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Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo

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Abstract

Purpose: The objective of this study was to determine the availability of the topical drug terbinafine (TBF) in human stratum corneum (SC) in vivo following its administration in formulations containing isopropyl myristate and ethanol. *Methods:* The ventral forearms of human volunteers were treated for 4 h with TBF, at a concentration equal to 1/4 saturation, in isopropyl myristate (IPM), in ethanol (EtOH) and in 50:50 v/v IPM/EtOH. At the end of the application period, the treated sites were carefully cleaned of excess vehicle and the SC was progressively removed by sequential tape stripping. TBF was quantified in the SC by: (a) extraction of the tape strips and subsequent HPLC analysis; and (b) attenuated total reflectance infrared spectroscopy (ATR-FTIR) of each sequentially exposed SC surface during the tape stripping procedure. *Results:* The concentration profile of TBF in the SC (i.e. drug concentration as a function of depth in the membrane) was fitted to the appropriate solution of Fick's second law of diffusion, allowing thereby the drug's SC/vehicle partition coefficient (K) and characteristic diffusion parameter (D/L^2, where D is the diffusivity of TBF in the SC of thickness L) to be deduced. *Conclusions:* While D/L^2 for TBF derived from the three vehicles remained essentially constant, the drug's partitioning into the SC was significantly higher from formulations containing ethanol. Both the semi-quantitative infrared data and the more rigorous HPLC results supported these deductions.

Keywords: Antimycotics; Topical vehicles; Topical administration; Topical bioavailability; Fourier transform infrared spectroscopy; Tape stripping

1. Introduction

The primary objective of a topical formulation for the treatment of a cutaneous disease is that the drug reaches the target site at the required

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concentration, and achieves its therapeutic action. Thus, the clinical efficacy of the formulation depends on the ability of the vehicle to release the drug which must then penetrate the stratum corneum (SC). These steps are a succession of diffusion and partitioning processes occurring in series, and it follows that the slowest event (the rate-limiting step) will determine the rate of local drug availability (Higuchi, 1960), and hence the clinical effect of the drug (Pershing et al., 1992; Treffel and Gabard, 1993).

In this context, the role of the vehicle is of particular importance: an optimized formulation will efficiently release the drug and, because of its composition, may also facilitate drug transport across the skin. However, in vivo assessment of the most important controlling factors of topical drug availability is not easily accomplished, despite the obvious utility of such for mechanistic understanding, rational formulation design, and preclinical evaluation.

In comparing different formulations of a particular drug, and to address whether the presence of certain vehicle components may enhance percutaneous absorption, there are physico-chemical 'rules' to follow so that data interpretation is unambiguous. For example, the formulation should initially contain the drug at the same thermodynamic activity whenever possible or, at the very least, the degree of saturation of the drug in the different vehicles should be known (Higuchi, 1960). It has then been shown that the key events, and contributions, of drug partitioning from the vehicle into the SC and of drug diffusion across this barrier layer can be separated and characterized (Guy and Hadgraft, 1987; Harrison et al., 1996; Pellett et al., 1997a).

In this study, the SC uptake and transport of a small, lipophilic drug, terbinafine (TBF, molecular weight = 291 Da, log [octanol/water partition coefficient] = 3.3), has been evaluated from three simple vehicles. TBF is a topical antimycotic with its site of action within the SC; it follows that an evaluation (and optimization) of its local bioavailability in this most superficial layer of the skin is pertinent to its ultimate clinical efficacy in vivo (Faergemann et al., 1991; Balfour and Faulds, 1992). To this end, we have determined the concentration profile of TBF across the SC of human volunteers using a tape stripping procedure and either HPLC or reflectance infrared spectroscopy (Higo et al., 1993; Pirot et al., 1997) to quantify the drug. The data have subsequently been interpreted using a straightforward diffusion–partition model of SC transport to reveal mechanistic information relevant to the efficient delivery of the drug.

2. Materials and methods

2.1. Chemicals

Terbinafine (TBF) as the free base was supplied by Novartis Pharma (Basel, Switzerland). It was dissolved at 1/4 saturation (equivalent thermodynamic activity), corresponding to 490 mg/ml in neat ($\geq 95\%$) isopropyl myristate (IPM, Siegfried, Zofingen, Switzerland), 193 mg/ml in analytical grade ($\geq 99.8\%$) absolute ethanol (EtOH, Fluka, Buchs, Switzerland), and 475 mg/ml in a 50:50 v/v mixture of the two solvents.

For extraction of TBF from the adhesive tape strips (see below) and for the HPLC analysis, all solvents (ultra-pure deionized water, acetonitrile, and tetrahydrofuran) were of HPLC grade (Sigma-Aldrich, Steinheim, Germany). Triethylamine and tetramethylammonium hydroxide pentahydrate were used as buffers.

2.2. Solubility of TBF in the vehicles

Saturated solutions of TBF were prepared by stirring an excess of drug in the appropriate vehicle for 24 h at room temperature (23 ± 1°C) in sealed 10 ml screw-cap vials. The resulting suspensions were centrifuged at 3000 rpm for 5 min, and the supernatants were subsequently filtered (MN GF-3 filter paper, Macherey-Nagel, Düren, Germany) and then transferred into clean vials.

The saturation solubility of TBF was measured by infrared (IR) spectroscopy (Nicolet 730, Madison, WI). Calibration plots were first constructed using series of known concentrations of TBF in EtOH, IPM and EtOH:IPM (50:50). The TBF absorbance at 774/cm (originating from the

aromatic C–H stretching vibrations) was used, and Beer–Lambert's law was accurately obeyed (absorbance versus concentration regressions always yielding r^2 values in excess of 0.99). TBF concentrations at saturation were determined by measuring the absorbance of 1:10 dilutions of the saturated solutions and back-calculation. Five replicate measurements in each solvent were made and revealed the following solubilities: IPM, 1.96 g/ml; EtOH, 0.77 g/ml, EtOH:IPM (50:50), 1.83 g/ml.

2.3. Experimental procedures

Three caucasian volunteers (two female, one male, 22–34 years) with no history of dermatological disease participated in this study, which was approved by the ethical commitee of the university hospital. Written consent was obtained from all subjects. The treated sites (7×1 cm^2) were non-hairy regions of the ventral forearm regions, 4 cm from the wrists.

Each treatment consisted of a 700 μl application of TBF solution, via a cellulose gauze (Tela, Basel, Switzerland) which was covered by an occlusive polyester film (Scotchpak, 3M, St. Louis, MN) and affixed to the skin with an adhesive (Opsite, Smith-Nephew, Hull, UK). After 4 h of contact, the 'patch' was removed and excess formulation gently removed using three dry cellulose swabs (without any solvent).

To determine the TBF concentration profile across the SC following drug application in the different vehicles, the barrier was first partially removed by sequential adhesive tape stripping (Scotch Book Tape, 3M, St. Louis, MN). Preweighed tapes were used which were re-weighed (Mettler AT 261 balance, Greifensee, Switzerland) after an SC layer had been detached to assess the amount of SC removed. From this mass, and knowing the area of the tape, and that the density of the tissue is about 1 g/ml (Anderson and Cassidy, 1973), it was possible to calculate the SC thickness removed as a function of stripping and hence the corresponding position (or depth) within the barrier. Periodic measurements of transepidermal water loss (Evaporimeter EP1, Servomed, Stockholm, Sweden) during the stripping process permitted the total thickness of the SC to be calculated (Kalia et al., 1996) and allowed all concentration profiles to be subsequently presented (and more efficiently compared, therefore) as a function of relative position (or depth) into the membrane.

Up to 20 strips were taken from each treated site on each volunteer, such that the SC was never completely removed. All tapes were subsequently analyzed for penetrated TBF; no strips were discarded and it was assumed, therefore, that any drug not removed by the surface cleaning process at the end of treatment, would eventually be bioavailable to the skin.

TBF in the skin was quantified in two ways. First, before each tape strip was removed, an infrared spectrum, using the attenuated total reflectance method (in conjunction with a 45° ZnSe internal reflection crystal (Spectra-Tech, Stamford, CT)) on the treated site in vivo was recorded (Higo et al., 1993; Pirot et al., 1997). Up to 15 spectra per site were taken. However, because it was not possible to internally calibrate these measurements, and because a constant contact between the skin and the reflection element, as a function of tape stripping, cannot be guaranteed, only relative amounts of TBF could be deduced. These values were obtained by dividing the area under the TBF absorption peak centered at 774/cm by the relatively constant area under the amide I and amide II absorbances originating from the carbonyl stretching and N–H bending vibrations, respectively, of the SC keratin (Mak et al., 1990; Pellett et al., 1997b).

Second, after re-weighing the tape strips,TBF was extracted quantitatively (16 h immersion in a 80:20 mixture of acetonitrile and triethylamine 0.72 M at pH 2.5), filtered (Nalgene 0.45 μm, Nalge, Rochester, NY) and finally analyzed by HPLC. The chromatographic system consisted of a model 600 pump, an autosampler 717 Plus (Waters-Millipore, Milford, MA), and a 12 cm Partisphere RP-18 column (Whatman, Clifton, NJ). The isocratic mobile phase was a 50:14.3:35.7 (v/v) mixture of acetonitrile, tetrahydrofuran and teramethylammonium hydroxide (1.59 mM at pH 7.8). Column effluent was monitored by a UV detector (Waters-Millipore, model 486, Milford,

MA) set at 280 nm. With a flow rate of 2 ml/min at room temperature, the TBF retention time was about 6 min. Peak recording and data processing were performed with the built-in system manager. TBF was determined using the AUC method and calibration plots generated with the neat compound. The detection limit was 0.5 μg/ml. Validation of the extraction revealed a TBF recovery efficiency of $96.6 \pm 1.9\%$ ($n = 5$).

2.4. Data analysis and modelling

The TBF concentration profiles (i.e. the drug concentration C_x as a function of position x and time t) were fitted to the appropriate solution of Fick's second law of diffusion, assuming: (i) that the SC behaved as a homogeneous, rate-limiting barrier to transport; (ii) that the concentration of TBF on the viable epidermal side of the SC could be assumed to be zero ('sink conditions'); and (iii) that, during the 4 h experiment, the concentration of TBF in the vehicle was not significantly depleted:

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

where C_v is the drug concentration in the vehicle and K is the SC-vehicle partition coefficient of TBF. The thickness of the SC is L and hence x/L reflects the relative depth of drug penetration into the membrane. The drug's diffusivity in the SC is designated D; it follows that D/L^2 can be considered as a characteristic (kinetic) transport parameter (units: per second) for the movement of TBF across the barrier. Thus, the best fits of Eq. (1) to the experimental results (infrared and HPLC) were produced (using Grafit software, version 3.03, Erithacus) and the values of K and D/L^2 which optimized these regressions were obtained.

Subsequently, together with the measured SC thickness (L), the effective TBF skin permeability coefficient (K_p) was deduced from Eq. (2), i.e.:

$$K_p = KL\frac{D}{L^2} = K\frac{D}{L}. \quad (2)$$

Finally, using the derived parameters, K and D/L^2, Eq. (1) was integrated between the limits $x = 0$ and $x = L$ (i.e. over the entire SC) to provide a 'local AUC', or extent of TBF absorption/uptake into the SC, as a means to compare the relative availability of the drug from the different vehicles.

3. Results and discussion

Representative concentration profiles of TBF, measured by reflectance IR spectroscopy and HPLC, respectively, delivered from three vehicles to one subject, are shown in Figs. 1 and 2. Note that the y-axis in Fig. 1 is given in arbitrary units (AU) as the spectroscopic method could not be quantified absolutely; on the other hand, the HPLC analysis permits absolute drug concentrations to be plotted. Example profiles are presented because the different amounts of SC removed from each volunteer with the tape stripping process means that the averaging of results obtained is not straightforward. Also shown in Figs. 1 and 2 are the best regression fits of the data to Eq. (1).

The mean ($\pm$SD) of the derived K and D/L^2 parameters for all subjects, for each vehicle, are in Tables 1 and 2 (IR and HPLC results, respectively). Again, because the IR does not allow absolute quantification of concentrations, the values of K in Table 1 must be considered as relative. However, as the D/L^2 parameter is extracted from the curvature of the profiles, both IR and HPLC methods yield 'real', absolute values of this kinetic constant.

Tables 1 and 2 additionally include the integrated 'AUC' values (relative, once more, for IR, absolute for HPLC) and the deduced permeability coefficients for TBF following its application in the three different vehicles. Because K_p includes the partition coefficient explicitly, only the values derived from HPLC can be considered quantitatively.

To facilitate comprehension of the results and the impact of different vehicles on TBF permeation into and through the SC, Fig. 3 summarizes and compares the K, D/L^2, AUC and K_p values derived from the IR and HPLC analysis.

Examination of Fig. 3B, and the corresponding data in Tables 1 and 2, shows (at least at the sensitivity of the measurement methods employed) that the diffusivity of TBF across the SC was not dependent upon the vehicle employed. In contrast, Fig. 3A reveals that the SC-vehicle partition coefficient in vivo was clearly a function of the properties of the vehicle and that K was inversely related to the solubility of TBF in the vehicle. This trend, of course, is observed in the 'AUC' results shown in Fig. 3C, and in the calculated permeability coefficients (Fig. 3D), which are directly dependent upon K.

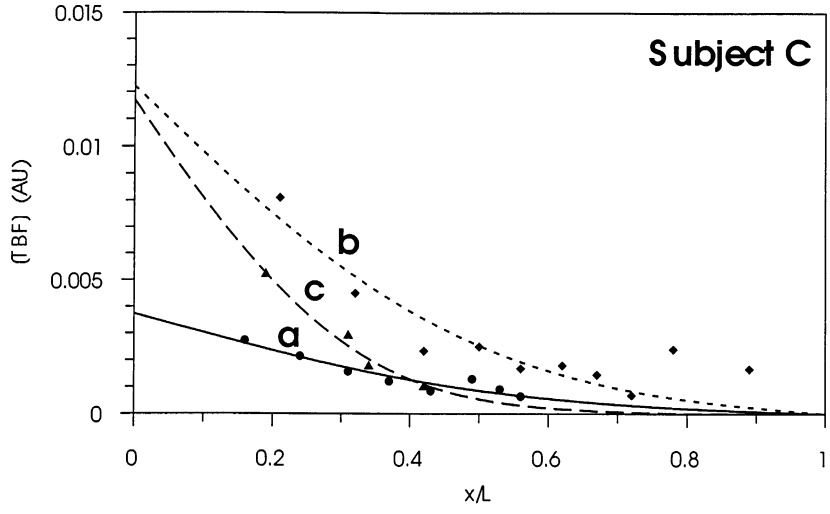

Fig. 1. In vivo concentration profiles of TBF across the SC of subject C after 4 h treatment with the drug in: (a) IPM; (b) EtOH; and (c) IPM:EtOH (50:50). The individual data points were evaluated by reflectance infrared spectroscopy during sequential tape stripping of the SC. The continuous curves represent the best fits of Eq. (1) to each set of data.

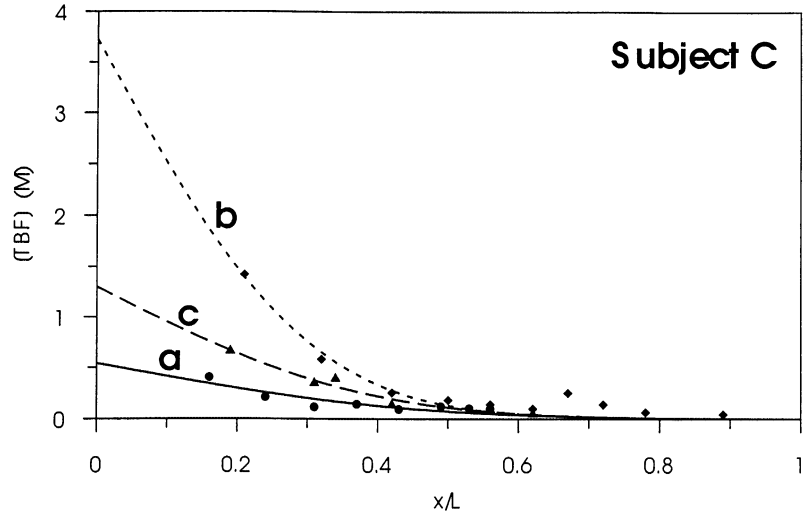

Fig. 2. In vivo concentration profiles of TBF across the SC of subject C after 4 h treatment with the drug in: (a) IPM; (b) EtOH; and (c) IPM:EtOH (50:50). The individual data points were evaluated by HPLC during sequential tape stripping of the SC. The continuous curves represent the best fits of Eq. (1) to each set of data.

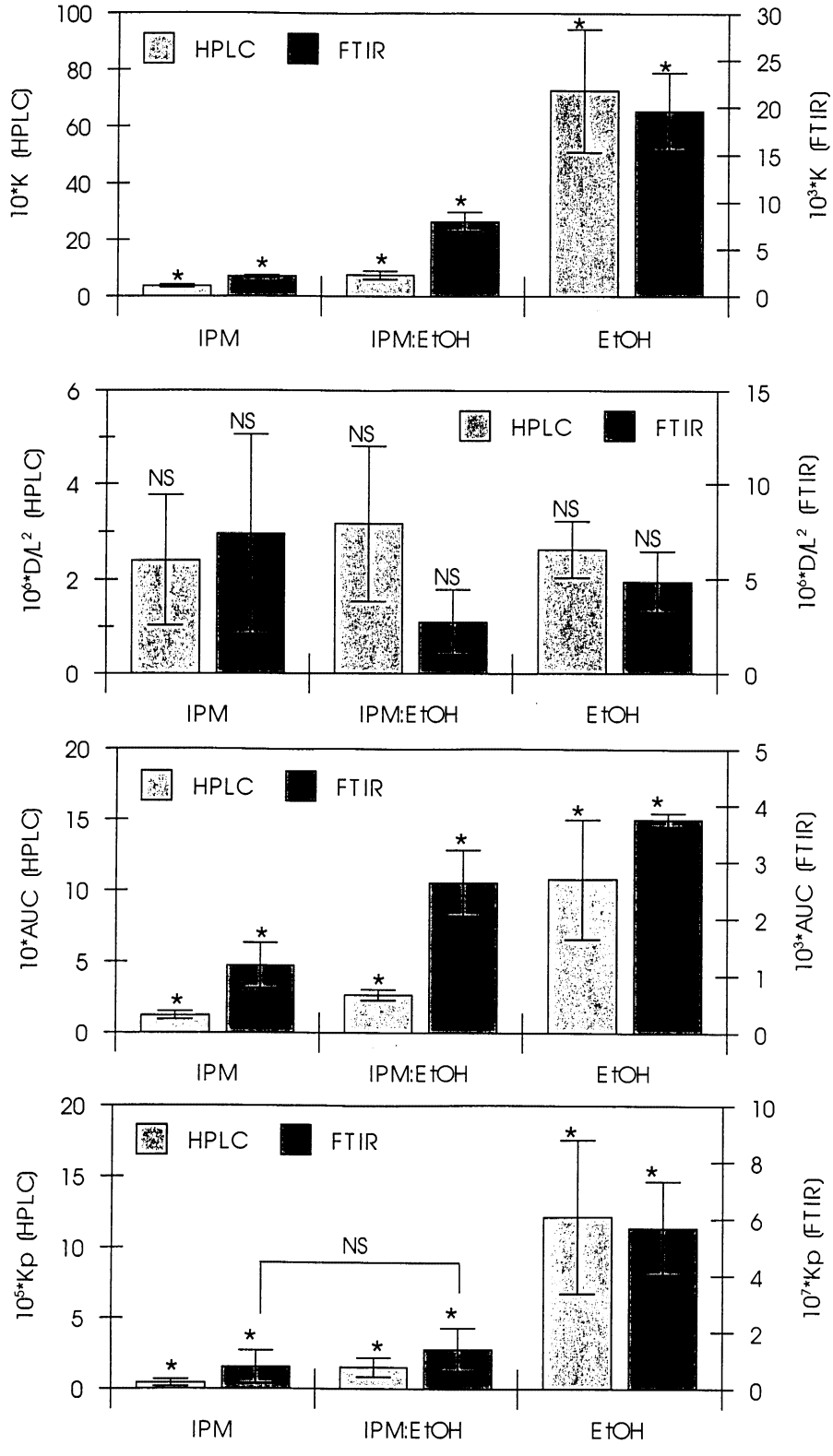

Fig. 3. (*Continued*)

Table 1
Deduced transport parameters of TBF across the SC from three vehicles[f]

Formulation	$10^3 \times K^{a,b}$	$10^6 \times [D/L^2]^{a,c}$(/s)	$10^3 \times \mathrm{AUC}^{b,d}$ (AU)	$10^7 \times$[App. K_p]^e (cm/h)
IPM	2.1 ± 0.1	7.4 ± 5.3	1.2 ± 0.4	0.8 ± 0.6
IPM:EtOH	7.9 ± 1.0	2.7 ± 1.7	2.6 ± 0.6	1.4 ± 0.8
EtOH	20 ± 4.1	4.9 ± 1.6	3.8 ± 0.1	5.7 ± 1.7

[a] Values deduced by fitting the observed TBF concentration profiles to Eq. (1).
[b] One-way ANOVA indicates that these values are significantly ($P<0.05$) different from one another.
[c] One-way ANOVA reveals no difference ($P>0.05$) between these values.
[d] AUC was determined by integrating Eq. (1) from $x=0$ to L using the fitted parameters for D/L^2 and K for each subject.
[e] An apparent permeability coefficient of TBF across the SC for each of the formulations was deduced from the determinations of D/L^2, K and L (the latter from TEWL measurements), i.e. $K_p = K \times L \times (D/L^2)$. The mean ($\pm$SD) L values were: IPM, 13 ± 2.2 μm; IPM:EtOH, 19 ± 3.7 μm; EtOH, 17 ± 2.9 μm, and were not significantly different. The K_p values are apparent because the IR results are only semi-quantitative, i.e. the K values are *relative* values only. Statistically, K_p of TBF for EtOH is quite different from either IPM or the IPM:EtOH mixture.
[f] Results based upon in vivo ATR-FTIR analysis.

Table 2
Deduced transport parameters of TBF across the SC from three vehicles[f]

Formulation	$10 \times K^{a,b}$	$10^6 \times [D/L^2]^{a,c}$ (/s)	$10 \times \mathrm{AUC}^{b,d}$ (AU)	$10^5 \times K_p{}^{b,e}$ (cm/h)
IPM	3.7 ± 0.5	2.4 ± 1.4	1.2 ± 0.3	0.4 ± 0.3
IPM:EtOH	7.4 ± 1.5	3.2 ± 1.6	2.7 ± 0.4	1.5 ± 0.7
EtOH	73 ± 22	2.6 ± 0.6	11 ± 4.2	12 ± 5.4

[a] Values deduced by fitting the observed TBF concentration profiles to Eq. (1).
[b] One-way ANOVA indicates that these values are significantly ($P<0.05$) different from one another.
[c] One-way ANOVA reveals no difference ($P>0.05$) between these values.
[d] AUC was determined by integrating Eq. (1) from $x=0$ to L using the fitted parameters for D/L^2 and K for each subject.
[e] The permeability coefficient of TBF across the SC for each of the formulations was deduced from the determinations of D/L^2, K and L (the latter from TEWL measurements), i.e. $K_p = K \times L \times (D/L^2)$. The mean ($\pm$SD) L values were: IPM, 13 ± 2.2 μm; IPM:EtOH, 19 ± 3.7 μm; EtOH, 17 ± 2.9 μm, and were not significantly different.
[f] Results based upon ex vivo HPLC analysis.

The value of this study with respect to local drug bioavailability following topical dosing to the skin is clear. The data presented are relatively easily and non-invasively obtained in vivo in human subjects. The experiments are of quite short duration, with the analytical chemistry being the most time-consuming element. Data analysis is uncomplicated and yields partitioning and diffusion parameters which can be immediately compared between vehicles, and manipulated to produce perhaps more practically useful measures for evaluation: i.e. the 'AUC' and the permeability coefficient, which together nicely account for the rate and extent of drug absorption into/through the SC.

Another feature worth pointing out is that the pattern of behaviour, and the conclusions to be

Fig. 3. Derived SC transport and uptake parameters for TBF in vivo, following drug application in IPM, EtOH, and IPM:EtOH (50:50), and deduced from concentration profiles measured by IR spectroscopy or HPLC (values presented are means $\pm$ SD; $n=3$): (A) SC/vehicle partition coefficients (K); (B) characteristic SC transport parameters (D/L^2); (C) TBF availability in the SC ('AUC' deduced from integration of Eq. (1)); (D) SC permeability coefficients (K_p). Note that the values of K, D/L^2, AUC and K_p from the HPLC measurements are quantitative; in contrast, while the IR data also yield an absolute value of D/L^2, the results for K, AUC and K_p are only 'effective' or relative values. Significant differences between the values were revealed by one way ANOVA, and are represented by an asterisk. The non-significantly different values are denoted by NS.

drawn, from either spectroscopic or chromatographic data are very similar, albeit that the latter method is not quantitative. This correlation between the methodologies employed is nicely illustrated in Fig. 4, in which the relative IR-deduced concentrations of TBF are compared with the absolute HPLC values for all subjects for each vehicle. The values of r^2 obtained (0.76, 0.90 and 0.74 for, respectively, IPM, EtOH and EtOH:IPM 50:50) attest to the coincidence between the measurements made and imply, when one has a compound with appropriate spectroscopic properties, that the IR approach may be a useful and efficient tool for assessing at least relative topical drug bioavailability.

Returning for a moment to K and D/L^2, the separation of these parameters further allows some mechanistic insight and direction for future experiment and for further formulation development. The changes, or lack thereof, in K and D/L^2 as the vehicle is altered (e.g. by the incorporation of a particular permeation enhancer) offer a quantitative means to optimize drug delivery under carefully controlled and realistic conditions. In the experiments reported here, for example, we cannot speculate on the potential drug diffusion-enhancing nature of EtOH and IPM (or a mixture thereof) across the SC. Perhaps, relative to an inert vehicle (such as water), all three formulations used increase the inherent D/L^2 of TBF through the SC (unfortunately, due to its very low aqueous solubility and the consequently low levels of TBF that can be taken up into the SC from an aqueous solution, our analytical methods are not yet sufficiently sensitive to answer this question), but we can be quite certain that D/L^2 for TBF is not dependent upon whether it is formulated in IPM, EtOH or IPM:EtOH (50:50).

On the other hand, our data point clearly to some sort of thermodynamic effect in that drug uptake into the SC was vehicle dependent. TBF had the same degree of saturation in all applied vehicles, i.e. the drug was administered at constant thermodynamic activity, which suggests, under ideal circumstances, that delivery would be the same from each solvent/cosolvent (Higuchi, 1960). The fact that EtOH and EtOH:IPM lead to greater TBF penetration into the SC suggests that another factor (or factors) may be playing a role (e.g. that the entry of EtOH into the SC permits a

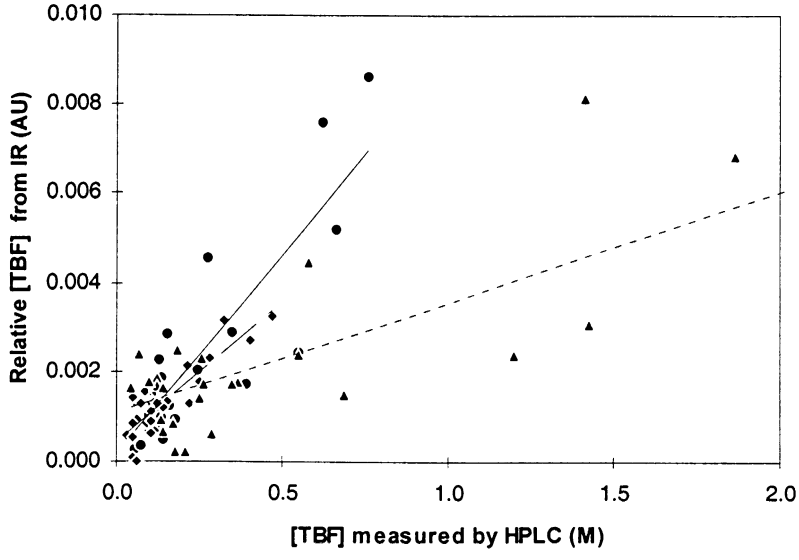

Fig. 4. Correlations between the absolute TBF concentrations measured in human SC ex vivo by HPLC and the relative values determined in vivo by IR spectroscopy following a 4 h application of the drug in either IPM (◆), EtOH (▲) or EtOH:IPM 50:50 (●). Each series (treatment) includes all the subjects and yields, respectively, a dashed, dotted or continuous linear regression with a r^2 value ranging from 0.74 to 0.90.

higher level of TBF to be taken up, or that the loss of EtOH from the vehicle, by its diffusion into the SC, creates a transiently supersaturated system from which drug delivery is more efficient). In any case, it seems plausible that the experimental approach described here may allow disparate observations in the literature, concerning the effects of IPM, EtOH and similar cosolvent systems on drug absorption across the skin (Sato et al., 1988; Sugibayashi et al., 1993), to be more clearly understood.

It also seems reasonable to suggest that the relatively short-contact strategy used in this work, and the derivation of key partition/diffusion parameters, may permit a much simplified approach to the evaluation of a drug's dermatopharmcokinetic profile as proposed recently by the U.S. Food and Drug Administration (Shah, 1998); that is, once K and D/L^2 have been determined at a certain time t, Eq. (1) and its integral may then be used to calculate/predict the drug's SC concentration profile and local availability at all other times. This hypothesis forms the basis of subsequent work from our laboratory.

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