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RESEARCH PAPER

Effect of Fatty Acid Diesters on Permeation of Anti-inflammatory Drugs Through Rat Skin

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ABSTRACT

Four fatty acid diesters (diethyl succinate, diethyl adipate, diethyl sebacate, and diisopropyl adipate) were used to study their enhancement effect on the permeation of four non-steroidal anti-inflammatory drugs (NSAIDs: ketoprofen, indomethacin, diclofenac sodium, and ibuprofen) through rat abdominal skin. With the diester pretreatment, drug permeation increased and the lag times decreased. No relationship was observed between the solubilities of the drugs in the diesters and the diester enhancement effects. The enhancement effect decreased with an increase of the drug lipophilicity, but increased with an increase of the lipophilic index of the diester up to about 3.5, after which the enhancement effect decreased or remained constant. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was employed to investigate the biophysical changes in the stratum corneum lipids caused by the diesters. The FTIR results showed that treatment of the skin with diesters did not produce a blue shift in the asymmetric and symmetric C–H stretching peak positions. However, all of the above diesters showed a decrease in peak heights and areas for both asymmetric and symmetric C–H stretching absorbances in comparison with water treatment.

These results suggested that the diesters were more effective for enhancing the penetration of hydrophilic drugs than lipophilic drugs, and the enhancing effect of

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lipophilic diesters was more effective than that of hydrophilic diesters. The enhancement effects of diesters may be due to their causing lipid extraction in the skin.

Key Words: *Fatty acid diester; FTIR; Lipophilicity; NSAIDs; Penetration enhancer; Percutaneous absorption*

INTRODUCTION

Transdermal drug delivery has received much attention recently. The stratum corneum, comprised of keratin-rich cells embedded in multiple lipid bilayers, is generally recognized as the primary barrier to drug delivery. Only a few materials, which tend to be lipophilic and have low melting points, are easily transported to the underlying viable aqueous tissue.[1] To overcome this skin impermeability, either chemical enhancers or physical methods, such as iontophoresis and electroporation,[2–6] have been suggested. In particular, much effort has been directed toward the search for enhancers in the development of practical transdermal therapeutic systems.

Fatty acid–alcohol esters are commonly used as adjuvants for cosmetics and pharmaceuticals. Several esters have been reported to enhance drug permeation.[7–11] We also reported that fatty acid diesters enhanced the permeation of diclofenac from oleaginous vehicles.[12] However, there have only been a few studies trying to systematically clarify the enhancement effect of fatty acid diesters on drug permeation.

Fourier transform infrared (FTIR) spectroscopy can be used to investigate the biophysical alterations taking place in the lipid bilayer after treatment with penetration enhancers. Of particular interest in this context are the IR absorbance regions near 2850 and 2920 cm^{-1} corresponding to symmetric and asymmetric methylene groups stretching, respectively.[13] A higher wavenumber shift in FTIR is an indication of an increase in gauche conformers.[13] And a broadening of the peaks is an indication of increased translational movement or mobility of lipid acyl chains.[14] On the other hand, decreases in the absorbances and the areas of the peaks have been linked to the extraction of the stratum corneum lipids.[15]

In the present study, we investigated the enhancing effects of four fatty acid diesters on the permeation of four non-steroidal anti-inflammatory drugs (NSAIDs) through rat abdominal skin. The relationships among the enhancement effects of the esters, the lipophilicities of the esters, and the drugs were studied. The biophysical changes in the stratum corneum by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy were also investigated to elucidate the effects of diesters on rat skin.

METHODS

Materials

Diclofenac sodium (Dc), ketoprofen (Ket), ibuprofen (Ibu), indomethacin (Ind), and the fatty acid diesters (diethyl succinate, D. Suc.; diethyl adipate, D.A.; diethyl sebacate, D.S.; diisopropyl adipate, D.I.A.) were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). The other reagents were of analytical grade and used without further purification.

Preparation of Test Vehicle

A slight excess amount of Dc, Ibu, or Ind suspended in a phosphate buffer (pH 6.0, 0.01 M) containing 30% ethanol was used for the experiments. For Ket, a 5 mM solution in the same buffer containing 30% ethanol was used because of its high solubility in the 30% ethanol solution. The pH of the vehicle was checked before and after the permeation study. No significant difference was observed between the pH of the vehicle before and after the permeation study.

In Vitro Permeation Study

After removal of abdominal hair from male Wistar rats (250 to 300 g) with electric clippers and an electric shaver one day before the penetration study, the abdominal skin was excised immediately before the experiment, after the animals had been sacrificed. Rats were used in accordance with the Guidelines for Animal Experimentation of Mukogawa Women's University, which are based on the Guidelines for Animal Experimentation of

the Japanese Association for Laboratory Animal Science. The fatty acid diester (15 μL) was applied to the skin 2 hr prior to the experiment. Any remaining enhancer on the skin was blotted with a Kimwipe after pretreatment and the skin was mounted in a Franz-type diffusion cell. In this study, 10 mL of 0.01 M phosphate buffer (pH 6.0) was used as the receptor medium, and 1 mL of the test vehicle was placed on the donor side. The surface area exposed for diffusion was 0.785 cm^2 (diameter = 1.0 cm). The receptor medium was kept at 37°C and stirred with a magnetic stirrer at 600 rpm.

Aliquots (0.1 mL) of the receptor medium were withdrawn periodically for 22 hr. Immediately after collection of the medium, 0.1 mL of fresh buffer was added. The concentration of the drug in the sample was determined by high-performance liquid chromatography (HPLC).

The flux rate of the drug was calculated by plotting the cumulative amount of drug penetrating the skin against time and determining the slope of the linear portion of the curve and *x*-intercept values (lag time) by linear regression analysis. Drug fluxes (J), at steady state, were calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which diffusion took place (0.785 cm^2 for the diffusion cells used in the present study). The permeability coefficient (P_e) was calculated by dividing the value of J by the drug concentration in the donor phase.

Enhancement ratios (ER) for the flux were calculated using the following equation:

$$\text{ER} = (P_e \text{ of diester pretreatment})/(P_e \text{ of water pretreatment})$$

Measurement of Drug Solubility in Fatty Acid Diesters

An excess amount of the drug was added to the diester. The mixture was then allowed to stand at 32°C for 24 hr under agitation. The suspension was filtered through a membrane filter with a pore size of 0.45 μm to obtain a clear fluid, and the drug concentration measured by HPLC.

Determination of Partition Coefficient

Octanol and buffer solution (pH 6.0, 0.01 M) were saturated with each other prior to use. Five milliliters of octanol with drug (1 mM) was mixed with 5 mL of buffer solution for 24 hr at 32°C. The mixture was then centrifuged and the drug concentration in the buffer measured by HPLC.

Analytical Methods

The drug was assayed using HPLC. The HPLC system consisted of a pump (880-PU, Jasco, Tokyo, Japan) and a detector (875-UV, Jasco), a 4.6× 250 mm^2 column packed with Nucleosil 100-5C_{18} (Macherey-Nagel, Germany), and an integrator (C-R3A, Shimadzu, Kyoto, Japan). The flow rate was 1.0 mL/min and the separation was performed at ambient temperature. The mobile phase composition (H_3PO_4:CH_3OH) and the ultraviolet (UV) detection were 23:77 and 282 nm for Dc, 23:77 and 282 nm for Ibu, 23:77 and 282 nm for Ind, and 23:77 and 282 nm for Ket, respectively.

Determination of Lipophilic Index of Fatty Acid Diesters

The lipophilic index was determined using the HPLC[16] described above with a UV detector operating at 210 nm. A mixture of methanol (80–60%) and distilled water (20–40%) was employed as the mobile phase. The elution time of a solvent (t_0) and the retention time of a fatty acid diester (t_R) were determined for each mobile phase composition. The $\log k'$ value defined by Eq. (1) was plotted against the methanol concentration in the mobile phase and the extrapolated $\log k'$ value to 0% methanol was obtained as an index of lipophilicity of the diester ($\log k'$):

$$\log k' = \log[(t_R - t_0)/t_0] \quad (1)$$

Preparation of Skin for ATR-FTIR Spectroscopy

The abdominal hair of male Wistar rats (250 to 300 g) was removed carefully with electric clippers and an electric shaver one day before the experiment. The abdominal skin was excised after the animals had been sacrificed, and the skin was cut to 1.0 cm in diameter. The skin was pretreated with fatty acid diesters for 2 hr at 32°C. The surface of the stratum corneum was rinsed with ethanol and water and gently wiped with Kimwipes.

ATR-FTIR Spectroscopic Study

The spectrum of the surface of the stratum corneum treated with fatty acid diesters in vitro was measured at ambient temperature. In the present study, an FTIR spectrophotometer (Spectrum 2000, Perkin-Elmer) was used to record the in vitro measurement. The spectrophotometer was equipped with an ATR accessory (Pike Technologies, Inc., Wisconsin), which supported a zinc selenide crystal plate ($8\times1\times0.4\,cm^3$, with a 45° incident angle). This ATR configuration results in an IR beam penetration depth of approximately 2 μm. All spectra reported were the average of 10 scans. The location of the IR absorbance peak maximum was determined to an accuracy of $0.1\,cm^{-1}$ using a center-of-gravity algorithm.

Statistical Analysis

The results are expressed as mean±standard deviation. Statistical significance was determined by Student's *t*-test or analysis of variance (ANOVA) (Bonferroni's method was used to compare individual data when a significant *F* value was shown), depending on the design of the experiments.

RESULTS

Physicochemical Properties of Drugs and Solubilities of Drugs in Fatty Acid Diesters

The physicochemical properties of drugs and the solubilities of drugs in fatty acid diesters (diesters) are shown in Tables 1 and 2, respectively. The solubilities of Dc, Ibu, and Ind in a phosphate buffer (pH 6.0, 10 mM) containing 30% ethanol were 1.9, 12.7, and 1.5 mM, respectively. The solubilities of drugs in diesters are markedly higher, 39–114-fold, than the vehicle on the donor side.

Drug Permeation

The permeation profiles of drugs through rat abdominal skin pretreated with diester from a suspension in phosphate buffer (pH 6.0, 10 mM) containing 30% ethanol except for Ket are shown in Fig. 1. For Ket, a 5-mM solution in the same buffer containing 30% ethanol was used for the experiment because of its high solubility in the 30% ethanol solution. Drug permeations markedly increased with diester pretreatment. However, different enhancement effects of diesters were observed on the permeation of NSAIDs. The calculated permeability coefficient (P_e), given in Table 3, shows the enhancement of drug permeation by the diesters to be D.S. = D.I.A. > D.A. > D.Suc. The lag time of the permeation, calculated from the steady-state flux and listed in Table 4, was shorter for all diester pretreatments.

Table 1

Physicochemical Properties of Anti-inflammatory Drugs

	Ket	Dc	Ibu	Ind
MW	254.29	318.13	206.27	357.81
pK_a	4.8[c]	3.9[d]	5.2[e]	4.5[e]
m.p.[a]	94	283–285	75–77	155–162
P_{oct}[b]	62.01	607.8	744.1	259.1

[a]Merck Index 10th Edition.
[b]Octanol/phosphate buffer (pH 6.0) partition coefficient (P_{oct}) measured at 32°C.
[c]Ref. 35.
[d]Ref. 36.
[e]Ref. 37.

Table 2

Solubilities of Drugs in Fatty Acid Diesters

	Solubility (mM)[a]			
	Ket	Dc	Ibu	Ind
D.Suc.	794.7	128.6	1453.1	87.4
D.A.	794.7	141.8	1453.1	106.8
D.S.	455.7	97.8	1389.1	58.2
D.I.A.	461.9	92.8	1223.2	57.8

[a]Solubility measured at 32°C.

Correlation of Diester Efficacy and Drug Properties

The correlation of the diester enhancement effect on drug permeation and drug physicochemical properties was investigated. No significant relationship was observed between the solubility of the drugs in the diesters and the enhancement effect (data not shown). Figure 2 presents the relationships

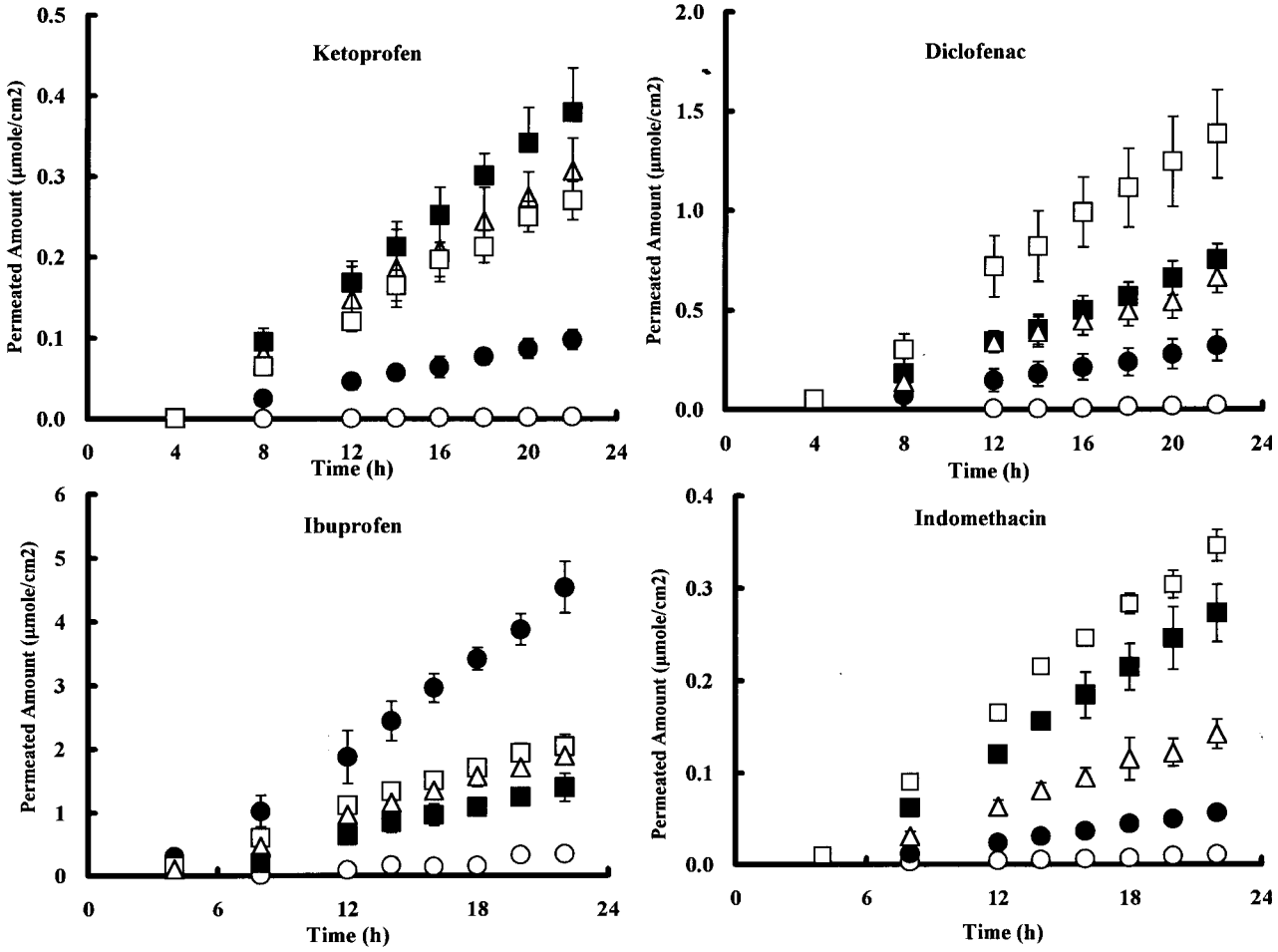

Figure 1. Permeation profiles of NSAIDs through the abdominal skin of rats. ○ Control, ● diethyl succinate, △ diethyl adipate, □ diisopropyl adipate, ■ diethyl sebacate. Each point represents the mean±S.D. (n=3–5).

Table 3

Permeation Parameters of Drugs in Rat Skin

	P_e (cm/hr)$\times 10^{-3}$ [a]				
	Cont.	D.Suc.	D.A.	D.S.	D.I.A.
Ket	0.05±0.01	1.1±0.1*	3.0±0.5*	4.2±0.6*	4.0±0.5*
Dc	2.4±0.5	6.2±0.7*	10.3±0.8*	20.8±2.1*	21.0±1.1*
Ibu	2.4±0.1	5.9±0.4*	8.2±0.9*	22.1±2.5*	18.9±1.2*
Ind	0.48±0.1	2.5±0.6*	5.5±0.8*	11.1±1.6*	12.3±0.3*

[a]The permeability coefficient was calculated from the steady-state flux rate and the initial concentration of each drug in the donor compartment. Each value represents the mean+S.D. (n=4–6).
*$p < 0.05$ compared to control.

Table 4

Permeation Parameters of Drugs in Rat Skin

	Lag time (hr)[a]				
	Cont.	D.Suc.	D.A.	D.S.	D.I.A.
Ket	10.1±1.0	3.2±0.5*	1.7±1.0*	3.8±1.0*	1.9±1.2*
Dc	8.7±0.3	3.8±2.3*	0.9±0.5*	3.5±2.2*	4.1±1.3*
Ibu	9.0±0.4	0.9±0.8*	2.3±1.1*	3.6±2.2*	2.9±2.4*
Ind	7.2±0.4	4.6±2.6	3.2±0.6*	3.7±0.3*	1.2±0.3*

[a]Lag times calculated from the straight lines in Figs. 1–4. Each value represents the mean±S.D. (n=4–6).
*$p < 0.05$ compared to control.

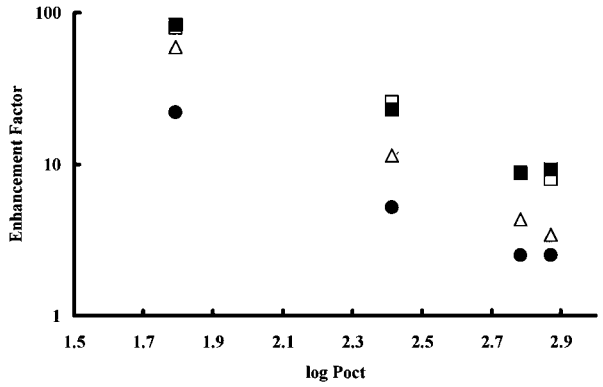

Figure 2. Relationship between the $\log P_{oct}$ of NSAIDs and the enhancement ratio of NSAIDs. ● Diethyl succinate, △ diethyl adipate, □ diisopropyl adipate, ■ diethyl sebacate. Each point represents the mean (n=3–5).

between the drug lipophilicities and the diester enhancement ratios. The enhancement ratios decreased as the drug lipophilicities increased.

To investigate the relationship between the diester lipophilicities and their enhancement effects, the lipophilic index of the diesters was measured.[17] Plots of the $\log k'$ values of diesters against the methanol concentration of the mobile phase showed a linear relationship (Fig. 3). The lipophilic indices were determined by the extrapolation of these plots to 0% methanol. Figure 4 shows the relationship between the lipophilic indices and the enhancement ratios of the diesters. The enhancement ratio increased as the diester lipophilic index increased until a value of about 3.5, after which the enhancement effect decreased or remained constant.

Effects of Diester on C–H Symmetric and Asymmetric Stretching Absorbance in ATR-FTIR

Fourier transform infrared studies were performed to gain insight into the effect of diesters on the biophysical properties of the stratum corneum. The FTIR spectra of the rat abdominal skin surface treated for 2 hr with water (control) or diesters are presented in Fig. 5. The prominent peaks near 2850 cm^{-1} and 2920 cm^{-1} result from asymmetric and symmetric stretching modes, respectively. Table 5 shows the absorbance peaks for both symmetric and asymmetric stretching absorbances. No significant changes were observed in both C–H asymmetric and symmetric stretching absorbance peaks due to diester treatment in comparison with water treatment. However, all of the diesters showed a decrease in peak heights and areas for both absorbances in comparison with water treatment (Table 6).

DISCUSSION

The physicochemical properties of drug, enhancer, and skin are the main factors that affect the percutaneous absorption of a drug, and a change in one of them is expected to alter their dynamic interactions and affect percutaneous drug permeation. In this study, pretreatment with diester was used to investigate its effect on the permeation of NSAIDs. This diester pretreatment was found to cause a marked increase in drug permeation (Fig. 1). The lipophilicity of the permeant is thought to play an important role in determining the

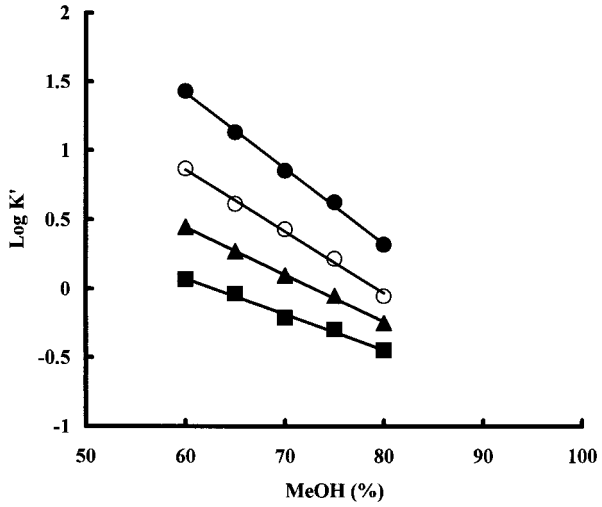

Figure 3. Relationship between $\log k'$ values of D.Suc. (■), D.A. (▲), D.S. (●), and D.I.A. (○) and methanol concentration (v/v, %) in the mobile phase.

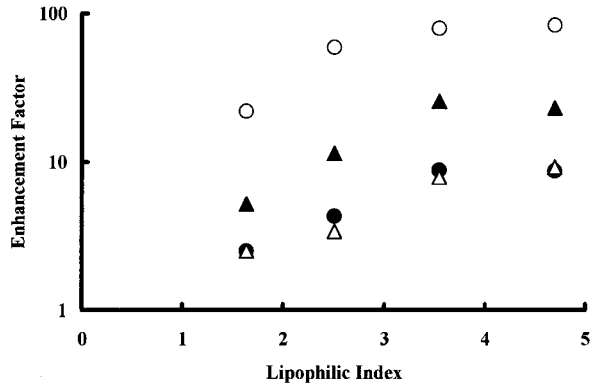

Figure 4. Relationship between the lipophilic index of diesters and the enhancement ratio of NSAIDs. ○ ketoprofen, ● diclofenac sodium, △ ibuprofen, ▲ indomethacin. Each point represents the mean ($n=3$–5).

enhancer-promoting activity on the permeation of the drug across the skin.[18–23] The enhancers produced the highest activity with the most hydrophilic drug, i.e., Ket, and the lowest activity with the most lipophilic drug, i.e., Ibu. An increase in drug lipophilicity was found to be associated with a decrease in the percutaneous permeation of the drug (Fig. 2). The same relationships were also observed with other kinds of enhancers.[23,24]

The lipophilicity of the enhancer is also thought to play an important role in determining the enhancer-promoting activity. Williams and Barry[25] found a linear relationship between the $\log P$ of the enhancer and the enhancement ratio of a drug using terpenes as enhancer. Recently, El-Kattan et al.[23] also suggested the same relationship. In our study, enhancement ratios increased with an increase of the lipophilic index of the enhancer up to 3.5, after which the enhancement ratios remained constant or decreased. These differences may be explained by the differences in thermodynamic activity of drugs and enhancers on the donor side, because El-Kattan et al.[23] used the drug solution including an enhancer as the donor side. The same maximum enhancement efficacies of enhancer were reported with N-alkylazacycloheptanones[26] and fatty acids.[27] To further elucidate the relationship among the lipophilicities of drugs and diesters and the enhancement ratios, these results were plotted against each other (Fig. 6). This figure clearly indicates that the important factor in enhancement by diesters is the lipophilicity of the permeant and the diester.

The mechanism of permeation enhancer action has been evaluated using differential scanning calorimetry, FTIR, and x-ray diffraction. In this study, FTIR was performed to clarify the effect of diesters on the biophysical properties of the stratum corneum. The most informative FTIR lipid absorbances for the stratum corneum are those originating from the hydrophobic alkyl chain.[28,29] Of these, the most extensively studied are the carbon–hydrogen stretching vibrations. The FTIR spectra of rat abdominal skin surface treated for 2 hr with water (control) or diesters are presented in Fig. 6. The prominent peaks near 2920 cm^{-1} and 2850 cm^{-1} result from asymmetric and symmetric stretching modes, respectively. Knutson et al.[30,31] reported that increases in the frequency of the C–H asymmetric stretching peak and band widths are due to transitions in stratum corneum lipids involving enhanced motional freedom of the hydrocarbon chains (i.e., increased lipid fluidity). Furthermore, the higher wavenumber shifts of the C–H asymmetric stretching vibrations are associated with an increase in the number of gauche conformers along the lipid hydrocarbon chains. Table 5 shows the absorbance peaks for both symmetric and asymmetric stretching absorbances. Compared with water, treatment of the stratum corneum for 2 hr with diesters did not result in shifts to higher frequency for the C–H symmetric and asymmetric stretching absorbance peaks.

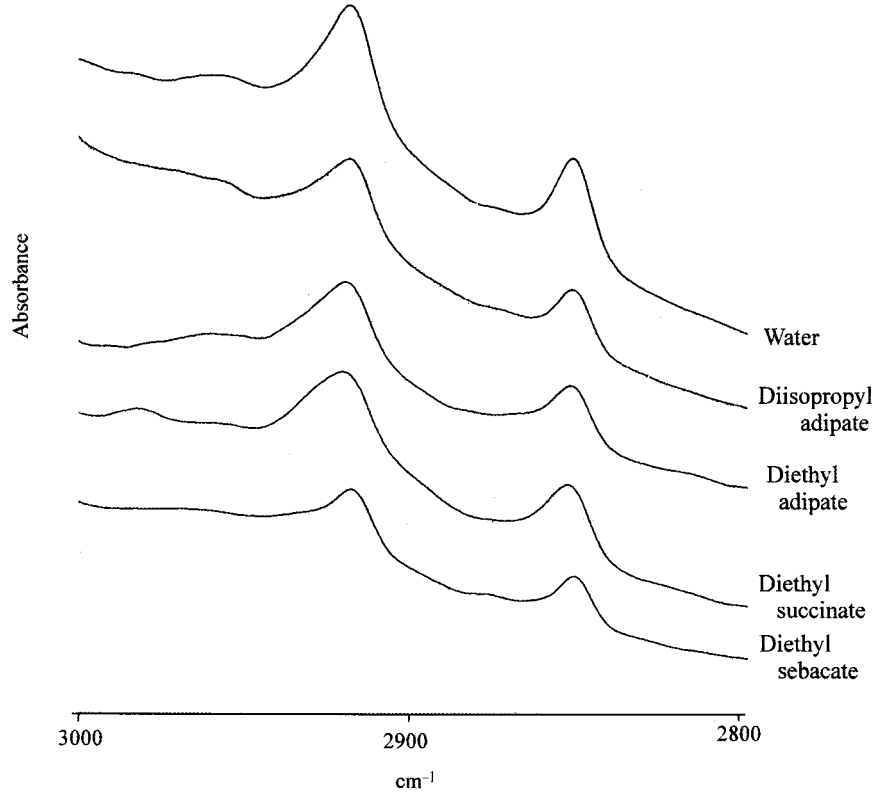

Figure 5. ATR-FTIR spectra of the stratum corneum of abdominal rat skin showing asymmetric and symmetric C–H bond stretching absorbances after diester treatment.

Table 5

Effects of Esters on the C–H Symmetric and Asymmetric Stretching Absorbance Shifts of Stratum Corneum Lipids of Rat Dorsal Skin In Vitro

	C–H Stretching Absorbances[a]	
	Symmetric (cm^{-1})	Asymmetric (cm^{-1})
Water	2849.66±0.27	2917.60±0.28
D.Suc.	2850.19±0.36	2918.02±0.72
D.A.	2850.06±0.18	2917.64±0.66
D.S.	2850.00±0.67	2917.94±0.44
D.I.A.	2849.89±0.24	2917.43±0.08

[a]Each value represents the mean±S.D. of four samples.

There are several reports that some compounds lead to lipid and/or protein extraction in the stratum corneum and enhance drug permeation.[32–34] These extractions can be evaluated by comparing the peak heights and areas of the symmetric and asymmetric C–H stretching absorbances before and after penetration enhancer treatment. Significant changes were observed in the absorbance peak heights and areas due to diester treatments in comparison with the treatment with water (Table 6). These results suggested that diesters affect the lipid extraction from the stratum corneum.

CONCLUSION

Our findings showed that pretreatment with diester can enhance the skin permeation of NSAIDs. The diesters were more effective for enhancing the permeation of hydrophilic drugs than lipophilic drugs, and the lipophilic diesters were more effective for enhancing drug permeation than the hydrophilic diesters. All of the diesters showed a decrease in peak heights and areas for both asymmetric and symmetric C–H stretching absorbances in

Table 6

Effects of Esters on Peak Height and Peak Area of the C–H Symmetric and Asymmetric Stretching Absorbance of Stratum Corneum Lipids of Rat Dorsal Skin In Vitro[a]

	Symmetric		Asymmetic	
	Peak Height	Peak Area	Peak Height	Peak Area
Water	0.091±0.011	1.075±0.188	0.125±0.011	2.565±0.181
D.Suc.	0.066±0.009*	0.776±0.134*	0.095±0.021*	2.093±0.473
D.A.	0.047±0.012*	0.504±0.140*	0.081±0.014*	1.766±0.304*
D.S.	0.048±0.012*	0.558±0.149*	0.069±0.020*	1.322±0.381*
D.I.A.	0.055±0.002*	0.608±0.034*	0.082±0.009*	1.542±0.287*

[a]Each value represents the mean±S.D. of four samples.
*$p < 0.05$ compared to water.

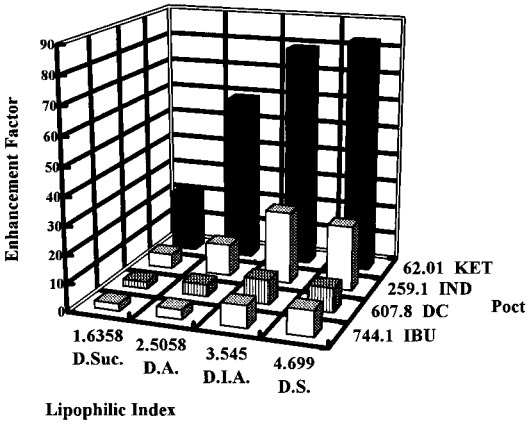

Figure 6. Relationship among the P_{oct} of NSAIDs, the lipophilic index of diesters, and the enhancement ratio of NSAIDs. Each point represents the mean (n=3–5).

comparison with water treatment. Therefore, the enhancement effects of the diesters may be due to their causing lipid extraction in the skin.

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