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News Release

FDA and AAPS Report of the Workshop on Principles and Practices of In Vitro Percutaneous Penetration Studies: Relevance to Bioavailability and Bioequivalence[1]

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INTRODUCTION

The purpose of the workshop was to review the relevant literature and to develop guidelines for *in vitro* percutaneous studies which can be used:

- as a quality control procedure to characterize the dosage form;
- to study the *in vitro* release and permeation rate characteristics of drug products during product development;
- to establish the drug delivery characteristics of topical formulations as one means of assuring lot to lot bioavailability equivalence; and
- to enable minor reformulations of topical products on which the bioavailability/bioequivalence has already been defined.

These guidelines have been developed on the basis of the existing literature. They should not stifle, but rather act as a focus for research in the area of *in vitro/in vivo* skin penetration. The consensus reached that human skin is preferred, and should be used for the above purposes, should in no way downgrade the employment of animal models (animal skin membranes) for product development work. Such studies, as well as studies on the utility of synthetic membranes, are specifically encouraged. Studies with animal skin membranes, studies with other than the recommended diffusion cell configurations (such as the horizontal method), skin metabolism studies, and other procedures, should be considered in preformulation studies, and may provide valuable information.

It is important at the outset to define what is meant by percutaneous absorption and by skin permeation, as these are subtly different.

Percutaneous absorption actually infers permeation of agents through the epidermis and into the deep layers of skin and general circulation *in vivo*, a total process that includes transport through the skin and local clearance. *Skin permeation* relates to the first part of the process, diffusion across the skin.

In percutaneous absorption either diffusion or clearance factors can, in principle, be rate controlling; however, with few exceptions skin permeation is the kinetically determining event. Thus, skin permeation observed *in vitro* is believed to reflect accurately the rate determining aspects of drug delivery in most instances and is, therefore, projected as a means of determining relative availability from dosage forms. In the context of these guidelines, percutaneous absorption is used interchangeably with skin permeation and is limited to the diffusion event associated with transdermal delivery.

The outline, which follows, provides a guide to methods of procedure in skin permeability research. The deliberations that led to the recommendations concerned situations where a given manufacturer is making minor modifications of a previously approved formula on one hand, and situations where markedly different formulations of a given drug from multiple manufacturers are to be compared on the other. The state of the art is such that the former appears possible today; the latter requires more experience with this application of the technology.

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[1] Washington, D.C., October 31 to November 1, 1986. The guidelines reflect the consensus of the authors and do not constitute an official endorsement by the sponsoring organizations.

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SKIN PERMEATION

Membranes

For *in vitro* percutaneous absorption studies, human skin should be used. This may be in the form of thinly dermatomed sections (≤0.5 mm thick) or epidermal sections isolated by gentle heat or other methods. The skin can be taken from any specified and rationalized anatomical site. However, in comparative studies, skin samples from the same body site should be utilized. It is preferable that comparisons be made on membranes prepared from the same piece of skin. Since multiple experiments on each formula have to be performed, the site may be chosen with consideration of the expansiveness of area of the tissue which can be harvested. Alternatively, skin over the intended site for clinical application of the drug delivery system (e.g., postaurical skin for testing a scopolamine delivery system) might be selected. In any case, the exact nature of the skin preparation used for permeation studies should be carefully documented. One should specify: if the skin tissue is a by-product of a surgical procedure or is excised; the manner of preparation of the membranes from the tissue; and the duration and conditions of storage of the skin prior to its use. One should also specify the gender, race, and age of the donor and any factors of the health of the donor which might influence the barrier qualities of the skin tissue. In the case of cadaver skin, the time elapsed between death and the skin harvest should be given. Any treatment of the cadaver prior to harvesting the skin should be recorded.

Fresh skin should be used when possible, as concerns about tissue viability and appropriate storage conditions are then minimized. If utilizing stored skin, the conditions of harvesting and storage should be described and the effects of storage ascertained. The latter may be accompanied by following the permeability of the drug of interest, or of a marker compound such as tritiated water, through the stored tissue and then comparing the penetration to that through fresh skin.

Human skin is notorious for its high level of barrier variability. In comparing drug delivery from two formulations using human skin, twelve (12) experiments for each formulation should be run and the average flux (the amount of drug penetration per unit of area and time) between the two formulations should be comparable. It is recommended that comparisons between two formulations be made *within* each piece of skin by preparing membranes in sets of two from the same skin sample, one to be used for each formula. The ratio of permeability across *sets* of paired results indicates the level or comparability in drug delivery, while averages across totally different skin sections do not. It is implicit that two formulations must be directly compared. Data from studies separated in time involving different skin samples normally cannot be used to determine equality or differences in drug delivery.

Cell Design

The *in vitro* diffusion cells should be made from inert, nonreactive materials (such as glass, stainless steel, teflon). Inertness (lack of absorption) to all components of the cell, including flow-through lines and the collection chambers themselves, should be demonstrated by experiment. It should also be shown that there is no loss of drug through its volatility during the permeation procedure. If volatility is a problem, a quantitative accounting of this must be made. The receptor medium should provide an effective sink for the penetrant. Ideally, it should, at the same time, contain a minimum volume to facilitate analysis because, in general, the more concentrated the drug in the collection medium, the easier its assay. The cell design should allow the receptor fluid to be well mixed and temperature controlled.

Receptor Fluid

For most studies, an isotonic solution buffered to pH 7.4 is a suitable and preferred receptor fluid. A different pH can be used if it can be justified. In all instances, the thermodynamic activity of drug in the receptor fluid should not exceed 10% of its thermodynamic activity in the donor medium so as to maintain a favorable driving force for permeation and assure reasonable and efficient collection of permeant. The receptor medium may need alteration from a strictly aqueous medium to attain this endpoint for hydrophobic compounds. This factor supercedes concern for maintaining a minimal receiver volume.

Hydrophobic compounds may be defined as compounds that have uniquely low solubilities in water (less than 10 mg/L) and solubilities in both water miscible (alcohol, propylene glycol) and water immiscible (ether, octanol, chloroform, hexane) solvents which are orders of magnitude larger. These compounds have large octanol/water partition coefficients which are on the order of 10^3 or higher. Such compounds have low tendency to partition into the receiver medium beneath the skin. It may be necessary to use a non-physiologic medium in which the drug is more soluble to efficiently elude such substances. When this is a concern, studies should be done either by (1) using a lipophilic receptor fluid that is without effect on the skin membrane, as demonstrated experimentally, or (2) using an isotonic solution containing an appropriate concentration of solubilizer for the hydrophobic compound. Flow-through cell designs also tend to minimize collection problems.

Temperature

The surface temperature of the skin should be maintained at 32 ± 1°C.

Dose

The dose per unit area should be equivalent to that normally applied in a single application. It is estimated that normal topical dosing would not lead to a layer of vehicle greater in depth than 50 μm, an amount roughly equal to the application of 5 mg of formulation per cm^2 of skin (assuming a formula density of 1.0).

Statistics

Appropriate statistics are to be utilized.

Occlusion vs. Open Application

If the product is to be left open in normal use, the appropriate test would be from open application. The applica-

tion might be occluded by a moisture impermeable material if occlusion is to be used in the clinical deployment of the drug delivery system.

Kinetic Analysis

Kinetic analysis should include, when appropriate and for each formulation tested, characterization of the delivery profile from time zero to essentially the point of exhaustion of drug from the drug containing application. Lag times and steady-state fluxes should be set forth in those instances in which the data lend themselves to such treatment. Whenever possible, the drug content in both the tissue and receptor at the end of the experiment should be noted and the total mass balance, which includes measurement of the residual drug in the skin, should be determined.

Data Presentation

In an *in vitro* experiment, in which a clinically relevant dose of formulation is applied, drug penetration will lead to accumulation of drug in the receptor phase with time. From this, the momentary rates of appearance of the drug per unit area can be evaluated and the maximum rate and the time to achieve the maximum rate can be determined and compared across different formulations. In situations for which steady-state kinetics are apparent, the apparent steady-state flux (slope) will be reported.

QUALITY CONTROL PROCEDURE

It is noted that for quality control purposes, membranes other than human skin may prove suitable. The Committee recommends that as part of an NDA application, the procedures and means for assuring batch-to-batch drug release equivalency for the topical dosage form be provided. When these dosage forms are heterogeneous and/or when the solubility state (fraction of the drug which is in true solution within the vehicle) is not well characterized, quality assurance procedures involving the release and permeation of the drug through suitable, reproducibility behaving, membranes are encouraged. In those few instances when the topical dosage forms involve total solution of a drug in a well characterized solvent system, data showing that the concentration of the drug is invariant from batch-to-batch is considered adequate.