J. Pharm. Pharmacol. 1996, 48: 1133–1137
Received December 28, 1995
Accepted March, 12 1996

Enhancing Effects of Unsaturated Fatty Acids with Various Structures on the Permeation of Indomethacin through Rat Skin

KAZUHIRO MORIMOTO, HIDEKI TOJIMA, TATSUO HARUTA, MASAO SUZUKI* AND MASAWO KAKEMI

*Department of Pharmaceutics, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki-City, Osaka 569-11, and *Oleochemical Research Lab., NOF Corporation, 1-56, Oohama-Cho, Amagasaki-City 660, Japan*

Abstract

Effects of straight-chain, *cis*-type, unsaturated fatty acids with various structures (alkyl chain lengths, numbers of double bonds, position of double bonds and *cis*- and *trans*-positional isomers) on the skin permeation of indomethacin were examined by using rat skins in-vitro. Furthermore, the disordering degrees of the intercellular lipid domain in the stratum corneum, which were treated with preparations of unsaturated fatty acids, were measured by the Fourier transform infrared (FT-IR) method using excised rabbit ear skins.

Unsaturated fatty acids enhanced the permeation of indomethacin through rat skins. These permeation-enhancing effects by unsaturated fatty acids were affected by changes of their alkyl chain length from C_{14} to C_{22}. The lag-times on the permeation of indomethacin were shortened by unsaturated fatty acids in the following order: $C_{20} = C_{18} = C_{22} < C_{16} < C_{14}$. These fluxes were increased by unsaturated fatty acids in the following order: $C_{20} > C_{22} = C_{18} = C_{16} > C_{14}$. Therefore, gondoic acid (*cis*-11-eicosenoic acid; $C_{20}H_{38}O_2$) mostly enhanced the skin permeation of indomethacin. However, the enhancing effects of unsaturated fatty acids (C_{18} chain) were not affected by their differences of position and numbers of double bonds.

These permeation-enhancing effects which were evaluated by flux were related to the degrees of wave-number shift in the frequency of the antisymmetric CH bond stretching absorbance (near 2920 cm^{-1}) on FT-IR spectra of the fatty acid-treated stratum corneum. Therefore, the perturbation increase of lipid domain in the stratum corneum by these fatty acids probably was the cause of the enhancing effects of permeation of indomethacin.

The transdermal delivery of drugs for topical treatment or systemic disorders has received much attention. The outer layer of the skin, the stratum corneum, is generally recognized as the primary barrier to transdermal delivery of drugs. The stratum corneum is a thin, heterogeneous structure comprising stacked layers of terminally differentiated and keratinized epidermal cells distributed in a complex, lamellar, intercellular lipid domain (Barry 1987, 1991). The stratum corneum intercellular lipids largely dictate the overall skin permeation properties.

Unsaturated fatty acids such as oleic acid (*cis*-9-octadecenoic acid), which contains a *cis*-double bond in the alkyl chain, were identified as enhancing the skin permeation of drugs and they have been shown to alter the barrier function of the stratum corneum by disordering structures of the lipid molecules (Barry 1987, 1991; Golden et al 1987; Mak et al 1990a,b; Ongpipattanakul et al 1991; Yamashita et al 1995). Mak et al (1990b) examined the action of oleic acid on the penetration of 4-cyanophenol into the human stratum corneum in-vivo focussing on the molecular motion of lipids domain by using fourier transform infrared/attenuated reflection (FT-IR/ATR). They reported that oleic acid caused the disordering of stratum corneum intercellular lipid domains and the weakening of the penetration barrier, which in turn resulted in the enhancement of 4-cyanophenol penetration.

In the present study, we investigated the enhancing effects of straight-chain unsaturated fatty acids of various structures (alkyl chain lengths, numbers of double bonds, position of double bonds and *cis*- and *trans*- positional isomers) on the permeation of indomethacin, a potent non-steroidal anti-inflammatory drug through rat skin in-vitro.

Correspondence: K. Morimoto, Department of Pharmaceutics, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki-City, Osaka 569-11, Japan.

Materials and Methods

Materials

Indomethacin (Sigma Chem. Inc., St Louis, MO) and Carbopol 1342 (B. F. Goodrich Chem. Co. Cleveland, OH, USA) were obtained commercially. Unsaturated straight-chain fatty acids with purity greater than 99·9% were supplied from NOF Co., Ltd., Amagasaki, Japan. These fatty acids are shown in Table 1. All other chemicals were of reagent grade.

Preparations

Table 1. Unsaturated fatty acids used in this study.

Myristoleic acid (*cis*-9-tetradecenoic acid): $C_{14}H_{26}O_2$
Palmitoleic acid (*cis*-9-hexadecenoic acid): $C_{16}H_{30}O_2$
Oleic acid (*cis*-9-octadecenoic acid): $C_{18}H_{34}O_2$
Gondoic acid (*cis*-11-eicosenoic acid): $C_{20}H_{38}O_2$
Erucic acid (*cis*-13-docosenoic acid): $C_{22}H_{42}O_2$
α-Linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid): $C_{18}H_{32}O_2$
α-Linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid): $C_{18}H_{30}O_2$
γ-Linolenic acid (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid): $C_{18}H_{30}O_2$
Arachidonic acid (*cis*-5, *cis*-8, *cis*-11, *cis*-14-eicosatetranoic acid): $C_{20}H_{32}O_2$
Asclepic acid (*cis*-11-octadecenoic acid): $C_{18}H_{34}O_2$
Petroselinic acid (*cis*-6-octadecenoic acid): $C_{18}H_{34}O_2$
Elaidic acid (*trans*-9-octadecenoic acid): $C_{18}H_{34}O_2$

Indomethacin (0·5 mg g^{-1}) and fatty acid (0·03 M) were dissolved in ethanol (24% w/w) and propylene glycol (12% w/w) and then mixed with gel base which was prepared with Carbopol 1342 (1% w/w) presoaked in water. The final pH (7·0) of the preparations was adjusted with ammonia solution.

In-vitro percutaneous permeation tests

Percutaneus permeation tests were determined by using the in-vitro permeation cell procedure (Franz type) (Morimoto et al 1990). Full-thickness abdominal skins of male Wistar strain rats weighing about 240 g (8 weeks old) were used. The hair of the abdominal area in rats was removed with an electric hair clipper and electric razor without breaking the skin, 1 day before the experiments. The extracted abdominal skin was mounted on the receptor phase compartment of the diffusion cell (available diffusion areas of 1·05 cm^2). The stratum corneum side was placed face upwards into the donor phase. The receptor phase contained 13 mL isotonic phosphate buffer (pH 7·4) at 37°C and was stirred with a magnetic bar at 500 rev min^{-1}. The drug preparation (1 g) was applied onto the skin surface. Samples (1 mL) were taken at an appropriate interval from the receptor phase and replaced immediately with fresh phosphate buffer (1 mL after each sample collection) to maintain the original volume. The concentration of indomethacin in the receptor phase was determined using a high-performance liquid-chromatographic (HPLC) method (Morimoto et al 1995).

The drug permeation through rat skins was expressed as a plot of the % dose of the cumulative amount permeating to the receptor phase of the diffusion cell as a function of time (t). The permeation parameters were calculated by using the following equations:

$$J = C \times D \times K/L \quad (1)$$

$$T = L^2/6D \quad (2)$$

where J is the mean flux of drug through rat skins, C is drug concentration in the preparation, D is diffusion constant within skin, K is the skin-gel preparation partition coefficient of the drug, L is the thickness of skin (18·4 μm) and T is the lag-time (Okamoto et al 1986).

Stratum corneum lipid fluidity tests

Stratum corneum lipid fluidity tests were determined by FT-IR spectroscopy using excised stratum corneum of rabbit (2–3 kg, male albino rabbits) ear skins. Stratum corneum sheets were separated from whole skin soaked in 2 M sodium bromide solution for 1 h (Scott et al 1986). The surface area of stratum corneum sheet for FT-IR measurement was 1·5 cm^2. The stratum corneum sheet samples were incubated in propylene glycol with or without fatty acid (0·03 M) for 2 h at 37°C. After the incubation, these sheets were washed in 20% ethanol solution for 10 s, spread on wire mesh and dried for several hours over a desiccant. All sheet samples were then placed for 1 day in a chamber maintained at 95% relative humidity and 22°C. Stratum corneum samples were equilibrated to a water content of 30% (w/w) under these conditions. Infrared spectra of the stratum corneum were obtained over the 300 to 2800 cm^{-1} region with a FT-IR spectrometer (Perkin Elmer 1720) equipped with a TGS detector. Change in the lipid fluidity of the stratum corneum was evaluated by higher wave number shift in frequency of the asymmetric CH bond-stretching absorbance (near 2920 cm^{-1}), which results primarily from methylene groups in the stratum corneum lipid acyl chains (Golden et al 1987; Mak et al 1990b).

Data analysis

All data were analysed by the interactive nonlinear least-squares regression analysis MULTI (Yamaoka et al 1981). Statistical significance was assessed with Student's paired t-test.

Results and Discussion

Fig. 1 shows the enhancing effects of various alkyl chain lengths (C_{14}–C_{22}) of *cis*-monounsaturated fatty acids (0·03 M) on the permeation of indomethacin through rat skin. Table 2 shows these permeation parameters of indomethacin through rat skins. The flux on indomethacin without fatty acid was very low. The fluxes were significantly increased by unsaturated fatty acids in the following order: $C_{20} > C_{22} = C_{18} = C_{16} > C_{14}$. The observed lag times were shortened by unsaturated fatty acids in the following order: $C_{20} = C_{18} = C_{22} < C_{16} < C_{14}$. Gondoic acid ($C_{20}$, *cis*-11-eicosenoic acid) mostly enhanced the skin permeation of indomethacin. Therefore, the permeation enhancing effects by unsaturated fatty acids were affected by changing alkyl chain length. Amounts of uptake of these unsaturated fatty acids into stratum corneum sheet of excised rabbit ear were in the following order: $C_{20} = C_{18} > C_{22} = C_{16} > C_{14}$ (data not shown). Uptake of these unsaturated fatty acids into the stratum corneum was related to the extent of the permeation-enhancing effect of the fatty acids.

Figs 2 and 3 show the enhancing effects of varying the numbers of double bonds in *cis*-unsaturated fatty acids with a C_{18} or C_{20} chain (0·03 M), on the permeation of indomethacin through rat skin. Table 3 shows these permeation parameters of

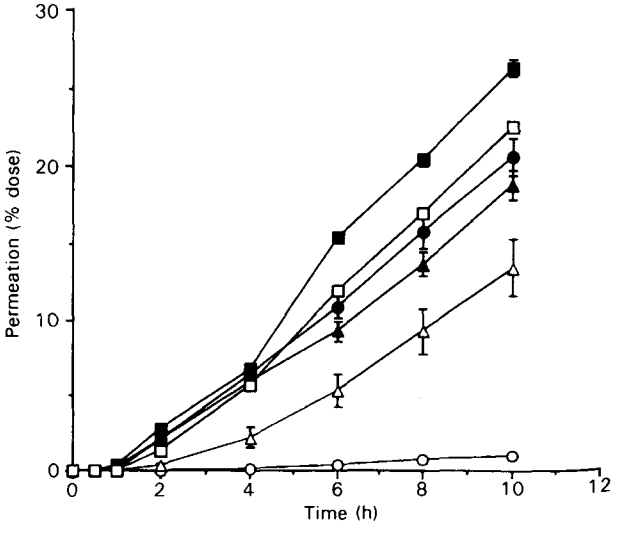

FIG.1. Effect of varying the alkyl chain lengths of *cis*-mono-unsaturated fatty acids (0·03 M) on the cumulative permeation of indomethacin through rat skin. ○ Control, △ myristoleic acid (C_{14}, *cis*-9), ▲ palmitoleic acid (C_{16}, *cis*-9), ● oleic acid (C_{18}, *cis*-9), ■ gondoic acid (C_{20}, *cis*-11), □ erucic acid (C_{22}, *cis*-13). Each point represents the mean ± s.e. of 4 experiments.

Table 2. Permeation parameters of indomethacin through rat skin from preparations containing unsaturated fatty acids with various alkyl chain lengths.

	Lag-time (h)	Flux (mg cm^{-2} h^{-1})	% Dose
Without fatty acid	$3{\cdot}80 \pm 0{\cdot}32^{c}$	$0{\cdot}60 \pm 0{\cdot}05^{c}$	$0{\cdot}96 \pm 0{\cdot}08^{c}$
Myristoleic acid (C_{14}, *cis*-9)	$3{\cdot}40 \pm 0{\cdot}45^{a}$	$8{\cdot}52 \pm 1{\cdot}19^{b}$	$13{\cdot}38 \pm 1{\cdot}86^{b}$
Palmitoleic acid (C_{16}, *cis*-9)	$2{\cdot}31 \pm 0{\cdot}17^{b}$	$11{\cdot}92 \pm 0{\cdot}65$	$18{\cdot}73 \pm 1{\cdot}02$
Oleic acid (C_{18}, *cis*-9)	$1{\cdot}55 \pm 0{\cdot}16$	$13{\cdot}06 \pm 0{\cdot}78$	$20{\cdot}50 \pm 1{\cdot}22$
Gondoic acid (C_{20}, *cis*-11)	$1{\cdot}34 \pm 0{\cdot}08$	$16{\cdot}68 \pm 0{\cdot}34^{a}$	$26{\cdot}18 \pm 0{\cdot}53^{a}$
Erucic acid (C_{22}, *cis*-13)	$1{\cdot}85 \pm 0{\cdot}09$	$14{\cdot}26 \pm 0{\cdot}11$	$22{\cdot}39 \pm 0{\cdot}17$

Each value represents the mean ± s.e. of 4 experiments. $^{a}P < 0{\cdot}01$, $^{b}P < 0{\cdot}005$, $^{c}P < 0{\cdot}001$ compared with oleic acid. % Dose is for the amount of drug permeated in 10 h.

Table 3. Permeation parameters of indomethacin through rat skin from preparations containing unsaturated fatty acids with various numbers of double bonds.

	Lag time (h)	Flux (mg cm^{-2} h^{-1})	% Dose
Without fatty acid	$3{\cdot}80 \pm 0{\cdot}32^{c}$	$0{\cdot}60 \pm 0{\cdot}05^{c}$	$0{\cdot}96 \pm 0{\cdot}08^{c}$
Oleic acid (C_{18}, *cis*-9)	$1{\cdot}55 \pm 0{\cdot}16$	$13{\cdot}06 \pm 0{\cdot}78$	$20{\cdot}50 \pm 1{\cdot}22$
Linoleic acid (C_{18}, *cis*-9, *cis*-12)	$2{\cdot}50 \pm 0{\cdot}21^{a}$	$13{\cdot}07 \pm 0{\cdot}36$	$20{\cdot}52 \pm 0{\cdot}57$
α-Linolenic acid (C_{18}, *cis*-9, *cis*-12, *cis*-15)	$1{\cdot}60 \pm 0{\cdot}28$	$13{\cdot}58 \pm 0{\cdot}51$	$21{\cdot}32 \pm 0{\cdot}81$
γ-Linolenic acid (C_{18}, *cis*-6, *cis*-9, *cis*-12)	$1{\cdot}77 \pm 0{\cdot}25$	$7{\cdot}98 \pm 0{\cdot}72^{b}$	$12{\cdot}53 \pm 1{\cdot}13^{b}$
Gondoic acid (C_{20}, *cis*-11)	$1{\cdot}34 \pm 0{\cdot}08$	$16{\cdot}68 \pm 0{\cdot}34^{a}$	$26{\cdot}18 \pm 0{\cdot}53^{a}$
Arachidonic acid (C_{20}, *cis*-5 *cis*-8, *cis*-11, *cis*-14)	$1{\cdot}32 \pm 0{\cdot}09$	$18{\cdot}75 \pm 0{\cdot}72^{b}$	$29{\cdot}44 \pm 1{\cdot}35^{b}$

Each value represents the mean ± s.e. of 4 experiments. $^{a}P < 0{\cdot}01$, $^{b}P < 0{\cdot}005$, $^{c}P < 0{\cdot}001$ compared with oleic acid. % Dose is for the amount of drug permeated in 10 h.

indomethacin through rat skin. The lag-times and fluxes for the permeation of indomethacin were not significantly different between oleic acid, α-linoleic acid and α-linolenic acid. However, the flux with γ-linolenic acid was significantly ($P < 0{\cdot}005$) decreased compared with those with oleic acid. The lag-time fluxes for the permeation of indomethacin were not significantly different between gondoic acid and arachidonic acid (C_{20} chain).

Fig. 4 shows the enhancing effects of various sites of double bonds of *cis*-unsaturated fatty acids with C_{18} chain (0·03 M) on the permeation of indomethacin through rat skin. Table 4 shows these permeation parameters of indomethacin through rat skin. The lag times and fluxes of oleic acid, asclepic acid and petroselinic acid on the permeation of indomethacin were not significantly different. *cis*-Monoenoic acid, which has the site of unsaturation centrally located, increased the salicylic

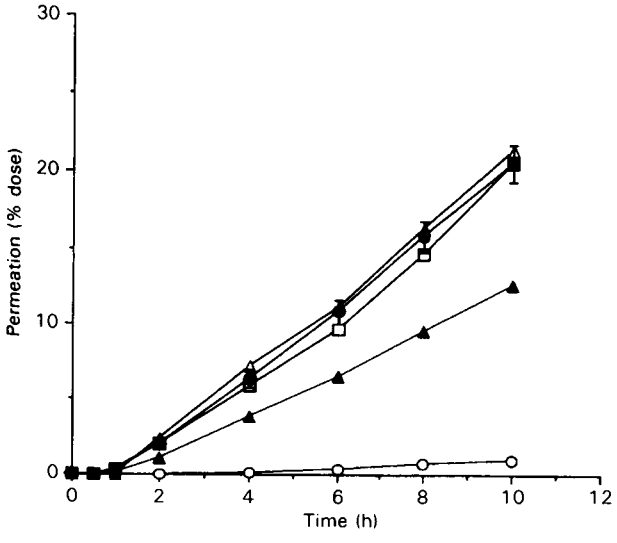

FIG. 2. Effects of varying the numbers of double bonds of *cis*-unsaturated fatty acids with a C_{18} chain (0·03 M) on the cumulative permeation of indomethacin through rat skin. ○ control, ● oleic acid (*cis*-9), □ linoleic acid (*cis*-9, *cis*-12), △ α-linolenic acid (*cis*-9, *cis*-12, *cis*-15), ▲ γ-linolenic acid (*cis*-6, *cis*-9, *cis*-12). Each point represents the mean ± s.e. of 4 experiments.

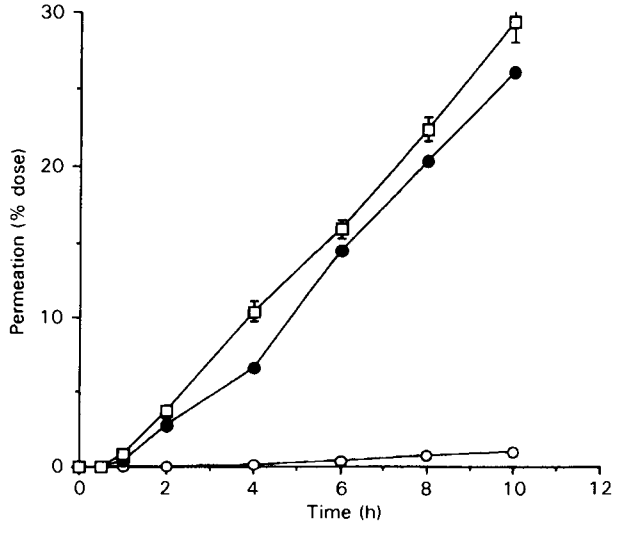

FIG. 3. Effect of varying the numbers of double bonds of *cis*-unsaturated fatty acids with a C_{20} chain (0·03 M) on the cumulative permeation of indomethacin through rat skin. ○ control, ● gondoic acid (*cis*-11), □ arachidonic acid (*cis*-5, *cis*-8, *cis*-11, *cis*-14). Each point represents the mean ± s.e. of 4 experiments.

Table 4. Permeation parameters of indomethacin through rat skin from preparations containing unsaturated fatty acids with various double bond positions and geometric isomers.

	Lag time (h)	Flux (mg cm^{-2} h^{-1})	% Dose
Without fatty acid	3.80 ± 0.32^{c}	0.60 ± 0.05^{c}	0.96 ± 0.08^{c}
Oleic acid (C_{18}, *cis*-9)	1.55 ± 0.16	13.06 ± 0.78	20.50 ± 1.22
Asclepic acid (C_{18}, *cis*-11)	2.50 ± 0.20^{a}	12.89 ± 0.90	20.23 ± 1.42
Petroselinic acid (C_{18}, *cis*-6)	1.81 ± 0.27	13.21 ± 0.84	20.73 ± 1.32
Elaidic acid (C_{18}, *trans*-9)	3.09 ± 0.16^{b}	4.75 ± 0.26^{c}	7.45 ± 0.45^{c}

Each value represents the mean ± s.e. of 4 experiments. $^{a}P < 0.01$, $^{b}P < 0.005$, $^{c}P < 0.001$ compared with oleic acid. % Dose is on the amount of drug permeated for 10 h.

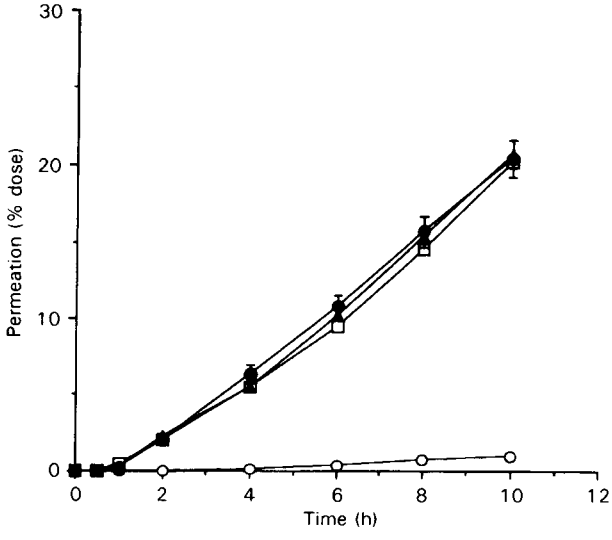

FIG. 4. Effect of varying the positions of double bonds of *cis*-unsaturated fatty acids with a C_{18} chain (0·03 M) on the cumulative permeation of indomethacin through rat skin. ○ control, ● oleic acid (*cis*-9), □ ascleptic acid (*cis*-11), ▲ petroselinic acid (*cis*-6). Each point represents the mean ± s.e. of 4 experiments.

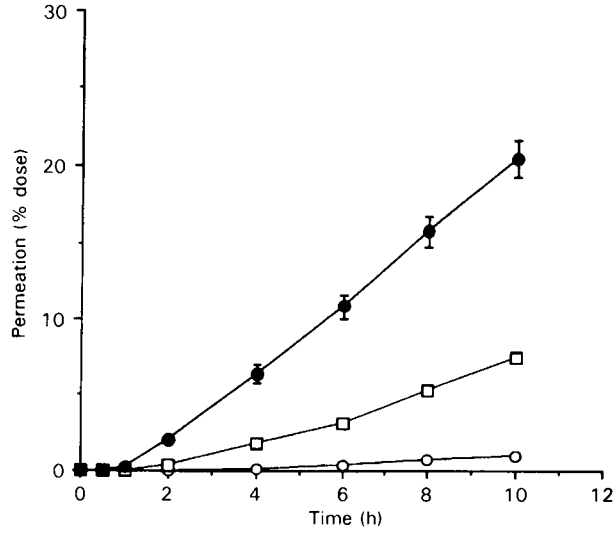

FIG. 5. Effect of geometric isomers (*cis* or *trans* type) of mono-unsaturated fatty acids with a C_{18} chain (0·03 M) on the cumulative permeation of indomethacin through rat skin. ○ control, ● oleic acid (*cis*-9), △ elaidic acid (*trans*-9). Each point represents the mean ± s.e. of 4 experiments.

acid flux through porcine skin (Golden et al 1987) to a greater extent. However, these results suggested that the enhancing effects of unsaturated fatty acids (C_{18} chain) except γ-linolenic acid were not affected by their differences of position and numbers of double bonds.

Fig. 5 shows the enhancing effects of *trans* and *cis* isomers of 9-octadecenoic acid with C_{18} chain (0·03 M) on the permeation of indomethacin through rat skin. Table 4 shows these permeation parameters. The lag time and flux with elaidic acid (*trans*-9-octadecenoic acid) were significantly ($P < 0.005$) prolonged and decreased, respectively, compared with those with oleic acid (*cis*-9-octadecenoic acid). The permeation-enhancing effect of elaidic acid was lower than that of oleic acid. This result is consistent with the enhancing effects of fatty acids with a series of *trans* and *cis* isomers of 9-octadecenoic acid on salicylic acid flux through porcine skin (Golden et al 1987).

The main barrier for penetration of drugs through the skin is the outermost layer, the stratum corneum. The stratum corneum is composed of keratinocytes embedded in lipid domains consisting of alternately hydrophilic and lipophilic layers. The

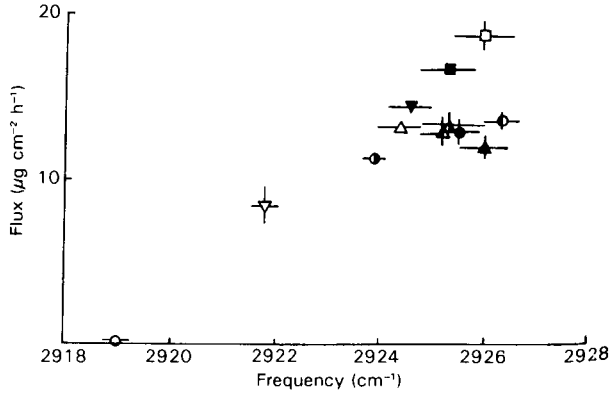

FIG. 6. Relationship between the wave number shift of C-H asymmetric stretching peak of the stratum corneum of rabbit ear skins and the fluxes on the permeation of indomethacin with *cis*-unsaturated fatty acids (0·03 M) through rat skin. ○ control, ▽ myristoleic acid, ▲ palmitoleic acid, ● oleic acid, ■ gondoic acid, ▼ erucic acid, △ linoleic acid, ◐ α-linolenic acid, ◐ γ-linolenic acid, ◨ arachidonic acid, ◭ ascleptic acid, ◭ petroselinic acid. Each point represents the mean ± s.e. of 4 experiments

evidence for the existence of distinct hydrophilic and lipophilic transport pathways was suggested from relationships between the permeability-partition coefficients of drugs (Barry 1987, 1991). Indomethacin, a relatively lipophilic compound (partition coefficient: log k = 1·5 in octanol/pH 7·4 phosphate buffer (Inagi et al 1981)) may permeate through a lipophilic route, the intercellular domain of stratum corneum. Treatment with various unsaturated fatty acids (0·03 M) resulted in a shift to higher frequency for the C-H asymmetric stretch peak near 2920 cm^{-1} on FT-IR spectra, which primarily results from the acyl chains of intercellular lipid in the stratum corneum lipid.

Fig. 6 shows the relationships between these changes in the peak frequency of the C-H asymmetric stretching vibration (near 2920 cm^{-1}) and the fluxes on the permeation of indomethacin through rat skin. The correlation (R) of fluxes on the permeation of indomethacin, was good (0·91). Golden et al (1987) also suggested that transdermal flux of salicylic acid was ultimately related to packing in the transcellular lipid domain of the stratum corneum which was treated with fatty acids (C_{18} chain). Ongpipattanakul et al (1991) reported that the conformational behaviour of the stratum corneum lipids and the added fatty acid in FT-IR experiments was resolved with the use of perdeuterated oleic acid. Yamashita et al (1995) reported that the basic mechanism of enhancement by oleic acid for the permeation of drugs is to increase their diffusivity and partitioning in the non polar route, irrespective of whether the in-vitro and in-vivo situation is considered. Therefore, the perturbation increase of the lipid domain in the stratum corneum by these unsaturated fatty acids may cause the enhancement of indomethacin permeation.

In conclusion, unsaturated fatty acids significantly enhanced the skin permeation of indomethacin. These permeation-enhancing effects of unsaturated fatty acids were affected by changes in their alkyl chain length. However, these enhancing effects were not affected by differences in their position and numbers of double bonds. These permeation-enhancing effects were related to the degrees of wave number shift in the frequency of the asymmetric C-H bond stretching absorbance (near 2920 cm^{-1}) in the FT-IR spectra of stratum corneum treated with fatty acids. Therefore, the increase in stratum corneum lipid disorder by these unsaturated fatty acids may cause the permeation of indomethacin to be enhanced.

References

Barry, B. W. (1987) Mode of action of penetration enhancer in human skin. J. Contr. Rel. 6: 85–97

Barry, B. W. (1991) Lipid-protein-partitioning theory of skin penetration enhancement. J. Contr. Rel. 15: 237–248

Golden, G. M., McKie, J. E., Potts, R. O. (1987) Role of stratum corneum lipid fluidity in transdermal drug flux. J. Pharm. Sci. 76: 25–28

Inagi, T., Muramatsu, T., Nagai, H., Terada, H. (1981) Mechanism of indomethacin partition between *n*-octanol and water. Chem. Pharm. Bull. 29: 2330–2337

Mak, V. H. W., Potts, R. O., Guy, R. H. (1990a) Oleic acid concentration and effects in human stratum corneum: non-invasive determination by attenuated total reflectance infrared spectroscopy in vivo. J. Contr. Rel. 12: 67–75

Mak, V. H. W., Potts, R. O., Guy, R. H. (1990b) Percutaneous penetration enhancement in vivo measured by attenuated total reflectance infrared spectroscopy. Pharm. Res. 7: 835–841

Morimoto, K., Nagayasu, A., Fukanoki, S., Morisaka, K., Hyon, S. H., Ikada, Y. (1990) Evaluation of polyvinyl alcohol hydrogel as sustained-release vehicle for transdermal system of bunitrolol-HCl. Drug. Dev. Ind. Pharm. 16: 13–29

Morimoto, K., Harura, T., Tojima, H., Takeuchi, Y. (1995) Enhancing mechanisms of saturated fatty acids on the permeations of indomethacin and 6-carboxyfluorescein through rat skin. Drug. Dev. Ind. Pharm. 21: 1999–2012

Okamoto, H., Komatsu, H., Hashida, M., Sezaki, H. (1986) Effect of β-cyclodextrin and di-*O*-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. Int. J. Pharm. 30: 35–45

Ongpipattanakul, B., Burnette, R. R., Potts, R. O., Francoeur, M. L. (1991) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm. Res. 8: 350–354

Scott, R. C., Walker, M., Dugard, P. H. (1986) In vitro percutaneous absorption experiments: a technique for the production of intact epidermal membranes from rat skin. J. Soc. Cosmet. Chem. 37: 35–41

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobiodyn. 4: 879–885

Yamashita, F., Koyaama, Y., Kitano, M., Takakura, Y., Hashida, M. (1995) Analysis of in vitro skin penetration enhancement by oleic acid based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum. Int. J. Pharm. 117: 173–179