movement. Ten or more

Figure 1: Schematic of experimental set-up for MD and OFM. Courtesy of Dr. Frank Sinner, Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft mbH, Graz, Austria, frank.sinner@joanneum.at

Figure 2: MD membrane has small micro-pores versus 100 µm open exchange areas in the OFM probe. Images courtesy of Dr. Frank Sinner, Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft mbH, Graz, Austria, frank.sinner@joanneum.at

Percutaneous Penetration in Diseased versus Healthy Skin

Whereas for systemically delivered drugs, in most cases the factors determining the rate and extent of absorption are not affected by the diseased state, this is rarely true for dermatological conditions. It is well known that drug absorption and distribution can be very different in healthy versus diseased skin, particularly in inflammatory skin diseases such as atopic eczema which are associated with impaired skin barrier function. Further, in inflammatory skin diseases there is increased blood flow to the skin and increased permeability of the vascular epithelium, which leads to an enhanced uptake and elimination from the skin. Conversely, vasoconstriction or decreased blood flow to the skin will lead to an increase in skin bioavailability. Therefore, the relevance of measurements of drug penetration in healthy skin may not be indicative of diseased skin, necessitating inclusion of measurements in patients with dermatological diseases to obtain relevant data of cutaneous BA.
adhesive film samples can be collected per test field. Following removal, the drug content is measured quantitatively in the individual or pooled tape strips. Since only the dead cells (corneocytes) in the outermost layers in the skin are removed, the procedure is only minimally invasive and relatively painless.

In 1998 the FDA issued a draft guidance which included DPK methodology as a primary means to document BA and bioequivalence (BE) of topically applied products. The guidance was subsequently withdrawn in the midst of controversy. The main reason for withdrawal was flaws in the recommended procedures for tape stripping, with contradictory results in inter-laboratory studies on the same products.

Despite difficulties encountered with the original procedure, a number of researchers are working to optimise this methodology, especially with regards to optimised analysis and presentation of results. There is a large inherent variability in the data collected due to differences in the number of corneocytes collected per adhesive tape, a factor which is particularly dependent on sampling depth in the SC, and the large variability of SC thickness, not only between subjects, but also between test sites within a subject. Ideally, results should be presented in a manner correlating to the depth profile of the SC and not simply to the number of strips collected.

A consensus on the usefulness and limitations of the DPK methodology for topical drug BA and BE will hopefully crystallise as more studies with a broader range of formulations and active substances are investigated. At present the method appears particularly promising for determining the local BA of drugs whose target site is in the SC, e.g. antifungal agents, UVA/UVB filters or antiseptics.

Microdialysis / Open-flow Microperfusion

Microdialysis (MD) and open-flow microperfusion (OFM) are methods for assessing penetration kinetics of drugs in the dermis and subcutaneous tissue. A feature of both is the ability to continuously monitor the extracellular concentration of a drug in real time. To do this a catheter is implanted into the dermis or subcutaneous tissue which allows for microperfusion with continuous sample collection.

In 1991 Anderson et al first described the use of MD sampling in the dermis to measure percutaneous penetration of ethanol. This is the only method that allows free, unbound drug in the skin to be sampled, a very important distinction as the level of unbound drug generally determines the pharmacodynamic response. For MD, a thin dialysis catheter with a semi-permeable membrane is implanted into the dermis or sub-dermis with the help of a guide needle. This catheter imitates the function of a small blood vessel: the catheter is attached to an inlet and outlet tube and is perfused with a physiological solution which equilibrates with the extracellular fluid, allowing substances to be exchanged by passive diffusion. The dialysis membrane is porous and is defined according to its molecular weight cut-off (e.g. 20kDa or 100 kDa). Only molecules smaller than the cut-off value can cross the membrane.

The insertion of the guide needle and catheter is a minimally invasive procedure and provokes minor tissue trauma with increased local blood flow, increased skin thickness and hyperemia. Sufficient tissue recovery to near baseline levels generally occurs after 60-90 minutes. After this time the values observed in the dialysate should reflect the concentration of the drug and its metabolites in the interstitial fluid. As an inflammatory response will occur after longer intervals, the length of a typical MD experiment is generally eight hours or shorter.

While MD and OFM are similar in many ways, there are several key differences. These are related to the nature of the dialysis catheter. The OFM probe is a mesh design catheter with openings of 100μm rather than a porous membrane. Because there is no limit in the size of molecules which can pass through the catheter, even middle and large sized molecules can be investigated. Further, both lipophilic and hydrophilic substances can be measured by OFM. MD is best for the detection of small, hydrophilic molecules and is not suited for very lipophilic substances. The other major difference between the methods is that whereas MD allows measurements of free, unbound drug, OFM only allows measurement of the total drug concentration including the protein-bound fraction. This can present a greater analytical challenge.

Further validation work is necessary for MD and OFM to be recognised by regulatory agencies as an option for BA and BE assessment. Standardised protocols must still be developed. A key issue is the reproducible insertion of the catheter to a consistent depth in the skin, a procedure which requires extensive training and practice. In addition, the extremely small dialysate volumes and low drug concentrations in the dialysate...
require highly sensitive analytical assays which can be very challenging.

**Confocal Raman Spectroscopy**

Confocal Raman spectroscopy is a more recent technological development which is currently under investigation to determine profiles of substances in the upper skin. The method is particularly attractive as it is non-invasive. A major drawback is that the molecule of interest must be present at a sufficient concentration and possess a unique Raman signature that permits its differentiation from the spectrum of other skin components. Further, at present it is only possible to measure relative rather than absolute concentrations using this method. It is too soon to predict whether this approach will be a real alternative for measurement of skin penetration.

**Fazit**

For dermatological product development it must be kept in mind that effectiveness and safety of a topical product is a composite of the active ingredient(s) and the vehicle. Therefore, the effectiveness of a product cannot be assessed solely on the ability of the active ingredient to penetrate into the skin. On the other hand, penetration into the SC and further distribution to the site of action in the skin is essential for pharmacological effects. The availability of standardised, routine methods to determine BA in the skin would be a huge boon to the development and optimisation of new topical formulations.

Determination of BA of topically applied drugs or BE between a new formulation and a marketed comparator usually require comparative clinical efficacy trials which are time-consuming and expensive. Currently the vasoconstriction or skin blanching assay for topical steroid preparations is the only acceptable BE method approved by the FDA and a number of other regulatory authorities. This situation will only change if alternative pharmacokinetic and pharmacodynamic models can be sufficiently validated, keeping in mind that whichever method is chosen, for regulatory acceptance it must correlate with the clinical outcome.

**References:**