Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man

Herkenne, Christophe; Naik, Aarti; Kalia, Yogeshvar; Hadgraft, Jonathan; Guy, Richard H.

Pharmaceutical Research, Vol. 23, No. 8, August 2006 (© 2006)
DOI: 10.1007/s11095-006-9011-8

Research Paper

Pig Ear Skin *ex Vivo* as a Model for *in Vivo* Dermatopharmacokinetic Studies in Man

Christophe Herkenne,[1,2] Aarti Naik,[1,2] Yogeshvar N. Kalia,[1,2] Jonathan Hadgraft,[3] and Richard H. Guy[1,4,5]

Received February 24, 2006; accepted March 14, 2006

Objective. The objective was to investigate pig ear skin as a surrogate for human skin in the assessment of topical drug bioavailability by sequential tape-stripping of the stratum corneum (SC). The potential benefits of *ex vivo* investigations are manifold: ethical approval is not required, multiple replicate experiments are more easily performed, and toxic compounds can be evaluated.
Materials and Methods. *Ex vivo* experiments on isolated pig ears were compared with *in vivo* studies in human volunteers. Four formulations, comprising the model drug, ibuprofen, in different propylene glycol (PG)-water mixtures (25:75, 50:50, 75:25 and 100:0), were compared.
Results. Derived dermatopharmacokinetic parameters characterizing the diffusion and partitioning of the drug in the SC *ex vivo* were consistent with those *in vivo* following a 30-minute application period. Further, the non-steady-state *ex vivo* results could be used to predict the *in vivo* concentration profile of the drug across the SC when a formulation was administered for 3 h (i.e., close to steady-state).
Conclusions. Taken together, the results obtained suggest that pig ear skin *ex vivo* has promise as a tool for topical formulation evaluation and optimization.

KEY WORDS: dermatopharmacokinetics; stratum corneum; skin; tape-stripping; topical drug bioavailability.

INTRODUCTION

There is an ongoing search to identify testing methods with which to optimize topical dosage forms and to assess topical drug bioavailability. While *in vitro* screening continues to play an important role (and is relatively inexpensive and simple to use), regulatory approval of drug delivery systems to the skin, with few exceptions, requires clinical trials to be performed. The latter, however, represent an onerous burden for the development of generic dosage forms and the U.S. Food & Drug Administration (FDA), in particular, has been examining whether surrogate measurements may be possible (17–19). On the one hand, for corticosteroids, the vasoconstriction assay (9) has been deemed an acceptable approach to compare innovator and generic products (11,12). On the other, for almost all other drugs used topically, the problem remains unresolved because an easily visualized pharmacodynamic response is not elicited (16).

As a consequence, various alternative techniques have been considered, of which microdialysis and stratum corneum (SC) tape-stripping are receiving most attention. While the former is technically more challenging, the potential reward is a drug concentration-time profile in a compartment presumed to be in close communication with the site of action of most dermatological drugs (8,20). The latter, in contrast, offers an apparently easy and quite non-invasive methodology for skin tissue sampling, and forms the basis of the FDA's so-called dermatopharmacokinetic (DPK) approach to the assessment of topical bioavailability and bioequivalence (19). However, validation and optimization of the procedure have not come quickly and the proposed guidance document has been withdrawn for re-evaluation. More recent work has addressed at least some of the important limitations of the DPK idea (4,5,13) and has proposed modifications to incorporate into an improved protocol.

The goal of the research described here is not only to contribute further to establishing the credibility of the DPK method, but also to demonstrate that useful and relevant measurements can be made on a surrogate, *ex vivo* skin model: the porcine ear. Pig skin is well-accepted as a decent facsimile of the human counterpart (14,15) and the availability of a validated model would allow extensive range-finding, developmental and optimization studies to be performed before conducting pivotal experiments in volunteers and/or patients.

[1] School of Pharmaceutical Sciences, University of Geneva, 30 quai E. Ansermet, CH-1211 Geneva 4, Switzerland.
[2] Centre Pharmapeptides, Parc d'affaires International, F-74160 Archamps, France.
[3] The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, UK.
[4] Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK.
[5] To whom correspondence should be addressed. (e-mail: r.h.guy@bath.ac.uk)

Thus, in this study, parallel DPK measurements have been made (a) on pig ears *ex vivo* and (b) in normal human subjects. Diffusion and partitioning parameters, which characterize the rate and extent of drug absorption, have been deduced and compared.

MATERIAL AND METHODS

Chemicals

S-(+)-Ibuprofen (Fluka, Buchs, Switzerland) was dissolved at saturation in various vehicles containing propylene glycol (PG) (Sigma–Aldrich, Steinheim, Germany) and deionized water. The composition of the formulations, and the corresponding saturation solubilities of the drug, were: (a) 25:75 v/v PG-water, 0.303 mg/mL; (b) 50:50 v/v PG-water, 2.55 mg/mL; (c) 75:25 v/v PG-water, 37.7 mg/mL; and (d) pure propylene glycol, 430 mg/mL. Solvents used for ibuprofen extraction and liquid chromatographic (HPLC) analysis were HPLC grade (Sigma–Aldrich). Citric acid monohydrate, sodium hydroxide (Sigma–Aldrich) and hydrochloric acid 37% (Fluka, Buchs, Switzerland) were used in buffer preparation.

The saturated solutions of ibuprofen were prepared by stirring an excess of drug in the corresponding vehicle at 20°C (±1°C) for 96 h in 30-ml vials. After centrifugation (15 min at 3,000 rpm), the supernatant was passed through a 0.45 μm nylon Acrodisc® filter (Pall, Basel, Switzerland). The saturation solubility of ibuprofen was measured by HPLC (see below) after adequate dilution. Three replicates were performed in each solvent. In addition, a two-fold supersaturated 75:25 PG-water formulation was prepared by dissolving the required quantity of ibuprofen in PG and then adding water. This system remained stable without precipitation for at least 3 h (the duration of the longest experiment performed).

Experimental Procedure

Twelve volunteers (9 female, 3 male, 24–46 years) with no history of dermatological disease participated in this study which was approved by the University of Geneva ethical committee. Informed consent was obtained from all subjects. The treated sites (4 × 5 cm) were non-hairy regions of the ventral forearm surface. Each treatment involved application of 1.9 mL of ibuprofen solution, impregnated on a cellulose gauze (Tela, Basel, Switzerland), covered by an occlusive polyester layer (Scotchpak, 3 M, St.Louis, MN) and affixed to the skin with an adhesive polyurethane film (Opsite, Smith & Nephew, Hull, UK). These applications could be considered as infinite doses from which negligible drug depletion was anticipated during the experiment. After the required time of contact (30 or 180 min), the patch was removed and excess formulation was gently removed using three dry cellulose swabs without any solvent.

Fresh pig ears were obtained from a local abattoir (SODEXA, Annecy, France); to ensure integrity of the skin barrier, ears were removed post-sacrifice before the carcass was exposed to the normal high-temperature cleaning procedure. Ears were washed with water, and any visible hairs were trimmed carefully with scissors. The formulations were applied to the ear in an identical fashion to that used for human volunteers.

SC Sampling Protocol

The ibuprofen concentration profile across the SC following application in the different vehicles was determined by sequential removal of this outer skin layer by tape-stripping (Scotch Book Tape, 3M, St. Paul, MN). The SC sampling site was delimited by a template which exposed an area smaller than that treated with the formulation. The template was centred over the drug application site immediately before tape-stripping began. The size of the opening in the template (2 × 2.5 cm) was smaller than the individual tape-strips used. Differential weighing (Mettler AT 261 balance, Greifensee, Switzerland) of tape-strips allowed the amount of SC removed to be estimated. From this mass, and knowing the area of the tape, it was possible to calculate the SC thickness removed (using a SC density of 1 g/cm^3 (3)) as a function of stripping, and hence the corresponding position (or depth, x) within the barrier. The apparent SC thickness (L) was determined as described elsewhere (6,7) from measurements of transepidermal water loss (TEWL) as a function of SC removed. This permits the drug concentration profile to be expressed as a normalized function of relative position within the SC (x/L) and facilitates the comparison of data originating from different subjects (1,2,7). The TEWL measurements were made at a site adjacent to the treated skin because residual PG volatilization provokes artefactually high TEWL readings (data not shown); furthermore, each TEWL measurement requires at least 2 min during which the drug continues to diffuse into the SC, thereby modifying the concentration profile. Ten to 16 strips were taken from each treated site on each volunteer/pig ear, the actual number depending upon the individual SC thickness; however, the SC was never completely removed. All tapes were subsequently analyzed for ibuprofen; no strips were discarded, and it was assumed that any drug not removed by the surface cleaning process at the end of the treatment would eventually be bioavailable to the skin.

Extraction and Analysis of Ibuprofen in the Tape Strips

After re-weighing, the tape-strips were rolled and placed in 1.5 ml HPLC vials. Ibuprofen was quantitatively extracted with a 90:10 mixture of acetonitrile and 1 M hydrochloric acid during 12 h. Validation of this procedure was evaluated by spiking tape-stripped samples of untreated SC with known amounts of drug in solution, chosen to bracket the expected range of concentrations to be found in the *in vivo* samples. It was not necessary to filter the tape-strip extraction solutions which were completely clear and protein-free (Biorad® protein assay, Hercules, CA). Ibuprofen was analyzed by HPLC using a Merck Lichrospher 100 RP18 (5 μm) column (Darmstadt, Germany) and a model 486 absorbance detector from Waters (Milford, USA) at 227 nm. The isocratic mobile phase was a 55:45 (v/v) mixture of acetonitrile and 0.1 M citrate buffer at pH 2.4. At a flow rate of 1.2 ml/min, and at room temperature, the retention time of ibuprofen was about 5 min.

Peak recording and data processing were performed with the built-in system manager. Ibuprofen was determined using the AUC method and calibration plots were generated with the neat compound. The quantification limit was 0.5 μg/ml.

Experimental Strategy and Data Analysis

Comparison of Formulations After a 30-Minutes Application Time

The SC concentration (C_x) *versus* depth (x) profiles of ibuprofen were fitted to the following solution of Fick's second law of diffusion:

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

The applicable boundary conditions are (a) the applied drug concentration (C_v) remains constant during the treatment period (t); (b) the viable epidermis acts as a perfect sink for the drug; and (c) the SC contains no drug at $t = 0$. The fitting generates values of K and D/L^2. The former is the SC/vehicle partition coefficient, a thermodynamic parameter reflecting the relative affinity of the drug for SC over the vehicle. The second parameter has units of $(\text{time})^{-1}$ and is a first-order kinetic constant describing drug diffusion across the SC. Integration of Eq. (1) across the SC thickness (i.e., from $x/L = 0$ to $x/L = 1$) provides an expression for the area under the SC concentration *versus* relative depth profile (AUC) which equals the total amount of drug present in the membrane divided by the volume of this compartment.

$$AUC = \int_0^1 C_x \mathrm{d}\left(x/L\right) = KC_v\left\{\frac{1}{2}-\frac{4}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2 Dt}{L^2}\right)\right\} \quad (2)$$

Prediction of the Amount of Ibuprofen in the SC at 3 h

The saturated and supersaturated (2 degrees of saturation, 2DS) drug formulations in 75:25 v/v PG-water were used in this experiment. The ibuprofen concentration-SC depth profiles at 30 min were evaluated independently using Eq. (1), generating best-fit values of K and D/L^2. From the D/L^2 values obtained, it was estimated that ~3 h (=[2.7·L^2]/6D) would be necessary to achieve steady-state transport. Then, using Eq. (2) with the K and D/L^2 values obtained from the 30-minute experiment, the AUC at 3 h was predicted. DPK experiments were subsequently performed with an application time of 3 h and the AUC was determined and compared to the prediction.

RESULTS AND DISCUSSION

It is first noted that the SC levels of ibuprofen following a 30-minute application of the 25:75 v/v PG-water formulation were very low and fell below the limit of HPLC detection after the first few tape-strips. Data (not shown) from these experiments were not considered further, therefore. Figure 1 summarizes the SC concentration-depth profiles both *in vivo* in human subjects, and *ex vivo* in pig ear skin, following treatment (again for 30 min) with the three other PG-water vehicles tested (50:50, 75:25 and 100:0, respectively). In both cases, variability was quite low, suggesting that the methodology is robust. It is also apparent that the drug was delivered into the SC more effectively as the percentage of PG in the formulation increased. Importantly, there was good visual consistency between the *in vivo* and *ex vivo* profiles, indicating that the pig ear may indeed be a useful model in this context.

A more objective evaluation of this conclusion was facilitated by fitting the individual profiles to Eq. (1) and comparison of the resulting partitioning and diffusivity parameters (K and D/L^2, respectively). Table I presents the average (±standard deviation (SD)) results for K and D/L^2 derived from fitting Eq. (1) to the individual profiles in Fig. 1. Also included in this Table are the values of AUC (again given as mean ± SD) calculated using Eq. (2) and the respective K and D/L^2 for each subject/pig ear. Immediately striking is that the diffusion kinetic is both independent of the formulation and statistically identical *in vivo* and *ex vivo*. The values of K and AUC, on the other hand, are significantly influenced by the nature of the formulation, a point discussed further below. Nevertheless, for each formulation, the *in vivo* and *ex vivo* results for K and AUC compare well. While there are some statistically significant differences noted (see Table I), the absolute values (human *versus* pig ear) never deviate by as much as a factor of 2; that is, it would be difficult to argue that this level of "disagreement" would be important in practice. The possibility that the significant differences observed were simply the result of differences in the SC thickness of human and porcine SC was examined by pooling more than 80 separate measurements of L in the two skins (Fig. 2). The thicknesses were log-normally distributed about median values of 11.0 and 8.2 μm for human and pig ear SC, respectively. *In vivo*, human SC thicknesses ranged from a minimum value of 7.8 μm to a maximum of 18.6 μm; for pig ear *ex vivo*, the range was 4.1 to 16.1 μm. While there is obvious (and not unexpected) variability in the values found, and despite a statistically significant difference between the human and porcine barriers, it is not possible to correlate systematically the small divergences in K and AUC in Table I to the differences in L. Lastly, for the 75:25 v/v PG-water formulation, the two-fold supersaturated vehicle performed as expected, resulting in identical diffusion and partitioning parameters as the simply saturated one, but producing a significantly, and approximately two-times higher, AUC.

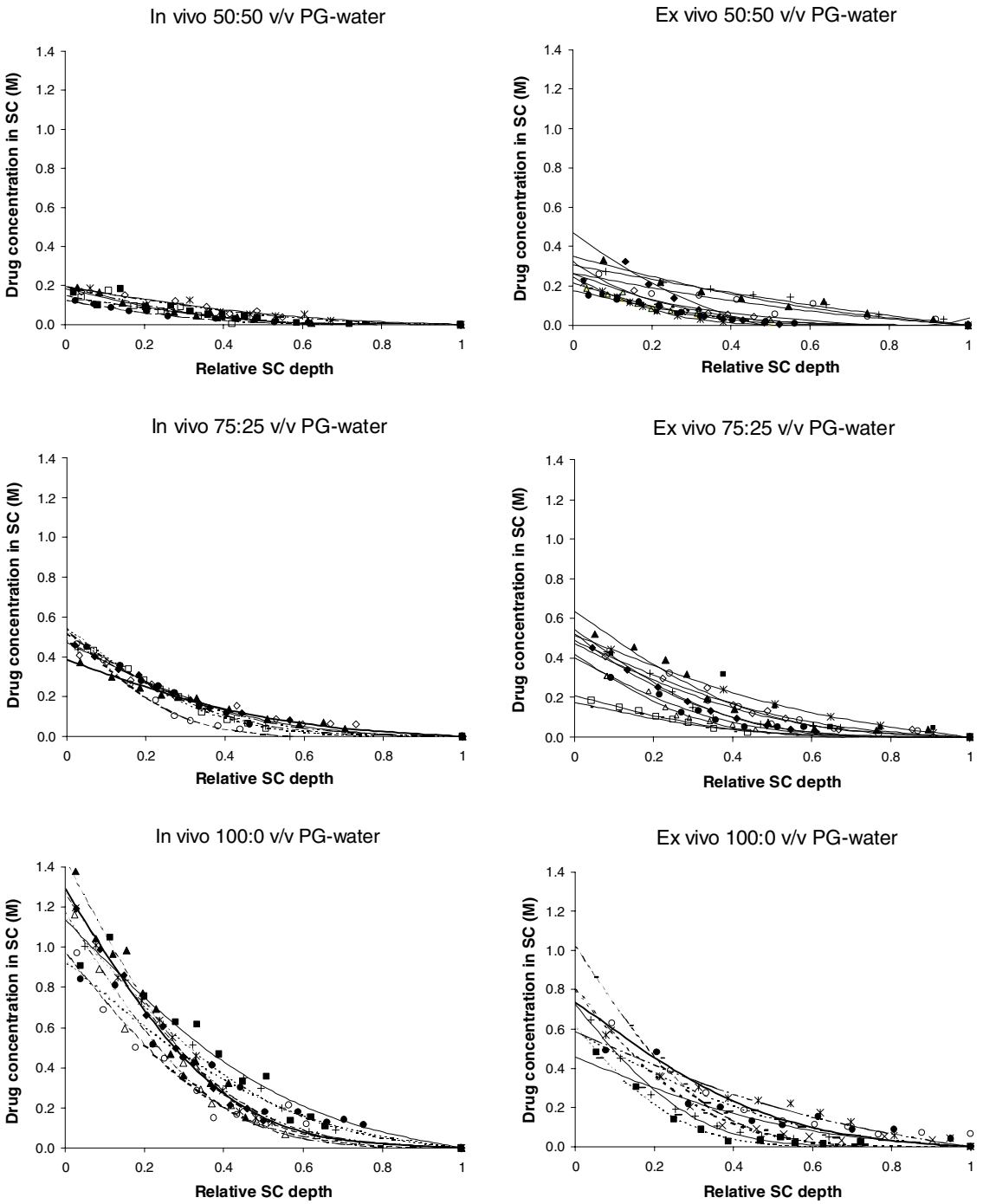

Fig. 1. *In vivo* human (*left panels*) and *ex vivo* pig ear (*right panels*) SC concentration *versus* relative depth profiles of ibuprofen following 30 min treatment with different PG-water formulations. The lines drawn through the data represent the best fits of Eq. (1) to each set of results.

From the values of K and D/L^2, together with the corresponding SC thickness (L) determined for each subject/pig ear, it is possible to deduce the permeability coefficient (K_p) of ibuprofen across the SC from each of the formulations tested using Eq. (3):

$$K_p = K\frac{D}{L^2}L = \frac{KD}{L} \tag{3}$$

Likewise, knowing K_p and the concentration of drug in the vehicle (C_v), the steady-state flux (J_{ss}) across the SC can be estimated:

$$J_{ss} = K_p C_v = \frac{KD}{L} C_v \tag{4}$$

The results are collected in Table II. Once again, there were no significant differences between either K_p or J_{ss} when

Table I. Diffusivity and Partition Parameters, and Calculated AUCs (Eqs. (1) and (2)) Describing Ibuprofen Uptake into SC Following a 30-Minute Application of Various PG-Water Vehicles either *in Vivo* in Human Volunteers or *ex Vivo* on Pig Ear Skin (Mean ± SD, n = 7–9)

Formulation	Test system	D/L^2 (h^{-1}) [a,b]	K[a]	AUC (M)[c]
50:50 PG-H_2O	*In vivo*	0.16 ± 0.07	14 ± 2[d]	0.05 ± 0.01[d]
	Ex vivo	0.16 ± 0.14	21 ± 6[d]	0.07 ± 0.04[d]
75:25 PG-H_2O	*In vivo*	0.12 ± 0.05	2.8 ± 0.3[d]	0.13 ± 0.02[d]
	Ex vivo	0.15 ± 0.07	3.0 ± 0.4[d]	0.16 ± 0.05[d]
75:25 PG-H_2O (2× supersat'd)	*In vivo*	0.19 ± 0.09	2.4 ± 0.4[d]	0.26 ± 0.05[d]
	Ex vivo	0.14 ± 0.03	2.7 ± 0.5[d]	0.28 ± 0.08[d]
100:0 PG-H_2O	*In vivo*	0.13 ± 0.06	0.56 ± 0.07[e]	0.33 ± 0.07[e]
	Ex vivo	0.14 ± 0.09	0.33 ± 0.08[e]	0.18 ± 0.05[e]

[a] Values from the best-fits of Eq. (1) to the results in Fig. 1.
[b] ANOVA reveals no significant differences between any of the D/L^2 values.
[c] Determined from Eq. (2) using the corresponding fitted D/L^2 and K for each subject/pig ear.
[d] For each formulation, each pair of values are not significantly different ($p > 0.05$, unpaired t-test).
[e] Values statistically different ($p < 0.05$, unpaired t-test).

comparing the *in vivo* human data with the corresponding *ex vivo* values; even when statistical significance was achieved, the 'real-life' impact of the difference would probably be negligible.

Differences in both K_p and J_{ss} were apparent, however, whether one considers either the *in vivo* or *ex vivo* results, between the various PG-water compositions examined. This observation was anticipated, of course, because of the differences in the SC-water partition coefficient and AUC reported in Table I. Theory predicts that the steady-state flux of a drug across a membrane from a saturated solution should be independent of the composition of the solution as long as the latter does not itself change in some way the properties of the barrier. In terms of Fick's first law, as expressed by Eq. (4), this means that the increases in C_v resulting from incorporation of progressively more PG into the formulation should be exactly balanced by a corresponding decrease in K, which can be defined as the ratio of drug solubilities in the SC and in the vehicle:

$$K = C_{SC}^{sat} / C_v^{sat} \quad (5)$$

Table I shows that K does indeed decrease as the percentage of PG in the vehicle increases, reflecting the fact that ibuprofen prefers the cosolvent to water. However, this decrease is less steep than the increase in drug solubility in the vehicle, and J_{ss} (and AUC) are therefore greater for the formulations with progressively higher levels of PG. This deduction can be appreciated by substitution of Eq. (5) into Eq. (4), recalling that all the vehicles studied in this work were saturated with drug. It follows that PG must be altering the solubility of ibuprofen in the SC; specifically, it appears that uptake of PG into the SC—which increases with increasing amounts of the cosolvent in the formulation—enhances the drug's solubility in the barrier (i.e., C_{SC}^{sat}) and results in improved penetration and transport. The literature provides support for this explanation, in that it has been shown that PG can replace water at its binding sites in the SC intercellular spaces (10) and that it is capable of effectively "dragging" drug into and through the SC, thereby increasing permeation (21).

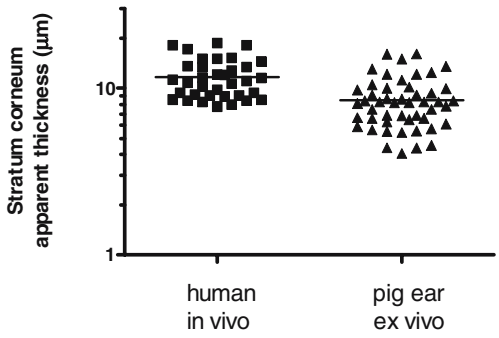

Fig. 2. Comparison between the apparent thickness of human SC *in vivo* (mean ± SD = 11.7 ± 3.2; n = 35) and that of excised porcine ear SC *ex vivo* (mean ± SD = 8.5 ± 3.0; n = 49). The individually displayed values are log-normally distributed and show a statistically significant difference (Mann–Whitney test, $p < 0.0001$).

Table II. Estimated Permeability Coefficients (K_p) and Steady-State Fluxes (J_{ss}) of Ibuprofen Across SC Following Drug Application in Various PG-Water Vehicles either *in Vivo* in Human Volunteers or *ex Vivo* on Pig Ear Skin (Mean ± SD, n = 7–9)

Formulation	Test system	10^4 K_p (cm h^{-1})[a]	J_{ss} (µg cm^2 h^{-1})[b]
50:50 PG-H_2O	*In vivo*	26 ± 10[c]	7 ± 3[c]
	Ex vivo	21 ± 14[c]	5 ± 4[c]
75:25 PG-H_2O	*In vivo*	4 ± 1[c]	15 ± 5[c]
	Ex vivo	4 ± 2[c]	13 ± 6[c]
75:25 PG-H_2O (2× supersat'd)	*In vivo*	5 ± 2[c]	33 ± 12[c]
	Ex vivo	3 ± 0.4[c]	21 ± 3[c]
100:0 PG-H_2O	*In vivo*	1.0 ± 0.5[d]	45 ± 21[d]
	Ex vivo	0.3 ± 0.1[d]	13 ± 6[d]

[a] Determined from Eq. (3) using the measured SC thickness (L) and fitted D/L^2 and K for each subject/pig ear.
[b] Determined from Eq. (4) using the appropriate C_v (see Materials and Methods) and the K_p values in this Table.
[c] For each formulation, each pair of values are not significantly different ($p > 0.05$, unpaired t-test).
[d] Values statistically different ($p<0.05$, unpaired t-test).

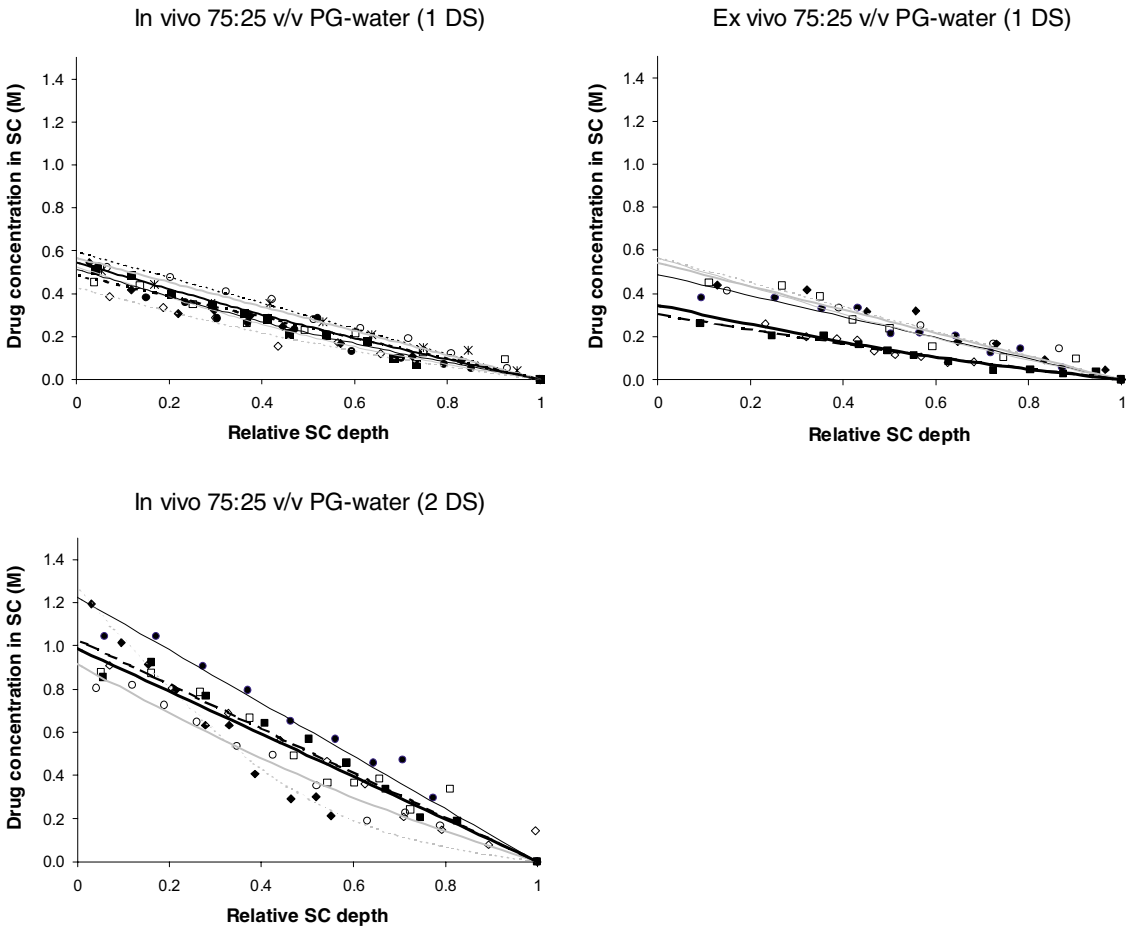

Fig. 3. SC concentration *versus* relative depth profiles of ibuprofen following 3 h treatment with a 75:25 v/v PG-water formulation in which the drug was present at either 1 or 2 degrees of saturation. The *left panels* show the results from human *in vivo* experiments (n = 6–7); the *right panel* presents the data from *ex vivo* measurements on isolated pig ears (n = 6).

In a final component of this study, the partitioning and diffusivity parameters derived from the experiment in which ibuprofen was applied in a 75:25 v/v PG-water vehicle (at both 1 and 2 degrees of saturation) were used, with Eq. (2), to predict the AUC when the duration of exposure was increased to 3 h (at which time, it was anticipated that drug transport would have reached close to steady-state). These predictions were then compared to the results of subsequently performed experiments (Fig. 3). Table III summarizes the outcome of this investigation and indicates that the predictions, both *in vivo* and *ex vivo*, and from a supersaturated formulation, were remarkably close to (and not significantly different from) the experimental results. It can be concluded once again, therefore, that pig ear skin *ex vivo* is a good model for the human barrier *in vivo*, and that relatively short-contact studies may be reliably extrapolated to predict an important fraction of the absorption phase of a DPK profile.

Table III. Comparison Between Predicted and Experimental Values of Ibuprofen AUC Following a 3-Hour Application of Saturated and Two-Fold Supersaturated 75:25 v/v PG-Water Formulations *in Vivo* in Human Volunteers and *ex Vivo* on Pig Ear Skin (Mean ± SD, n = 7–9)

Formulation	Test system	Predicted AUC at 3 h $(M)^a$	Experimental AUC at 3 h $(M)^b$	Deduced K 3 h $(M)^c$
75:25 PG-H_2O (saturated)	*In vivo*	0.24 ± 0.02[d]	0.25 ± 0.04[d]	3.0 ± 0.3[e]
	Ex vivo	0.26 ± 0.04[d]	0.23 ± 0.07[d]	2.9 ± 0.9[e]
75:25 PG-H_2O (2× supersat'd)	*In vivo*	0.41 ± 0.06[d]	0.49 ± 0.07[d]	3.1 ± 0.4[e]

[a] Calculated using Eq. (2) and the corresponding mean (±SD) K and D/L^2 values determined following a 30-minute application of the same formulations (see Table I).
[b] Determined by graphical integration of the SC concentration *versus* relative SC depth data.
[c] Determined from the y-axis intercepts of the profiles in Fig. 3 and the corresponding values of C_v for each formulation.
[d] No significant difference between predicted and experimental values ($p > 0.05$, unpaired t-test).
[e] Values are not statistically different from the corresponding results determined after a 30-minute application of the same formulations (see Table I) ($p > 0.05$, unpaired t-test).

CONCLUSIONS

This research has revealed three significant findings: [1] The *ex vivo* pig ear model appears to be a quantitative and reproducible model for DPK studies of topical bioavailability, producing data that are comparable to those observed *in vivo* in human subjects. An important caveat here is that the *ex vivo* system lacks, of course, a functioning cutaneous circulation; as a consequence, while the approach will be representative of short-contact exposures, it may be less adequate for longer application times, particularly for drugs of high lipophilicity (where the isolated pig ear may not provide appropriate 'sink' conditions). [2] Formulations containing high PG content apparently result in significant uptake of the cosolvent into the SC, and this can lead to (a) an increased saturation solubility of a drug (such as ibuprofen) in the membrane, and (b) as a result, enhanced delivery into and through the skin. [3] Short-contact DPK experiments can be used to derive diffusion and partitioning parameters that are subsequently able to predict drug penetration into the SC following longer periods of application.

ACKNOWLEDGMENTS

We thank Leo Pharmaceutical Products (Denmark) for financial support. We are indebted to the following individuals for stimulating discussion, suggestions, comments and criticism of the work performed: Drs. A. Jorgensen, E. Didriksen, A. Fullerton, V. Shah and, in particular, Professor Annette Bunge from the Colorado School of Mines (Golden, CO, USA).

REFERENCES

1. I. Alberti, Y. N. Kalia, A. Naik, J. D. Bonny, and R. H. Guy. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum *in vivo*. *Int. J. Pharm.* **219**:11–19 (2001).
2. I. Alberti, Y. N. Kalia, A. Naik, and R. H. Guy. Assessment and prediction of the cutaneous bioavailability of topical terbinafine *in vivo* in man. *Pharm. Res.* **18**:1472–1475 (2001).
3. R. L. Anderson and J. M. Cassidy. Variations in physical dimensions and chemical composition of human stratum corneum. *J. Invest. Dermatol.* **61**:30–32 (1973).
4. D. P. Conner: Differences in DPK Methods. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_05_conner/index.htm, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, 2001.
5. T. J. Franz. Study #1, Avita Gel 0.025% *vs* Retin-A Gel 0.025%, Advisory committee for pharmaceutical sciences meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, 2001.
6. 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).
7. Y. N. Kalia, I. Alberti, A. Naik, and R. H. Guy. Assessment of topical bioavailability *in vivo*: the importance of stratum corneum thickness. *Skin Pharmacol.* **14**:82–86 (2001).
8. A. L. Krogstad, P. A. Jansson, P. Gisslen, and P. Lonnroth. Microdialysis methodology for the measurement of dermal interstitial fluid in humans. *Br. J. Dermatol.* **134**:1005–1012 (1996).
9. A. W. McKenzie and R. B. Stoughton. Method of comparing percutaneous absorption of steroids. *Arch. Dermatol.* **86**:608–610 (1962).
10. R. Panchagnula, P. S. Salve, N. S. Thomas, A. K. Jain, and P. Ramarao. Transdermal delivery of naloxone: effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin. *Int. J. Pharm.* **219**:95–105 (2001).
11. L. K. Pershing, B. S. Silver, G. G. Krueger, V. P. Shah, and J. P. Skelley. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. *Pharm. Res.* **9**:45–51 (1992).
12. L. K. Pershing, L. D. Lambert, V. P. Shah, and S. Y. Lam. Variability and correlation of chromameter and tape-stripping methods with the visual skin blanching assay in the quantitative assessment of topical 0.05% betamethasone dipropionate bioavailability in humans. *Int. J. Pharm.* **86**:201–210 (1992).
13. L. K. Pershing. Bioequivalence assessment of three 0.025% tretinoin gel products: dermatopharmacokinetic *vs* clinical trial methods, advisory committee for pharmaceutical sciences meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, 2001.
14. N. Sekkat, Y. N. Kalia, and R. H. Guy. Biophysical study of porcine ear skin *in vitro* and its comparison to human skin *in vivo*. *J. Pharm. Sci.* **91**:2376–2381 (2002).
15. N. Sekkat, R. H. Guy. Biological models to study skin permeation. In B. Testa, H. Van de, Waterbeemd G. Folkers, R. H. Guy (eds.), *Pharmacokinetic Optimisation in Drug Research*, Wiley-VCH and VHCA, Zürich, 2001, pp. 155–172.
16. V. P. Shah, G. L. Flynn, R. H. Guy, H. I. Maibach, H. Schaefer, J. P. Skelly, R. C. Wester, and A. Yacobi. *In vivo* percutaneous penetration/absorption. *Pharm. Res.* **8**:1071–1075 (1991).
17. S. Shah, D. Hare, S. V. Dighe, R. L. Williams. Bioequivalence of topical dermatological products. In V. P. Shah and H. I. Maibach (eds.), *Topical Drug Bioavailability, Bioequivalence and Penetration*, Plenum, New York, 1993, pp. 393–413.
18. V. P. Shah. Challenges in evaluating bioequivalence of dermatological drug products. In C. Surber, P. Elsner, and A. J. Birhcer (eds.), *Exogenous Dermatology. Curr Prob Dermatol*, Karger, Basel, 1995, pp. 152–157.
19. 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.
20. I. Tegeder, U. Muth-Selbach, J. Lötsch, G. Rüsing, R. Oelkers, K. Brune, S. Meller, G. R. Kelm, F. Sörgel, and G. Geisslinger. Application of microdialysis for the determination of muscle and subcutaneous tissue concentrations after oral and topical ibuprofen administration. *Clin. Pharmacol. Ther.* **65**:357–368 (1999).
21. L. Trottet, C. Merly, M. Mirza, J. Hadgraft, and A. F. Davis. Effect of finite doses of propylene glycol on enhancement of *in vitro* percutaneous permeation of loperamide hydrochloride. *Int. J. Pharm.* **274**:213–219 (2004).