ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 265–271

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Codiffusion of propylene glycol and dimethyl isosorbide in hairless mouse skin

Emilio Squillante*, Thomas Needham, Anita Maniar, Serpil Kislalioglu, Hossein Zia

Department of Applied Pharmaceutics, University of Rhode Island, Kingston, RI 02881, USA

Received 19 December 1997; accepted 23 March 1998

Abstract

The in vitro percutaneous fluxes of propylene glycol (PG), *cis*-oleic acid (OA) and dimethyl isosorbide (DI) were determined and their effect on nifedipine (N) flux and lag time evaluated. PG, OA and DI flux through hairless mouse (HM) skin was measured in vitro by beta-scintigraphy and N permeation was measured by HPLC under finite and infinite dose conditions. Evaluation of each of the solvents separately showed that pure DI possessed the inherent ability to traverse the skin (12% in 24 h). For the tested formulation after 24 h, 57% of the PG and 40% of the DI had permeated across the skin with nearly linear permeation between 4 and 18 h and the relative order of permeation was PG > DI > N. DI permeation was further aided in the presence of PG and OA. N flux was dependent on concomitant solvent permeation. Over a 24-h test period a dose dependent response was observed for N, with 4.9–15.6 mg of N delivered from the lowest and highest doses, respectively, and the highest dose yielding zero-order flux of 146 (g/h per cm^2).

Keywords: Nifedipine; Dimethyl iscsorbide; Propylene glycol; Oleic acid; Hairless mouse skin; Percutaneous

1. Introduction

The dihydropyridine calcium channel blockers are used extensively for the treatment of cardiovascular disease and their physicochemical properties have caused them to receive attention as potential candidates for transdermal delivery. Nifedipine (N) is firmly entrenched as a baseline treatment for angina and hypertension. N has a short biological half life and high first pass metabolism requiring high oral daily doses that must be given frequently. These problems could be avoided if a transdermal patch with a surface area of 20 cm^2 could be designed to deliver N through the skin at a target rate of 0.033 mg/cm^2 per h.

The skin is a heterogeneous laminate with differential influence on overall skin permeability, the passage through which can be envisioned as a series of partitions starting in the lipophilic environment of stratum corneum lipids, the viable epidermis and on through the increasingly hydrophilic regions of the dermal, subcutaneous and adjacent systemic circulation. N possesses a peculiar solubility profile in that it appears to be a neutral compound with neither strong lipophilic nor hydrophilic character [1] and this profile presents formidable challenges in the development of a transdermal dosage form. Strata beneath the stratum corneum will present a significant diffusional resistance to drugs with poor aqueous solubility, resulting in accumulation in the well hydrated regions of the skin. This stasis may be offset by codiffusion of drug with an appropriate vehicle.

Enhancers such as propylene glycol (PG), *cis*-oleic acid (OA), and dimethyl isosorbide (DI) act by various mechanisms to perturb diffusional pathways through the skin. Efforts to clarify the effect of PG upon drug penetration

* Corresponding author. Tel.: +1 401 8742152; fax: +1 8742181; e-mail: aii101@uriacc.uri.edu

0939-6411/98/$19.00
PII S0939-6411(98)00030-7

through skin show that results depend strongly on experimental conditions [2–4]. Proposed mechanisms of PG enhancer action include alteration of barrier function by its effects on a-keratin structure or a PG induced increase in the solution capacity within the SC [5]. This latter solvent effect is of particular interest, since PG will decrease the significant barrier to permeation that exists for drugs with poor aqueous solubility, such as estradiol and arildone [6]. DI is a unique liquid with amazing solubility properties, inertness, and a low order of toxicity whose physical characteristics and solvent properties are superior in many cases to propylene glycol or ethyl alcohol [7]. The effects of DI on the skin and the extent to which DI permeates the skin are not a matter of public record and it was felt that much could be accomplished if the skin kinetics of this solvent could be characterized.

Previous work showed that N flux across hairless mouse skin was enhanced from formulations containing N suspended in mixtures of propylene glycol, *cis*-oleic acid, and dimethyl isosorbide i.e. 'PG:OA:DI' [8]. Due to the extremely low aqueous solubility of N these solvents were selected for their potential to readily pass through the skin, to solubilize and thereby facilitate percutaneous drug absorption. Mixtures of DI and OA accelerated N perfusion through mouse skin when used alone, but acted synergistically in PG-based cosolvent mixtures. Also, the results of that study indicated that differences in flux and lag time were mainly affected by the compositional mole fraction ratio of cosolvent vehicles, in particular DI, in the donor formulations. An optimization of the results of that work identified a formulation containing PG:OA:DI at the 84%, 10% and 6% (mol%) ratio yielded low lag time and nearly zero-order delivery expected from a saturated solution of N over 24 h. The amount of N delivered in vitro was considerably higher than that calculated to be necessary to maintain requisite therapeutic levels for similar flux rates in vivo. This formulation is used in the present study which seeks to gain a better understanding of the basis of N permeability enhancement by characterizing in vitro the percutaneous absorption of all non-drug formulation components through hairless mouse (HM) skin.

2. Materials and methods

Propylene glycol labeled with carbon 14 ([^{14}C]PG) was supplied as 250 pCi/ml aqueous solution from ICN Biomedicals, Costa Mesa, CA. Labeled *cis*-oleic acid ([^{14}C]OA) from NEN Research Products, Boston, MA was supplied as a 500 pCi/ml ethanolic solution. Dimethyl isosorbide (1,4:3,6-dianhydro-2,5-di-o-methyl−D-glucitol, CAS 5306-85-4) was a gift from ICI. [^{3}H]Dimethyl isosorbide ([^{3}H]DI), 1 mCi/ml in ethanol, was custom labeled by NEN Research Products. *cis*-Oleic acid (Sigma), Scinitsafe Plus 50% and all other materials were obtained from Fisher Scientific.

2.1. Kinetic studies

We adopted the convention of expressing the levels of the factors (i.e. the relative amounts of formulation components) in terms of mole fraction. The in vitro permeation of N was investigated as follows: to a cosolvent mixture of 10 mol% oleic acid, 6 mol% dimethyl isosorbide and 84 mol% propylene glycol was added excess suspended N. The suspension was equilibrated, filtered, and dual labeled with appropriate amounts of either [^{14}C]PG or [^{14}C]OA and [^{3}H]DI. Equimolar amounts were drawn up into tared syringes (total of 10 mmol of cosolvent). Female hairless mice (26–28 g, 8–12 weeks of age) were the progeny of four females and one male SKH-1 obtained from Charles River Laboratories. A separate fresh skin sample was used for each individual cell. Following cervical dislocation, whole thickness, intact skin from the ventral region was removed using blunt dissection and immediately placed on the diffusion cells in contact with the receptor phase. The donor formulation was applied to skin mounted in vertical Franz cells (17 ml half cell volume and 4.9 cm^2 cross-sectional area) equipped with screw threads and sealed with a phenolic cap and septum (Vanguard International, Neptune, NJ).

Sloan et al. [9] discussed how the application of regular solution theory to predict the partitioning process requires the adoption of three essential features in the experimental design: (1) apply only saturated solutions to the skin so that the thermodynamic activity of drug would remain constant throughout the study period; (2) after each initial application period, a 24-h washout period is used to determine the degree of accumulation in the skin and (3) after the washout period, a second application of saturated drug solution is applied to determine the degree of damage to the barrier properties of the skin. This is important, because if drug flux measured prior to and directly after the washout period remains invariant, then the diffusion coefficient is invariant, in which case the absorption potential of a drug is related to its partition coefficient. The effect of polyethylene glycol 400 (PEG400) on N flux was found to be insignificant. Reapplication of suspensions of N in pure PEG400 after the 24-h washout period showed N flux did not change and, on this basis, PEG400 was considered to be a 'non-interacting' vehicle. Incorporation of 40% PEG400 increased the equilibrium saturation solubility of N from 0.006 mg/ml to 0.462 mg/ml. Therefore, sink conditions were promoted by employing an aqueous solution containing polyethylene glycol (40% PEG 400 and 60% of 0.9% sodium chloride) as the receptor fluid (RF) medium.

At predetermined times the entire contents of receptor compartment were withdrawn, filtered through 0.45 μm filters, 1 ml of filtered receptor fluid placed in an amber vial, and analyzed by an HPLC method. The responses of interest, i.e. lag time ($t_{1,N}$) and steady state flux (J_N) represent the average values taken over four replicates of each formulation. Stratum corneum was removed by stripping the

abdominal skin 20 times with Scotch tape (3M, St. Paul, MN) prior to excision.

Mass balance, skin retention and skin kinetics of the other formulation components were performed in an identical manner except that the donor was dual labeled with [^{3}H]DI and either [^{14}C]OA or [^{14}C]PG prior to application to the skin. At the conclusion of the experiment, the contents of the donor compartment were rinsed three times with cold methanol, diluted to volume in volumetric flasks and the activity of a 0.5 ml aliquot was added to a scintillation cocktail and counted to determine residual disintegrations per minute (DPMs) remaining in the donor. Following the methanol rinsing, the donor compartment was washed three times with water. The skin was removed from the cell, the circular area of diffusion was cut out, patted between tissue, weighed, transferred to tared vials, its weight recorded, 15 ml 0.3 N NaOH added, and incubated at 70°C for 24 h. Upon cooling, 1.0 ml skin digest was neutralized with 1 ml 0.3 N HCl and an aliquot counted to determine residual DPMs in the skin. Skin permeation kinetics and mass balance of PG, OA and DI were determined by liquid scintillation counting.

2.2. Scintillation counting

Scintillation cocktail (Scintisafe Plus 50%) 15 ml was added to samples (approx. 0.3 g), vortexed, allowed to stand overnight and counted the next day in an LS 5000TD scintillation counter (Beckman Instruments, Fullerton, CA). Automatic quench correction was performed using quench standards prepared from calibrated sources (Dupont Isotope Division) using nitromethane as the quenching agent [10]. Chemical quenching was studied by adding increasing concentrations of N to a stock solution of fixed activity. The sampling scheme was designed such that the amount of N never exceeded either: (a) 10% of the saturation solubility of N in receptor fluid or (b) levels at which chemical quenching affected the liquid scintillation assay. DPMs were converted to microcuries (mCi) using the relation, 1 mCi = 2.22×10^6.

2.3. Statistical analysis

Results are reported as the mean ± SD. All data analyses and statistical calculations were performed using Sigma Plot 3.0 (SPSS, Chicago, IL). A minimum *P*-value of 0.05 was used as the significance level for *t*-tests.

3. Results

3.1. Assay validation of N

All separations were performed on a Baseline 810 Chromatography Workstation (Dynamic Solutions Division of Millipore, CA) and Waters Model 484 Tunable Absorbance Detector set at 238 nm. A Hibar RT 250-4 LiChrosorb RP-18 (10 (m) column protected by a LiChrocart RP-18 (5 mm) precolumn (EM Separations, NJ) was kept at 37°C in a Model CH-30 HPLC Column Heater (FIAtron Laboratory Systems, WI). An isocratic mobile phase was prepared as follows: filtration of HPLC grade acetonitrile, methanol and water individually through 0.45 μm Teflon filters; measured in a ratio of 1:1:2 by volume, respectively; sonicated and used at a flow rate of 1.3 ml/min.

Following five runs of N standard curves on different days, a composite standard curve was obtained from the Baseline 810 software. The assay was linear over the range of this composite line ($R^2 = 0.0999$). Within- and between- run results on a stock reference solution were determined and the values for accuracy were within ±5%.

3.2. Solubility experiments

The solvent saturation solubilities of N (Cs) in the solvents were: DI (85 mg/ml), OA (0.33 mg/ml), PG (14 mg/ml) and water (0.006 mg/ml). N solubility in the PG:OA:DI optimized mixture was 13.3 mg/ml. The partition coefficient determination yielded a log K_{oct} of 3.31.

3.3. Characterization of non-drug components

Previous studies resulted in the development of an optimized formulation. Fig. 1 relates the penetration profile of PG, DI and N on a molar scale and shows the penetration profiles of PG and DI through excised hairless mouse skin after application of 10 mM dose of the ternary cosolvent containing 10 mol% OA and 6 mol% DI in 84 mol% PG. The optimized formulation provided a low lag time, nearly zero-order flux over 24 h and significantly higher than desired absorption rates of N. This formulation was used to characterize the movement of drug and excipients through mouse skin by radiolabeling with [^{3}H]DI and [^{14}C]PG and applying to the donor side. Aliquots of receptor fluid were sampled as a function of time, the apparatus disassembled, and the total amount of components in the var-

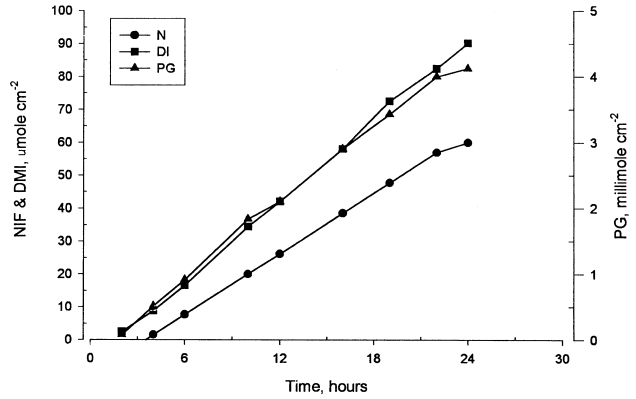

Fig. 1. Cumulative amount of permeated of nifedipine (●), dimethyl isosorbide (■), and propylene glycol (▲, *y*-axis-2) from optimized donor through hairless mouse skin through 25 mm cells, $n = 3$.

Table 1

Permeation of ^{14}C-labeled propylene glycol across hairless mouse skin

Time (h)	Average cumulative activity (mCi ± SD)	Cumulative percent dose permeated (%)
2	0.07 ± 0.02	1.1
4	0.46 ± 0.10	7.1
6	0.82 ± 0.14	12.6
10	1.65 ± 0.29	25.5
14	2.43 ± 0.35	37.5
18	3.03 ± 0.35	46.7
24	3.70 ± 0.32	57.1

Table 2

Permeation of [^{3}H]dimethyl isosorbide across hairless mouse skin

Time (h)	Average cumulative activity (mCi ± SD)	Cumulative percent dose permeated (%)
2	0.38 ± 0.27	1.1
4	1.29 ± 0.84	7.1
6	2.42 ± 1.38	12.6
10	5.03 ± 1.93	15.3
14	8.08 ± 1.79	37.5
19	10.59 ± 1.48	46.7
24	13.15 ± 1.26	57.1

ious compartments determined at the 24 h mark. The movement of N, PG and DI from the donor compartment into the receptor fluid through mouse skin is shown in Fig. 1. The lag times for the two solvents were not noticeably different in mouse skin.

3.3.1. Propylene glycol

The average cumulative activity of 14C-labeled PG in receptor fluid and the percentage of PG permeating over 24 h from the donor are shown in Table 1. PG readily traversed the skin in the first 24 h with 57% of the initially applied PG diffusing out of the donor into the receptor medium. As shown in Fig. 2, permeation of PG was linear between 4 and 18 h. The average amount of PG retained in the skin was 0.38% per 0.335 g of wet tissue. Pure PG is reported to readily permeate the skin. PG generally acts as solvent and has the added advantage that it readily permeates the skin and in doing so may carry the drug molecule across.

In a separate experiment, an equimolar amount of radiolabeled N in PG suspensions was applied. Mass balance studies following application of N suspended in ^{14}C-labeled PG yielded an average recovery of 96.9% of total applied PG but in this instance only 4% (as opposed to 57%) of the PG dose traversed mouse skin in a 24-h period.

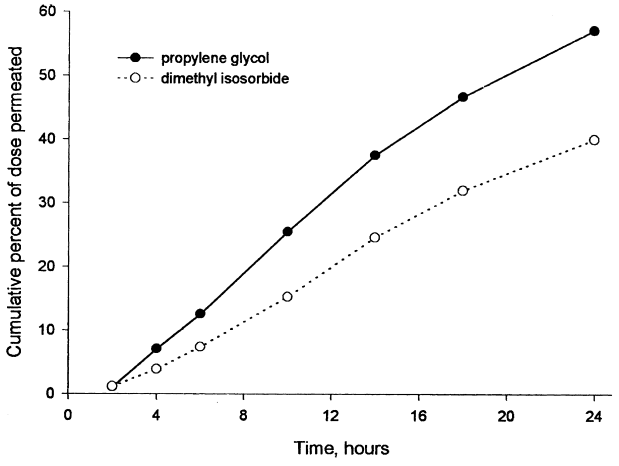

Fig. 2. Permeation of propylene glycol and dimethyl isosorbide through hairless mouse skin from cosolvent mixture initially composed of 10 mol% OA, 6 mol% Dl, 84 mol% PG.

3.3.2. Oleic acid

^{14}C-Labeled OA did not appear in significant amounts in the receptor phase when its flux was measured from the optimized formulation. Skin retention measurements at 24 h yielded an average of 2.2% of OA retention per 0.53 g of wet tissue. Mass balance studies on the formulation spiked with 14C-labeled OA yielded an average recovery of 92.2% of the total initially applied activity.

3.3.3. Dimethyl isosorbide

The cumulative activity of [^{3}H]DI in RF and the cumulative percent permeated are shown in Table 2. DI readily permeated the skin from the optimized formulation. Up to 40% of the applied dose of DI had moved into the RF in 24 h. Fig. 2 shows that the amount permeated was approximately linear (r = 0.9985) with time. The lag time calculated from the regression equation was 1.6 h. The average amount of DI retained in the skin was 0.4% per 0.354 g of wet tissue.

In a separate experiment, 2.0 g of pure DI was spiked with [^{3}H]DI (6.356 mCi/g) and applied to the skin. The cumulative activity in the donor and cumulative percent permeated are shown in Table 3. The permeation was much lower (12.4%) than the 40% achieved with the formulation. Fig. 2 shows that DI permeation declines with time. The amount of DI retained in the skin was 0.01% per 0.14 g wet tissue. DI had no effect on PG permeation: removal of DI from the formulation to form a 10 mol% OA in PG binary suspension showed no difference in the percent of dose recovered in receptor at 24 h following equimolar doses of PG for the

Table 3

Permeation of [^{3}H]dimethyl isosorbide from pure dimethyl isosorbide across hairless mouse skin

Time (h)	Average cumulative activity (mCi ± SD)	Cumulative percent dose permeated (%)
2	0.22 ± 0.02	1.7
4	0.47 ± 0.05	3.7
8	0.87 ± 0.08	6.7
12	1.15 ± 0.09	9.0
20	1.51 ± 0.12	11.9
24	1.61 ± 0.13	12.6

binary mixture (26.1%) versus the DI-containing ternary mixture (26.7%).

4. Discussion

The mechanism by which an enhancer is purported to exert an accelerant effect upon a drug molecule is frequently oversimplified. Such work suffers from a lack of full knowledge of the change(s) within the dosage form that take place as drug absorption proceeds. Kinetic studies on all components show that it is equally important to recognize the ability of enhancer to potentiate the activity of other enhancer(s) and/or excipients. Kinetic studies which evaluated all constituents of the formulation placed in the donor compartment demonstrate the additive effect of using multiple components in a transdermal formulation. Following equimolar applications of pure solvent, only 12% of the DI dose and 4% of the PG dose traversed mouse skin when the pure DI or PG suspensions were applied individually, whereas the percent recovery of the applied dose in the receptor phase of both DI (40%) and PG (26%) increased significantly in the presence of OA. Although DI permeates the skin (Table 3), its permeation was enhanced by PG and/or OA. These results show that PG and DI, in the presence of OA, increase their efficiency as accelerants, which, in turn, increase the skin permeation of N.

The effectiveness of these permeation enhancers to increase the skin permeability of N was related to the amount of vehicle applied and the ability of the vehicle to readily permeate the skin. When a comparison was made of the intrinsic ability of the various solvents to pass through the skin, it appeared that permeation enhancement of N was related to the accelerated permeation of cosolvent vehicle. Azone, polyethylene glycol 400, DI and PG, are very good solvents for nifedipine. However, azone [11] and polyethylene glycol 400 [12] are hardly absorbed through the skin. Although both are good solvents for N, azone and PEG400 had little or no effect on the skin permeability of N when applied as pure solvents [13]. The saturation solubilities of N in azone:OA mixtures or PEG400:OA mixtures should be less than in pure azone or pure PEG400. The N-enhancing effects of OA in non-permeating vehicles (e.g. azone and PEG400) were measurable but marginal and only slight improvement in N flux was observed when these suspensions were applied to mouse skin. However, when azone and PEG (poor inherent skin permeabilities) are replaced by DI and PG (good inherent skin permeabilities), N flux is dramatically improved, because N release is promoted from vehicles which permeate the skin.

Our observation that N release is somehow linked to the ability of vehicle to traverse the skin is seen also with other drugs of poor aqueous solubility [14]. Megrab et al. [15] reported on the bidirectional flux of PG across SC and the uptake of PG and estradiol under infinite dose conditions. They showed, on a molar basis, that the water content of human stratum corneum drops sharply in the presence of PG and suggested that PG readily displaces water in the stratum corneum. Water was thought to make lipid layers less tightly packed and PG-induced dehydration should have a deleterious effect on drug mobility through the skin. The observed flux enhancement of estradiol purportedly arose from a raised PG concentration in the SC with subsequent increases in estradiol solubility in this membrane. These authors suggested that the increase in estradiol solubility overcomes the decreased diffusivity caused by PG-induced dehydration. The DI kinetics described here show that DI readily permeates the skin where it probably acts by the same mechanism described by Megrab et al. [15] for PG, i.e. the displacement of SC water by PG and DI increasing N solubility in the SC lipids.

N has a very poor saturation solubility in water (aqueous solubility of N was measured as 6 mg/l). Therefore, based on this poor aqueous solubility, one would predict that N diffusion through hydrated skin would be likewise be poor, since diffusion through an environment consisting in large part of hydrogen bonded water molecules would be unfavorable. The replacement of epidermal water with solvent such as DI or PG (saturation solubility of 85 and 15 mg/ml, respectively) would likely improve the N diffusion. A concomitant codiffusion of cosolvent with drug permeation has been ascribed as a discriminating, significant feature of enhancer permeation [16]. Bendas et al. described the dual effect by which PG acts as both cosolvent and enhancer for glucocorticoid permeation from PG/water hydrogels [17]. PG penetrated rapidly into excised human skin and the degree of both effects was attributed to the physicochemical properties of drug in the PG/water mixtures: PG functioned as a cosolvent for lipophilic betamethasone, whereas PG acted both as cosolvent and enhancer for the more hydrophilic hydrocortisone.

We believe that DI and PG function as carriers through the epidermis via a mechanism for N flux enhancement that is analogous to that attributed to betamethasone flux. Both drugs possess poor water solubility. N flux is dependent not only on the reported ability of OA to disrupt the fluidity of stratum corneum lipids [18], but also on the ability of donor vehicles to readily pass the stratum corneum. Apparently, a synergistic acceleration of cosolvent permeation exists, augmented by the combined, additive effects of membrane perturbing and intrinsic solvating properties of cosolvent vehicle.

Donor applied to skin from which its stratum corneum had been mechanically stripped showed only slightly less resistance to N diffusion than intact skin. Fig. 3 shows the penetration profiles of N through excised hairless mouse skin following removal of the stratum corneum by skin stripping. Although the extent of N permeation was greater from stripped skin, the lag time and initial rate were indistinct as compared to intact skin. The significant resistance to N permeation through tape-stripped epidermis suggested that in addition to SC, viable epidermis presented a signifi-

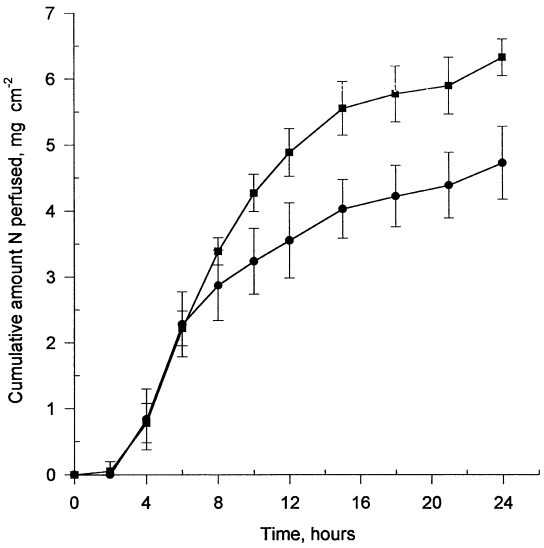

Fig. 3. Penetration profile of nifedipine following single application of 10 mmol of 10 mol% OA, 6 mol% DI and 84 mol% PG across excised intact (●) or stripped (■) mouse skin.

cant diffusion barrier because of the very low aqueous solubility of N.

It has been shown that significant delivery can be achieved to local subcutaneous structures following topical administration because the cutaneous microcirculation does not always act as a perfect sink [19]. Estradiol and progesterone accumulation was found in the region under topical application, whereas accumulation was not seen after subcutaneous administration, demonstrating slow exchanges between blood and tissue that were distinct from the classic 'reservoir' effect. These compounds, like N, have low aqueous solubilities and large partition coefficients. It is well recognized that the partition coefficient is crucially important in promoting a high initial concentration of the agent within the upper strata of the epidermis. For the case of a permeant with a tissue-water partition coefficient greater than 400, a 200 mm layer of dermis will display a diffusional resistance comparable to that of stratum corneum 10 mm thick [20]. In our experiments, the N octanol-water partition coefficient was determined using *n*-octanol/phosphate buffer pH 7 at 37°C, and found to give a log K_{oct} of 3.31. This value lies between a range of values reported in the literature of 3.14 in *n*-octanol/water [21], 2.49 in *n*-octanol/phosphate buffer [22], and 4.00 [23].

Based on these considerations, and the results of the skin stripping experiments, we postulate a three-fold mechanism to explain the solvent dependent enhancement of N flux: (1) N permeates into the lipid domain of the SC by a passive diffusive process accelerated by the lipid-disrupting properties of OA; (2) OA further accelerates N permeation by facilitating DI and PG penetration into the skin beneath the stratum corneum; and (3) the stasis caused by the more hydrophilic environment of the lower strata is unfavorable to N solubility and this impedance is offset by higher DI and PG levels in the skin, which solubilize N in the sub-stratum corneum regions.

In conclusion, permeation characteristics of all donor constituents were described in an attempt to clarify the mechanism of accelerant effects. Both DI and PG readily permeate hairless mouse skin, whereas OA showed no appreciable permeation in the presence of these solvents. In addition to the intrinsic solvent properties for N and the combined effects of OA on membrane perturbation, DI and PG acted as carriers for N and, as such, promote N permeation across hairless mouse skin. Flux of DI, PG, and N was enhanced by OA. Skin stripping experiments showed that epidermal layers other than the stratum corneum represent a locus of significant resistance to N diffusion. The synergistic acceleration of cosolvent flux was attributed to the effect of OA on DI and PG permeation which, in turn, created conditions that favor partition of drug from the viable epidermis into the dermal layers.

Acknowledgements

In addition to the generous donation of reagents by the various suppliers mentioned in the body of this report, the authors acknowledge the interest and support of Dr. Rainer Hoffman and the partial support by a grant by LTS-Lohmann Therapie-Systeme, GmbH&Co. KG, Andernach, FDR.

References

[1] E. Squillante, T.E. Needham, H. Zia, Int. J. Pharm. 159 (1997) 171–180.
[2] M. Goodman, B.W. Barry, Int. J. Pharm. 57 (1989) 29.
[3] M. Goodman, B.W. Barry, J. Invest. Dermatol. 91 (1988) 323–327.
[4] J. Hilton, B.H. Woollen, R.C. Scott, T.R. Auton, K.L. Trebilcock, M.F. Wilke, Pharm. Res. 11 (1994) 1396–1400.
[5] B.W. Barry, Molec. Aspects Med. 12 (1991) 195–241.
[6] E.J. Baker, J. Hadgraft, Pharm. Res. 12 (1995) 993–997.
[7] Imperial Chemical Industries, Effect of Arlasolve DMI on stratum corneum, Scientific Documentation, ICI Specialty Chemicals, Delaware, 1992.
[8] E. Squillante, Doctoral dissertation, University of Rhode lsland, 1993.
[9] K.B. Sloan, S.A.M. Koch, K. Siver, F.P. Flowers, J. Invest. Dermatol. 87 (1986) 244–252.
[10] L. Dahlgren, pers. commun. Beckman Instruments, Product Support, 2500 Harbor Blvd. Fullerton, CA, 1995.
[11] J. Wiechers, B. Drenth, J. Jonkman, R. de Zecuw, Int. J. Pharm. 47 (1990) 43–49.
[12] S.B. Ruddy, B.W. Hadzija, Influence of molecular size on the iontophoretic transport of polymeric nonelectrolytes through hairless rat skin. AAPS Eastern Regional Meeting Podium Session, 1991.
[13] E. Squillante, T. Needham, H. Zia, Int. J. Pharm., submitted.
[14] A. Ganem-Quintanar, C. Lafforgue, F. Falson-Rieg, P. Buri, Int. J. Pharm. 147 (1997) 165–171.
[15] N.A. Megrab, A.C. Williams, B.W. Barly, J. Cont. Rel. 36 (1995) 277–294.
[16] L.K. Pershing, L.D. Lambert, K. Knutson, Pharm. Res. 7 (1990) 170–175.

[17] B. Bendas, U. Schmalfub, R. Neubert, Int. J. Pharm. 116 (1995) 19–30.
[18] R.O. Potts, V.H.W. Mak, R.H. Guy, M.L. Francoeur, Adv. Lipid Res. 24 (1991) 173–210.
[19] J.P. Marty, R.H. Guy, H.I. Maibach, Percutaneous penetration as a method of delivery to muscle and other tissue, in: R.L. Bronaugh, H.I. Maibach, (Eds.), Percutaneous Absorption, Mechanisms, Methodology, Drug Delivery, 2nd Edn., Dekker, New York, 1989.
[20] R.J. Scheuplein, Skin permeation, in: S.A. Jarrett (Ed.), The Physiology and Pathophysiology of the Skin, Academic Press, New York, 1978, pp. 1693–1730.
[21] I. Diez, H. Colom, J. Moreno, R. Obach, C. Peraire, J. Domenech, Pharm. Sci. 80 (1991) 931–934.
[22] D.M. McDaid, P.B. Deasy, Pharm. Acta Helv. 71 (1996) 253–258.
[23] S.L. Ali, Nifedipine, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, Vol. 18, Academic Press, San Diego, CA, 1983, pp. 222–288.