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Report

Structure/Effect Studies of Fatty Acid Isomers as Skin Penetration Enhancers and Skin Irritants

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Comparisons were made of branched vs unbranched saturated fatty acids and *cis* vs *trans* unsaturated fatty acids as skin penetration enhancers and primary skin irritants. Skin penetration studies used naloxone base as the diffusant, propylene glycol as the vehicle, and human skin. Maximum naloxone flux was with C_{9-12}-branched and unbranched fatty acids. For C_{5-14} fatty acids, branched and unbranched isomers had similar effects. One branched C_{18} fatty acid isomer (C_{16}-branched isostearic acid) was more effective in enhancing skin penetration than a differently branched (C_2-branched isostearic acid) or unbranched C_{18} isomer (stearic acid). There was no significant difference between *cis* and *trans* unsaturated C_{16-18} fatty acid isomers in their effects on naloxone flux, and all unsaturated fatty acids were more effective enhancers than the corresponding saturated isomers. Several of these fatty acid/propylene glycol vehicles were evaluated in a rabbit primary skin irritation test. Irritation indices were poorly correlated with the effectiveness of the vehicles in enhancing naloxone flux. It was possible to enhance naloxone skin penetration greatly with a vehicle with only minimal skin irritation potential.

KEY WORDS: penetration enhancer; absorption promoter; fatty acids; skin penetration; skin irritation.

INTRODUCTION

Fatty acids can have potent skin penetration enhancing effects. Drugs that have shown increased skin permeability in the presence of fatty acids include salicylic acid (1), acyclovir (2), betamethasone-17-benzoate (3), mannitol and hydrocortisone (4), naloxone (5), indomethacin (6), nitroglycerin (7), and others. The mechanism by which fatty acids increase skin permeability appears to involve disruption of the densely packed lipids that fill the extracellular spaces of the stratum corneum. Alteration of the physical structure of stratum corneum lipids can be assessed using differential scanning calorimetric (DSC) and infrared spectroscopic (IR) techniques (8). Oleic acid treatment of stratum corneum decreased the phase transition temperatures of the lipids, as shown by DSC and IR, indicating increased motional freedom or fluidity of the lipids (9). Those changes were proportional to changes in permeability to salicylic acid (9).

The lipids in the intercellular spaces of the normal stratum corneum are arranged in multiple bilayers and consist mostly of ceramides, free fatty acids, triglycerides, and sterols (10,11). The acyl groups of the ceramides, free fatty acids, and triglycerides are principally 16 or more carbon atoms in chain length and are saturated (10,11). This affords close packing of the hydrophobic tails within the bilayer structure. It was proposed that because of its kinked structure (the kink due to the *cis* double bond), oleic acid creates gaps in the packed lipid structure and thus reduces diffusional resistance (12). Studies using model phospholipid membranes in which the acyl groups were long, saturated chains have similarly shown packing disruption upon incorporation of lipids with unsaturated acyl groups (13,14). Other studies have shown that packed lipid structures are also disrupted by shorter, saturated acyl chains or those with branched side chains (15,16).

Most of the studies on fatty acid penetration enhancers have focused on oleic acid. However, in two studies (5,17) it was shown that the most effective saturated fatty acids were those with C_{10}–C_{12} chain lengths, and for C_{18} fatty acids unsaturation increased the penetration enhancing effects. These structure/effect relationships are consistent with the model membrane studies and suggest that short acyl chains or unsaturated acyl chains may disrupt the packing of the long, saturated acyl chains of stratum corneum lipids. In this report the effects of linear and branched isomeric forms of fatty acids of various chain lengths were compared, as it was expected that branched isomers could be more disruptive of membrane lipid packing. Branched fatty acids have not been previously reported as membrane penetration enhancers. To study further the effects of fatty acid isomer structure on skin permeability, the effects of *cis* and *trans* isomers of several unsaturated fatty acids were also compared.

A second aspect of these studies was to compare the skin irritation potential of fatty acid isomers and to see whether penetration enhancement is related to irritation. This is very important, since practical use of penetration

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enhancers requires careful balancing of their benefits and risks, i.e., penetration rates and irritation.

MATERIALS AND METHODS

Materials. Sources and structures of the linear and branched saturated fatty acids are given in Fig. 1. The unsaturated fatty acids, palmitoleic, palmitelaidic, elaidic, linoleic, and linoelaidic acids were all obtained from Sigma. Oleic acid was from Emery (Emersol 213). Naloxone base was from DuPont Pharmaceuticals.

Skin Penetration. In vitro skin penetration rates were measured using glass diffusion cells in which human skin was clamped into a position separating donor and reservoir compartments. The reservoir was stirred and maintained at 37°C using a circulating water jacket or a dry block heater. The reservoir solution was saline. The entire reservoir volume (7–9 ml) was removed at the sampling times and replaced with drug-free saline. The donor vehicle volume was 0.5 ml. The donor chamber was sealed from the atmosphere with Parafilm. The diffusional surface area was 1.8 cm^2. Human skin specimens, dermatomed to an approximate thickness of 0.4 mm, were obtained from an organ bank. The skin donor population was comprised of 11 males and 2 females, of which 11 were Caucasian. The average (±SD) age was 31 ± 15 years. Skin was from the thigh and calf areas.

The vehicles used were propylene glycol and either 0.5 *M* or 10% fatty acid in propylene glycol. The fatty acid/propylene glycol mixtures were prepared at 50–60°C to melt and dissolve the adjuvant. Clear solutions were obtained, except for myristic acid, stearic acid, and isostearic acid (C_2 branch), each of which was semisolid at room temperature. Naloxone base was then added to these vehicles in excess of saturated solubility to maximize skin penetration. These suspensions were used for skin penetration experiments. Naloxone concentrations in the reservoir were determined using high-performance liquid chromatography (HPLC), as previously described (5). Naloxone flux was calculated as the slope of the linear portion of amount transferred vs time plots and this was normalized to a 1-cm^2 surface area. Statistical comparisons of average flux values were done using *t* tests. There were three to seven diffusion experiments per group.

Linear	Branched
Valeric $CH_3(CH_2)_3COOH$ (Sigma)	Isovaleric $(CH_3)_2CHCH_2COOH$ (Sigma)
	"Neopentanoic" $(CH_3)_3CCOOH$ (Exxon)
Heptanoic $CH_3(CH_2)_5COOH$ (Celanese)	"Neoheptanoic" (Exxon) See "Neodecanoic," with $R=(CH_2)_2CH_3$
Pelargonic $CH_3(CH_2)_7COOH$ (Celanese)	"Neononanoic" (Exxon) See "Neodecanoic," with $R=(CH_2)_4CH_3$
	Trimethylhexanoic (CTC) $(CH_3)_3CCH_2CH(CH_3)CH_2COOH$
Capric $CH_3(CH_2)_8COOH$ (Sigma)	"Neodecanoic" (Exxon) $R-C(R')(R'')-COOH$ where, $R=(CH_2)_5CH_3$, $R',R''=CH_3$ } 31%; $R<(CH_2)_5CH_3$, $R''>CH_3$, $R'=CH_3$ } 67%
Lauric $CH_3(CH_2)_{10}COOH$ (Sigma)	"Neo 12-14" (Exxon) See "Neodecanoic", with $R=(CH_2)_{7\text{-}9}CH_3$
Myristic $CH_3(CH_2)_{12}COOH$ (Emery)	
Stearic $CH_3(CH_2)_{16}COOH$ (Sigma)	Isostearic (C_2-branch, CTC) $CH_3(CH_2)_8CH(CH_2CH_3)COOH$
	Isostearic (C_{16}-branch, Pfaltz and Bauer) $(CH_3)_2CH(CH_2)_{14}COOH$

Fig. 1. Linear and branched fatty acids studied as skin penetration enhancers.

Irritation Testing. Primary skin irritation tests were performed on several of the fatty acid/propylene glycol vehicles using New Zealand white rabbits. Hair was clipped from the midback area and some sites were abraided by making slight epidermal incisions with a needle. The abrasions were deep enough to penetrate the epidermis but not deep enough to induce bleeding. The solution or semisolid formulations (0.5-ml vol) were applied to an intact skin area and an abraided skin area on each of six rabbits. Application was via a 1-$in.^2$ surgical gauze pad which was taped down with adhesive tape. After a 6-hr exposure period, the application was removed and residual formulation was sponged from the skin with a moistened towel. Visual irritation assessments were made 0.5, 1, 2, 24, and 72 hr later. Each application site was scored for erythma and eschar formation and edema formation as follows.

Skin reaction	Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

For each rabbit, the eight scores for the 24- and 72-hr readings (erythema and eschar formation, edema, intact skin, abraided skin) were added and divided by 4 to give an irritation index (0–8 scale) which was averaged for all rabbits.

RESULTS

One objective of this study was to determine whether branched fatty acids were more effective skin penetration enhancers than the linear fatty acid with the same number of carbon atoms. Naloxone was used as the model diffusant, as

Table I. Effects of Unbranched and Branched Fatty Acids of Various Chain Lengths on Naloxone Skin Penetration Rates[a]

Fatty acid	Naloxone flux (μg/cm² hr) Mean	SE	Enhancement factor
None	3.6	1.2	
C_5			
Valeric	8.7	3.8	2
Isovaleric	6.5	2.3	2
Neopentanoic	12.9	5.2	4
C_7			
Heptanoic	43.3	16.8	12
Neoheptanoic	29.5	11.3	8
C_9			
Pelargonic	148.0	44.3	41
Neononanoic	127.9	22.1	36
Trimethylhexanoic	73.6	22.4	20
C_{10}			
Capric	111.4	37.3	31
Neodecanoic	145.7	63.1	40
C_{12-14}			
Lauric	136.1	42.6	38
ECR 903	62.0	26.1	17
Myristic	62.3	4.5	17
C_{18}			
Stearic	17.1	3.4	5
Isostearic (C_2 branch)	29.1	11.2	8
Isostearic (C_{16} branch)	60.4	8.9	17

[a] Vehicles contained 0.5 *M* fatty acid in propylene glycol.

an extension of our previous study (5) of fatty acids and other skin penetration enhancers. Propylene glycol was shown previously to be the most effective vehicle when fatty acids are used as skin penetration enhancers (5). The structures of the fatty acids examined are shown in Fig. 1. Average results comparing linear and branched fatty acids are reported in Table I. There were large specimen-to-specimen variations in skin penetration rates, typical of human skin penetration studies. Therefore, whenever possible, all vehicles were tested on skin from the same donors. The rank order of skin permeability from the various donors was generally constant. All fatty acids, except the C_5 acids, significantly increased naloxone flux relative to the propylene glycol control containing no adjuvant. An enhancement factor was calculated as the ratio of average flux values in the presence and absence of adjuvant. The effects of unbranched fatty acids on naloxone skin penetration rates were maximal with C_9–C_{12} chain lengths (flux increased 20- to 40-fold), similar to our previous results (5). The effects of the branched fatty acids were not significantly different from those of the unbranched fatty acids of the same carbon number, with one exception. Isostearic acid branched at a position distant from the carboxylic acid functional group (C_{16} branch) was significantly more effective in enhancing naloxone flux than was stearic acid. However, this was not the case for the isostearic acid branched in a position proximal to the carboxylic acid (C_2 branch). This demonstrates the possibility that branched fatty acid isomers may have different effects on skin permeability than unbranched isomers, depending on the position and/or chain length of the branch, but that there is no general trend for an effect of branching.

The other aspect of fatty acid isomeric structure that was examined was the effect of the geometric configuration about the double bond(s) of unsaturated fatty acids. Three pairs of *cis/trans* isomers were studied. Results are summarized in Table II. There were no significant differences between the effects of *cis* and those of *trans* isomers for any isomeric pair. All unsaturated fatty acids increased naloxone flux more than the saturated fatty acid of the same chain length.

Several of the linear and branched saturated fatty acids and a pair of *cis* and *trans* unsaturated isomers were evaluated in a 6-hr exposure, skin irritation test on rabbits. Results are given in Table III. The propylene glycol control vehicle produced no perceptible erythema or edema in any rabbit. There were differences in skin irritation indices between lauric acid and neodecanoic acid and between oleic acid and elaidic acid, even though their effects of enhancing naloxone skin penetration were similar. This shows that is is possible to separate skin penetration enhancing effects from skin irritation effects. The correlation between penetration enhancement and irritation, which is probably more common, was seen for stearic and isostearic (C_{16}-branch) acids. Overall, there was no correlation between the naloxone flux and the irritation index for these fatty acid/propylene glycol vehicles (Fig. 2).

DISCUSSION

We have assumed, as the literature has suggested, that fatty acids increase skin permeability by disrupting the packed structure of the lipids in the extracellular spaces of the stratum corneum. Studies on model lipid membranes in-

Table II. Comparison of *cis* and *trans* Unsaturated Fatty Acids as Enhancers of Naloxone Skin Penetration

Fatty acid (10% in propylene glycol)		Naloxone flux (μg/cm² hr)	Enhancement factor
16:1Δ9 *cis*	Palmitoleic	138.4 ± 7.9	38
16:1Δ9 *trans*	Palmitelaidic	99.8 ± 13.8	28
18:1Δ9 *cis*	Oleic	51.0 ± 10.1	14
18:1Δ9 *trans*	Elaidic	77.6 ± 14.0	22
18:2Δ9,12 *cis*	Linoleic	103.0 ± 14.1	29
18:2Δ9,12 *trans*	Linolelaidic	85.8 ± 12.4	24

Table III. Skin Irritation Indices After a 6-hr Exposure of Rabbits to Various Fatty Acid/PG Vehicles

Vehicle	Primary skin irritation index (0–8 scale)
A. Propylene glycol	0.0
B. 10% lauric acid in PG	0.7
C. 10% neodecanoic acid in PG	0.0
D. 10% stearic acid in PG[a]	0.2
E. 10% isostearic acid (C_{16} branch) in PG	2.6
F. 10% oleic acid in PG	2.3
G. 10% elaidic acid in PG[a]	0.7

[a] Semisolid.

dicate that unsaturated or branched acyl chains disrupt the packing of longer, saturated acyl chains. The acyl chains of the stratum corneum lipids of normal skin are predominantly saturated C_{16} or longer chains and they are packed tightly together. Unsaturated fatty acids are more disruptive of skin lipid packing than are saturated fatty acids of the same chain length, if skin permeability is used as an indicator. We found no difference in penetration enhancement between *cis* and *trans* isomers of unsaturated fatty acids (Table II). Golden *et al.* (9) previously showed that 18:1Δ6 *cis* and *trans* acids were equivalent as skin penetration enhancers for salicylic acid but that for 18:1Δ9 isomers the *cis* isomer (oleic) was approximately 1.6-fold more effective than the *trans* isomer (elaidic), and for 18:1Δ11, the *cis* isomer was 5.0-fold more effective than the *trans*.

Branched fatty acids had not been studied previously as skin penetration enhancers. The results summarized in Table I showed an effect of branching only for one C_{18} fatty acid. This could be because the lower-chain length unbranched fatty acids are already maximally disruptive of lipid packing, so branching does not increase the disruptive effect. Stearic acid, being like the hydrocarbon tails of normal stratum corneum lipids, probably does not disrupt the packed structure. In this case, however, branching could disrupt packing and C_{16}-branched isostearic acid increased skin penetration. The effect of branching was apparently related to the position and length of the branch, since another branched C_{18} isostearic acid, branched at the C_2 position, was equivalent to stearic acid as a penetration enhancer. Some published data also suggest that linear, short-chain, saturated fatty acids are maximally disruptive. Undecylenic acid (11:1Δ10) was no more effective in increasing naloxone skin penetration than capric acid (10:0) or lauric acid (12:0) (5). Similarly, structure/penetration enhancing activity studies of azacycloalkane-type enhancers for 6-mercaptopurine showed no effect of branching or unsaturation for C_{10-15} acyl groups (18).

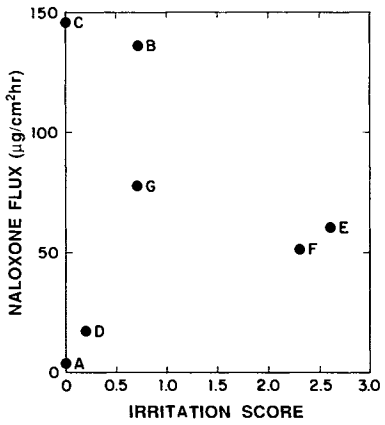

Fig. 2. Correlation between irritation indices and effects on naloxone skin penetration for various fatty acid/PG vehicles. Vehicles are as described in Table III.

In developing formulations for transdermal or topical delivery where skin permeability is manipulated, it is essential to minimize the skin irritation potential while optimizing penetration. For the few vehicles evaluated in this study there was no correlation between the naloxone skin penetration enhancement and the irritation indices of the vehicles. The importance of this is that some penetration enhancers can increase permeability, but not at the expense of causing irritation! These are characteristics of an ideal enhancer. It should be pointed out that these irritation studies were performed with a 6-hr application period and more severe irritation would be expected with longer application times. In a one-subject comparison of lauric acid/PG and neodecanoic acid/PG vehicles applied to human skin *in vivo*, lauric acid was quite irritating after a 6-hr exposure, but neodecanoic acid produced only slight erythema after a 24-hr exposure. The reasons why neodecanoic acid was less irritating than lauric acid, and why elaidic acid was less irritating than oleic acid, are not yet known.

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