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Enhancement effects in the permeation of Alprazolam through hairless mouse skin

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Summary

Alprazolam (ALP) is an anxiolytic, antidepressant agent, having suitable features for the development of a transdermal medication. The objectives of this preliminary study were to determine· (a) whether ALP is absorbed in vitro through hairless mouse skin; (b) whether it is metabolized during diffusion, and (c) the influence of some chemicals on ALP penetration through skin. ALP permeates through hairless mouse skin in vitro No degradation product of the drug resulted during skin permeation experiments, therefore, ALP was assumed to diffuse unchanged across the skin. Oleic acid (OLA), linoleic acid (LNA), linoleic acid diethanolamide (LNDA), coconut fatty acid diethanolamide (CNDA), lauric acid diethanolamide (LRDA), bis(2-hydroxyethyl)cocamine (HECA) and isopropyl lanolate (IPL) were evaluated with respect to their skin-permeation enhancing effect either as neat solvents or combined with propylene glycol (PG). All the vehicles excepting IPL and PG were more effective than OLA in enhancing transdermal absorption of ALP. The most effective was HECA followed by LNDA, CNDA and LNA/PG (8.5 1 5, w/w) The effects of skin pretreatment with HECA, LNA, LNDA and CNDA on the percutaneous absorption of ALP from a drug suspension in IPL were also investigated. For all the pretreatment vehicles ALP flux from IPL through pretreated skin was greater than that from IPL or OLA through untreated skin. In order to facilitate the interpretation of the absorption results, the stratum corneum/water, whole skin/water and *n*-octanol/water partition coefficients of the drug were determined

Introduction

Alprazolam (ALP; 8-chloro-1-methyl-6-phenyl-4H[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine) is an anxiolytic, antidepressant agent. When orally administered it is readily absorbed in man; the daily dosage is 0.5 mg three times a day. At steady state the average peak plasma concentration of ALP is 23.50 ng/ml. ALP falls in the group of benzodiazepines with an intermediate elimination half-life; the mean half-life is 10–12 h. It is extensively metabolized in man by oxidation and the metabolites are of no clinical significance (Greenblatt et al., 1983; Dawson et al., 1984).

The potential of using the intact skin as a port for transdermal drug delivery to achieve systemic

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effects has recently been recognized. Several therapeutic systems for rate-controlled transdermal administration of drugs for systemic medication have been successfully developed. The advantages of the transdermal administration route, such as a better control of blood levels, reduced incidence of systemic toxicity, absence of hepatic first-pass metabolism, etc., are well known (Ledger and Nichols, 1989). Such advantages, together with the possibility of avoiding the development of dependence connected with the prolonged use of ALP, make it attractive to use the skin as an alternative route for the administration of this drug. In the present paper, a preliminary study is reported the objectives of which were to determine: (a) whether ALP is absorbed in vitro through hairless mouse skin; (b) whether it is metabolized during diffusion; and (c) the influence of some chemicals on the penetration of ALP through skin. Chemicals were evaluated with respect to their skin-permeation enhancing effect either as neat solvents or combined with propylene glycol (PG).

Materials and Methods

Materials

Upjohn SpA (Milano, Italy) generously provided 8-chloro-1-methyl-6-phenyl-4H[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (Alprazolam) (ALP) and 8-chloro-6-(2-chlorophenyl)-1-methyl-4H[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (Triazolam) (TRI). Oleic acid (OLA) was purchased from E. Merck (Darmstadt, Germany). Linoleic acid diethanolamide (LNDA) (Comperlan F), coconut fatty acid diethanolamide (CNDA) (Comperlan KD), and lauric acid diethanolamide (LRDA) (Comperlan LD) were supplied by Henkel HGaA (Düsseldorf, Germany). Bis(2-hydroxyethyl)cocamine (HECA) (Ethomeen C12) was obtained from Akzo Chemie Italia. Linoleic acid (LNA) and crude trypsin from porcine pancreas (Type II) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Isopropyl lanolate (IPL) (Amerlate P) was supplied by Amerchol (NJ, U.S.A.).

Membranes for the permeability measurements were dorsal sections of full-thickness skin from hairless mice, 6–10 weeks old. Four sections were obtained from each animal. Stratum corneum was prepared from full-thickness skin according to a procedure described in a previous paper (Nannipieri et al., 1990). Dorsal sections of full-thickness skin, dermis side down, were floated for 2 h at 37 °C in a 2% trypsin solution in phosphate-buffered saline at pH 8. The skin was then floated in normal saline where the stratum corneum was carefully separated from the epidermis by traction.

Percutaneous penetration experiments

The diffusion rates of ALP across excised skin were measured using diffusion cells based on the Franz design (Franz, 1975). The experiments were carried out at 30 °C. Skin, supported by a perforated cellophane membrane, was placed, dermal side down, between the donor and the receptor phase of the diffusion cell (cross-sectional area 1.13 cm^2). The receptor phase was approx. 8 cm^3 normal saline, containing 0.035% formaldehyde as preservative (referred to henceforth as NSF). The donor phase consisted of a 0.2 ml (or 0.3 g, for semisolid preparations) aliquot of a drug suspension in the test vehicle. Suspensions were prepared by carefully levigating the drug into the oil; prior to use, they were stored at 30 °C for at least 24 h. To maintain pseudosink conditions throughout the experiments, the receptor phase was completely removed at measured time intervals and replaced by an equal volume of fresh prethermostatted NSF. Comparisons between ALP suspensions in different vehicles were performed with tissue sections obtained from the same animal. Each comparison was repeated at least three times with different animals.

Permeability measurements through pretreated skin were carried out as follows. After the tissue had been mounted in the diffusion cell, in contact with the receptor phase, a 0.5 ml (or 0.5 g) aliquot of the test vehicle was applied to the donor side of the skin surface. The skin was kept in contact with the vehicle for 24 h. Three changes of the receptor phase were made during this period. At 24 h the bulk of the test vehicle was

removed from the skin by a gentle flow of water; then, the residual vehicle and clinging water were removed by passing through filter paper. Finally, a 0.3 g aliquot of an ALP suspension in IPL was applied to the donor side while the receptor phase was replaced by fresh NSF. The receptor phase was removed at measured time intervals for determination of ALP concentration. In each run the permeation through pretreated skin was compared with that through untreated skin from an ALP suspension in the same vehicle as that used for the pretreatment. Each run was performed with tissue sections obtained from the same animal. Each comparison was repeated three times.

Partition coefficient determinations

n-Octanol / NSF *n*-Octanol and water were presaturated with each other. Equal volumes of octanol and aqueous drug solution were tumble-mixed at 30 °C for 24 h. After separating the phases the aqueous solution was analysed for drug concentration. Drug concentration in *n*-octanol was evaluated on the basis of loss of drug from the aqueous phase.

Stratum corneum / NSF Stratum corneum was dried in a desiccator and the dry weight was used for calculating the partition coefficient. The stratum corneum (about 30 mg) was placed in a screw-capped vial and supplemented with 6.0 ml of drug solution in NSF. The vial was gently tumbled at 30 °C. The equilibrium concentration of the aqueous phase was determined after 24 h; missing drug was assumed to have entered the stratum corneum. The stratum corneum/NSF partition coefficient was expressed as the ratio of molal to molar concentrations.

Full-thickness skin / NSF Full-thickness skin was gently sandwiched between filter paper to remove clinging water, weighed (about 0.5 g), placed in a screw-capped vial and 5.0 ml of ALP solution in NSF was added. The vial was gently tumbled at 30 °C for 30 h. The equilibrium concentration of the aqueous phase was determined and missing drug was assumed to have entered the skin. The full-thickness skin/NSF partition coefficient was expressed as the ratio of molal to molar concentrations.

Assay method

ALP concentration in aqueous samples was assayed by gas chromatographic analysis according to the following procedure. A 4.0 ml sample was pipetted into a stoppered test tube containing 8.0 g of freshly distilled chloroform. The tube was vigorously shaken for 3 min and centrifuged at 4000 rpm for 20 min. The supernatant aqueous phase was carefully removed by suction. A 7.0 g sample of the underlying organic solution was combined with a known volume of a TRI solution in benzene, which was used as internal standard, containing 0.4 mg/ml of TRI. The resulting solution was evaporated to dryness at 35–40 °C under a stream of nitrogen. The residue was redissolved in a few microliters of chloroform, of which about 5 μl were injected into the chromatograph.

The analytical instrument was a C. Erba gas chromatograph equipped with a flame-ionization detector and contained a 3 mm × 150 cm glass column packed with 1.3% OV on 80-100 Chromosorb W. The column was maintained at a temperature of 280 °C. The detector and injector port were maintained at a temperature of 300 °C. The carrier gas was nitrogen at a flow rate of 30 ml/min. Under the chromatographic conditions described above, the approximate retention times were 6.4 and 7.5 min for ALP and TRI, respectively.

Results

Before studying the vehicle effect on the penetration of ALP through skin, the possibility of chemical or enzymatic degradation of the drug during skin penetration experiments was assessed by adding a drug suspension in normal saline (or in NSF) to the donor side of the skin surface; the receptor phase consisted of normal saline (or NSF). In these experiments, the receptor phase was not renewed so that the drug was in contact with both the skin and the solvent for as long as possible; at 48 h the receptor phase was analysed by gas chromatography. Fig. 1 shows representative chromatograms recorded during such experiments. Since, as observed in Fig. 1, no degradation product resulted from gas chromatographic

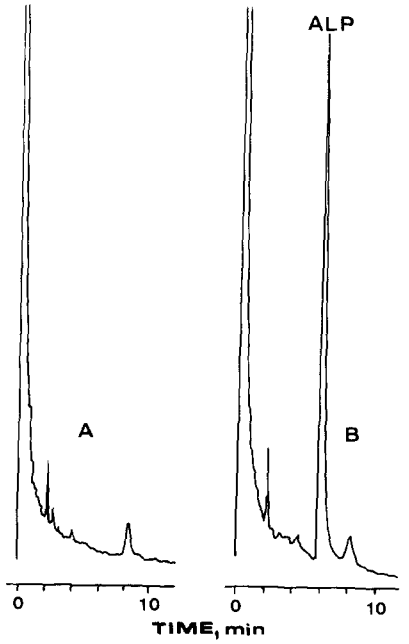

Fig 1 Representative chromatograms of extracts of receptor phase collected during transport experiments through skin: (A) from drug-free normal saline (or NSF) and into normal saline (or NSF), or (B) from drug-containing normal saline (or NSF) and into normal saline (or NSF).

analysis, in either the presence or absence of formaldehyde, ALP can be assumed to diffuse unchanged across whole hairless mouse skin.

Comparison between the percutaneous penetration of ALP from the vehicles under study was based on flux values; drug flux, expressed as the mass of drug passing across 1.0 cm^2 of skin per unit time, was calculated as described previously (Di Colo et al., 1989). Since drug saturated vehicles were used, the thermodynamic activity of the drug did not change with varying vehicle. The absolute flux values from hairless mouse skin are generally not of great practical importance. In fact, while this animal skin provides a good model in percutaneous penetration studies, it is usually considered more permeable than human skin. On the basis of these considerations and because of the well known considerable degree of variability between skin samples from different animals, flux data were not pooled but rather relative flux values were calculated by using skin sheets obtained from the same animal.

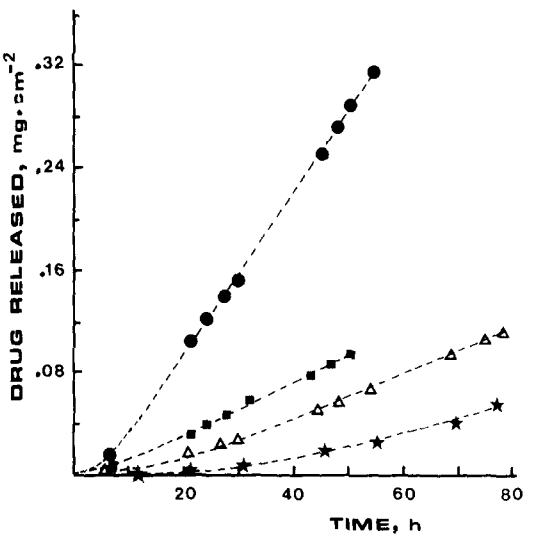

Fig 2 Typical permeation curves of ALP through hairless mouse skin from a drug suspension in PG (⋆), IPL (△), OLA (■) or LNA (●).

ALP suspensions in neat vehicles

Representative permeation curves are shown in Figs 2 and 3. OLA, owing to its generally recognized promoting action on percutaneous absorption of polar and non-polar drugs (Barry, 1987; Francoeur et al., 1990), was used as the

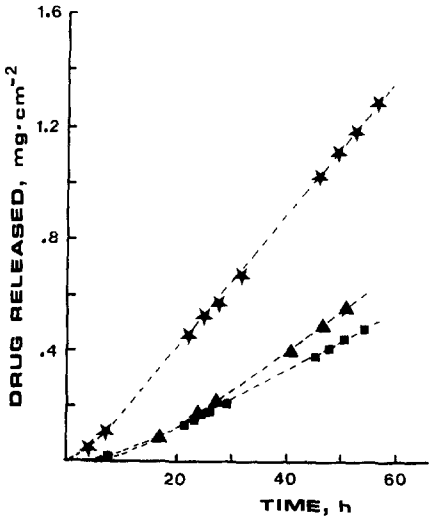

Fig. 3. Typical permeation curves of ALP through hairless mouse skin from a drug suspension in CNDA (■), LNDA (▲) or HECA (⋆).

reference vehicle. The chemicals to be compared with OLA were LNA, two diethanolamides of fatty acids, LNDA and CNDA, a tertiary alkanolamine, HECA, a lanolin derivative, IPL, and PG. IPL had previously been shown to exert a moderate accelerant action on the transdermal absorption of a highly lipophilic molecule (Nannipieri et al., 1990). The relative flux values, which are listed in Table 1, indicate that all the vehicles except PG and IPL were more effective than OLA in enhancing the permeation of ALP. The enhancement effect of the vehicles increased in the order IPL = PG < OLA < LNA < LNDA = CNDA < HECA. Lag-time values were not very reproducible and varied from 3 to 12 h for the two fatty acids (OLA and LNA) and the amine (HECA), and from 8 to 20 h for the two amides (LNDA and CNDA) and IPL. As can be observed in Fig. 2, a steady state was not attained with PG.

ALP suspensions in two-component vehicles

All the chemicals, with the exception of LNA and lauric acid diethanolamide (LRDA), were fully miscible with PG. Since LRDA is a solid with slight solubility in PG, it was suspended in PG. As for LNA, this acid was saturated with 15% PG. The permeation behaviour of ALP from each binary system was directly compared with that from the respective neat enhancer through a skin sample from the same animal. Relative flux data are given in Table 1. Inspection of the data shows that only the combination of LNA and PG increased ALP flux significantly, i.e., 2.2-fold over that resulting from neat LNA and, hence, approx. 4.6-fold over that in the case of neat OLA. When the percentage of PG in CNDA/PG or LNDA/PG mixtures was increased above 25%, a reduction in the drug flux resulted. Also, the results of a single run, which are not reported in Table 1, indicated that percentages of PG greater than 25% had either no influence or a lowering effect on ALP flux from HECA/PG or LRDA/PG mixtures. Moreover, none of the two-component vehicles except LNA/PG modified the lag-time values which had been observed for neat vehicles. The lag time for LNA/PG was half that observed for neat LNA.

TABLE 1

Permeation data of ALP through skin from neat vehicles and two-component vehicles

Vehicle (w.w)	n [a]	Neat vehicles		Two-component vehicles	
		Flux ± SD ($\times 10^3$) (mg cm^{-2} h^{-1})	Relative flux ± SD [b]	Reference vehicle	Relative flux ± SD [c]
OLA	3	3 3 ± 0.6	1		
LNA	4		2 1 ± 0.25		
LNA/PG (8 5 1.5)	3			LNA	2 21 ± 0.30
CNDA	3		6 3 ± 0.50		
CNDA/PG (7 5 : 2 5)	3			CNDA	1 25 ± 0.10
CNDA/PG (1 1)	3			CNDA	0 96 ± 0 54
CNDA/PG (2.5 · 7.5)	4			CNDA	0 40 ± 0.18
LNDA	3		7 8 ± 1 25		
LNDA/PG (7 5 2.5)	3			LNDA	1 25 ± 0 07
LNDA/PG (2.5 · 7 5)	3			LNDA	0.70 ± 0 25
LRDA/PG (7 5 2.5)	4			LNDA	1 19 ± 0 35
HECA	3		16 9 ± 0 48		
HECA/PG (7 5 2.5)	4			HECA	0.87 ± 0 14
PG	3		0 45 ± 0 18 [d]		
IPL	4		0 53 ± 0 17		

[a] Number of determinations.

[b] Flux relative to OLA ± standard deviation

[c] Flux relative to the respective reference vehicle ± standard deviation.

[d] The steady state was not reached (see Fig 2); the reported flux value was calculated at 70 h

Permeation of ALP through pretreated skin

The effects of pretreatment on the skin permeation of ALP from a drug suspension in ILP, a poor enhancer (see Table 1), were investigated. The duration of the pretreatment period was 24 h, i.e., a time equal to or longer than the lag times observed in the permeation experiments through untreated skin. The permeation profiles reported in Fig. 4 show an initial burst effect, which is particularly evident for pretreatment with HECA; subsequently, the flux declines in time until a nearly stationary value is achieved. Experimental relative flux was calculated by comparing the steady-state flux through pretreated skin with that through untreated skin from an ALP suspension in the pretreatment vehicle (reference vehicle, in Table 2). In Table 2, the experimental relative-flux data are compared with those calculated on the basis of the ratio between ALP fluxes, which are reported in Table 1, through untreated skin from IPL and from the proper reference vehicle, respectively. Comparison between the two sets of data indicates that pretreatment of the skin always increased the later flux of ALP from IPL; nevertheless, each flux value for pretreatment was lower than the flux through untreated skin from an ALP suspension in the corresponding reference vehicle. For example, as demonstrated by the data in Tables 1 and 2, ALP flux from IPL for pretreatment with HECA was 40% of that through untreated skin from ALP in HECA, 12.9-fold over that obtained for ALP from IPL through untreated skin, and 6.76-fold over that in the case of ALP from OLA through untreated skin. It is worth noting that for all the pretreatment vehicles ALP flux from IPL and through pretreated skin was greater than that from OLA through untreated skin.

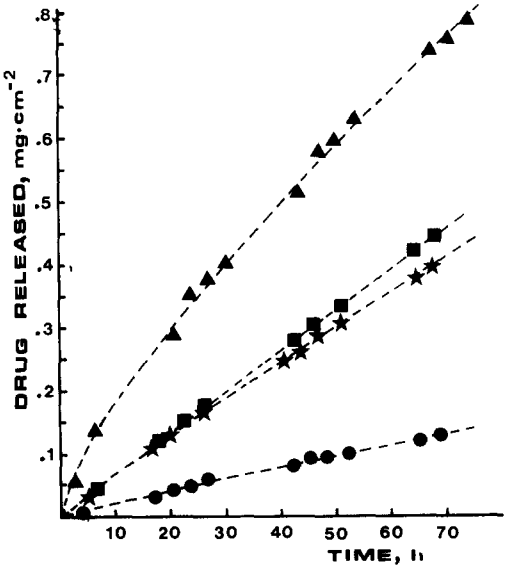

Fig 4. Typical permeation curves of ALP from a drug suspension in IPL and through hairless mouse skin pretreated with LNA (●), LNDA (★), CNDA (■) or HECA (▲)

TABLE 2

Permeation data through skin pretreated with neat vehicles

Pretreatment vehicle (step 1) [a]	Permeation vehicle (step 2) [b]	Reference vehicle	Relative flux ± SD [c]	
			Experimental [d]	Calculated [e]
LNA	IPL	LNA	0.6 ± 0.11	0.252
HECA	IPL	HECA	0.4 ± 0 10	0 031
LNDA	IPL	LNDA	0 3 ± 0 07	0 068
CNDA	IPL	CNDA	0 5 ± 0 12	0 084

[a] In step 1 skin was treated over 24 h with the pretreatment vehicle alone, which was then removed.

[b] In step 2 a permeation experiment through pretreated skin was run with an ALP suspension in IPL (permeation vehicle)

[c] Relative flux ± standard deviation ($n = 3$)

[d] Permeation data from step 2 were compared with those through untreated skin from ALP suspensions in the reference vehicle

[e] These values were determined from the data in Table 1, each value corresponds to the ratio between ALP fluxes through untreated skin from IPL and from the reference vehicle, respectively

Discussion

Although ALP permeates through whole mouse skin in vitro, lag times are very prolonged with both neat and two-component vehicles; moreover, no correlation was found between lag-time values and enhancement effect. Owing to the great complexity of the skin membrane, investigation of the factors affecting lag times is a difficult task. As a consequence, the discussion will be limited to a few comments. Generally, for

a heterogeneous membrane, such as the skin, the chief factors that contribute to prolonged lag times should be: (a) low diffusion coefficients of the penetrant molecule in the different phases of the polyphasic membrane; (b) adsorption of the penetrant molecule on active sites of the membrane (Flynn and Roseman, 1971); (c) progressive change in skin barrier properties, when enhancement occurs.

Frequently, the skin is viewed as a bilayer barrier with a lipid horny layer in series with the aqueous viable tissues of the dermis-epidermis layer. From a comparison between the stratum corneum/NSF and whole-skin/NSF partition coefficients, which are listed in Table 3, it appears that ALP shows, as expected, stronger affinity to lipid stratum corneum than to aqueous tissues. Moreover, as shown in Fig. 5, the linearity of the stratum corneum/NSF partition isotherm, indicates a simple dissolution mechanism of the drug in the stratum corneum (Giles et al., 1960; Chandrasekaran et al., 1976), whereas the curvature of the partition isotherm for whole skin is probably indicative of the cooperative adsorption of ALP on active sites of dermis-epidermis layer (Giles et al., 1960). In a separate experiment, the flux and lag time of ALP from a drug suspension in NSF through full-thickness skin were compared with those through the dermis-epidermis layer; this layer was isolated by repeated stripping of full-thickness skin with cellophane tape, as described by Ghanem et al. (1987). ALP flux through the dermis-epidermis layer was 6.5×10^{-3} mg cm^{-2} h^{-1}, i.e., 36-times greater than that through whole skin, while the lag time was 1.0 h for aqueous

TABLE 3

n-Octanol / NSF, stratum corneum / NSF and whole skin / NSF partition coefficients of ALP

	Partition coefficient
n-Octanol/NSF	132 [a]
Whole skin/NSF	0.69–2.77 mg/g [b]
Stratum corneum/NSF	8.9 ml/g [c]

[a] The partition coefficient did not change in the presence of 35.4×10^{-3} M LNA in octanol.
[b] The partition isotherm was not linear (see Fig 5).
[c] The partition isotherm was linear (see Fig. 5).

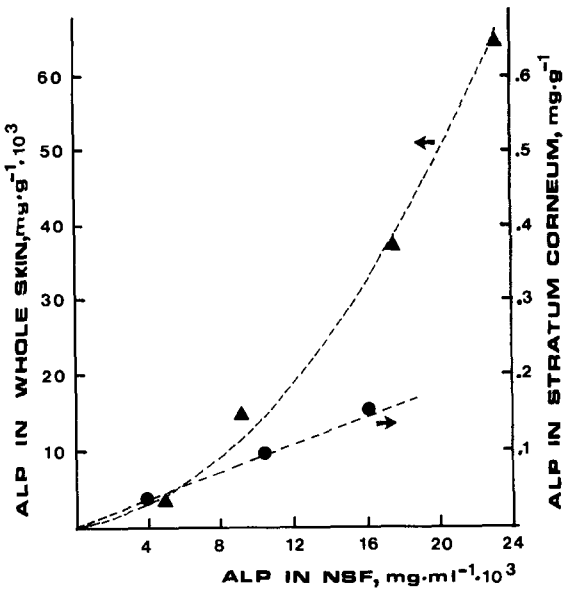

Fig. 5. Stratum corneum/NSF (●) and whole skin/NSF (▲) partition isotherms of ALP. Equilibrium concentration of the drug in the tissue vs equilibrium concentration of the drug in the aqueous vehicle

tissues and 24 h for whole skin. In conclusion, the foregoing results suggest that: (a) the horny layer is the major rate-limiting barrier for ALP; (b) the diffusion coefficient of ALP in the horny layer is lower than that in the dermis-epidermis layer; and (c) despite the hydrophobic character of ALP and its adsorption on active sites of the dermis-epidermis tissues, this layer provides a minor contribution to lag times. Therefore, the chief factors leading to long lag times, such as a low diffusion coefficient, etc., are within the horny layer. In particular, the absence of a lag time in the permeation profiles reported in Fig. 4 for pretreated skin indicates that progressive change in the barrier properties of the stratum corneum as a result of the effect of an enhancer in the course of experiments with untreated skin makes a considerable contribution to the increase in time for onset of the steady state.

Although the mode of action of the enhancers in promoting ALP flux is beyond the scope of the present investigation, some suggestions can tentatively be put forward for LNA, in particular. Recently, a mechanism has been proposed in

which the unsaturated fatty acid OLA is heterogeneously dispersed within stratum corneum lipids, in which it forms a number of permeable fluid-like channels (Walker and Hadgraft, 1991) or permeable interfacial defects (Ongpipattanakul et al., 1991). Also, according to Barry (1991), OLA can interact with stratum corneum lipids thus disrupting the organization of their structures, increasing their fluidity and, consequently, increasing drug flux. The two mechanisms together could be proposed to explain the accelerant action of LNA. Indeed LNA, reportedly, diffuses through and accumulates within the horny layer (Hoelgaard and Mollgaard, 1982; Wepierre et al., 1986), where it might form a separate phase. A possible, albeit indirect factor confirming the dual mode of action of LNA appears to be the trend of ALP flux which was observed in penetration experiments through pretreated skin. As can be deduced from Fig. 5, ALP flux decreased with time until a pseudo-stationary state had been reached; the initial decrease in flux might be related to the loss of the fraction of free or fluid LNA which is within the tissue and possibly diffuses in and out of the skin more easily than does the fraction of the acid tightly bound (clustered) to the lipids. This tightly bound LNA should be responsible for the residual enhancing effect related to the pseudo-stationary flux. Hence, the formation of clustered LNA could prevail at low LNA concentrations within the horny layer, while free or fluid LNA could form at higher acid concentrations. Although the release profiles reported in Fig. 4 for HECA, CNDA and LNDA show trends similar to that for LNA, the present permeation data alone do not suffice to justify the suggestion of a dual sorption mechanism for these chemicals as well.

Although ion pairing has been proposed as a possible mechanism of increasing drug partitioning into the skin and, hence, drug skin permeation, we found no evidence of drug and LNA forming more lipophilic ion pairs. Indeed, we investigated such a possibility by measuring ALP partitioning into *n*-octanol in either the absence or presence of LNA; as shown in Table 3, the octanol/NSF partition coefficient was not affected by the acid. Nevertheless, we cannot exclude ion-pair formation within such a different environment as that of the skin lipids.

When PG was added to LNA so as to obtain a saturated solution, the binary mixture was more effective than neat LNA in both enhancing ALP flux and reducing lag time. The result is consistent with the findings of many investigators who reported that combination of a number of vehicles with PG is more effective than the vehicle alone. According to Barry (1991), PG may accumulate in the stratum corneum and promote partitioning of the main enhancer. Nevertheless, the synergy shown by PG failed when it was combined with the other enhancers under study. Although the subject is very complex, the divergent behaviour of the mixtures HECA/PG, CNDA/PG and LNDA/PG could find an explanation in the chemical potential of the enhancer in the binary mixture in contact with the skin. Indeed, in contrast to the LNA/PG mixture, the other mixtures consisted of completely miscible components. Consequently, the solubilizing effect of PG reduces the chemical potential of HECA, CNDA and LNDA, thus decreasing the driving force for their partitioning into the horny layer. Our suggestion is consistent with the observations of Hori et al. (1990), who pointed out that the binary mixtures are more effective when their components have different physicochemical properties.

In conclusion, the most effective vehicle in enhancing the transdermal absorption of ALP was HECA followed by LNDA, CNDA and LNA/PG. Skin pretreatment with enhancer has shown potentially interesting aspects. Essentially, skin pretreatment is a technique suitable for the study of the reversibility of the enhancing effect. In the present work, the enhancing effect decreased gradually in time after the removal of the enhancers. Although prompt reversibility is generally demanded, nevertheless the lack of a prompt recovery of skin properties might turn out to be advantageous in practice. In fact, one can cautiously speculate about such an advantage of human skin pretreatment as the achievement of quite high flux values coupled with very short lag times by application of a non-irritant vehicle on pretreated skin. Nevertheless, owing to the limitations implicit in the use of hairless mouse skin,

which is more susceptible to chemical perturbations than human skin, it should be recognized that the good results obtained with the animal skin might not be strictly relevant to the clinical situation.

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