

The Cadaver Skin Absorption Model and the Drug Development Process*

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ABSTRACT Drug absorption through the skin can be assessed simply by the use of in vitro mounted human cadaver skin, and the method has been extensively utilized by industry in the development of topical and transdermal drugs. Although the Food and Drug Administration has not formally accepted this approach, examination of various applications of the technique during the past 40 years demonstrates its value as a valid surrogate for in vivo measurements of bioavailability and bioequivalence. Newer data, in particular, reveal the sensitivity of the model and recommend its wider use in all phases of the drug development process.

INTRODUCTION

A crucial element in the development of topical and transdermal drug products is evaluation of the percutaneous absorption of the active pharmaceutical ingredient (API). Generally, the goal for topical products is to maximize cutaneous delivery of the API, whereas for transdermal products the goal is to achieve a specific rate of absorption sufficient to achieve a systemic blood level within the therapeutic range for its intended indication. In either situation success depends on identifying the *optimum* formulation from a large number of prototypes—an onerous task for most therapeutic classes if screening must be conducted in human subjects. Only the topical glucocorticoid products are unique because a pharmacodynamic endpoint (vasoconstriction) exists by which their percutaneous absorption can be relatively easily assessed in human subjects (1, 2).

To circumvent the need for time-consuming and expensive human pharmacokinetic studies as a screening mechanism for formulation optimization and to allow formulation development to proceed during the preclinical phase of drug development (without the need for animal safety data and submission of an investigational new drug application), pharmaceutical scientists have made extensive use of a simple in vitro model that relies on excised human skin mounted in diffusion chambers (3–6). The basis of the model is the fact that the barrier properties of skin are preserved after death, and, as a result, the rate of drug absorption measured in vitro accurately mimics the in vivo state. Although much of these data reside only in corporate archives, sufficient data can be found in the literature published during the past four decades to clearly illustrate the utility, versatility, and power of the model. What is puzzling, however, is that the Food and Drug Administration (FDA) has not made greater use of data generated by this highly relevant model in their evaluation of new drug applications (NDAs) and abbreviated new drug applications (ANDAs). In the past although some reviewers have requested cadaver skin data in order to address specific review issues, others have claimed the model is not generally accepted and have suggested that data derived from the model, although supplemental, serve no pivotal purpose in the review process. The latter argument seems incongruous given the vital role the cadaver skin model plays within sponsor companies in the development of the products under review. Tacit acceptance of the method by industry based on successful transfer of products from labora-

tory to market argues strongly for the validity of the model. The purpose of this *Stimuli* article is to examine the utility of the cadaver skin model at various points within the drug development process.

PRECLINICAL PHASE—TOPICAL DRUG PRODUCTS

Formulation Development

The first important class of topical therapeutic agents that had a major impact on dermatologic practice was the glucocorticoids, many of which were introduced in the 1950s and 1960s (7, 8). At this time use of in vitro-mounted human skin to study percutaneous absorption was just evolving, and the model had not been applied to drug development. Similarly the importance of the vehicle in drug delivery was not well understood, and products that contained various strengths of the same API (e.g., 0.025%, 0.1%, or 0.5% triamcinolone acetonide, and 1.0% or 2.5% hydrocortisone) were marketed with the mistaken belief that increased concentration would lead to increased efficacy. Years later, after the human vasoconstrictor (VC) assay was developed, the different product strengths for both triamcinolone acetonide and hydrocortisone were shown to be of equal potency (9).

Rational vehicle design came a decade later and is illustrated by the work done at Syntex Laboratories during the development of the first high-potency glucocorticoid, fluocinonide (10). The work established not only the importance of drug concentration in the vehicle, specifically soluble drug concentration, but also the ability of the drug to leave the vehicle and enter the stratum corneum (partitioning). Of particular interest is the fact that an integral part of the drug development scheme was the use of the cadaver skin model as a means to validate the theoretical considerations and chemistry involved in the formulation process. For the first time formulation scientists demonstrated the pivotal role of the cadaver skin model as an intermediate step between chemistry and clinic. Subsequently, this format has become the blueprint for the development of other glucocorticoid preparations as well as topical formulations, in general (11, 12).

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Line Extensions and Enhanced Formulations

Because both clinical and marketing requirements often dictate the need for different forms of the same topical drug (e.g., cream, ointment, gel, solution, lotion, or foam), the situation created by line extensions plainly exemplifies the worth of the cadaver skin model. Development of the first product sets a benchmark for the interrelationship of drug absorption, efficacy, and toxicity by virtue of the animal and human safety and efficacy testing that preceded product approval. If the bioavailability of each successive product approximates that of the first product, one can be assured that the efficacy and systemic safety profile of the new products also will approximate that of the first. Direct comparison of potential new formulations with the original formulation in the cadaver skin model can help establish the bioavailability relationship between the two. There should be no need for additional long-term animal tests if the bioavailability of the new product does not lead to systemic exposure that falls outside of the boundary established as safe for the first product. Nor should there be

need for an animal/human pharmacokinetic bridging study to establish the relationship between new and old formulation because the bridge can be established *in vitro*.

Alternatively, formulators may develop a new topical formulation of an old drug with the specific objectives of enhanced bioavailability and increased efficacy. Because the original formulation can serve as a benchmark for comparative *in vitro* bioavailability studies of the “improved” formulations, use of the cadaver skin model can increase the likelihood of clinical success before the company initiates the pivotal clinical trial. A case in point is the development of Luxiq Foam, a new delivery form (a low-residue, thermolabile foam) of an old drug, betamethasone valerate (13). Comparison of the new foam formulation with a traditional lotion formulation of the drug in the cadaver skin model demonstrated a three-fold increase in absorption from the foam in the first 12 h. When the two products were tested clinically, the foam product showed 50% improvement in the treatment of scalp psoriasis, affirming the validity of the *in vitro* results (Figure 1).

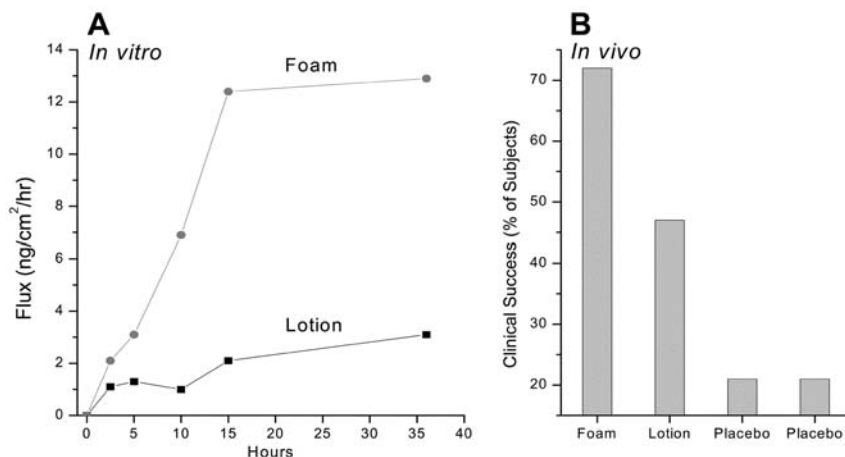


Figure 1. A: Rate of absorption of betamethasone valerate through human cadaver skin from Luxiq Foam versus the reference listed drug. The drug concentration in both products was 0.12% when expressed as betamethasone valerate. B: Results from a 28-day clinical trial of the same two products used in the treatment of scalp psoriasis, showing the percentage of subjects judged to be clear or almost clear (Investigator’s Global Assessment of Efficacy) at end of treatment. (Redrawn from data of reference 13.)

PRECLINICAL PHASE—TRANSDERMAL DRUG PRODUCTS

The development of transdermal products is similar to that of topical products insofar as the rate of percutaneous absorption of the API is a critical factor, and its measurement *in vitro* through cadaver skin plays a dominant role in early development. Data about the adequacy of the flux for systemic delivery, or the need to search for an enhancer, can be obtained quickly. A clear illustration of the importance of the cadaver skin model in this process was evident during the development

by Alza Corp. of the first transdermal system, scopolamine (Transderm-Scop) (Figure 2): “Our conclusions from these studies are that if one follows the permeation of drugs through human skin *in vitro*, one can measure the permeability of the skin, the maximum transdermal flux, the extent of binding by the skin tissue, and the factors which affect the percutaneous absorption and transport. The results obtained with scopolamine and ephedrine concerning transdermal permeation *in vitro* accurately predicted the situation which pertains *in vivo*” (14).

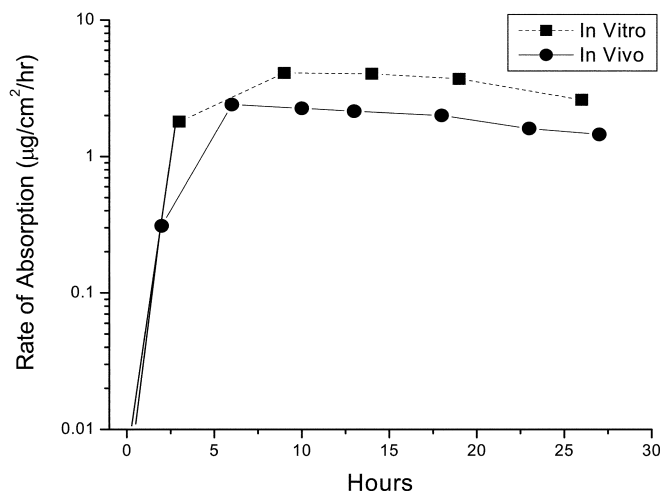


Figure 2. Comparison of the rate of absorption of scopolamine from the transdermal system measured in vitro in the cadaver skin model and in vivo in human subjects. The in vivo rate was determined by measuring scopolamine loss from the patch. (Redrawn from data of reference 14.)

Support for the validity of the Alza conclusion and demonstration of the excellent agreement between in vitro and in vivo data are illustrated in the development by TheraTech Inc. of transdermal testosterone (Androderm) and estradiol

(Alora) (15). When expressed as average cumulative absorption, the rate of absorption profiles obtained in vitro were virtually identical to those subsequently obtained in vivo (Figure 3).

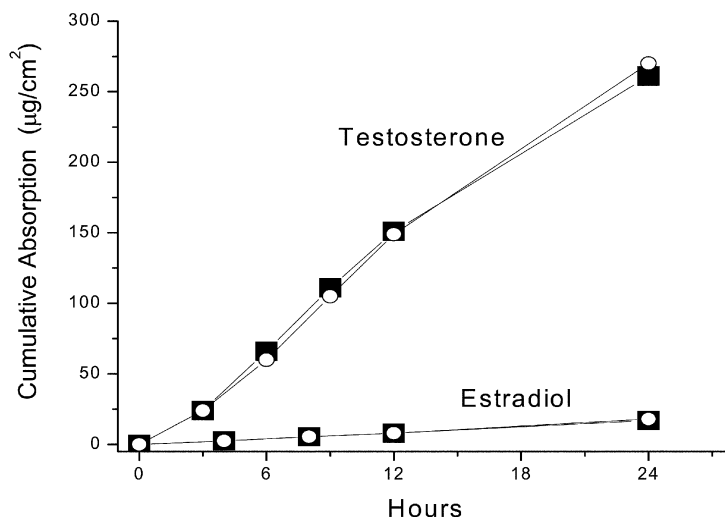


Figure 3. Cumulative absorption of testosterone and estradiol from transdermal systems determined in vitro (■) in the cadaver skin model and in vivo (○) in human subjects. In vivo absorption was determined by measuring drug loss from the patch. The estradiol system is intended for twice-weekly use, and data were collected to 96 h. Differences between the in vitro and in vivo data occurred in the last 48 h (in vitro > in vivo) but were readily explainable by the conditions of the experiment in which the area available for diffusion in vitro was only 67% of the total patch area. This led to a lower rate of drug depletion in vitro than in vivo because part of the patch was not in contact with the skin. (Redrawn from data of reference 15.)

PHASES I–III

Although the most frequent use of the cadaver skin model takes place during the preclinical phase of drug development, an obvious outcome of that work is establishment of a benchmark for the bioavailability of the specific formulation(s) that will enter toxicological and clinical evaluation. A confounding factor during many topical drug development programs is that changes are made to the initial formulation as development

progresses, raising the possibility that bioavailability also may change. This of course raises questions about the adequacy of the clinical and toxicology studies at the time of NDA submission. Clearly, however, this is a situation in which a direct comparison of the to-be-marketed product with the initial formulation in the cadaver skin model could easily address this issue in a timely and cost-effective manner in lieu of an animal or human pharmacokinetic bridging study or the need to repeat long-term animal tests.

This problem is exemplified by a relatively recently approved topical drug product, eflornithine (Vaniqa) (16). Development of the drug began at Gillette Medical Evaluation Laboratories, then moved to Bristol-Myers Squibb. Throughout development a number of formulations were tested, and even the manufacturing process changed when the product moved from one company to the other. To check on the relative bioavailability of the product following various formulation and manufacturing process changes, formulators conducted a series of in vitro permeation studies. The last study, done just prior to initiation of the Phase III trials, assessed product made by three different processes to verify that the bioavailability of product made by the new manufacturing process—the most efficient process—was not inferior to that of the other two (Table 1). The data demonstrated equivalent drug delivery for all three processes and confirmed that the expected clinical results from the pivotal Phase III trials should be equivalent to results seen in earlier trials. If not, at least the bioavailability of the formulation could not be questioned.

Table 1. Eflornithine absorption through human cadaver skin from three batches of product, each manufactured by a different process.*

Mfg Process #1	Mfg Process #2	Mfg Process #3
0.11 ± 0.05	0.14 ± 0.09	0.12 ± 0.04

* Average data (± standard error of the mean) are expressed as % dose absorbed at 48 h ($n = 24$ skin sections/product). Differences between products were not statistically significant by paired t -test ($p < 0.05$).

Because this and the prior examples cited demonstrate not only the utility of the cadaver skin model but also the reliability and sensitivity of the data provided, it can be argued that this is the best method by which to assess the relative bioavailability of topical formulations throughout the entire drug development process. Even after the preclinical phase of drug development and the reformulation that commonly occurs as development progresses, use of the model can extend through scale-up and postapproval changes. A consensus statement of a workshop jointly sponsored by the American Association of Pharmaceutical Scientists (AAPS), FDA, and USP stated that “diffusion cell measurements” may be useful as part of a multi-tiered approach to justify major changes in a formulation and to avoid the need for additional clinical evaluation (17). The authors believe that sufficient data now exist to support

use of the cadaver skin model as the essential, if not the sole, test for documenting the equivalent bioavailability of a succession of related formulations.

An obligatory element in the formal acceptance of this model for regulatory purposes will be the establishment of a standardized methodology, as has been done in Europe (18). Measurement of a drug’s permeation in vitro requires that a number of diverse variables be adequately controlled, and for those new to the field the model can at first appear complex and difficult to use. Highly variable data sporadically are obtained, which adds to concern about the reliability of the model. Development of a guidance document might help resolve these critical issues.

GENERIC DRUG DEVELOPMENT

Reverse Engineering

Use of the cadaver skin model in the development of generic topical products is a logical extension of the rationale underlying its use in the development of innovator products. In one approach multiple formulations are tested to determine which one supports the greatest bioavailability, and in another approach multiple formulations are tested to determine which one most closely matches the reference listed drug (RLD). The importance of establishing the degree of concordance between test and reference products before the company conducts the pivotal bioequivalence study cannot be overemphasized because clinical trials are the basis for approval of most generic dermatologics, and the cost of failure is high.

A case in point is a problem encountered by one company during development of an antifungal cream. The company encountered analytical difficulties when it attempted to determine the exact concentrations of two co-solvents in the innovator product. To circumvent the problem the company’s formulators made three best-guess formulations in which they varied the concentration of the co-solvents and determined the relative bioavailability of the API from each using the cadaver skin model (Figure 4A). None of these three formulations matched the RLD, but the data narrowed the possibilities and guided the formulators as they made and tested three more formulations (Figure 4B). This time one of the test formulations demonstrated comparable bioavailability, and it was subsequently shown by clinical trial to be bioequivalent to the RLD (data on file, PRACS-Cetero).

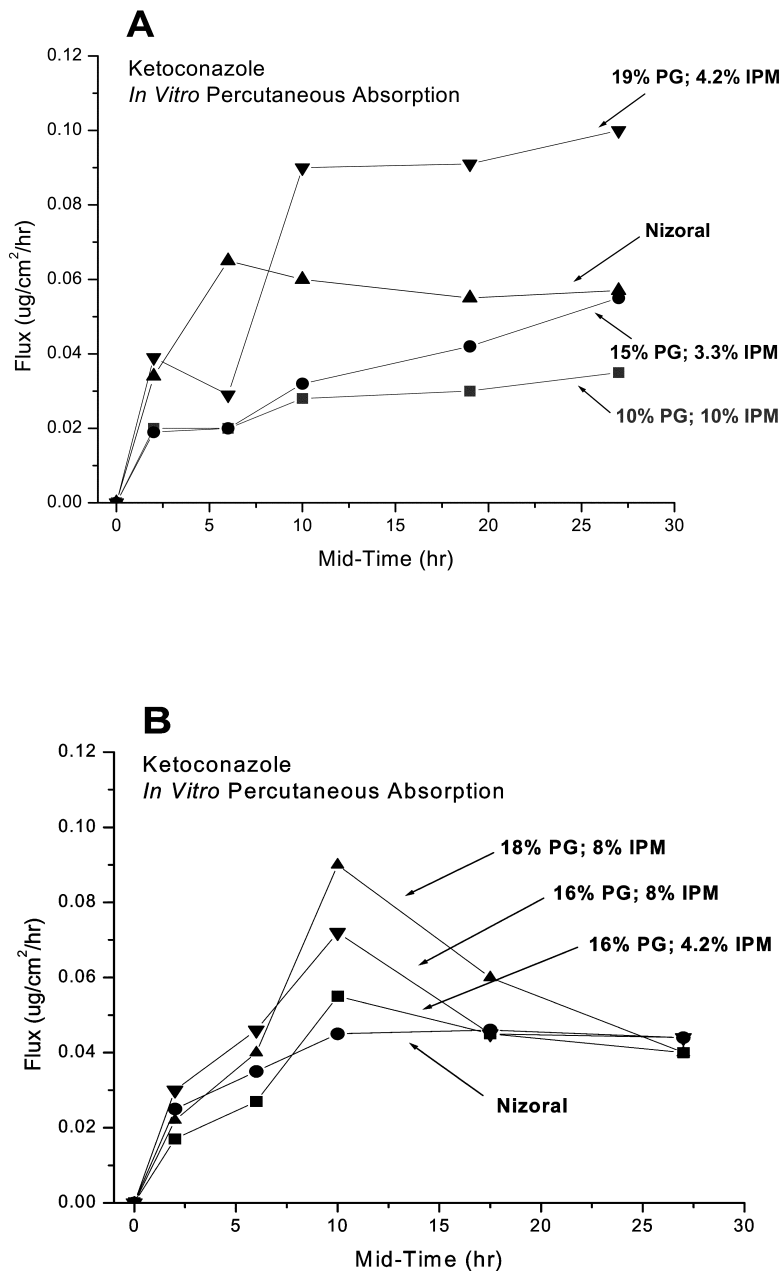


Figure 4. Screening of test formulations of ketoconazole in the cadaver skin model to determine which best matches the RLD (Nizoral). A. Ketoconazole bioavailability in the first three formulations tested failed to match the innovator product. B. Ketoconazole bioavailability from one of second batch of three test formulations closely approximated the RLD. (Data on file, PRACS-Cetero.)

A somewhat different situation that also illustrates the utility and power of the cadaver skin model in the development of generic drugs occurred during the elaboration of a topical corticosteroid product. Because proof of bioequivalence relies on pharmacodynamic assay rather than clinical trial, the test formulation was run against the RLD in the VC assay. In three sequential VC studies the test formulation failed to demonstrate bioequivalence to each of three separate lots of the reference product. Surprisingly, the single test formulation was low in two studies but high in the third.

These unusual results apparently were explained by a comparison of the API's bioavailability from multiple lots of reference product (different from the lots used in the VC study) in

the cadaver skin model (Table 2). Two different lots of test material gave virtually identical results. However, one lot of the reference material was not equivalent to the other two. The inability to demonstrate bioequivalence in the prior VC studies may have been caused by variability of the RLD (data on file, PRACS-Cetero). This also demonstrates that the human cadaver skin model can be used to preselect test and reference lots whose in vitro bioavailability are most similar and, in effect, increase the likelihood of success in the pivotal BE study. This will be discussed further in the next section.

Table 2. Total absorption of diflorasone diacetate through human cadaver skin at 48 h (mean \pm standard error of the mean). Both lots of test material were equivalent to RLD lot #3 but not to RLD lots #1 or #2. (Data on file, PRACS-Cetero.)

	Generic #1	Generic #2	RLD #1	RLD #2	RLD #3
Ng Absorbed	198.7 \pm 47.3	194.7 \pm 59.9	111.4 \pm 26.7	117.6 \pm 11.1	172.9 \pm 32.5

Bioequivalence

The prior examples illustrating the advantage of the cadaver skin model in generic drug development raise a question about its potential use as a bioequivalence testing method. Indeed, this has been a subject of discussion in the past at meetings jointly sponsored by AAPS and FDA (19, 20). Although insufficient data were available at that time to make a compelling argument for its use as a bioequivalence test method, and regulatory interest in other bioequivalence methods has subsequently sidetracked further consideration, new data continue to emerge and strongly support use of the cadaver skin model as a bioequivalence method.

As became evident a number of years ago during discussions of the use of stratum corneum tape stripping (DPK) as a potential bioequivalence method (21), several critical hurdles must be overcome before FDA will accept any surrogate bioequivalence method: (A) the test system must be able to show that products proven to be bioequivalent by FDA-approved methods (e.g., clinical trial or VC assay) are equivalent in the model, and (B) the test system must be able to show that products proven *not* to be bioequivalent by FDA-approved methods also are not equivalent in the model. Meeting the first of the two criteria can be achieved because many bioequivalent topical products are available to test; the second criterion is problematic because finding bioequivalent products is difficult. However, because the primary purpose of the latter requirement is to demonstrate sufficient sensitivity of the

method to differentiate small differences between similar products, clinical trial results are not necessarily the only, or even the best, yardstick to use. Two examples illustrate the ability of the cadaver skin model to meet these two critical criteria.

Two AB-rated generic tretinoin gels, 0.01% and 0.025% (Spear Pharmaceuticals) were approved on the basis of clinical trials in subjects with acne vulgaris. These same two generic products were also compared to the RLDs (Retin-A, Johnson & Johnson) in the cadaver skin model and were found to be bioequivalent (22). In the model system all four products were tested side-by-side in the same donor skins, making it possible not only to demonstrate equivalence within strengths but also to demonstrate inequivalence between strengths, an internal validation of the sensitivity of the method (Table 3). Unfortunately, the clinical trials of the same four products were conducted as separate trials, low strength versus low strength and high strength versus high strength, which made it impossible clinically to corroborate bioequivalence. However, more than 30 years of clinical experience has taught physicians that the two strengths are not equivalent in at least one important aspect, local irritation, a highly significant adverse reaction that was noted during the initial clinical trials of the original Retin-A products and continues to be an issue with topical tretinoin use (23, 24).

Table 3. Tretinoin absorption measured in the cadaver skin model from Spear Tretinoin Gel 0.01% and 0.025% versus Retin-A Gel 0.01% and 0.025%. The primary pharmacokinetic parameters calculated were: (A) total absorption at 48 h (Total Abs, ng), (B) maximum rate of absorption (F_{max} , ng/cm²/h), and (C) time when maximum rate of absorption occurred (T_{max} , hr). The primary analysis was based on natural log-transformed data. The estimated error standard deviation was used to compute the 90% confidence intervals (CI) for the ratios of the means (Test/Reference) of the listed parameters (19).

	Test	Reference	Test/Reference	90% CI
0.01% Tretinoin Gel				
Total Abs	2.9961	2.9742	1.02266	97.07, 107.46
F_{max}	0.5492	0.5716	1.03789	92.53, 115.05
T_{max}	3.5957	3.5726	1.04300	92.23, 116.37
0.025% Tretinoin Gel				
Total Abs	3.4921	3.4709	1.02798	95.14, 110.45
F_{max}	0.9058	0.8840	1.11481	95.08, 127.88
T_{max}	3.6642	3.7248	0.98389	97.26, 99.52

A second example that demonstrates the utility of the cadaver skin model as a surrogate bioequivalence test comes from the therapeutic class that contains the largest number of generic topical drugs, the glucocorticoids. Five approved generic products were evaluated in the model during their development stage and were compared to the corresponding

reference products (data on file, PRACS-Cetero). The lots of test and reference product evaluated in vitro, with one exception, were the same lots subsequently evaluated in the pivotal human vasoconstrictor study leading to ANDA approval. Thus, these data offer a unique opportunity to examine in vitro/in vivo correlation (Table 4).

Four of the five approved generic products were found to be equivalent to their corresponding reference standard in the cadaver skin model, in agreement with the VC data. However, one product, mometasone furoate ointment, was not found to be equivalent. Though the result may at first appear to demonstrate a failure of the model, the data are actually an indication of the greater sensitivity of the cadaver skin model relative to the VC assay. This is plainly illustrated by comparison of the VC scores and absorption data for the alclome-

tasone cream and ointment products. The two products are virtually identical by VC assay, but the cadaver skin model demonstrates a >15-fold difference in bioavailability of the API from the ointment product. A similar trend is seen with halobetasol cream and ointment. Although the VC scores of ointment and cream are very similar, a two-fold difference in bioavailability is found in the cadaver skin model.

Table 4. Total absorption (ng/48 h) of API (halobetasol propionate, alclometasone dipropionate, or mometasone furoate) measured in the cadaver skin model and the same products tested in the VC assay (area under the blanching curve). Identical lots of test and reference products were used in both the cadaver skin and VC assays, with the exception of halobetasol propionate cream. The VC data shown are from the pivotal trials that resulted in ANDA approval. (Data on file, PRACS-Cetero.)

	Total Absorption			VC Response		
	Test	Reference	Test/Reference	Test	Reference	Test/Reference
Halobetasol Cream	110.4	96.9*	1.14	33.1	30.7	1.08
Halobetasol Ointment	246.7	256.4	0.96	28.6	28.5	1.00
Alclometasone Cream	4.53	4.39	1.03	18.5	16.8	1.10
Alclometasone Ointment	67.0	70.0	0.96	16.0	17.4	0.92
Mometasone Ointment	213.4	338.7	0.63	13.7	12.3	1.11

* Average of 3 reference lots.

SUMMARY

The human cadaver skin model is a powerful and sensitive tool by which to accurately quantitate a drug's rate of percutaneous absorption, and it has application within multiple areas of the drug development process. The area of greatest application historically has been preclinical development, where it is used principally to screen and select the optimum formulation for further development. However, as new data continue to emerge to support the validity of the model as a surrogate for in vivo measurements of bioavailability and bioequivalence, its application within other phases of the drug development process have become evident. These include: (A) reformulation during Phases I–III, (B) development of line extensions and products with enhanced delivery, (C) scale-up and postapproval changes, (D) generic drug development, and (E) use as a surrogate for bioequivalence. Greater recognitions by FDA of its standing as a scientifically sound test method and acceptance of its use as a valid surrogate for more costly and time-consuming human/animal tests would help to simplify the development of both new and generic topical/transdermal drug products.

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