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In vitro and in vivo enhancement of skin permeation with oleic and lauric acids

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Summary

The effect of two fatty acids (oleic and lauric) on the transport of the cationic drug naphazoline, neutral caffeine, and an anionic model drug salicylate, across excised human skin was studied using Franz diffusion cells. Oleic and lauric acids both increased the in vitro skin permeation of all penetrants. Oil/water partitioning data and rotating diffusion cell measurements, in the presence of the fatty acids, suggested that the enhanced flux of the cationic naphazoline could be accounted for by an increase in lipophilicity through ion pairing with the carboxylate anion of the acid. Caffeine and sodium salicylate were incapable of forming ion pairs consequently, increases in skin permeability are also due to a disruption of the stratum corneum. This conclusion was further supported by (a) increased transepidermal water loss, and (b) increased in-vivo skin permeation of the non ion-pairing methyl nicotinate, at skin sites pretreated with the fatty acids.

Introduction

For drugs to be effective topical agents they should penetrate the skin as readily as possible. In transdermal drug delivery the excellent barrier properties of skin often limit the amount of drug that can penetrate. Since many drugs are weak acids or bases, they exist as charged species at physiological pH and their partitioning into skin is hindered by their intrinsic charge. Passive diffusion of charged molecules across a membrane as lipophilic as the stratum corneum (the outermost layer of skin) is a slow process. It is possible to reduce the resistance to transdermal flux either by disrupting the skin structure itself or by increasing the lipophilicity of the ionised permeant, eg. through ion-pair formation (Barker and Hadgraft, 1981).

Oleic acid and lauric acid have previously been employed to increase the permeation of polar and non-polar compounds across skin (Aungst et al, 1986; Cooper, 1984). Thermal analysis (differential scanning calorimetry) of human skin, following pretreatment with oleic acid, reveals a decrease in the temperature of phase transitions associated with stratum corneum lipids (Golden et al., 1987). This fluidisation process may well explain the oleic acid induced increase in stratum corneum penetration of a number of molecules.

Oleic acid and lauric acid can also facilitate the flux of cationic β-blockers across a lipoidal membrane (Green and Hadgraft, 1987). At pH values above the pK_a, the fatty acid present at the membrane surface will ionise and the subsequently formed carboxylate anion will form an ion pair with the cationic permeant arriving from the aque-

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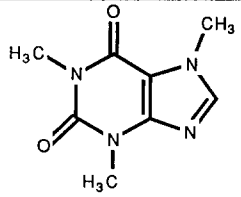
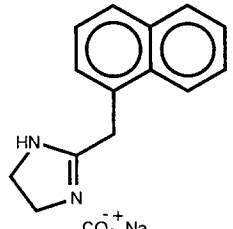
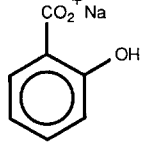
TABLE 1

Chemical structure pKa and spectroscopic data for the model permeants

Compound	Structure	p*K*a (Moffat, 1986)	Wavelength of maximum absorption (nm)
Caffeine		< 1.0 [a]	273
Naphazoline		10.9	280
Sodium salicylate		3.0	298

[a] Perrins (1965).

ous donor phase. It is possible, therefore, that oleic acid exerts its 'enhancing action' on the skin permeation of cationic molecules in a two-fold manner, i.e. through fluidisation of the intercellular skin lipids and by ion pair formation.

In this study in vitro and in vivo methods were employed to assess the effect of two fatty acids on the skin permeation of 4 model drugs. Physicochemical data from the rotating diffusion cell (RDC) and from oil/water pH partition studies were used to identify ion pairing between the fatty acids and the various permeants. The model permeants chosen for in vitro skin diffusion studies included the base naphazoline, the weak base caffeine, and the anionic substrate salicylate (Table 1). Each drug was chosen because of its ease of analysis and because each exists predominantly in a charged or uncharged form at pH values used in the experiment. Methyl nicotinate, a neutral topical vasodilator, was employed for monitoring in-vivo skin permeation. This compound crosses the skin relatively easily and causes erythema, the time course and magnitude of which can be conveniently followed using laser Doppler velocimetry (LDV) (Guy et al., 1983).

Further experiments, to assess possible disruption to the barrier function of the skin caused by oleic acid and lauric acid measured, their effect on transepidermal water loss (TEWL). TEWL has previously been employed to show perturbations of the stratum corneum after pretreatment with surfactants (Murahata et al., 1986; Van der Valk et al., 1984) and other penetration enhancers such as dimethylsulphoxide, dimethylformamide and dimethylacetamide (Baker, 1968).

Materials and Methods

Materials

Lauric acid was specially pure biochemical grade; formic acid and sodium salicylate were

GPR grade; potassium dihydrogen phosphate and acetic acid were AnalaR grade. All reagents were supplied by BDH, with the exception of HPLC grade methanol and isopropyl alcohol (Rathburns Chemicals Ltd), heptane sulphonic acid (Fisons reagent). Methyl nicotinate and caffeine B.P anhydrous were supplied by Sigma Chemical Company and May and Baker, respectively. Oleic acid (99 + % Gold label) and naphazoline hydrochloride were obtained from Aldrich Ltd. Isopropyl myristate was supplied by Croda Chemicals Ltd. Cellulose nitrate 0.2 μm pore size membrane filters were obtained from Whatman Ltd.

Methods

Measurement of distribution coefficients

The partitioning of each drug between isopropyl myristate (IPM) and aqueous phosphate–citrate buffer (McIlvane, 1980), at 32°C, was studied using a filter probe technique (Tomlinson, 1982) at pH 8.0 and 7.4. The ionic strength of each buffer was adjusted to 0.6 M with sodium chloride and was presaturated with IPM overnight before use. The drug concentrations in the aqueous phase were continually monitored spectrophotometrically via a flow cell, set at the appropriate wavelength (Table 1).

The distribution coefficient (D_c) of each drug was calculated using Eqn. 1:

$$D_c = \frac{C_0 - C_t}{C_t} \frac{V_{aq}}{V_{org}} \quad (1)$$

where C_0 and C_t were the concentrations in the aqueous phase initially and after partitioning, respectively. V_{aq} and V_{org} were the volumes of phosphate–citrate buffer and IPM respectively.

The effect of fatty acids on the oil/water distribution coefficient of each solute was determined by the addition of oleic acid and lauric acid to the IPM phase at a concentration of 0.1 M. Four determinations of the distribution coefficients were made for each drug.

Determination of model membrane permeability coefficients

Certain cations, in the presence of oleic acid and lauric acid, can be transported across a lipoidal membrane. This facilitated transfer has been shown to occur when the pH of the donor phase (a) is greater than the pKa of the acid, and (b) exceeds that of the receptor (Green and Hadgraft, 1987). In this subsequent study on the diffusion of model drugs we have utilised previous experimental conditions (Fig. 1) required for the facilitated transport mechanism to operate. Under these conditions the protonated basic drug cation HB^+ ion-pairs with the carboxylate anion of the fatty acid (A^-) at the donor/membrane interface. The neutral ion pair then diffuses down its concentration gradient to the opposite interface where it dissociates and releases the cation.

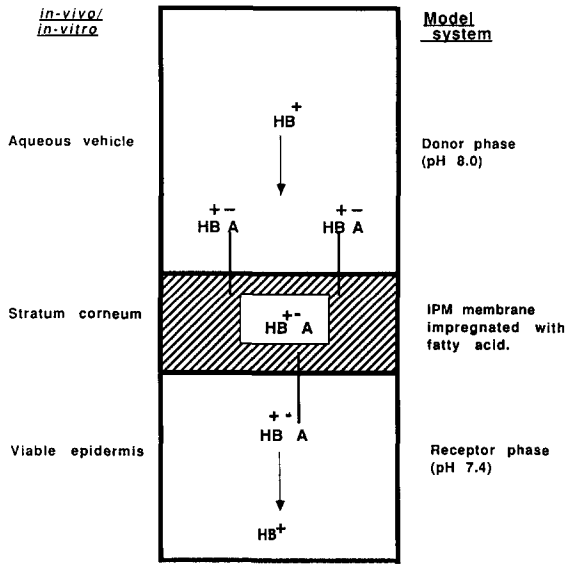


Fig. 1. Conditions required to facilitate the transport of a cation (HB^+), from an aqueous donor phase across an IPM membrane impregnated with fatty acid (A^-), to an aqueous receptor phase.

The transfer of each molecule from the aqueous phosphate-buffered donor phase at pH 8.0 (40 cm^3), across an IPM membrane, into the pH 7.4 phosphate-buffered receptor phase (100 cm^3) was measured using the RDC (Albery et al., 1976). The experimental techniques have been described by Hadgraft et al. (1985). The membrane was a 0.2 μm cellulose nitrate membrane filter impregnated with IPM. The fatty acids were incorporated into the membrane as 0.1 M solutions in IPM. All experiments were performed at 32°C.

The steady-state flux (J) of the drug across the membrane is given by

$$J = kAC_d$$

where A is the area of the filter, C_d the concentration of the drug in the donor compartment and k the apparent permeability coefficient.

Preparation of full-thickness skin membranes

Full-thickness human skin was obtained at autopsy from the abdominal region. The average age of the caucasian donors was 79 ± 12 years. The subcutaneous fat was removed from the dermis by blunt dissection and swabbing with pH 7.4 phosphate-buffered saline. The stratum corneum was cleaned gently using pH 8.0 phosphate-citrate buffer. The skin was either used immediately or stored frozen for a maximum of 1 week before use.

Measurement of skin permeation

The diffusion rates of each compound across excised human cadaver skin were measured using a diffusion cell based on the Franz design (Franz, 1975). Full-thickness skin was placed, dermal side down, between the donor and receptor phase of the diffusion cell (cross-sectional area 1.8 cm^2) and clamped into position. The receptor phase was approximately 4.5 cm^3 pH 7.4 phosphate-buffered saline, containing 0.001% phenylmercuric nitrate as preservative. The receptor phase was degassed prior to use by filtration through a 0.45 μm Millipore filter. The cells were allowed to pre-equilibrate for 1 h in a water bath at 37 °C. 50 μl of an ethanolic solution of the fatty acid (0.1 M) was applied to the surface of the skin and left in contact for 2 h. After this period, the alcohol had evaporated to leave the fatty acid deposited in the outer layers of the skin. The drug was then added to the covered donor compartment as a 0.05 M (caffeine 0.01 M) solution (1 ml) in pH 8.0 phosphate-citrate buffer. Control cells were set up in which the skin surface was treated with 50 μl of ethanol.

Samples (0.5 ml) were removed from the receptor phase at regular intervals and replaced by fresh prethermostatted pH 7.4 phosphate-buffered saline. The samples were stored protected from light until assay by HPLC. Experiments were performed in triplicate using skin samples from the same donor.

The amount of drug diffusing across the skin was plotted as a function of time and linear regression analysis was used to determine the gradient of the steady state portion of the profile.

Eqn. 2 was used to determine permeability coefficients, K_p (cm h^{-1})

$$J = K_p A C_d \quad (2)$$

where J (mol hr^{-1}) is the steady state flux of the permeant traversing the skin of cross-sectional area A cm^2 and C_d is the drug concentration in the donor phase.

High performance liquid chromatography (HPLC)

Drug concentrations in the receptor phase of the skin diffusion cell were determined using HPLC. The analytical system consisted of a fixed volume Rheodyne sample injector with a 100 μl loop, and an LDC computing integrator and a UV detector (LDC Spectromonitor III, Milton Roy) set at the appropriate wavelength specified in Table 1. A dual piston pump (model IIIG constametric, LDC) was used to propel the mobile phase through an Apex 1, 5μm particle size octadecyl silica reverse-phase chromatographic 250 mm × 4.6 mm i.d. column (Jones chromatography). Table 2 lists the other chromatographic parameters.

Transepidermal water loss (TEWL)

For each subject 3 points on the mid-line of the volar aspect of the left and right forearm, approximately 5 cm apart were identified. The subjects were normal, healthy adults aged between 22 and 33 years from whom informed consent (approved by the UCSF Committee on Human Research) was obtained. 10 μl of oleic acid was applied to one site of each arm; the site was covered by a Hill-top chamber (Hill-top Research), cross-sectional area 2.5 cm^2, from which the inner absorbent pad had been removed. The pretreated sites were covered in order to localise and prevent accidental removal of the fatty acid. The control was a similar Hill-top chamber covering an untreated adjacent site. The patches were removed

after 4 h and any remaining oleic acid or perspiration was gently removed with a tissue. The sites were left unoccluded for a further hour. After this period, transepidermal water loss (TEWL) was determined at pretreated, control and at (the third) adjacent uncovered (baseline) sites on both arms, using a model EP1C evaporimeter (Servomed, Sweden). The evaporimeter measures the vapour pressure gradient of water adjacent to the skin surface (Nilsson, 1977). The evaporation rate of water is then computed directly from Fick's law of diffusion. The unventilated Teflon capsule, situated at the end of the probe, contains the sensors for measuring partial water pressure. The capsule is placed on the skin sites and the probe gently taped into position. A period of 2 to 5 minutes was allowed for stabilisation of the evaporimeter. Prior to the TEWL measurements, subjects were seated in a room of reasonably constant temperature ($23 \pm 2°$ C) for 15 min. During this period, skin temperature and ambient relative humidity were monitored throughout and were found to be (mean $\pm$ S.D.) $32 \pm 2°$ C and $45 \pm 10\%$, respectively. In additional experiments 10 μl lauric acid (0.5 M in acetone) was applied. Control sites were treated with 10 μl acetone. The top section of the applied Hill–top chambers were removed in order to allow evaporation of the acetone. The patches were removed after 4 h and the sites gently wiped. TEWL was determined immediately afterwards.

Laser Doppler velocimetry (LDV)

An aqueous solution of methyl nicotinate (10 mM) was applied for 15 s to a ventral forearm site via 1 cm diameter Al-test circular patches (Imeco ab, Sodertasse, Sweden). The onset of action, time to peak response and peak height of the subsequent erythemal responses (Ryatt et al., 1986) were measured using a laser Doppler flowmeter (Perimed, Sweden). In this non-invasive technique, a 5 mW helium–neon laser produces a monochromatic light source at a wavelength of 632.9 nm. The light is carried to and from the skin surface via a fibre optic within a cylindrical probe. The monochromatic light is reflected back by red blood cells moving through the dermal microcirculation and by stationary tissue. Light striking moving red blood cells is reflected back at a Doppler-shifted frequency proportional to the number of erythrocytes multiplied by their velocity. The velocimeter converts the Doppler-shifted component of the backscattered radiation to a voltage output. This parameter correlates linearly with the cutaneous blood flow (Stern et al., 1977).

In the study design, the order of experiments was such that tolerance to, or potentiation of, nicotinate effects would be highlighted. There were, furthermore, at least 5 days between successive nicotinate application on the same subject. No systematic nor significant attenuation or enhancement of vasodilator effect was detected in the subjects considered.

The results of the TEWL and LDV experiments were analysed using a two column paired *t*-test, with each subject serving as its own control.

Results and Discussion

The permeation of all model drugs across excised human skin was enhanced in the presence of the fatty acids (Fig. 2). The increase in the permeability coefficient of naphazoline (Fig. 3a) can be accounted for in part by the partitioning data in Table 3. The cation ion pairs with the carboxylate anion of the fatty acids and causes, consequently, an increase in partitioning into the oil phase. The data in Table 4 show the effectiveness of lauric acid and oleic acid in facilitating, by an ion pair mechanism, the transfer of naphazoline across an IPM membrane in the RDC.

The movement of sodium salicyclate and caffeine, from an aqueous phase, into and across IPM was unaffected by the fatty acids. This was expected with salicylate since it cannot ion-pair with the similarly charged carboxylate anion. Caffeine is neutral at pH 8.0 and its inability to form ion-pairs with oleic acid and lauric acid is also expected. The ability of the fatty acids to facilitate the transport of sodium salicylate (Fig. 3b) and caffeine (Fig. 3c) across human skin appears not to involve a change in physicochemical properties of the permeant, therefore, but rather an interference in the barrier property of the skin itself.

TABLE 2

Reverse-phase chromatographic conditions required to analyse skin permeants

Drug	Mobile Phase	Detector wavelength (nm)	Flow rate (ml/min)	Retention time (min)
Naphazoline	0.005 M heptane sulphonic acid in 950 ml methanol, 5 ml acetic acid and 45 ml distilled water	280	2.5	3.7
Caffeine	0.005 M heptane sulphonic acid in 400 ml methanol, 5 ml acetic acid and 595 ml distilled water	273	1.0	4.0
Sodium salicylate	342 ml isopropyl alcohol, 10 ml formic acid to 1 litre with 0.1 M potassium dihydrogen orthophosphate (aq)	298	1.0	4.5

Pretreatment of skin in vivo with both fatty acids produced a small but significant increase ($P < 0.05$) in TEWL (Table 5). This is indicative of perturbation to the barrier function of the stratum corneum. Further evidence for a disruption of the horny layer is provided by the LDV study. In this experiment it was found that application of the neutral, and hence non ion-pairing, methyl nicotinate (Table 3) to sites pretreated with the fatty acids, resulted in a significant decrease ($P < 0.05$) in (a) time of onset of erythema, and (b) time to peak response, and a significant increase ($P < 0.05$) in the intensity of the resulting erythema (Table 6). These results imply an increase in the rate and extent of methyl nicotinate penetration following skin pretreatment with the fatty acid. Application of oleic and lauric acid alone did not cause an increase in the LDV reading. In two subjects (results not included in Table 6) the distinctive erythema was only observed at sites pretreated with oleic acid. These subjects were not used in the lauric acid study. No significant difference between LDV measurement of erythema was noted for left and right arms. In

TABLE 3

Effect of oleic and lauric acid on the oil–water distribution coefficients (D_c) (mean ± S.D.) between IPM and phosphate–citrate buffer at 32 °C

Compound	IPM/buffer (pH 7.4)	Oleic acid in IPM/buffer (pH 7.4)	Lauric acid in IPM/buffer (pH 7.4)	IPM/buffer (pH 8.0)
Caffeine	0.12 ± 0.02	0.11 ± 0.01	0.12 ± 0.02	0.12 ± 0.02
Naphazoline hydrochloride	0.02 ± 0.01	0.36 ± 0.04	0.45 ± 0.06	0.03 ± 0.01
Sodium salicylate	N.D.	N.D.	N.D.	N.D.
Methyl nicotinate	2.26 ± 0.21	2.19 ± 0.20	2.40 ± 0.27	2.36 ± 0.26

Distribution coefficients for IPM were not determined with the fatty acids above pH 7.4 since this caused emulsification of the aqueous phase. N.D., Not detectable

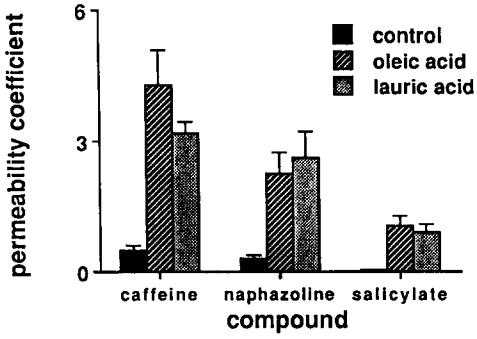


Fig. 2. Effect of oleic acid and lauric acid on the mean permeability coefficient (K_p) (cm $h^{-1} \times 10^{-3}$) ± S.D. of various drugs across excised human skin (donor phase: 0.05M, pH 8.0 phosphate–citrate buffer; receptor phase: pH 7.4 phosphate-buffered saline).

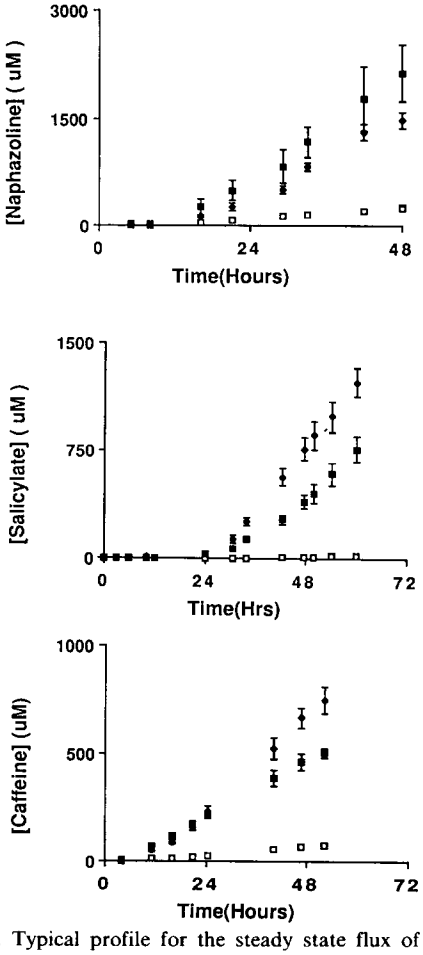


Fig. 3. Typical profile for the steady state flux of (a) naphazoline, (b) salicylate and (c) caffeine across human skin (□) and skin pretreated with 0.1 M oleic acid (◆) and 0.1 M lauric acid (■). Each value is the mean ± S.D.

TABLE 4

Effect of fatty acids on the mean apparent permeability coefficient (k) of various cationic drugs (concentration in donor phase, 25 mM) across an IPM membrane in the RDC

Drug	Membrane constituent		
	IPM	0.1 M oleic acid in IPM	0.1 M lauric acid in IPM
Caffeine	111.7 ± 15.1	125.9 ± 18.0	104.6 ± 12.9
Naphazoline hydrochloride	6.7 ± 0.8 [a]	185.6 ± 18.9	201.0 ± 32.7
Sodium salicylate [a]	N.D	N.D	N.D

Values are in $nM \cdot s^{-1}$, mean ± S.D. N.D: Transport not detectable

[a] Starting concentration in the donor phase 0.3 M.

addition, there was no significant difference in TEWL or LDV readings for control and baseline sites of each arm. These results suggest that any occlusive effect resulting from application of the Hilltop chamber, has dissipated after the 1 h removal period.

Oleic acid appears to be a better enhancer than lauric acid. The *cis* double bond at C_9 in oleic acid (*cis*-9-octadecenoic acid) causes a kink in the alkyl chain (Fig. 4), which is more likely than lauric acid to disrupt the ordered array of the predominantly saturated straight chain skin lipids (Golden et al. 1987).

In conclusion, the data reported here suggest that oleic acid and lauric acid can be employed to

TABLE 5

The effect of oleic acid and lauric acid on TEWL (g/m^2h)

	Mean TEWL (± S.D) of control site	Mean TEWL (± S.D) of pretreated site	*n*
Oleic acid			
Right arm	3.0 ± 0.7	4.1 ± 0.7	13 [a]
Left arm	3.0 ± 0.6	3.9 ± 0.9	13 [a]
Lauric acid			
Right arm	2.9 ± 0.8	3.6 ± 0.8	8 [b]
Left arm	2.8 ± 0.8	3.5 ± 1.0	8 [b]

[a] 8 females, 5 males, age 26 ± 5 years

[b] 5 females, 3 males, age 27 ± 5 years.

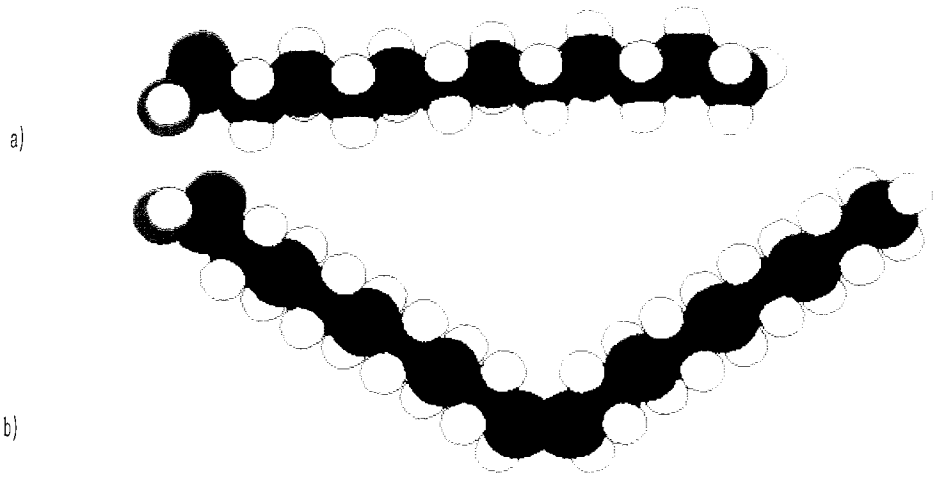


Fig. 4. Chemical structure of (a) lauric acid (b) oleic acid. Oxygen atoms are coloured grey, carbon black and hydrogen white.

TABLE 6

LDV-assessed effects of oleic acid and lauric acid on the vasodilative response (mean ± S.D) of methyl nicotinate

	Onset of action (min)		Time to peak response (min)		Peak Height (mV)		*n*
	Control	Pretreated	Control	Pretreated	Control	Pretreated	
Oleic acid							
Right arm	7.4 ± 4.6	2.4 ± 1.0	11.6 ± 4.8	6.6 ± 2.1	523 ± 340	773 ± 280	8 [a]
Left arm	7.1 ± 3.0	2.5 ± 1.2	10.6 ± 3.8	6.7 ± 1.6	393 ± 227	701 ± 228	
Lauric acid							
Right arm	4.4 ± 1.4	2.1 ± 1.0	8.9 ± 1.3	6.0 ± 1.7	494 ± 304	658 ± 361	8 [b]
Left arm	4.7 ± 2.2	2.0 ± 0.9	9.0 ± 3.0	6.4 ± 2.1	350 ± 235	636 ± 317	

[a] 6 females, 2 males age 25 ± 3 years.
[b] 5 females, 3 males age 27 ± 4 years.

increase the permeability of human skin to a number of charged and uncharged molecules. This appears to be due to disruption of the stratum corneum structure. However, in the case of naphazoline, penetration enhancement may additionally be caused by ion pair formation with the fatty acid and a consequent increase in partitioning into the stratum corneum.

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