In Vitro Human Skin Barrier Modulation by Fatty Acids: Skin Permeation and Thermal Analysis Studies

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Purpose. This study aims to elucidate the skin permeation enhancement and the skin perturbation effects of a number of fatty acids, i.e. straight-chain saturated (SFA), monounsaturated (MUFA) and polyunsaturated acids (PUFA).

Methods. The skin permeation enhancement effects were studied using human stratum corneum (SC) and *p*-aminobenzoic acid (PABA) as a model permeant. The fatty acids in propylene glycol (FA/PG) were applied according to a pre-treatment/co-treatment protocol. The perturbation effects were studied using differential thermal analysis (DTA) on SC after pretreatment with FA/PG.

Results. SFA with 6 to 12 carbons exhibit a parabolic correlation between enhancement effect and chain-length, with a maximum at nonanoic-decanoic acids (with 9 and 10 carbons). Nonanoic and decanoic acids exert barely noticeable effects on the thermal behaviour of SC, suggesting that they easily mix with the skin lipids. All *cis*-6-, 9-, 11- or 13-octadecenoic acids (MUFA) enhance the permeation of PABA to the same extent. DTA revealed that the *cis*-9- and 13-isomers form a separate domain containing mostly the pure fatty acids within the SC lipids and suppress the lipid transitions at 70°/80°C. PUFA—linoleic (LA), α-linolenic (ALA) and arachidonic acids—enhance PABA permeation stronger than MUFA but additional double bonds do not further increase the degree of enhancement. LA and ALA form separate domains but do not completely suppress the SC lipid transitions at 70°/80°C. Increase in the enthalpy changes of 70°/80° transitions linearly correlates to the decrease in the permeability coefficients, suggesting that an increased perturbation of the skin lipids not necessarily has to yield an increased PABA permeation.

Conclusions. The enhancement effects of fatty acids on the PABA penetration through SC are structure-dependent, associated with the existence of a balance between the permeability of pure fatty acids across SC and the interaction of the acids to skin lipids.

KEY WORDS: fatty acid; skin permeation enhancement; diffusion study; differential thermal analysis.

INTRODUCTION

The use of saturated and unsaturated fatty acids as enhancers for drug permeation is of interest for both topical and transdermal drug delivery (1). This class of enhancers has the advantage of being one of the endogenous compounds in human skin lipids, including stratum corneum (SC) (2). Fatty acids have already been known to play an important role in determining the properties of biological membranes (3).

The most frequently used *in vitro* method for studying drug permeation is the simulation of diffusion across excised skin to mimic the *in vivo* penetration process. For this purpose a new flow-through diffusion cell has been developed (4), which has been used in this study. As model permeant, *p*-aminobenzoic acid (PABA) was selected, because of the ease in analysis and its use as topical ultraviolet absorber. The *in vitro* permeation study aims to assess the enhancing capability of several series of fatty acid from different subclasses—saturated, mono-unsaturated and polyunsaturated—and furthermore to establish a possible structure-activity relationship from the results.

In the past thermal studies on SC have been carried out to investigate the components of SC (5,6) and to get insight in the mode of action of penetration enhancers on SC (7). In this study thermal analysis is used to assess the effects of fatty acids on the thermal behaviour of SC. The combination of the data from diffusion experiments and thermal analysis should give more insight into the mode of action of these acids as skin penetration enhancers.

MATERIAL AND METHODS

Preparation of Stratum Corneum Samples

SC sheets were isolated by trypsination from fresh human breast or abdominal skin obtained by surgical operation. A detailed procedure is described elsewhere (6). Prior to use, the sheets were cleansed and stored above silica gel in nitrogen atmosphere at room temperature.

Dehydrated SC samples were prepared by placing SC sheets for 24 h at 50°C in a closed vessel above phosphorus pentoxide (Baker, Deventer, The Netherlands) 10 mg/cm^3 vessel volume.

Hydrated SC samples were prepared by equilibrating SC sheets for 24 h at room temperature in a closed vessel above a 27% w/v sodium bromide (Merck, Darmstadt, Germany) solution in purified water.

Treatment Solutions

The solutions for the pre-treatment and co-treatment of SC were propylene glycol (PG; Baker, Deventer, The Netherlands), or 0.16 M fatty acid in PG. The following fatty acids were used in the skin permeation study: (i) *saturated* (SFA): hexanoic, heptanoic, octanoic, nonanoic, decanoic, undecanoic and lauric acids; (ii) *mono-unsaturated* (MUFA): *cis*-octadecenoic acids with double bonds at position: 6, 9, 11 or 13; (iii) *poly-unsaturated* (PUFA): linoleic (18:2), linolenic (18:3), arachidonic (20:4) acids. The acids involved in the thermal study were nonanoic, decanoic, *cis*-9-octadecenoic (oleic), *cis*-13-octadecenoic, linoleic and linolenic acids. The concentration 0.16 M was used since it is below the maximum solubility of all fatty acids used in this study. All acids were purchased from Aldrich Chemie, Bornem, Belgium and were of the highest purity.

Pre-Treatment of Stratum Corneum for Skin Permeation Studies

The SC sheet was pre-treated by allowing a contact with the penetration enhancer solution just on the anatomical surface side (300 μl per 2.54 cm^2 surface area) for 24 h at 32°C. The

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solution was put into a Hill-Top chamber (outer diameter 25 mm, opening diameter 18 mm, Hill-Top Research, Cincinnati, Ohio, USA) placed upside down. An adequate amount of medical silicone adhesive (Type 355, Dow Corning, Midland, Michigan, USA) was applied on the double rim at the edge of the chamber. The SC sheet was then laid covering the whole opening of the chamber and got in contact with the adhesive on the rim. After the attachment of the sheet to the rim had been secured, the chamber was turned over, so the SC sheet lay under the chamber and came into full contact with the pretreating solution (the contact area equals the inner area of the chamber). During the pretreatment the SC sheet was simultaneously hydrated by floating it (on the dermal side) on the surface of phosphate buffered saline (PBS) (pH 7.4) (without stirring).

Pre-Treatments for Differential Thermal Analysis

The SC sheet, either dehydrated or hydrated prior to pretreatments, was submerged (without stirring) in penetration enhancer solutions (2 ml/10 μg SC) for 24 h at 32°C. Then the SC sheet was dried by pressing it manually between 2 pieces of nylon wire-netting wrapped with tissue-paper repeatedly until the sheets did not wet the paper anymore.

Skin Permeation Study of p-Aminobenzoic Acid (PABA)

The flow-through system consisted of diffusion cells, a multichannel peristaltic pump (JPS-8, Ismatec, Zurich, Switzerland), a circulating water-bath set at 32°C and fraction collectors (Retriever II, ISCO, Lincoln, NE, USA). The diffusion cells were assembled with a set of membranes mounted between the donor and acceptor chambers. The membrane set consisted of a SC sheet and a sheet of dialysis membrane (Diachema, molecular weight cutoff 5000 Da, Dianorm, München, Germany) as supporting layer. The acceptor solution, PBS (pH 7.4) was transported through the acceptor chamber with a flow rate of 5 ml/h and collected in a fraction collector. 400 μl/chamber of donor solution, i.e. 25 g/l PABA (Aldrich, Bornem, Belgium) solution in 0.16 M fatty acid in PG (the same fatty acid as in the pre-treatment solution), was injected into the donor chambers. The solutions were equilibrated at 32°C throughout the experiment by placing the vessels in the water-bath, where the cells were immersed during the study. The experiment started immediately after the donor solution was applied. Samples were collected every hour during a 20-hour period.

PABA was analyzed in an HPLC system (Spectra Physics, San Jose, USA) connected to a fluorescence detector (JASCO 821-P, Tokyo, Japan). The excitation and emission wavelengths were set at 265 and 336 nm, respectively. A reversed-phase column was used (Asahipak, OD-50, Hewlett Packard, Santa Clara, USA) at room temperature. The mobile phase used consisted of 0.014 M borate buffer (pH 9.0, according to European Pharmacopoiea 2nd ed.)/methanol (85:15 v/v). In the collection tubes, 0.1 N NaOH was added to each sample (about 2% v/v of the estimated sample volume), to maintain the pH at around 11.0 as suggested in (8). The materials to make solutions were purchased from Merck, Darmstadt, Germany and Baker, Deventer, The Netherlands.

Differential Thermal Analysis

SC samples (dehydrated, hydrated or penetration enhancer-pretreated), each weighing 10–30 mg, were placed into medium pressure stainless steel crucibles made by Mettler, Greifensee, Switzerland, and hermetically sealed to avoid water evaporation during the analysis. Differential thermal analysis was performed using Mettler TA 3000 Thermal Analysis System with a Low Temperature Cell, with an empty pan as reference. Samples were subjected to the following thermal analysis cycle: cooling from 20° to −130°C, then equilibrating isothermally for at least 5 minutes to achieve a stable condition at −130°C, followed by heating from −130° to 120°C. The rate for both cooling and heating was 2°C/min. The transition temperatures were determined by taking the temperature corresponding to the top of peaks on the heating curves. The heating curves were constructed by plotting the heat flow values, which have been normalised using sample weight (as mW/mg), against temperatures.

RESULTS

Skin Permeation Study

In this study, the enhancer solution was applied in two sequential steps: (i) the application of the enhancer solution (without the drug) on the SC membrane for 24 hours (pre-treatment), followed by (ii) the application of the drug dissolved in the enhancer solution on the pre-treated SC during the skin permeation experiment (co-treatment). This procedure is called the pre-treatment/co-treatment protocol (9). The use of the pre-treatment/co-treatment protocol has been suggested in order to standardize the difference in the thermodynamic activity among the acids as well as the lag time to reach the steady state condition of the permeation flux.

Table I lists the results of skin permeation experiments with PABA as model permeant. The permeability coefficient of PABA from a PG solution was lower than from an aqueous PABA solution. There are several reasons for this: (i) PABA may have greater affinity to PG than to SC, therefore the use of PG as a solvent reduces the partitioning of PABA from donor solution into SC, (ii) PABA absorbed in SC may remain in PG and thus show a lower partition into SC lipid, (iii) in the presence of PG in SC, PABA may have greater affinity to non-lipid components of SC and therefore be retained. When PABA was applied from PG solution containing fatty acids, the presence of fatty acids, which in most cases are less polar than PABA, decreases the polarity of the PG solution and may result in a driving force for PABA to partition into SC (change in the thermodynamic activity). This will increase the permeation flux of PABA in addition to the permeation enhancement caused by the intrinsic enhancing capability of the fatty acids.

The influence of straight-chain SFA on the permeation of PABA is shown in Figure 1A. For fatty acids with 6 to 9 carbon atoms (hexanoic, heptanoic, octanoic and nonanoic acids), steady-state conditions of PABA flux were not reached during the 21-hour diffusion experiments (see Figure 1D). Nevertheless, we observed that the enhancement capacity of fatty acids increased with increasing chain-length with a sharp increase between 8 and 9 carbons. In contrast, for acids with 10 to 12 carbon atoms (decanoic, undecanoic and lauric acids) steady state conditions were reached within the period of experiments. The permeability of PABA induced by decanoic, undecanoic and lauric acid decreased with increasing chain length. Hence, the relation between the permeability of PABA from fatty acid/

Table I. Diffusion Parameters of PABA Permeation Across Human Stratum Corneum Following Pre-Treatment/Co-Treatment Protocol

Treatment solution/solvent for donor solution	n	J_{ss} (μg/cm²/h)	P (10^{-8} cm/s)	*ER*
water	15	2.57 ± 0.19	28.55 ± 2.11	1
PG	5	0.28 ± 0.14	0.31 ± 0.16	0.01
saturated:				
hexanoic acid/PG	5	44.91[a] ± 34.09	49.90[a] ± 37.88	1.2[a]
heptanoic acid/PG	6	74.46[a] ± 34.64	82.73[a] ± 38.49	1.6[a]
octanoic acid/PG	6	81.64[a] ± 52.71	90.71[a] ± 58.57	2.6[a]
nonanoic acid/PG	7	837.84[b] ± 190.30	930.93[a] ± 211.44	32.1[a]
decanoic acid/PG	5	790.16 ± 225.90	877.95 ± 251.00	30.3
undecanoic acid/PG	7	520.78 ± 22.93	578.64 ± 25.48	25.1
lauric acid/PG	5	92.02 ± 11.12	102.24 ± 12.36	3.5
monounsaturated:				
cis-6-octadecenoic (petroselinic) acid/PG	8	454.63 ± 79.72	504.84 ± 88.58	17.4
cis-9-octadecenoic (oleic) acid/PG	6	405.78 ± 95.40	450.87 ± 106.00	15.8
cis-11-octadecenoic (vaccenic) acid/PG	7	310.75 ± 65.97	345.28 ± 73.30	11.9
cis-13-octadecenoic acid/PG	6	428.73 ± 47.98	476.37 ± 53.31	16.4
polyunsatured:				
linoleic acid (*all cis* 9,12-octadecadienoic acid)/PG	8	657.85 ± 132.47	730.94 ± 147.19	25.2
linolenic acid (*all cis* 9,12,15-octadecadienoic acid)/PG	7	596.81 ± 119.83	663.12 ± 133.14	22.9
arachidonic acid (*all cis* 5,8,11,14-eicosatetraenoic acid)/PG	8	565.25 ± 97.79	628.05 ± 108.66	21.7

Note: J_{ss}: flux at steady state condition; P: permeability coefficient, obtained by dividing J_{ss} with concentration difference between donor and acceptor chamber, i.e. 2.5 g/l (in water) or 25 g/l (in PG); ER: Enhancement ratio, obtained by dividing the permeability coefficient of a treatment against the one of PABA in water with no pretreatment; PABA: *p*-amino benzoic acid; PG: propylene glycol.
[a] Not in steady state.
[b] At 20 h. Flux was increasing with a rate of 64.18 μg/cm²/h².

PG solutions as function of the fatty acid chain-length is bell-shaped with a maximum enhancement at 9 and 10 carbon atoms.

The influence of straight-chain MUFA on the permeation of PABA was studied using an isomeric series of *cis*-octadecenoic acids, with the double-bond at the 6th, 9th, 11th or 13th position counted from the carboxyl head group. Figure 1B displays the permeability coefficient as a function of the position of the double bond. There is no significant difference in the effects of these acids on the permeability of PABA. In all cases, the steady state conditions were reached. In general the enhancement factors are approximately 15, compared to the permeation of PABA dissolved in water.

Figure 1C shows the influence of the number of double bonds (in *cis*-conformation) in straight-chain PUFA with respect to the permeability coefficient of PABA. Compared to MUFA (with only one double bond), PUFA—linoleic, α-linolenic and arachidonic acid with respectively 2, 3 and 4 double bonds—produced a significantly higher increase in PABA permeation across SC. There was no significant difference in effects among the PUFA. In all experiments with these acids the steady state conditions were reached.

Thermal Analysis

The thermal transitions of SC were measured using differential thermal analysis after 24-hour pre-treatments of PG or of fatty acids in PG. The treatments were performed on either dehydrated SC or hydrated SC. Several thermal transitions of SC were reported to be present between −130° and 120°C. According to a number of references these transitions are located at approximately −10°C, 40°C, 70°C, 80°C and 100°C (5,6). The first three transitions are assigned to the SC lipids, the one at 80°C originates from protein-associated lipids, whereas the transition at 100°C belongs to the denaturation of protein and can only be observed when the hydration level of SC is high enough (more than 10%) (10).

Dehydrated Samples

The temperature changes of the thermal transitions after the treatments of dehydrated SC with fatty acids dissolved in PG (0.16 M) are presented qualitatively in Figure 2A. After the pre-treatment of PG the lipid transition at −10°C disappeared. Furthermore, the 70° and 80°C transitions observed in untreated SC were shifted to lower temperatures as reported before (6,7) and the total enthalpy changes involved in these two transitions was reduced by 25% (see Table IIA). Dehydrated SC pretreated by SFA/PG showed some transitions in the low temperature region: upon the application of nonanoic acid/PG two transitions at −8° and +2°C were observed, whereas treatment of decanoic acid/PG resulted in only a single peak at −14°C. The enthalpy of this transition after the treatment of decanoic acid was higher than after the treatment of nonanoic acid. Additionally, both fatty acid treatments shifted the transitions at 70° and 80°C to slightly lower temperatures at the same extent. However, in comparison to untreated samples, decanoic acid decreased the sum of the enthalpy changes of these transitions, whereas nonanoic acid increased it.

In contrast to nonanoic and decanoic acids, the native thermal transition of MUFA, e.g. oleic acid (*cis*-9-octadecenoic acid) and *cis*-13-octadecenoic acid, in PG could be recognized in the thermal profile of treated SC. Furthermore, the subzero peak at −10°C, which was absent by the application of PG alone, was again present after these treatments. The temperature of this transition was shifted to a lower value after the treatment of oleic acid/PG, but not after the treatment of 13-octadecenoic

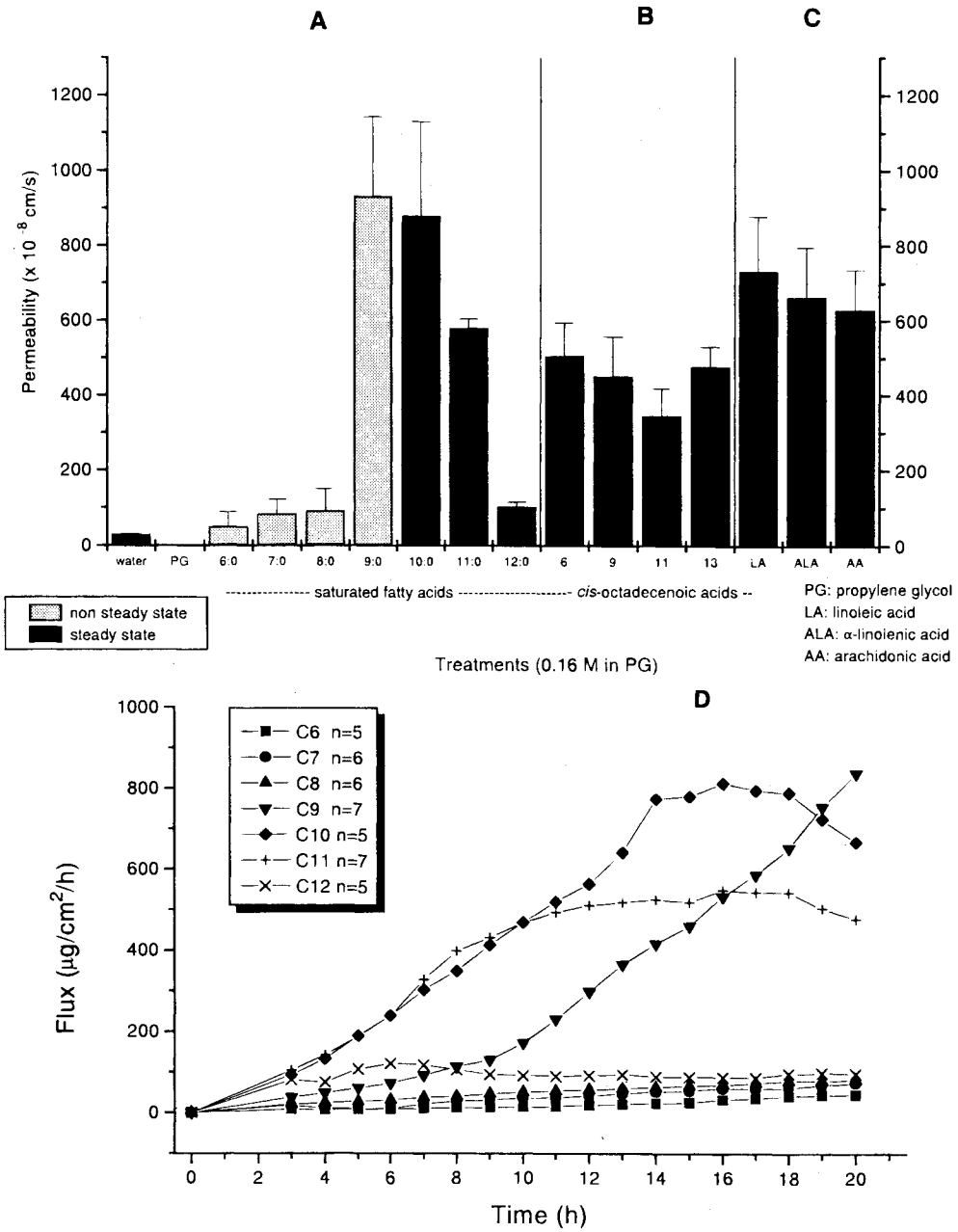

Fig. 1. Permeability of PABA in fatty acid/PG solution across human stratum corneum following the pre-treatment/co-treatment with fatty acid/PG: (A) saturated acids; (B) monounsaturated acids; (C) polyunsaturated acids. (D) Permeation flux of PABA versus time in the presence of saturated fatty acid and PG.

acid in PG. Both treatments tremendously reduced the enthalpy of the transitions at 70° and 80°C.

After the treatment of PUFA, e.g. linoleic and linolenic acids, in PG the transition at −10°C remained present. Additionally, a transition (melting) peak was observed at a lower temperature, that corresponded to the transition of the respective pure acid solutions in PG. The DTA scan of SC treated with linoleic acid in PG also produced an additional peak at −1°C, which was not visible after the α-linolenic acid in PG treatment. The transition temperatures at 70° and 80° were shifted to lower temperatures by linoleic acid in PG to the same extent as by PG alone, but unlike with PG, the enthalpy of the transitions did not change in comparison to the untreated control. After the treatment of α-linolenic acid in PG both transitions almost completely disappeared.

Hydrated Samples

Figure 2B displays the effects of fatty acid treatments on hydrated SC. The thermal behaviour of hydrated SC is characterized by the presence of an additional transition at −20°C caused by the unbound water and an additional transition assigned to protein at +100°C. These transitions were reduced after a 24-hour treatment of PG alone. In contrast to dehydrated

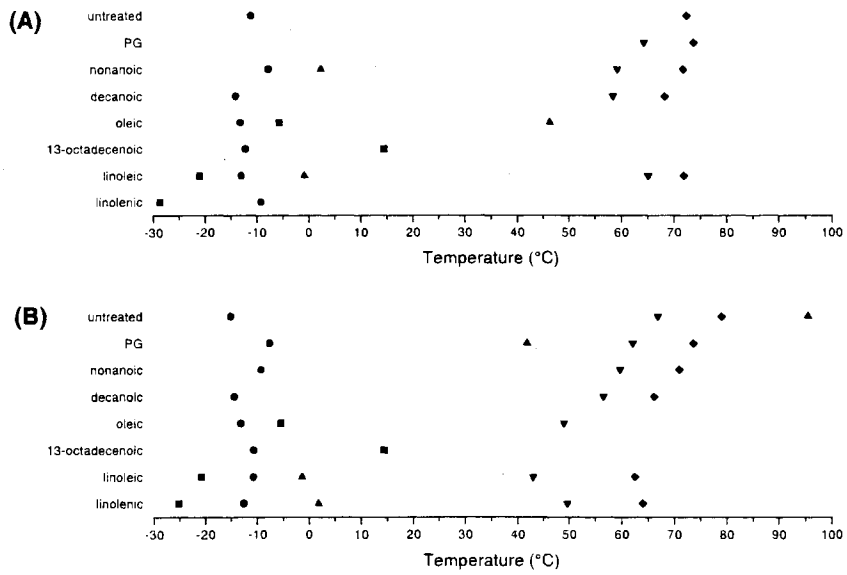

Fig. 2. Transition temperatures of (A) dehydrated and (B) stratum corneum after pretreatments using fatty acids dissolved in propylene glycol (PG) and PG as reference. Different symbols show the classification of transition temperatures: at around (●) −10°C, (▼) 70°C, (◆) 80°C; (■) of pure fatty acid; (▲) additional peak.

SC, PG did not suppress the lipid transition at −10°C in hydrated SC, showing the higher affinity of PG towards water than towards lipids (6). The shift of the transitions at 70° and 80°C to lower temperatures was similar to that observed in the dehydrated samples. Following the treatments of nonanoic and decanoic acids the transition at −10°C was still visible and the transitions at 70° and 80°C were slightly shifted to lower temperatures compared to the effects of PG alone. Decanoic acid increased the enthalpy of transition at −10°C and decreased the enthalpy of transitions at 70° and 80°C, whereas nonanoic acid did the opposite, just as in case of dehydrated SC (see Table IIB).

Treatments of 9- and 13-octadecenoic acids showed consistently the subzero transition at −10°C and the peak based on the transition of pure solutions. The transitions at 70° and 80°C were almost entirely suppressed. This is consistent with the observations on dehydrated samples.

The transitions of linoleic and linolenic acids in PG were also visible next to the transition at −10°C. Additionally, a transition at around 0°C was observed in both treatments. The transitions at 70° and 80°C were shifted to lower temperatures, accompanied with a high enthalpy reduction by α-linolenic acid in comparison to linoleic acid; this was also observed in the dehydrated samples. In general, the results on the hydrated SC are in line with those observed in previous experiments on dehydrated SC.

Table II. Changes in Enthalpy of Transitions$\Delta_i(\Delta H)$ Compared to Control Samples

A. Dehydrated stratum corneum

Treatment	T ≈ −9°C	T ≈ 70° + 80°C
PG	−100	−26.1
nonanoic/PG	−22.4	38.7
decanoic/PG	26.0	−15.4
oleic/PG	−8.4	−100
13-octadecenoic/PG	−27.4	−100
linoleic/PG	−49.6	9.5
α-linolenic/PG	2.1	−100

B. Hydrated stratum corneum

Treatment	T ≈ −9°C	T ≈ 70° +80°C
PG	−96.8	−25.8
nonanoic/PG	−96.1	8.4
decanoic/PG	−93.6	−57.1
oleic/PG	−95.4	−100
13-octadecenoic/PG	−96.8	−100
linoleic/PG	−97.0	−25.0
α-linolenic/PG	−95.9	−72.4

DISCUSSION

Saturated Fatty Acids: Effect of Chain Length

The increase in PABA permeation through human SC in vitro with respect to the chain-length of SFA show a parabolic correlation with a maximum at acids with 9 and 10 carbon atoms, i.e. nonanoic and decanoic acid, respectively. Although in the case of acids with 6 to 8 carbons the steady state of PABA permeation was not reached, it is reasonable to assume that the permeation rate of PABA will not reach the values found for acids with 10 and 11 carbons. A similar correlation has previously been observed in other skin permeation studies. Some of them are compiled in Table III. It has been proposed that the acids with a certain chain-length, i.e. around 12 carbons, possess an optimal balance between partition coefficient or solubility parameter and affinity to skin (11). Shorter chain fatty acids would have insufficient lipophilicity for skin permeation,

Table III. The Parabolic Correlation Between the Permeation Enhancement and the Hydrocarbon Chain-length of Enhancers

	Max.[a]	Remarks
Influence of drugs on enhancement by fatty acids		
p-aminobenzoic acid	9–10	this study
naloxone	9–12[b]	11 not studied
propranolol	12–14[c]	9,11 not studied
tegafur	12[d]	9,11 not studied
indomethacin	12[e]	<12 not studied
Influence of functional head groups		
fatty acids -COOH	9–14	see above
fatty acid salts, *e.g.* -COONa	12[f]	
fatty alcohols -OH	10–12[b]	
azone analogues -$NCO(CH_2)_5$	12[g]	
alkyl trimethylammonium halides - $N^+(CH_3)_3)$ X^-	12[h]	X:halides
Influence of the permeation of pure enhancers		
fatty acids	3[i]	
fatty acid salts, potassium	12[j]	
fatty acid salts, sodium	8[k]	>8 not studied

[a] The hydrocarbon chain-length of enhancers exerting maximum permeation enhancement effects.
[b] B. J. Aungst, N. J. Rogers, E. Shefter. *Int. J. Pharm.* **33**:225–234 (1986).
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[j] F. R. Bettley. *Br. J. Dermatol.* **75**:113–116 (1963).
[k] S. Del Terzo, C. R. Behl, R. A. Nash. *Pharm. Res.* **6**:85–90 (1989).

whereas longer chain fatty acids would have a much higher affinity to lipids in SC and thereby retarding their own permeation and that of other permeants. The parallel effect with the permeation enhancement suggests that the mode of action of SFA as enhancers is dependent on their own penetration across the SC/skin. This enhancement capacity can as well be explained by a "push-pull" mechanism suggested by Kadir *et al.* (12), in which the "push" effect from the excess free energy of the drug in the donor phase containing the fatty acids and the "pull" effect originates from the permeability coefficient of pure fatty acids across the skin. An optimum balance between the "pull" and the "push" forces will produce an optimum enhancement effect. This can explain the phenomenon that acids with 10 or more carbons produce a steady enhanced flux of the permeant—in this study PABA—while the ones with 9 or less carbons cause a steady increase in permeation flux (within the 21-hour period). This phenomenon cannot be explained only by the change of lipophilicity (leading to the change in thermodynamic activity) in the donor solution containing the permeant and the fatty acids, as the change of lipophilicity has a linear correlation with the fatty acid chain length. The limit of permeability is apparently reached in the fatty acid with 10 carbon atoms, whereby the lipophilicity (for the penetration into the skin lipids) is high, but the affinity to lipids also becomes high enough to retard the flux. In this situation, the assumption supporting the "push" mechanism, i.e. the vehicle does not perturb the skin, is no longer valid. By a decreasing "pull" force, the enhancing effect decreases, too.

It was observed by thermal analysis, that when applied to human SC, SFA dissolved in PG do not produce a distinctive extra transition of pure solution to the lipid phase transitions of SC. This suggests that these acids are not present in large separated domains in SC. The temperature decrease of the SC phase transitions at 70° and 80°C induced by these fatty acids may give the clue that these acids predominantly modulate the gel-liquid transformation properties and/or lamellar ordering of SC lipids. The increase in transition enthalpies induced by nonanoic acid and the decrease in enthalpy induced by decanoic acid cannot satisfactorily be explained yet, since there is no supporting evidence available from other members containing less or more carbon atoms.

Mono-Unsaturated Fatty Acids: Effect of the Position of Double Bond

cis-Octadecenoic acid series, especially oleic acid (with a double bond at 9th position), has been the focus of many investigations since the 1980's (13). The double bond has been proposed to cause the formation of "holes" or "kinks" in the lipid structure to allow water permeation across the skin (14). The "kinks", as mobile free volumes, allow small molecules to migrate across a hydrocarbon phase of a membrane together with them (15). The position of this "kink" along the chain may have influence on the mobility of the alkyl chain in the hydrocarbon phase. In this study, however, the permeation of PABA across SC was enhanced by MUFA to a similar extent. The similarity in the enhancing capability among these acids is interesting knowing that there is a relatively big difference of physical properties among the acids, *e.g.* the difference of melting points between oleic acid (*cis*-9-octadecenoic acid) and *cis*-13-octadecenoic acid is 20°C. The results imply that only the presence of a double bond, and not its position, counts for the enhancement capacity. Thermal analysis also shows that these acids exhibit the same changes in the thermal behaviour of SC. Both oleic and 13-octadecenoic acids form separate domains resulting in a transition peak of pure fatty acid solution (in PG). The reduction in the enthalpy change of the lipid transitions at 70° and 80°C suggests that the lipid organization is strongly perturbed. The formation of separate domains by oleic acid within SC lipids has been reported (16) and visualized (17). These facts can explain the mode of action of MUFA as penetration enhancers.

Polyunsaturated Fatty Acids: Effects of the Number of Double Bonds

The enhancement factor of PUFA in PG to the permeation of PABA across human SC was found to be at least 20. The highest enhancement factor was achieved by linoleic acid which contains two double bonds. Addition of more double bonds slightly decreased the enhancement ratio. The similar relationship between the number of double bonds and the enhancement ratio of fatty acids have been reported for naloxone (18) and alprazolam (19). Polyunsaturated fatty alcohols have also been found to show a similar enhancement pattern (18).

Thermal analysis of human SC following the treatment of linoleic and linolenic acid shows a moderate suppression of the

lipid phase transitions at 70° and 80°C and the appearance of the transition peak of the native acids in PG solution. The dissimilarity of the effects on the enthalpy of transitions at 70° and 80°C between these two acids indicates that the PUFA do not work in the same way, although the permeation studies showed a similar enhancement capacity.

It has been suggested that fatty acids with 20 or more carbons are only slightly more hydrophobic than those with 18 carbons (20), implying that the change in physical properties of long chain acids is not as pronounced as that in the shorter chain ones. This fact may be the reason that arachidonic acid with 20 carbons yields a comparable enhancement effect as the 18-carbon acids.

Correlation of Skin Permeation Enhancement to Skin Perturbation

An attempt has been made to correlate the enhancing effect and the skin perturbation effect of the fatty acids involved in this study. The permeation enhancement effect can be represented by the permeability coefficient, K_p, of PABA in the presence of the respective fatty acids and the thermal analysis by the change of enthalpy, $\Delta_r(\Delta H)$, which is calculated according to the following equation:

$$\Delta_r(\Delta H) = \frac{\Delta H_{\text{treated}} - \Delta H_{\text{untreated}}}{\Delta H_{\text{untreated}}} \times 100\%$$

Figure 3A and 3B show the plot of these two parameters in dehydrated and hydrated SC, respectively. A correlation coefficient $r = 0.86$ was found between the combined change in enthalpy of the lipid phase transitions at 70° and 80°C and the permeability coefficients, suggesting a rather strong relation between the lipid perturbation and enhancing effect. The higher the perturbation, the lower the enhancing effect. The following explanation may be given for this fact: A smaller decrease in enthalpy allows a higher enhancement of the PABA flux, whereas a larger decrease in enthalpy, related to higher perturbation, may hinder the penetration. This may indicate that in a high perturbed skin lipid system, the permeant may be trapped inside and therefore may have a lower capability to permeate through the skin. While this may only happen in the case of PABA, it can give an important message that the high degree of perturbation by an enhancer in the skin lipid organization may not always result in a better performance of the enhancer.

CONCLUSIONS

The enhancement effects of fatty acids on the penetration of PABA through human SC are structure-dependent. SFA show a parabolic pattern of enhancement, with a maximum at nonanoic-decanoic acids, as an indication that a balance exists between the permeability of pure acids and the affinity to skin lipids. These acids easily mix with the skin lipids and their effects on the thermal behaviour of SC are hardly noticeable. Homologous series of MUFA enhance the PABA flux to about the same extent. These acids have in common the formation of separate domains containing pure acids and suppressing the skin lipid phase transitions at 70° and 80°C. PUFA enhance

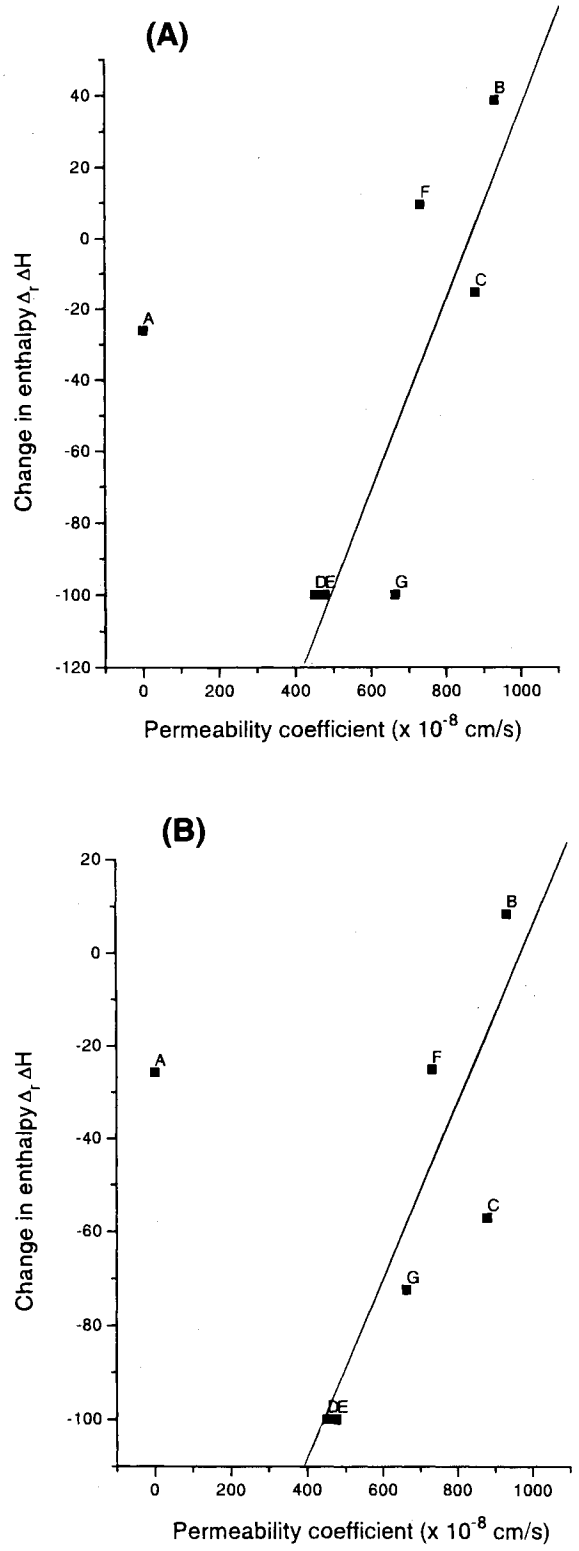

Fig. 3. Correlation between permeability coefficient, K_p, against combined change in enthalpy, $\Delta_r(\Delta H)$, of the transitions at 70° and 80°C in (A) dehydrated and (B) hydrated SC. Linear regression: (PG not included) (A) $f(x) = 0.27\,x - 232.92$; $r = 0.8640$ and (B) $f(x) = 0.19\,x - 185.68$; $r = 0.8623$. A: PG, B: nonanoic, C: decanoic, D: oleic, E: *cis*-13-octadecenoic, F: linoleic, and G: α-linolenic acid.

more than MUFA, but additional double bonds do not further elevate the degree of enhancement. Their effects on thermal behaviour of SC are comparable to MUFA, except they do not completely suppress the phase transition peaks at 70° and 80°C.

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