Assessment and Prediction of the Cutaneous Bioavailability of Topical Terbinafine, *In Vivo*, in Man

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INTRODUCTION

The *in vivo* effectiveness of a topical, dermatological formulation depends on the bioavailability of the drug within the skin at the site of action. Unlike oral drugs, or those delivered transdermally for systemic effect, the amount of active agent reaching the general circulation after topical application is a measurement of questionable relevance with respect to its *local* bioavailability (1). In addition, the level of a dermatological drug, which can be found in the blood is invariably very small and difficult, if not impossible, to quantify easily (2). While local, 'skin' concentrations of topical drugs can be obtained from biopsies of the site of application, this approach is very invasive and is unacceptable for routine use. Other practical alternatives have not been abundantly proposed (3), and the vasoconstriction assay for corticosteroids (4) remains the only method (albeit imperfect itself) with significant credibility.

Recently, the U.S. Food and Drug Administration has suggested a new, so-called dermatopharmacokinetic (DPK), approach (3,5). The idea is to evaluate topically applied drug levels in the stratum corneum (SC) *in vivo* as a function of time post-application and post-removal of the formulation, and to generate a SC concentration versus time profile from which such "classical" measures as maximum drug level, time to reach this maximum, and the area under the curve can be obtained (i.e., in an analogous fashion to blood level measurements for an orally administered drug). The DPK method assumes that: (a) in normal circumstances, the SC is the rate-determining barrier to percutaneous absorption, (b) the SC concentration of drug is directly related to that which diffuses into the underlying viable epidermis, and (c) SC drug levels are more useful and relevant for assessing local, dermatological efficacy than plasma concentrations.

Whereas methodological and validation issues for the DPK technique remain to be answered, it is clear that there is an attractive logic behind this idea. However, examination of the draft guidance (5) reveals that this methodology will be labor-intensive even for relatively simple evaluations of bioequivalence. Our objective, therefore, is to begin an examination of whether experiments (following, in general, a DPK methodology) of relatively short duration can be analyzed to produce physicochemical parameters that describe drug transport in the SC and which can therefore be used to *predict* drug uptake as a function of time.

Specifically, using the antimycotic drug, terbinafine (molecular weight = 291 Da; log(octanol/water partition coefficient) = 3.3) (6,7), we have measured, *in vivo*, in man, the SC concentration versus depth profile following a 0.5-h exposure to a simple formulation. The data have been analyzed mathematicaly to yield the SC/vehicle partition coefficient (K) of the drug and its characteristic diffusion parameter (D/L^2, where D is the drug's diffusivity across the SC of thickness L). With these values, the mathematical model has been used to predict the integrated quantity of drug in the SC following treatment periods of 2 and 4 h, and the predictions have then been compared to experiment. In addition, the longer-time data have been evaluated independently to determine whether K and D/L^2 are sensitive to the duration of drug treatment.

MATERIALS AND METHODS

Chemicals

Terbinafine (TBF) was from Novartis Pharma (Basel, Switzerland). It was dissolved at 500 mg/mL in a 50:50 v/v mixture of isopropyl myristate (≥95%; Siegfried, Sofingen, Switzerland) and absolute ethanol (≥99.8%; Fluka, Buchs, Switzerland). The high TBF concentration was chosen so as to minimize drug depletion from the formulation during the experiment, and to facilitate its analytical detection. Deionized water, acetonitrile and tetrahydrofuran (Sigma-Aldrich, Steinheim, Germany) were HPLC grade. Buffers, triethylamine (TEA) and tetramethylammonium hydroxide pentahydrate (TMAH), were also from Sigma-Aldrich.

Human Subjects

Four healthy volunteers (2 female, 2 male, 24–41 years) without history of dermatological disease participated in this study, which had received ethical approval. Written consent was obtained. The drug application sites (area = 7×1 cm^2) were on the volar forearm, 4 cm from the wrist.

Treatment Protocol

700 μL of TBF formulation (i.e., 100 μL/cm^2 = 50 mg TBF/cm^2) were applied on a cellulose patch (Tela, Basel, Switzerland), which was then affixed to the skin via adhesive polyurethane (Opsite, Smith-Nephew, Hull, UK) under an occlusive polyester film (Scotchpak, 3M, St. Louis, MN). After 0.5, 2, or 4 h, the patch was removed and the excess formulation was cleaned by gently blotting the skin with three dry cellulose swabs.

SC Sampling Protocol

SC is the proposed sampling compartment for the DPK approach. Cutaneous bioavailability was therefore assessed

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by measuring drug concentration in the SC, by sequential removal, and subsequent drug analysis within, successive layers of the barrier (see below). This procedure is relatively non-invasive and painless, and was easily performed by simply applying an adhesive tape (Scotch Book Tape, 3M, St. Paul, MN) to the treated site, pressing it firmly to the skin, and then removing it. The SC sampling site was delimited by a template which left only the previously treated "window" of (7×1) cm^2 of skin exposed. No tape-strips were discarded: all drug not removed in the surface cleaning process was considered "bioavailable".

Up to 20 SC layers were removed. To check skin barrier function, transepidermal water loss (TEWL) measurements were performed (Evaporimeter EP1, Servomed, Stockholm, Sweden) during the stripping procedure, which was stopped if TEWL reached 50 g/m^{2}h. Each tape was carefully weighed before and after stripping on a 10-μg precision balance (Mettler AT261, Greifensee, Switzerland) to determine the mass and thickness of the SC layer removed (8). The amount of TBF on each strip could then be converted to a concentration within that layer of the SC. The depth of drug permeation into the SC, and the total thickness of this membrane, were deduced from the TEWL measurements during the sequential tape-stripping process, using the approach which has been described in detail elsewhere (8,9).

Extraction and Analysis of TBF in the Tape Strips

To extract the TBF, the tapes were placed in polypropylene vials with 7 mL of 80:20 v/v acetonitrile and TEA (0.72 mM) at pH 2.5. After 16 h agitation, the supernatant was passed through a 0.45 μm filter (Nalgene, Rochester, NY) and then analyzed by HPLC. The adhesive matrix of the tapes did not dissolve, and the extracted impurities did not interfere with the drug's chromatographic peak. Validation of the extraction was carried out by spiking tape-stripped samples of untreated SC with 100 μL of a 10 mg/mL solution of TBF. Drug recovery was 96.6 ± 1.9% (n = 5).

TBF was determined using isocratic HPLC (Waters-Millipore (Milford, MA) model 600 pump, autosampler 717 Plus and model 486 UV detector at 280 nm, used in conjuction with a 12-cm Partisphere RP-18 column (Whatman, Clifton, NJ)). The mobile phase was acetonitrile, THF and TMAH (1.59 mM, pH 7.8) (50:14.3:35.7 v/v). At a flow rate of 2 mL/min and at room temperature, the retention time of TBF was ~6 min. A calibration curve was generated with the pure compound. The detection limit was 0.5 μg/mL.

Experimental Strategy and Data Analysis

The SC concentration (C_x) versus normalized depth (x/L) profile of TBF was first determined following a 0.5-h treatment of the skin; the data were fitted to the appropriate solution of Fick's second law of diffusion (10):

$$C_x = K\,C_v\left(1 - \frac{x}{L} - \frac{2}{\pi}\sum_{n=1}^{3}\frac{1}{n}\sin\left(n\pi\frac{x}{L}\right)\exp\left(-\frac{D}{L^2}n^2\pi^2 t\right)\right) \quad (1)$$

which assumes that the applied drug concentration (C_v) remains constant for the treatment period (t) and that the viable epidermis is a perfect sink for the drug. The third boundary condition is that the SC contains no drug at $t = 0$. The fitting permitted K and D/L^2 values to be deduced. Subsequently, Eq. (1) can be integrated across the SC thickness (i.e., from $x/L = 0$ to $x/L = 1$) to provide an effective AUC (units = mol/L or M) of the drug in the SC at any time t:

$$\text{AUC (M)} = \int_0^1 C_x\,d\left(\frac{x}{L}\right) = KC_v\left(\frac{1}{2} - \frac{4}{\pi^2}\left(\exp\left(-\frac{D}{L^2}\pi^2 t\right) + \frac{1}{9}\exp\left(-9\frac{D}{L^2}\pi^2 t\right)\right)\right) \quad (2)$$

This overall uptake is the metric to be evaluated in the proposed DPK method as a function of time. We therefore used Eq. (2) and the K and D/L^2 values from the 0.5-h experiment to predict AUC at $t = 2$ and $t = 4$ h. These predictions were then compared with experiments performed on the same subjects. In addition, the SC concentration versus depth profiles at 2 and 4 h were evaluated independently using Eq. (1), in each case generating best-fit values of K and D/L^2 which could then be compared with the results at $t = 0.5$ h to reveal any time-dependent changes in these key transport/uptake parameters.

RESULTS AND DISCUSSION

The SC concentration of TBF versus depth profiles for four subjects following a 0.5-h treatment are in Fig. 1. Because different subjects had different SC thicknesses and because the tape-stripping process rarely, if ever, permits a reproducible amount of SC to be removed either per strip, or per subject, the concentration profiles are presented as a function of normalized SC depth to facilitate comparison between the results.

The best fits of Eq. (1) to the data are drawn through the individual points in Fig. 1. The deduced values (mean ± SD) of K and D/L^2 from these measurements were 0.77 (± 0.34) and 2.28 (± 0.87) $\times 10^{-5}$ s^{-1}, respectively. These parameters were then used with Eq.(2) to predict AUC following 2 and 4 h of TBF application and, finally, relevant experimental data were obtained to compare with the predictions. This comparison is shown in Table I, and indicates that no significant difference ($P > 0.05$) between experiment and prediction, at either 2 or 4 h was observed.

The individual concentration profiles of TBF at 2 and 4 h

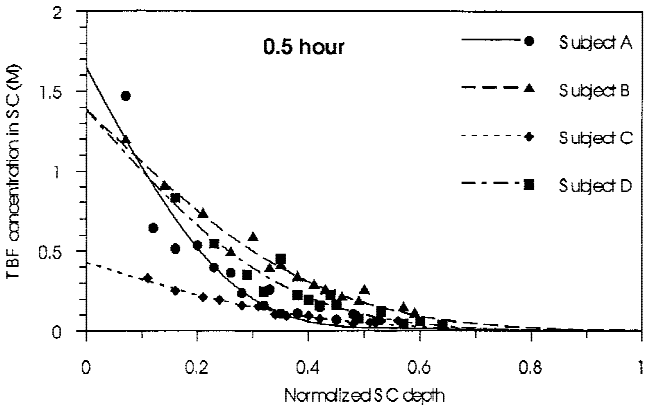

Fig. 1. Concentration profile of TBF across human SC *in vivo* following a 0.5-h application in a vehicle containing 50:50 v/v ethanol/IPM under occlusion. The lines of best fit of Eq. (1) through the individual experimental data points are shown.

Table I. Comparison between the Experimentally Determined Values of AUC (Mean ± SD; n = 4) following 2 and 4 Hours of TBF Application, and the Predictions Based upon K and D/L^2 Results Determined from the Data Obtained after a 0.5-Hour Application

Treatment time (h)	Experimental 10^1* AUC (M)	Predicted 10^1* AUC (M)
0.5	2.60 ± 1.09	—
2	4.28 ± 1.81	4.76 ± 1.95[a]
4	4.17 ± 1.03	5.21 ± 2.68[a]

[a] Experimental value is not significantly different ($p > 0.05$) from the corresponding predicted result.

are presented in Fig. 2A and 2B, respectively. Compared to Fig. 1, it is seen that the drug permeates further into the SC with increasing application time and that the profiles tend toward linearity for the longest contact period. As previously reported (11), we also found that, with increasing exposure time, more SC was removed from all subjects by the same number of tape-strips. At $t = 0.5$ h, 58 ± 5% of the SC was taken off in 20 strips; at 2 and 4 h, the amounts removed were 74 ± 9% and 81 ± 9%, respectively. The combined effects of occlusion, and of vehicle penetration into the SC, on the cohesivity of the SC are probably important factors contributing to this observation.

The continuous lines through the data in Fig. 2A and 2B represent the independent best fits of Eq. (1) to each profile. The resulting values of K and D/L^2 obtained from the data at

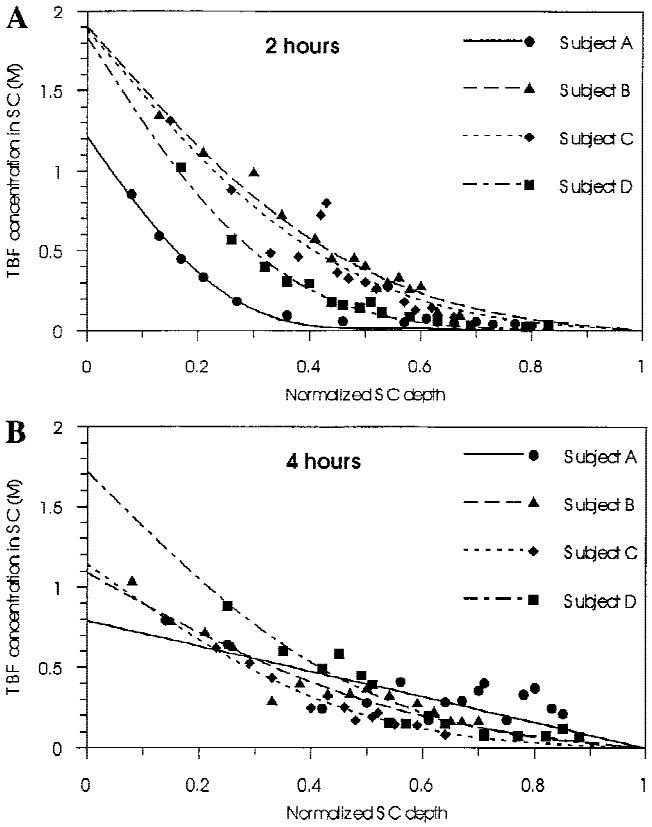

Fig. 2. Concentration profile of TBF across human SC *in vivo* following either a (A) 2-h or (B) 4-h application in a vehicle containing 50:50 v/v ethanol/IPM under occlusion. The lines of best fit of Eq. (1) through the individual experimental data points are shown.

Table II. Values of K and D/L^2 (Mean ± SD; n = 4) Deduced from the Best Fit of Eq. (1) to the SC Concentration Profiles of TBF Shown in Figures 1 and 2

Treatment time (h)	10^5*D/L^{2a} (s^{-1})	K[b]
0.5	2.28 ± 0.87	0.77 ± 0.34
2	0.69 ± 0.37	1.08 ± 0.21
4	0.57 ± 0.12	0.84 ± 0.22

[a] ANOVA ($p < 0.05$) indicates that the value at 0.5 hr is significantly greater then the values at both 2 and 4 hr; however, the data at 2 and 4 hr are not significantly different from one another.

[b] ANOVA indicates no significant differences between these three values.

2 and 4 h, together with those deduced by the same analysis at 0.5 h, are in Table II. An interesting effect is apparent at short application times, in that the diffusion parameter (D/L^2) is significantly higher at 0.5 h than at 2 and 4 h (when it appears constant). A possible explanation is that ethanol, a significant component of the vehicle used, rapidly and significantly enters the SC post-application of the dosage form and transiently facilitates TBF transport in the outer layers of the barrier. After this initial "burst" effect, it appears, the drug diffusion slows to a more moderate pace. Clearly, from a mechanistic point of view, this result warrants further investigation. On the other hand, the SC/vehicle partition coefficient of TBF is not influenced by the contact time between the skin and the formulation, the results suggesting a rapid equilibriation of TBF at the interface.

With respect to the study's more practical objective, however, it is nevertheless remarkable that, despite the elevated D/L^2 value derived from the $t = 0.5$ h application, the predicted AUC results at longer times are in good agreement with the experimental findings (Table I). Although based on a limited set of observations, it is tempting to conclude that a short-time experiment may therefore provide sufficient information with which to construct a major part of a drug's DPK profile. This deduction is supported, at least in part, by some earlier work in our laboratory targeted at the facile prediction and assessment of dermal exposure to toxic chemicals (12). Of course, these preliminary observations require additional work to be performed, ideally examining other drugs and other vehicles. Also, while we have so far addressed the "uptake" aspects of the DPK profile, it will be important also to consider the "elimination" phase of drug from the SC post-removal of the delivery system (as is also predicated in the draft FDA guidance document (5)).

In conclusion, we believe that the results of this preliminary work offer a practical and potentially insightful approach to quantify and, ultimately, optimize topical drug bioavailability. The method may prove sufficiently sensitive to detect subtle vehicle-induced effects on skin permeation and be pragmatic enough to have utility in efficient cutaneous bioavailability/bioequivalence studies.

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REFERENCES

1. S. Borsadia, A. H. Ghanem, Y. Seta, W. I. Higuchi, G. L. Flynn, C. R. Behl, and V. P. Shah. Factors to be considered in the evaluation of bioavailability and bioequivalence of topical formulations. *Skin Pharmacol*. **5**:129–145 (1992).
2. R. H. Guy, J. Hadgraft, and H. I. Maibach. A pharmacokinetic model for percutaneous absorption. *Int. J. Pharm*. **11**:119–129 (1982).
3. V. P. Shah, G. L. Flynn, A. Yacobi, H. I. Maibach, C. Bon, N. M. Fleischer, T. J. Franz, S. A. Kaplan, J. Kawamoto, L. J. Lesko, J. P. Marty, L. K. Pershing, H. Schaefer, J. A. Sequeira, S. P. Shrivastava, J. Wilkin, and R. L. Williams. Bioequivalence of topical dermatological dosage forms–methods of evaluation of bioequivalence. *Pharm. Res*. **15**:167–171 (1998).
4. B. W. Barry. Bioavailability of topical steroids. *Dermatologica* **152**:47–65 (1976).
5. V. P. Shah. Topical dermatological drug product NDAs and ANDAs: in vivo bioavailability, bioequivalence, in vitro release, and associated studies. US Dept. of Health and Human Services, Rockville, 1998, pp. 1–19.
6. J. Faergemann, H. Zehender, T. Jones, and I. Maibach. Terbinafine levels in serum, stratum corneum, dermis-epidermis (without stratum corneum), hair, sebum and eccrine sweat. *Acta Derm. Venereol*. **71**:322–326 (1991).
7. J. A. Balfour and D. Faulds. Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. *Drugs* **43**:259–284 (1992).
8. Y. N. Kalia, F. Pirot, and R. H. Guy. Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. *Biophys. J.* **71**:2692–2700 (1996).
9. F. Pirot, E. Berardesca, Y.N. Kalia, M. Singh, H.I. Maibach, and R.H. Guy. Stratum corneum thickness and apparent water diffusivity: facile and noninvasive quantitation *in vivo*. *Pharm. Res.* **15**:490–492 (1998).
10. J. Crank. Diffusion in a plane sheet. In: The mathematics of diffusion. Oxford University Press, London, 1975, pp. 44–68.
11. J. C. Tsai, M. J. Cappel, N. D. Weiner, G. L. Flynn, and J. J. Ferry. Solvent effects on the harvesting of stratum corneum from hairless mouse skin through adhesive tape stripping *in vitro*. *Int. J. Pharm*. **68**:127–133 (1991).
12. F. Pirot, Y. N. Kalia, A. L. Stinchcomb, G. Keating, A. L. Bunge, and R. H. Guy. Characterization of the permeability barrier of human skin in vivo. *Proc. Natl. Acad. Sci. USA* **94**:1562–1567 (1997).