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Review

The Bioavailability of Dermatological and Other Topically Administered Drugs

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The literature addressing determination of the bioavailability of dermatological and other topically administered drugs has been reviewed. The various methods employed, their advantages and drawbacks, have been identified and evaluated. The state of the art and the success of topical bioavailability assessment are discussed in the light of the information presented. It is concluded that, although current methodology ensures the responsible use of topical medicaments, the techniques are, on the whole, quantitatively inadequate. A number of recommendations are proposed as possible improvements to the approaches now undertaken, and specific measurements for drugs in different therapeutic categories are suggested. The ultimate objective of this survey is to catalyze the establishment of straightforward, objective, quantitative, and reproducible methods to evaluate topical bioavailability and to reduce significantly, thereby, the incidence of bioinequivalence and pharmacological inactivity observed following drug dosing to the skin.

KEY WORDS: topical drugs, bioavailability; percutaneous absorption; pharmacology, cutaneous; bioequivalence, topical.

INTRODUCTION

Application of the term "bioavailability" to topical dosage forms presents, first, a question of definition. Most simply, the bioavailability of a topical drug may be considered to be its relative absorption efficiency (1–4). Unfortunately, a very general definition such as this begs a further series of questions: Absorption efficiency relative to what? Relative to another drug, to the same drug administered via a different route, or to the same agent delivered from a standard vehicle preparation? Absorption where? To the stratum corneum, to the epidermis or dermis, or into the systemic circulation? The subject is clearly complex and is characterized by an absence of consistency in approach (3–7). We may state at the outset, therefore, that the straightforward concept and measurement of bioavailability for oral drug administration cannot, in most cases, be applied to topical dosage forms.

The topical delivery of therapeutic agents serves two primary functions.

(i) To treat local skin disease or discomfort. Drugs falling in this category comprise, by far, the majority of transcutaneously delivered substances.

(ii) To treat systemic disease. A limited number of drugs are currently included in this group, although there is, of course, a great deal of transdermal drug delivery research in progress.

The latter class of agents and dosage forms presents a rather conventional bioavailability problem which can be dealt with using established procedures. The former, on the other hand, confronts the generality of the bioavailability definition head-on.

Historically, the approach to the determination of topical bioavailability has centered around the evaluation of basic criteria selected (predominantly) from a choice of three:

(a) To what extent does the drug, when delivered from its topical dosage form, elicit a designated pharmacological effect?

(b) To what extent does the drug penetrate through skin tissue from the applied vehicle phase? and

(c) To what extent is the drug released from the delivery system into an appropriate receptor phase?

In practice, bioavailability is expected to be correlated with the level and duration of persistence of drug in the "biophase," which includes the site of drug action (3,8). When one considers bioavailability (F) from an oral dosage form, for example, it is generally accepted that circulating blood levels (and areas under plasma concentration–time curves, etc.) will adequately reflect the time course of drug presence within the biophase and that they can be used, therefore, to evaluate F. As we show, this approach is also acceptable for topical dosage forms in category ii above, i.e., for transdermal drug delivery systems (bandages, "patches," ointments) designed to treat disease of systemic origin. For dermatotherapeutics, however, this procedure (a) cannot be employed routinely (because circulating levels of the topical medicament are too low to be analyzed by conventional techniques) (9) and (b) is of questionable relevance because the biophase is within the skin at the applica-

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tion site (3); it becomes almost impossible, therefore, to measure drug concentrations at the site of action and show that these amounts are related to drug levels in the bloodstream. In consequence, the above approaches (a–c) have assumed major roles in the bioevaluation (bioavailability and bioequivalence) of topical drug dosage forms.

The objectives of this review are

(i) to present the pertinent literature and information concerning the evaluation of topical bioavailability;
(ii) to describe the key methods of topical bioavailability determination revealed by the literature search;
(iii) to identify those approaches, used to evaluate topical drugs, which are well validated and show acceptable reliability; and
(iv) to suggest how, in the future, the problems of assessing the bioavailability of topical drugs should be addressed.

This article addresses these aims in the following way: having identified the sources of information on which this review is based, methods for evaluating the bioavailability of dermatological drugs (and drugs delivered transdermally to elicit systemic pharmacological effects) are described. The procedures for the assessment of bioavailability are critically evaluated and their attributes and limitations are indicated. We conclude by proposing a number of recommendations which, it is believed, would significantly improve the quality of topical bioavailability determination. Finally, two appendices offer (a) specific suggestions of bioavailability measurement for drugs in different therapeutic classes and (b) a sampling of examples in the literature of topical bioinequivalence.

INFORMATION—SOURCES AND ACQUISITION

Essentially conventional procedures were followed in obtaining the information on which this report is based. Pertinent literature searches, of course, provided the bulk of the material analysed.

Letters stating the purpose of our efforts were mailed to approximately 100 recognized investigators working in skin penetration, dermatopharmacology, and topical biopharmaceutics. Responses were received from a significant number of those canvassed. Replies frequently included either a list of references or reprints of relevant articles.

Finally, much attention was paid to a limited number of in-depth treatises focusing upon dermatological formulations and percutaneous absorption. These works were excellent sources of both extensive literature citations and informed comment on the subject of bioavailability. Outstanding among these are B. W. Barry's monograph "Dermatological Formulations: Percutaneous Absorption" (3) and the review "Skin Absorption" by H. Schaefer *et al.* which appears in "Normal and Pathologic Physiology of the Skin" (4).

DETERMINATION OF THE BIOAVAILABILITY OF DERMATOLOGICAL DRUGS

When a drug preparation is applied to diseased skin, the purpose is to induce a therapeutic response. The occurrence of this response and its time of onset, duration, and magnitude depend upon the relative efficiency of three sequential processes:

(i) release of the drug from the vehicle,
(ii) penetration of the drug through the skin barriers, and
(iii) activation by the drug of the desired pharmacological effect.

To determine topical bioavailability, therefore, requires that we possess techniques capable of assessing these three events for the drug/vehicle combination.

Drug Release from Topical Vehicles

Release rates of drugs from topical vehicles have been well studied (10–20) despite the generally accepted fact that liberation of the medicament from the formulation is not usually rate-determining for drug penetration into the skin. Drug release studies invariably involve simple *in vitro* methods, extrapolation of the results from which to the *in vivo* situation may be questionable. For example, a classic release test may involve measurement of drug diffusion out of the vehicle into some type of "sink" receptor phase. This receiving medium may be aqueous or lipophilic (e.g., isopropyl myristate) (21–24) and may be separated from the vehicle by a model synthetic membrane (e.g., silastic). While these experiments are useful for comparing formulations under carefully controlled and reproducible conditions in the laboratory, they may bear little relation to delivery kinetics *in vivo* because the skin is frequently the input-rate controlling barrier. These studies may also neglect the effects that different vehicles may have on the permeability of the skin, e.g., increased hydration with occlusive ointments, or the penetration-promoting potential of low molecular weight solvents (8,25,26). Of course, in the design and formulation of a topical dosage form, considerable attention must be paid to the physical and chemical properties (stability, rheology, solubility, phase characteristics, elegance, etc.) (3). Without careful efforts to ensure that the delivery system is functional a priori, subsequent evaluations of topical bioavailability may prove futile.

Methods for Studying Percutaneous Penetration

The measurements, which are made most frequently to assess topical bioavailability, involve determination of how much of an applied drug actually penetrates skin and how fast (3,4). A wide variety of experimental approaches has been developed, therefore, to answer these questions. However, it must be stated at the outset that (a) there is continuing debate about the factors that influence percutaneous absorption (27–38), and (b) there is as yet no single, *generally* accepted technique for percutaneous absorption determination, and as a result, there are conflicting opinions concerning the rationale by which an experimental model is selected. The primary division of the methods available is *in vivo* versus *in vitro*. The former category involves the use of living skin of humans or experimental animals *in situ*. The latter employs an isolated skin (or artificial) membrane mounted in a simple diffusion cell. In discussing topical bioavailability from this standpoint, we consider the advantages and disadvantages of these two approaches and indicate their limitations and weaknesses with respect to the objec-

tive of assessing the rate and extent of drug delivery to the cutaneous site of action (3,4,39–42).

In Vivo. If concensus does exist among researchers in percutaneous absorption, then the point of agreement is that the best way to learn about topical availability in humans is to measure penetration in human subjects (3,4,9). Unfortunately, these measurements are difficult and often indirect. In some cases, though, it has been possible to administer a drug topically, to determine, with a sensitive assay, a plasma concentration–time profile, and then to compare this with the corresponding data following parenteral delivery (e.g., Refs. 38 and 43–50). Classic bioavailability can now be assessed. More typically, an experiment involving human subjects adopts the following procedure (9,51–61): the drug is obtained with a radiolabel (usually ^{14}C or ^{3}H) and is dissolved or dispersed in the vehicle of interest. The dosage form is administered and absorption is assessed by monitoring the rate of excretion of radioactivity in the subject's urine over a several-day period. This approach has been widely used and has provided invaluable information concerning percutaneous absorption in humans. However, from the standpoint of more formal bioavailability assessment, the approach has some obvious flaws. First, the investigator measures absorption indirectly by counting radiolabel in the urine; thus, the chemical nature of the excreted product is not usually known. When there is metabolism, what fraction occurs in the skin (62,63)? Is the drug excreted only in the urine? A correction for this can be established by parenteral administration of the radiolabeled drug. Of course, this control measurement must assume that the metabolic profile and biodisposition of the drug are somewhat independent of the administration route (9). Little experimental verification for this assumption, however, is available. Because penetration is frequently slow, these measurements must be made over prolonged periods of time; even then, it is possible that long-term sequestering of the drug within, for example, deep skin or muscle tissues beneath the application site may complicate and reduce the precision of the final analysis of the data (64).

At this point, we must again ask: What do these measurements tell us about the bioavailability of the drug within the skin? Given that absorption must take place, at least through the stratum corneum, in order for a therapeutic effect to occur, then the information is of relative importance; quantitative utilization of the results for bioavailability determination is not established. More direct observations of drug at the application site and within the skin have been proposed (4). For example, again following the administration of a radiolabeled drug, surface disappearance can be monitored with an external Geiger-Müller counter (65). Limitations here include a lack of knowledge about the depth into the skin which the device can see and no information with respect to the quenching characteristics of different topical preparations. In an alternative approach, radiolabeled drug is applied in its vehicle for a designated time (or series of times). At the end of this period, the remaining dosage form on the surface is removed and the skin is then repeatedly "stripped" with adhesive tape until the stratum corneum has been largely excised (4,33). The individual "strips" can then be counted and a crude concentration profile, at a specific time, established. This procedure has the disadvantage that each strip does not remove the same amount of skin; it becomes more difficult to remove tissue as one proceeds into the skin. Once the stratum corneum has been destroyed, adhesion of subsequent strips is very poor. This difficulty can be partially circumvented by taking a punch biopsy of the application site at a chosen time post-administration. With a radiolabeled substrate, autoradiography may then be possible and deeper localization of drug can be determined. More recently, this invasive, difficult, and not generally accessible technique, which provides at best only semiquantitative information, has been refined and improved. Rougier and colleagues (68–70) have shown, in hairless rats and in humans, that a relationship exists between so-called "stratum corneum reservoir function" and *in vivo* percutaneous absorption. They compare penetrant urinary excretion levels after 96 hr following a 30-min topical exposure with the corresponding amount of compound located, by tape-stripping, within the stratum corneum after, again, a 30-min application. The two measurements appear to be highly correlated for a diverse range of chemicals, vehicles, and administration times.

Some of the limitations with human experimentation can be circumvented using animal models but then another rhetorical question immediately emerges: What animal represents the best model for human skin penetration? (69,70). Many species have been employed including the following: rhesus and squirrel monkey, rat, mouse, guinea pig, rabbit, miniature and weanling pig, and hairless dog, hairless (fuzzy) rat, and hairless mouse (71–78). An interesting and exciting development is the successful implantation of excised human skin onto the back of an athymic nude mouse (79,80). There have been a number of studies designed to determine which, if any, of a number of animals bears the closest relationship to humans (72,73). It has been suggested that the skins of the monkey (81–91) and of the pig show the most similar permeability characteristics to the dermal barrier of humans (71–73,79,80). These investigations are, of necessity, limited in scope and this conclusion is based, therefore, upon comparative measurements made in a rather small number of animals with a narrow range of permeants. Many experiments will, as a result, continue to be performed on the less expensive, more common, and more easily handled laboratory animals (rats, rabbits, mice, guinea pigs). This will be true despite the clear physiological differences between the skins of these animals and that of humans and despite the fact that potentially damaging pretreatment of these animals' skins (clipping, shaving, or depilation) is necessary before the absorption experiment can be carried out. What are the advantages of an animal experiment? In certain instances, it may prove possible to obtain more specific pharmacokinetic data and to analyze for the applied drug in the bloodstream. More detailed investigation of the localization of the drug within the skin beneath the application site is also possible. Tissue can be excised in reasonable amounts down to the muscle, then sectioned and assayed for drug content (64). Larger doses can be used to improve resolution of the results. In the long term, it is clearly desirable to develop reliable and predictive models for human skin penetration, and animal experimentation within reasonable bounds is an acceptable approach. The drawbacks associated with animal measurements of percu-

taneous absorption are various. First, and perhaps most importantly, the lack of agreement as to the "best" animal model reduces the confidence associated with this type of data. Second, considerable care must be taken to ensure that the animal is incapable of interfering with the administered dose. Third, we have poor understanding of the biochemical differences between human and animals' skins and little knowledge, therefore, as to how these differences will influence penetration and local bioavailability. Finally, the state of our current understanding of the suitability of the various models is such that measurements in humans are almost invariably required at a later stage.

In Vitro. Dermatological drugs are designed to treat diseases within the skin. An opportunity exists, therefore, with the topical route of drug administration, to monitor the rate and extent of absorption in skin tissue isolated from the complete physiological system (i.e., the rest of the body). Carefully controlled experiments under standard laboratory conditions are thus possible (3,92,93). In general, *in vitro* skin absorption experiments are performed using simple glass diffusion cells which are divided into donor and receptor compartments by the excised skin membrane. Diffusion cells exist in a variety of styles and configurations; almost all, however, have certain basic characteristics. (i) The cells have a water jacket so that studies at constant temperature can be conducted. (ii) The receptor phase is stirred (usually magnetically) to ensure complete mixing of its contents. (iii) The receiving chamber has at least one sampling port through which small aliquots of the receptor phase can be withdrawn at designated time periods; cells of most recent design permit continuous sampling via a flow-through configuration. (iv) The donor compartment is constructed such that various vehicle phases can be administered without difficulty and permits occlusion of the skin surface if desired; more sophisticated cells also allow the surface loss of penetrant (through evaporation, for example) to be measured.

The nature of the skin membrane for *in vitro* experiments is again varied. Both excised human skin from cadavers (or patients undergoing plastic or amputation surgery) and a multiplicity of animals' skins have been and are being used (92,93). Once the skin has been excised, further manipulations and sectioning may be performed before experimentation. The literature includes reports of studies using one or more of the following: full-thickness skin (subcutaneous fat removed), split-thickness skin, heat-separated epidermis, heat-separated dermis, isolated stratum corneum, and tape-stripped skin. There are, in addition, a number of investigations that utilize model membranes, the properties of which are suggested to mimic certain characteristics of actual skin tissue (e.g., cellulose filters impregnated with oil or lipid phases, synthetic zeolites, eggshell membranes, silastic, and other polymeric systems) (94).

The argument in favor of *in vitro* methodology centers upon the general assumption that the stratum corneum, which is a principal barrier to drug input via the skin, is (to all intents and purposes) a dead tissue layer (38). Hence, skin excision cannot change its diffusional resistance. While this is a persuasive and reasonably sound hypothesis, the absence of a blood supply (usually extensive) and the compromised viability of the epithelial cells of the epidermis are cause for concern (95,96).

We should also consider more closely the choice of *in vitro* skin tissue. Obviously full-thickness skin from a human should be most representative of the *in vivo* barrier. Unfortunately, the sources of cadaver skin, for example, are often not constant and are rarely consistent between different laboratories. Intraspecimen and interspecimen variabilities for *in vitro* absorption behavior (expressed as a percentage standard deviation) have been estimated to be 45 and 65%, respectively. Contributory factors to this variability probably include donor age, sex, health history, race, the anatomic site from which the skin was taken, the time postmortem that the skin was removed, and the storage conditions of the skin between excision and experiment. As a result, animal skin is frequently used for *in vitro* measurements. Improvements in variability are immediately accessible because the factors listed above can be much more closely controlled. As with *in vivo* animal models, the skin of many species has been used, and the suitability of the various models is subject to the same debate and concerns (92,93,95–98).

In terms of bioavailability, *in vitro* experiments can contribute significantly to the assessment of a topical dosage form (3). The *in vivo* use of the delivery system can be mimicked and the penetration rate and extent followed over time (3,97,98). Formulation variables and their ability to change absorption profiles can be screened efficiently with the *in vitro* approach. Steady-state transport experiments utilizing effectively infinite doses can be employed to determine fundamental parameters which characterize skin permeation, e.g., diffusion and partition coefficients (93). These, in turn, together with careful structure–activity experiments, may be used prospectively to predict potential drug availability (3,93,99). There have been reports in the literature to suggest that simple and short-duration *in vitro* experiments can be predictive of *in vivo* absorption over a several-day period (97,98). If further validation supports these data, then we may expect considerable weight to be added to the value of the *in vitro* approach. The technique, in addition, possesses the advantage that analysis of the skin is possible at various time points postapplication of drug (via sectioning and radioactivity counting procedures) (4,100). Furthermore, the *in vitro* approach permits specific chemical assays of the receptor phase, thereby providing the ability to identify biotransformations during the absorption process. The latter methodology is developing at this time and is requiring much more careful attention to be paid to the nature of the receptor medium and what should be done to maintain tissue viability over a 24- to 48-hr period postexcision (101). Thus, while the *in vitro* state of the art may not (and will probably never) be such that *in vivo* experiments are no longer necessary, important advances are taking place that will significantly raise the confidence with which penetration data from these studies are viewed.

In concluding this section, it is instructive to list those areas pertinent to percutaneous absorption in which our understanding is poor and more work is required.

(a) What is the dominant route of penetration of drugs through the skin? It is not clear whether "shunt" diffusion

via the hair follicles is important in humans, for example. There is debate as to the relative importance of an intercellular lipid pathway across the stratum corneum. Is the route of penetration sensitive to the physicochemical properties of the drug (102–107)?

(b) Where do drugs bind within the skin and to what extent does this binding influence local bioavailability (108)? For example, when radioactivity is measured in a skin section, what are the relative proportions of bound and free drug (i.e., how much active drug is available)? The "reservoir" effect of corticosteroids is accepted dogma but little is known about the occurrence of similar behavior by other drug classes (3).

(c) What is the significance of skin metabolism on percutaneous penetration (62,63)? The efficiency of the enzymatic systems in skin are not well quantified. Topical prodrugs have been synthesized and tested but the extent to which such agents may be used successfully *in vivo* is not yet known (101).

(d) How does skin age, condition, and anatomic site affect topical bioavailability? We know that the permeability of the skin of premature infants is greater than that of full-term newborns because the former have only a rudimentary and poorly developed stratum corneum (109–113). Almost no information exists concerning percutaneous penetration (114–118) in elderly subjects in whom obvious physiological and biological skin changes take place (119). Present demographic shifts in the population demand that this subject be addressed soon. Most of what is known about skin absorption has been learned from experiments on healthy skin. There are very few reports concerning percutaneous penetration through diseased tissue (4,9). What differences exist in absorption at different anatomic sites (52)? The limited information available indicates that profound differences may be expected with obvious effects on bioavailability.

(e) Vehicle–skin interactions have been studied in some detail but we are far from completely understanding this area. In particular, the development of agents capable of enhancing percutaneous absorption will necessitate careful reevaluation of our knowledge (3,8,25,26,120–128). In this respect, the level and potentiation of skin hydration warrant considerable further study (129).

Bioassays for Topical Drugs

The most direct method to determine bioefficacy is to administer the drug to the patient and observe how quickly and safely the disease is revolved. Such an approach is independent of the mechanism by which the drug acts but is rather subjective in nature. For drugs exhibiting a narrow therapeutic index, furthermore, this attack may be undesirable. Alternatively, if the drug elicits a well-defined bioresponse, which is measurable and which is believed to be correlated with its therapeutic effect, then these pharmacodynamics can be used to assess the agent's effectiveness. Unfortunately, with dermatological drugs, common indicators such as blood-pressure changes and cardiac-output alterations cannot be used because the drugs are rarely administered in sufficient quantities to be systemically active. It is therefore necessary to identify bioresponses that can be measured within the skin (of either humans or an animal model) or in an appropriate skin-cell culture.

In reviewing the literature, it is clear that the majority of skin bioassays for dermatological drugs depends upon evaluation of a visual cutaneous vasoresponse (2,3,130–139). In other words, it is known or assumed that the drug's vasoactivity is connected to, or is a basic component of, its pharmacological effect. Both vasodilatation (skin reddening or erythema) and vasoconstriction (whitening or blanching) have been used in this way. Unfortunately, this approach must be inherently subjective in that the assessment of redness or blanching is made by an observer. As a result, although intralaboratory variation may be reasonable, interlaboratory differences in the assessment of identical preparations may be significant. Of the various bioassays which have been described, the vasoconstriction test for corticosteroids (3) is the most ubiquitous and important given the success of these agents in dermatochemotherapy.

Much effort has been devoted to the development of a standard vasoconstrictor assay (2,3,130–138). Variables in designing such a test include the duration of contact of steroid preparation with skin, site of application, desirability of occlusion, time(s) of vasoconstriction assessment, room lighting, use of a standard steroid preparation as a control, scale of measurement (e.g., 0 to 4, where 0 = normal skin, no pallor; 1–4 = pallor of increasing intensity and definition), randomization, and double-blind procedures. Complete profiles of response versus time have been obtained in which the pharmacodynamic changes are expressed as a percentage of a theoretical maximum response. These profiles can be utilized for bioequivalence evaluations and bioavailability determinations. Similar types of experiment can be used to study tachyphylaxis following multiple dosing, to screen novel synthetic steroidal compounds, to optimize formulations, to develop dosing regimens, and to rank existing dosage forms on the market (3,130–138).

Concern about the subjectivity of the vasoconstriction (and other vasoresponse) assays remains, however. There have been attempts, therefore, to replace the visual assessment with an objective, instrumental, observer-independent approach. Included among these methodologies are thermography, reflectance spectroscopy, reflectometry and colorimetry, and procedures sensitive to alterations in cutaneous blood flow (xenon washout, photoplethysmography, and laser Doppler velocimetry) (139). While successful applications have been reported, no single approach has yet been sufficiently well validated such that it competes with the vasoconstriction assay. The simplicity and speed of the latter represent perceived advantages which will be difficult for alternative approaches to overcome.

BIOAVAILABILITY AND TRANSDERMAL DRUG DELIVERY

Transdermal drug delivery has undergone a recent period of rapid growth and interest. The initial success of the nitroglycerin devices has provoked considerable activity in this field. The potential benefits and drawbacks of delivering systemically acting drugs via the skin have been well documented. There is no doubt that the route of administration is

particularly attractive for very potent drug molecules for which oral delivery is not optimal (e.g., inconvenient dosing regimen, large first-pass effect) (47–49,140–148).

Bioavailability evaluation for these drug/delivery systems appears to represent a rather straightforward problem compared with topical dosage forms for local dermatotherapy. Conventional approaches can be followed in determining the rate and extent of drug availability from the device. In these situations, drug levels in the plasma can be measured by specific chemical assay. Information will have been obtained about the plasma concentration–time profile of the drug following parenteral or oral dosing. In some cases (e.g., nitroglycerin), alternate topical delivery systems, which are not designed to provide constant input (such as a conventional ointment), will also be available for comparative purposes. Bioavailability from the transdermal system can be determined, therefore, with quite reasonable accuracy.

Of greater concern at this time are questions relating to pharmacodynamics. In other words, is the attainment of a particular target steady-state drug level a guarantee of biological efficacy? Are all candidate drugs for transdermal delivery pharmacologically suited to continuous administration (149,150)? The answers to these questions are not yet established but it is clear that these issues must be addressed when molecules are formulated in transdermal delivery systems.

Attention must also be paid to the dermatological aspects of transdermal drug delivery and the ramifications of prolonged contact between a topical (and invariably occlusive) device and the skin. How does the barrier function of the skin change under these conditions? What can be done to minimize irritant and allergic responses (47,148)? These unknowns together with those identified earlier concerning percutaneous absorption require further study and resolution.

DISCUSSION AND CONCLUSIONS

It is clear that determination of the bioavailability of topically applied drugs is difficult. Despite the multiplicity of approaches that have been suggested for bioavailability evaluation, no single procedure has achieved either general acceptance or general applicability to a wide range of drugs. However, this is not to say that topical drugs are currently used either irresponsibly or unsafely. The methodologies highlighted in this review are targeted to the fundamental question of bioavailability assessment. The approaches seek to establish the following.

(i) Does the formulation release the active ingredient(s) at an appropriate rate?
(ii) How much compound is absorbed into the skin and across the dermal barrier into the body?
(iii) Is the drug delivery system capable of eliciting a pharmacodynamic response, which can be correlated with its therapeutic effect?
(iv) When a topically administered compound is designed to elicit a central effect, can true bioavailability be measured?

These basic requirements are precisely those necessary to show safety and efficacy. It is encouraging, therefore, to be able to report that no key component is conspicuously absent from the work reviewed.

Among dermatological drugs, the largest class (the corticosteroids) has been subject to the most intensive investigation. The bioassays, which have been developed for these agents, although predominantly subjective, are most often put forward as successful examples of topical bioavailability assessment (3). While this may be a debatable point, there is strong support (130,131) for the fact that steroid potencies can be ranked very adequately by these techniques. For other drug categories, less direct (and more diverse) approaches have to be taken and careful extrapolation from model test systems may be required for bioavailability evaluation. For all drugs, we believe, the development and implementation of objective (and ideally noninvasive) pharmacodynamic monitoring instrumentation should be actively sought. In this way, it may be possible to resolve the frustrating paradox that drug effect can frequently be observed within the skin but that the quantification of drug levels eliciting the response cannot be directly measured.

Further, an obvious long-term goal must be the establishment of standard testing procedures for topical drugs (see Appendix 1), particularly with respect to skin permeability determination. Much of the lack of concensus in the approach to topical bioavailability centers around the absence of a validated, reliable, and accessible test system for the measurement of percutaneous absorption. A major reason for this inadequacy is our incomplete understanding of the skin penetration process and, thus, our inability to decide which types of absorption measurements are most relevant. We suggest, therefore, that increased comprehension of the percutaneous absorption process and of the relationship(s) between drug structure and penetration forms an important research objective of direct applicability to topical bioavailability evaluation.

In summary, it may be stated that topical bioavailability is measurable but that, on the whole, present procedures are lacking in sophistication and generality. Significant examples of bioavailability inequivalency, furthermore, can be identified (see Appendix 2). Simple resolution of the problems is not accessible because fundamental research questions remain unanswered. We believe that the level of awareness in the scientific community is now sufficiently high that we may expect to witness important progress in the forseeable and not too distant future.

RECOMMENDATIONS

Finally, we identify specific suggestions, the implementation of which would, we believe, facilitate the establishment of rational bioavailability testing of dermatological drugs.

(i) Recent studies suggest that the corticosteroid vasoconstriction assay is capable of very adequately ranking the potencies of these drugs and does so with few inconsistencies (3,130,131). However, the assay has never been truly validated against other measures of "bioavailability." *We suggest, therefore, that the vasoconstriction assay be validated using* in vivo *percutaneous absorption measurements in humans.* The simple radiolabel approach (9,51–61) can be employed for this purpose provided that parenteral administration control data are also acquired in the same subjects.

(ii) A significant effort should be instigated to resolve the many problems and disagreements associated with *in vitro* measurements of skin absorption and topical bioavailability (92,93). *A reproducible, reliable, relevant, and accessible* in vitro *system would greatly rationalize the future determination of topical drug bioavailability.* Attainment of such a system, however, will require a major concerted effort by investigators in the field. *A sensible starting point would be the organization of a comprehensive workshop (151) at which leading researchers could discuss present methodologies and address the following points of contention.*

(a) What criteria must the skin membrane satisfy? Human or animal (92,95,96)? If animal, what species? Age of skin? Anatomic site? Storage method, temperature, duration?

(b) How should the *in vitro* diffusion cell be designed? Static system or flow-through? If flow-through, what flow rate should be used?

(c) At what temperature are *in vitro* experiments to be performed? What is an appropriate duration for an *in vitro* experiment?

(d) What should be the composition of the receptor fluid? How often should the receptor phase be replaced or sampled?

(e) What is the correct skin component to use in these experiments? Full-thickness tissue, split-thickness epidermis, stratum corneum? If a separated skin component is to be employed, what method of preparation is appropriate?

(f) What exactly are we going to measure in the *in vitro* experiment? Do we want kinetics following topical administration of a relevant finite dose or is a permeability coefficient (i.e., an infinite dose parameter) required (97,98)? In either case, how should we handle the data and to what extent do we need *in vivo* validation of the approach?

(g) Should independent verification of the integrity of the skin be mandatory? If so, how should this be assessed? For example, if we routinely measure the permeability of tritiated water, within what bounds must our observations fall to be considered "satisfactory"?

(iii) In terms of the availability of topical drugs, the measurement of primary interest is the level of therapeutic agent within the skin or within a specific tissue layer of the skin (4). *We recommend, therefore, exploration of procedures that are specifically designed to assay drug levels in skin tissues* (4,152–157). We should pursue the "skin-scraping" technique used to assess the efficacy of antifungal agents (e.g., griseofulvin) and their delivery systems (152–155). We should consider the utility, on a routine basis, of the skin-stripping and sectioning procedures employed to evaluate penetrant concentration profiles across the skin (4,156,157). We should also investigate whether the noninvasive external counting method (65) can be validated and quantified for this purpose.

(iv) We advocate the careful implementation of standard *in vitro* release testing systems. In comparing generic products to the innovator's original formulation, it is essential to establish at the outset of an evaluation that the derivative preparations are at least equivalent in their performance when assessed in a simple *in vitro* release test. *It is clear that changing the formulation of a topical drug can dramatically alter its bioavailability* (3,5–8). The thermodynamic activity of the drug in the delivery system is perhaps one of the most critical indicators of potential efficacy and is one that must be considered seriously for even the smallest change in formulation. Again, designation of the most appropriate test systems will require broad discussions and consultations. We strongly urge that this forum and that proposed for *in vitro* absorption methodology be initiated as soon as possible.

APPENDIX 1: RECOMMENDED "BIOAVAILABILITY" TESTS FOR DERMATOLOGIC DRUGS

Antibacterials. Determine the skin distribution by tape-stripping or skin-scraping methods. Explore the possible utility of the external counting procedure. Activity assessment should use corium disks on innoculated culture plated after application of drug to the epidermal skin surface.

Antifungals. For topical agents, use the same approaches as those for the antibiotics. Skin scrapings can also be employed to assess orally administered griseofulvin, for example.

Antivirals. Biopsy, skin section, and assay for drug in the various tissue layers should be used. A meaningful *in vitro* measurement is very important for this class of drug. *In vivo* kinetics and a parenteral control would be beneficial, also. Consideration should be given to the possible biotransformation of these agents within the skin (i.e., one should be aware of a possible cutaneous first-pass effect).

Ectoparasiticides. External counting and tape-stripping should be used. *In vivo* kinetics are probably advisable because of possible systemic side effects.

Corticosteroids. Vasoconstriction assay mandatory. *In vivo* kinetics and skin sectioning are also advisable until unequivocal validation of the blanching assay has been established. Some consideration should be given to the ramifications of multiple repetitive dosing of these drugs on the barrier function of the skin.

Keratolytics and Other Destructive Agents. External counting and tape-stripping or skin scraping should be used. For potent molecules, *in vitro* or *in vivo* assessments of percutaneous absorption should be made. Multidosing situations must be carefully considered for these agents.

Acne Preparations. Determine local concentrations with sectioning techniques. Monitor *in vivo* absorption kinetics for these drugs. Possibly evaluate a pharmacodynamic response by quantification of erythema (visually or objectively using, for example, laser Doppler velocimetry).

Miscellaneous. In general, for other drug classes (including local anesthetics, antihistamines, sunscreens, and agents affecting pigmentation), skin sectioning to measure drug disposition within the skin layers is suggested. External counting, stripping, and scraping are also sensible so that the compound's ability to penetrate the stratum corneum may be assessed. Clearly, for some of these categories of therapeutic agent, pharmacologic indicators can be used advantageously, e.g., a sunscreen's substantivity can be determined by its continued ability to inhibit uv-induced erythema, and a local anesthetic's duration of action can be measured by its inhibition of tactile sensation.

For *all* topical drugs, *in vivo* penetration kinetics are desirable and permit the likely time course of the drug's local profile to be interpolated. These measurements also

allow potential systemic effects to be estimated. Precise local bioavailability measurements require a standard *in vitro* procedure that will permit both absorption kinetics and local disposition to be found. The absence of such a procedure at the present time is, in our opinion, the major cause of the uncertainty associated with topical bioavailability determination.

APPENDIX 2: EXAMPLES OF BIOAVAILABILITY INEQUIVALENCY

Numerous instances of bioavailability inequivalency may be identified in the literature. The following list is intended to be illustrative rather than exhaustive and is designed to indicate the diversity of chemical species for which inequivalency has been demonstrated.

(i) The antifungal effect of topical griseofulvin (1%) is extremely sensitive to the formulation in which it is delivered to the skin (158). An aqueous-based cream vehicle (Aquacare), for example, proved almost completely ineffective against *Trichophyton mentagrophytes* infections. Poor solubility and inadequate drug delivery into the stratum corneum were implicated as the major reasons for this inefficiency.

(ii) The care which is necessary when interpreting human vasoconstriction measurements following the topical delivery of corticosteroids has been discussed by Haigh and Kanfer (131). In addition to warning against ill-conceived extemporaneous dilution (see below), the authors caution that new formulations containing well-established steroids should not be considered bioequivalent if the vehicle is constituted with novel adjuvants and/or excipients. They suggest that comparative bioavailability data obtained with the vasoconstriction assay may provide valuable information to regulatory bodies concerned with new product registration.

(iii) Bioavailability inequivalence has also been highlighted by research in the area of contact dermatitis. Fischer and Maibach (159–161) have shown that materials formulated in petrolatum vehicles for patch testing are inconsistently bioavailable, and local responses are subject, therefore, to wide variability.

(iv) An important illustration of stability effects upon bioavailability leading to inequivalence was reported by Ryatt *et al.* (162). They showed that the topical availability (determined by the blanching assay and by reflectance spectroscopy) of betamethasone-17-valerate in emulsifying ointment decreased with the age postpreparation and that the time course of the decay was sufficiently rapid to warrant concern over the routine use of the medication.

(v) Extemporaneous formulations (*viz.*, dilution) of topical preparations provide multiple examples of bioinequivalence (3,163–171). Little attention is invariably paid to the stability and/or activity of the resulting medication. The corticosteroids and the antibiotics are the most frequently diluted drug classes and appear remarkably sensitive to indiscriminate dilution.

(vi) The particle size of a solid drug incorporated into a topical formulation can exert a distinct effect upon the subsequent *in vivo* cutaneous bioavailability. For example, using a vasoconstrictor assay, Barret *et al.* (172) showed that micronized fluocinolone acetonide in white soft paraffin demonstrated significantly better bioavailability than coarse particles of the drug in the same vehicle.

(vii) At a recent American Burn Association meeting (1982), it was reported (*Am. Soc. Hosp. Pharm.*, May–June, 1983, p. 20) that two supposedly equivalent ointment preparations of silver sulfadiazene show quite distinct *in vivo* antimicrobial efficacy and that routine *in vitro* testing does not reveal the discrepancy.

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