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Enhancing Mechanisms of Saturated Fatty Acids on the Permeations of Indomethacin and 6-Carboxyfluorescein through Rat Skins

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ABSTRACT

Effects of saturated straight-chain fatty acids at various chain lengths (C_8-C_{18}) on the permeation of indomethacin, a relatively lipophilic compound and 6-carboxyfluorescein, a hydrophilic compound were examined using rat skins in in vitro. Furthermore, the disordering degrees of intercellular lipid domain in stratum corneum, which were treated by preparation containing saturated fatty acids were measured by FT-IR method using excised rabbit ear skins. Capric acid (C_{10}), lauric acid (C_{12}) and myristic acid (C_{14}) within series of

saturated fatty acids (0.07 M) showed the enhancing effects on the skin permeations of indomethacin and 6-CF. The permeation enhancing effects by these saturated fatty acids (C_8-C_{18}) except for capric acid (C_{10}), were relative to the degrees of wavenumber shift in the frequency of the asymmetric CH bond stretching absorbance (2920 cm^{-1}) on FT-IR spectra of the fatty acid treated stratum corneum. Therefore, the perturbation increase of lipid domain in stratum corneum by these fatty acids probably was the cause of the enhanced effects of permeation of indomethacin and 6-CF. On the other hand, capric acid appears to enhance the permeations of these two drugs by separate mechanisms.

INTRODUCTION

The transdermal route has many advantages for administration of drugs in local and systemic therapy. 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 comprised of stacked layers of terminally differentiated and keratinized epidermal cells distributed in a complex, lamellar, intercellular lipid domain (1). The stratum corneum intercellular lipids largely dictate the overall skin permeation properties.

Long chain unsaturated fatty acids (e.g. oleic acid) were identified to enhance skin permeation of drugs and they have been revealed to alter the barrier function of the stratum corneum by disordering structures of the lipid molecules (1). Mak et al and Potts et al 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) (2-3). 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. However, there were a few reports which deal with enhancing effects of saturated fatty acids on the skin permeations of drugs. Aungst et al examined the enhancing effects of saturated fatty acids ranging from heptanoic (C_7) through stearic acid (C_{18}) on naloxone permeation across human skin and obtained the maximum enhancing effect by lauric acid (C_{12}) (4). Furthermore, they reported the enhancing effects of fatty acids and amines on the skin permeation of various compounds including indomethacin.

In the present study, we investigated the effects of saturated straight-chain fatty acids at various chain lengths (C_8-C_{18}) on the permeation of indomethacin, as a relatively lipophilic compound through rat skins in vitro. Furthermore, we discussed the relationship between the enhancing effects on the skin permeation of these drugs and the disordering degree of stratum corneum lipid domains which was measured by FT-IR method using excised rabbit ear skins.

MATERIALS AND METHODS

1. Materials Indomethacin (Sigma Chem. Inc, St. Louis, Mo), 6-CF (Easterman Kodak Co. Rochester, NY) and Carbopol™ 1342 (B.F. Goodrich Chem. Co. Cleverland, Oh) were obtained commercially. Saturated straight-chain fatty acids were obtained from Nacalai Tesque, Inc. (Kyoto) or Nippon Oil & Fats Co., Ltd. (Tokyo). All other chemicals were of reagent grade.

2. Preparations Indomethacin or 6-CF (0.5 ug/ml), and fatty acids (0.03-0.14 M) were dissolved in ethanol (24% w/w) and then mixed with gel base which was prepared with Carbopol 1342 (1%w/w) presoaked in distilled water. The final pH (pH 7.0) of the preparation was adjusted with ammonia water.

3. In Vitro Percutaneous Permeation Percutaneus permeation tests were determined by using the in vitro permeation cell procedure (Franz type) (5). Full thickness abdominal skins of male Wistar strain rats weighing about 240 g were used. The hair of abdominal area in rats was removed with electric hair clipper and electric razor without breaking the skin one day before the experiments. The extracted abdominal skin was mounted on the receptor phase compartment of diffusion cell (available diffusion areas of 1.05 cm^2). The stratum corneum side faced upward into the donor phase. The receptor phase containing 13 ml isotonic phosphate buffer (pH 7.4) at 37 °C and stirred with 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 receptor phase and fresh fluid (1 ml) was added back to the receptor phase to maintain the original volume.

The concentration of indomethacin was determined by the high performance liquid chromatographic (HPLC) method (6). The 6-CF was determined by the fluorescene measurement method (7). The drug permeation through rat skins were expressed as plots 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: $J=C\ D\ K/L$ (Eq. 1), $T=L^2/6D$ (Eq. 2), where J is the mean flux of drug through rat skins, C is drug concentration in preparation, D is diffusion constant within skin, K is the skin-gel preparation partition coefficient of drug, L is the thickness of skin (1.84 mm) and T is the lag time (8).

5. Stratum Corneum Lipid Fluidity Tests Stratum corneum lipid fluidity tests were determined by FT-IR method using stratum corneum sheet of excised rabbit (2-3 kg, male albino rabbit) ear skins. Stratum corneum sheets could be separated

from whole skins soaked in 2 M sodium bromide solution for 1 h (9). The surface area of stratum corneum sheet for FT-IR measurement was 1.5 cm^2. The stratum corneal sheet samples were incubated in the propylene glycol with or without fatty acid (0.07 M) for 2 h at 37 °C. After the incubation, these sheets were washed in ethanol for 10 s, spread on wire mesh and dried for several hours over a desiccant. All sheet samples were then placed 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 300 to 2800 cm^{-1} region with a FT-IR spectrometer (Perkin Elmer 1720) equipped with a TGS detector. Change in lipid fluidity of stratum corneum was evaluated by higher wavenumber shift in frequency of the asymmetric CH bond stretching absorbance (2920 cm^{-1}), which results primarily from methylene groups in the stratum corneum lipid acyl chains (2).

6. Uptake of Fatty Acids by Stratum Corneum To examine the uptake of saturated fatty acid by stratum corneum, the stratum corneum sheet of excised rabbit ear was mounted on the diffusion cell described in permeation tests. The surface side of stratum corneum was placed upward facing the donor phase. The gel preparation (1 g) containing fatty acid (0.07 M) was applied onto the surface area of stratum corneum for 4 h. After application, the preparation was removed from surface side of stratum corneum and the stratum corneum was washed with 50% ethanol solution and homogenized in distilled water (1 ml). Saturated fatty acids in stratum corneum were extracted by the method of Folch et al (10) and the concentrations of saturated fatty acids were determined by HPLC method of D'Amboise and Gendreau (11) .

7. Lipophilic Indexes of Fatty Acids Lipophilic indexes (log k) of saturated fatty acids were determined by HPLC method of Okamoto et al (12). A HPLC system equipped with an UV detector operating at 224 nm was used in a reverse-phase model. LiChrospher™ 5 C_8 packed column (4.6 x 150 mm; 10 um, Cica-Merck) was used. A mixture of methanol (95-85%) and distilled water (5-15%) was employed as a mobile phase. The elusion time of a solvent (t_0) and the retention time of a fatty acid (t_n) were determined at each of the mobile phase composition. The log k' value defined by Eq. 3 was plotted against the methanol concentration in the mobile phase and the extrpolated log k' value to 0% methanol was obtained as an index of lipophilicity of the fatty acid (log k'_0).

$$\log k' = \log (t_r - t_0) \quad \text{Eq. 3}$$

8. Data Analysis All data were analyzed by the interactive nonlinear least-squares regression analysis MULTI (13). Statistical significance was assessed with Student's paired t test.

RESULTS

Figure. 1 shows the effects of saturated fatty acids (0.07 M) of various chain lengths (C_8-C_{18}) on the permeations of indomethacin and 6-CF through rat skins. Tables 1 and 2 show the permeation parameters of indomethacin and 6-CF through rat skins, respectively. The lag times on the permeations of indomethacin were shortened by saturated fatty acids in the following order; $C_{12} < C_{14} \leq C_{16} = C_{10} \leq C_{18} < C_8$. The lag times on the permeations of 6-CF were shortened by saturated fatty acids in the following order; $C_{12} = C_{14} < C_{10} < C_{16} = C_8 < C_{18}$. The fluxes of indomethacin were increased by saturated fatty acids in the following order; $C_{12} > C_{14} > C_{10} > C_{16} > C_{18} > C_8$. The fluxes of 6-CF were increased by saturated fatty acids in the following order; $C_{12} > C_{10} = C_{14} > C_{16} = C_8 > C_{18}$.

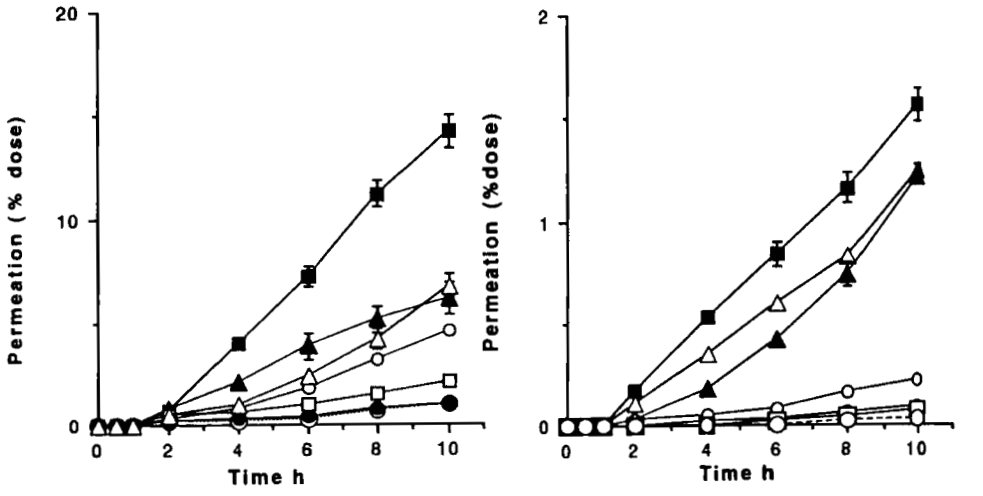

Figure 1

Effects of saturated fatty acids (0.07 M) at various chain lengths on the cumulative permeation of indomethacin and 6-CF through rat skins.

--○--control, ● caprylic acid(C_8), ▲capric acid(C_{10}), ■ lauric acid (C_{12}), △myristic acid(C_{14}), ○ palmitic acid(C_{16}), □stearic acid(C_{18}).

Each point represents the mean ± S.E. of 4 experiments.

Table 1 Parameters of Permeation of Indomethacin through Rat Skin from Gel Preparations containing Various Saturated Fatty Acids (0.07 M)

	Lag time (h)	Flux ($ug/cm^2/h$)	%Dose*
control	3.80±0.03	0.602±0.054	0.96±0.08
caprylic acid (C8)	3.11±0.32NS	0.634±0.104NS	1.01±0.10NS
capric acid (C10)	2.75±0.14c)	3.056± 0.034c)	6.11±0.78c)
lauric acid (C12)	1.79±0.82a)	8.924±1.040c)	14.28±0.96c)
myristic acid (C14)	2.65±0.36a)	4.235±0.128c)	6.78±0.57c)
palmitic acid (C16)	2.73±0.24b)	2.851±0.546b)	4.56±0.30c)
stearic acid (C18)	2.85±0.23b)	1.333±0.185b)	2.11±0.28b)

NS was not significant difference.
a) $p<0.05$, b) $p<0.01$ and c) $p<0.001$ compared with control.
*%Dose of amount of drug permeated for 10 h.
Each value is a mean ± SE (N=4).

Table 2 Parameters of Permeation of 6-CF through Rat Skin from Gel Preparations containing Various Saturated Fatty Acids (0.07 M)

	Lag time (h)	Flux ($ug/cm^2/h$)	%Dose*
control	3.87±0.19	0.017±0.004	0.026±0.006
caprylic acid (C8)	3.12±0.10a)	0.053±0.003c)	0.085±0.005c)
capric acid (C10)	2.35±0.04c)	0.774±0.023c)	1.239±0.038c)
lauric acid (C12)	1.11±0.08c)	1.053±0.082c)	1.565±0.079c)
myristic acid (C14)	1.25±0.20c)	0.757±0.042c)	1.213±0.007c)
palmitic acid (C16)	3.12±0.07b)	0.138±0.014c)	0.222±0.002c)
stearic acid (C18)	3.72±0.23NS	0.045±0.004b)	0.070±0.002c)

NS was not significant difference.
a) $p<0.05$, b) $p<0.01$ and c) $p<0.001$ compared with control.
*%Dose of amount of drug permeated for 10 h.
Each value is a mean ± SE (N=4).

Therefore, lauric acid (C_{12}) enhanced the skin permeations of indomethacin and 6-CF the most.

Figure. 2 shows the effects of various concentrations of lauric acid (C_{12}) on the permeations of indomethacin and 6-CF through rat skins. The highest enhancing effects were obtained at 0.07 M lauric acid. Permeations of indometacin and 6-CF with 0.14 M lauric acid significantly decreased compared to those with 0.07 M lauric acid ($p<0.05$ and $p<0.001$, respectively).

Treatment with saturated fatty acids (0.07 M) caused changes in the peak frequency of the CH asymmetric stretching vibration (2920 cm^{-1}) on FT-IR spectra, which results primarily from methylene groups in the stratum corneum lipid acyl chains. Figures. 3 and 4 show the relationships between these changes and the fluxes on the respective permeations of indomethacin and 6-CF through rat skins. The correlation

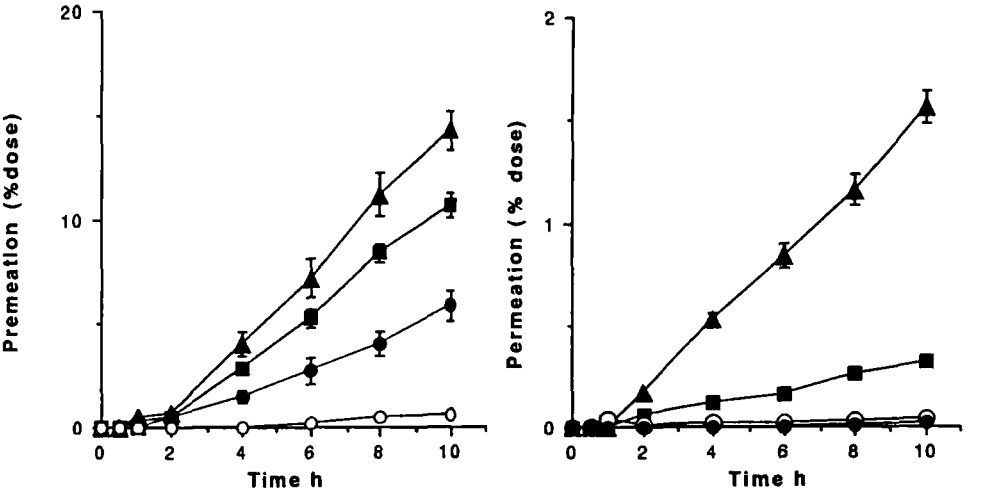

Figure 2

Effects of concentrations of lauric (C_{12}) acid on the cumulative permeation of indomethacin and 6-CF through rat skins.
○ control, ● 0.03 M, ▲0.07 M, ■0.14 M.
Each point represents the mean ± S.E. of 4 experiments.

coefficients obtained for indomethacin and 6-CF were 0.914 and 0.728, respectively. When the points of capric acid (C_{10}) on indomethacin and 6-CF were avoided, the correlation coefficients were improved to 0.956 and 0.902, respectively.

Figure. 5 shows the relationships between the lipophilicities of saturated fatty acids (log k') and the uptakes of saturated fatty acid by stratum corneum. Saturated fatty acids of longer chain lengths showed higher lipophilicities and had higher uptakes by stratum corneum. Therefore, this correlation coefficient was 0.834 .

DISCUSSIONS

The main barrier for penetration of drugs through the skin is the outermost layer, the stratum corneum. The stratum

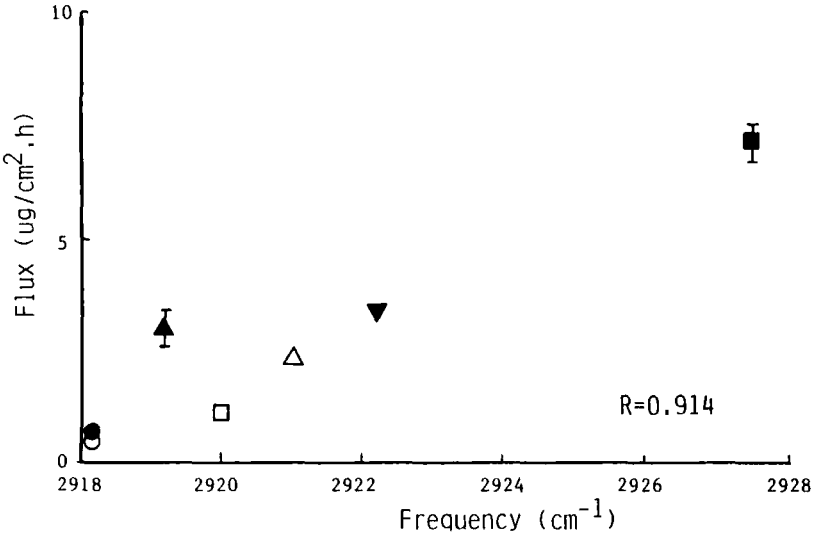

Figure 3

Relationship between the wavenumber shift in the frequency of CH asymmetric stretching peak of the stratum corneum of rabbit ear skins and the fluxes of indomethacin through rat skins.

○ control, ● caprylic acid(C_8), ▲ capric acid(C_{10}), ■ lauric acid(C_{12}), ▼ myristic acid(C_{14}), △ palmitic acid(C_{16}), stearic acid(C_{18}).

Each point represents the mean ± S.E. of 4 experiments.

corneum is composed of keratinocytes embedded in lipid domains consisting of alternately hydrophilic and lipophilic layers. The evidence for the existence of distinct hydrophilic and lipophilic transport pathways were suggested from relationship of permeability-partition coefficient of drugs (1). Indomethacin, a relatively lipophilic compound (partition coefficient; log k = 1.5 in octanol/pH 7.4 phosphate buffer solution (14)) may be absorbed through lipophilic route, which is intercellular domain of stratum corneum. While, 6-CF, a

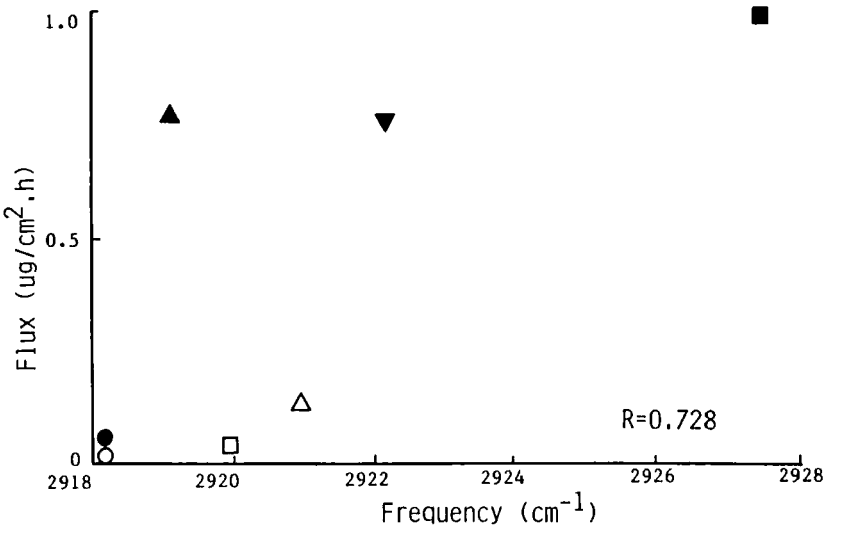

Figure 4

Relationship between the wavenumber shift in the frequency of CH asymmetric stretching peak of the stratum corneum of rabbit ear skins and the fluxes of 6-CF through rat skins.
○control, ●caprylic acid(C_8), ▲capric acid(C_{10}), ■lauric acid(C_{12}), ▼myristic acid(C_{14}), △palmitic acid(C_{16}), □stearic acid(C_{18}).
Each point represents the mean ± S.E. of 4 experiments.

hydrophilic compound (partition coefficient; log k= -2.13 in octanol/pH 7.4 phosphate buffer solution (7)) may be absorbed through hydrohilic route (15), which is a transcellular route. However, there are lipophilic enviroment of interdigitaing cell layers which must be overcome by 6-CF. It is expected that this lipophilic layer will not be the determing factor for skin permeation of 6-CF.

In this study, the largest enhancement in the permeation of indomethacin and 6-CF was obtained by lauric acid (C_{12}). However, the enhancing effects of caprylic acid (C_8) and stearic

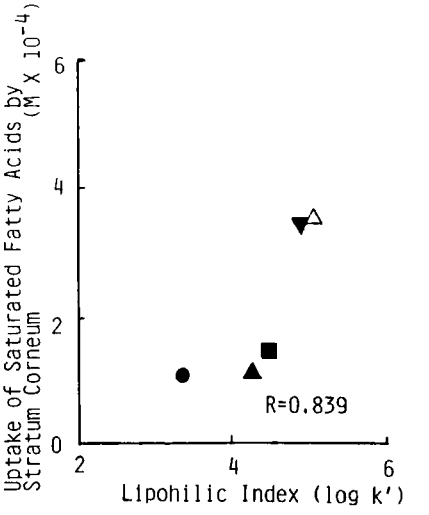

Figure 5

Relationship between the lipophilicities of saturated fatty acid (log k') and the uptake of the saturated fatty acid into stratum corneum of rabbit ear skins.

●caprylic acid(C_8), ▲ capric acid(C_{10}), ■ lauric acid(C_{12}), ▼ myristic acid (C_{14}), △palmitic acid(C_{16}).

Each point represents the mean ± S.E. of 4 experiments.

acid (C_{18}) were very low on the permeation of indomethacin. Effect of fatty acids on the permeation of naloxone through human cadaver skins was reported by Aungst et al.(4).

The permeation enhancing effects by saturated fatty acid, which were evaluated by fluxes on the skin permeations of these drugs and 6-CF was correlatable with the degrees of wavenumber shift in the frequency of the asymmetric CH bond stretching absorbance, which were induced by all saturated fatty acids except for capric acid (C_{10}). Therefore, saturated fatty acids except capric acid increased the perturbation olipid domain in stratum corneum. Not only might this result cause

the permeation enhancing effects of indomethacin, a lipophilic compound but also those of 6-CF, a hydrophilic compound.

Enhancing effect by capric acid (C_{10}) on the permeation of indomethacin and 6-CF was relatively high. However, low degree of wavenumber shift in the frequency of the asymmetric CH bond stretching absorbance, was induced by capric acid. Therefore, this main factor of the permeation enhancing effect did not cause an increase of the perturbation of lipid domain in stratum corneum. Aungst et al. reported the effects of fatty acids on skin permeation of solvent (15). The enhancing permeation rate of propylene glycol by capric acid was about about 5 times greater than that found in the presence of lauric acid. The permeation rate of the solvent can be an important factor influencing the permeation of drugs (15-16). The results of increased solvent penetration into the skin may include increased drug solubility in the skin and increased barrier disruption if the solvent itself is a permeation enhancer.

Saturated fatty acid of longer chain lengths has higher lipophilicities and had higher uptakes into stratum corneum. However, permeation enhancing effects of saturated fatty acids did not correlate with these factors. Therefore, amount of uptakes of saturated fatty acid into stratum corneum were not relate to the degree of permeation enhancing effect of them.

In conclusion, capric acid (C_{10}), lauric acid (C_{12}) and myristic acid (C_{14}) mostly enhanced the skin permeations of indomethacin and 6-CF out of a series of saturated staight-chain fatty acids. The perturbation increase of lipid domain in stratum corneum by these fatty acids except capric acid (C_{12}) caused the enhancing effects of permeation of indomethacin and 6-CF. On the other hand, capric acid enhanced the permeations of these two drugs by separate mechanisms. The skin irritation by these fatty acids was lower than that of oleic

acid (16). Therefore, these fatty acids may be useful as skin permeation enhancer of indomethacin and other related drugs.

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