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Effect of lipophilic multicomponent system on the skin permeation of ketotifen fumarate

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Abstract

The effect of a lipophilic chemical enhancer system on the skin permeation of ketotifen fumarate (KF) was investigated in vitro using excised hairless rat skin. Organic acids, alcohols and oleaginous solvents were selected as fundamental components of the system. Among the several lipophilic systems, a ternary component vehicle consisting of L-lactic acid, ethanol and isopropyl myristate (IPM) (3:10:87) exhibited a significant skin penetration-enhancing effect. The rank order of the effect on KF skin permeation for oleaginous components containing ethanol or L-lactic acid/ethanol was IPM > ethyl oleate > diisobutyl adipate > oleic acid. This order was similar to that on ethanol flux. The KF permeation was thus enhanced and regulated by the ethanol permeation. The KF flux from various alcohol/IPM systems was increased, with an increase in the solubility parameter of alcohols. L-Lactic acid in the alcohol/IPM systems did not have a marked effect on the alcohol flux, whereas it increased the KF flux. Addition of several organic acids into the ethanol/IPM system (1:10:89) shortened the lag time of KF permeation, and the effect may be related to these chain lengths and functional groups.

Keywords: Transdermal drug delivery; Penetration enhancer; Multicomponent system; Lipophilic vehicle

1. Introduction

Low skin permeability of drugs is a significant problem which must be overcome when developing a transdermal drug delivery system (TDDS). The use of chemical enhancers is one of the

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simplest and most convenient methods to increase the permeability (Walters and Hadgraft, 1993). A good enhancer creates many kinds of TDDS which can lead to innovations in current drug therapy. Much effort has been applied to finding effective enhancers and to elucidating their mechanism and site of action (Kadir et al., 1987; Barry, 1991).

We have developed a new multicomponent aqueous enhancer system consisting of *l*-menthol, ethanol and water (MEW system) (Morimoto et al., 1993). This system showed a synergistic effect of *l*-menthol and ethanol on the in vitro permeability of morphine hydrochloride (Morimoto et al., 1993) and several cardiovascular agents (Kobayashi et al., 1993) through hairless rat and human skin. A similar multicomponent aqueous system containing *d*-limonen, ethanol and water was reported by Okabe et al. (1989). Several multicomponent lipophilic systems such as Panasate-800/ethanol (Lee et al., 1993), ethanol/ethyl acetate (Catz and Friend, 1990) and isopropanol/isopropyl myristate (IPM) (Pardo et al., 1990) were also reported to significantly facilitate skin permeation of different drugs.

In the present study, we developed a multicomponent lipophilic system according to the above information using ketotifen fumarate (KF, Fig. 1) as a model drug; this drug exhibits low skin permeability without any enhancers due to its relatively high hydrophilicity. KF is a candidate drug for TDDS because of its superior pharmacological action against asthma at even a low dose. The oleaginous solvent such as IPM was the main component for the lipophilic system, because IPM and the relative compounds were known to exhibit a high skin penetration-enhancing effect by themselves (Aungst et al., 1986; Sato et al., 1988). An alcohol such as ethanol and an organic acid like L-lactic acid were also selected as additives to the systems. Ethanol is broadly used as pharmaceutical additive for topical formulations and shows skin penetration-enhancing effect as stated above. Organic acid was used in expectation of its increasing KF solubility in the multicomponent vehicle and skin barrier.

2. Materials and methods

2.1. Materials

KF was supplied by Sandoz (Tokyo, Japan). IPM, ethanol, L-lactic acid and other reagents were commercial products and used without further purification.

2.2. In vitro skin permeation study

Abdominal skin excised from hairless rat (WBN/ILA-Ht, male, weight about 180 g, Life Science Research Center, Josai University, Saitama, Japan) was used as a skin sample. Two kinds of diffusion cells, a vertical type (Sugibayashi et al., 1992) and a side-by-side (2-chamber) type (Okumura et al., 1990) were used for the in vitro skin permeation studies. The skin sample was carefully mounted between the cell body and the cell cap of a vertical diffusion cell (effective diffusion area: 0.95 cm^2, cell body volume: 4.5 ml) or two half-cells of a 2-chamber diffusion cell set (effective diffusion area: 0.95 cm^2, cell volume: 2.5 ml). Drug solution (0.1% KF) or suspension (about twice the solubility) in each solvent system was applied on the stratum corneum side of the vertical cell (0.15 ml) or 2-chamber cell (2.5 ml), and the dermis side was filled with 4.5 or 2.5 ml of distilled water. The permeation experiment was done at 37°C. Sample solution was withdrawn from the dermis side chamber at predetermined times. The same volume of distilled water was

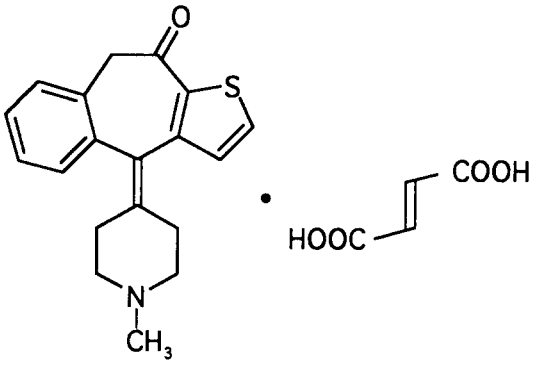

Fig. 1. Structure of ketotifen fumarate (KF).

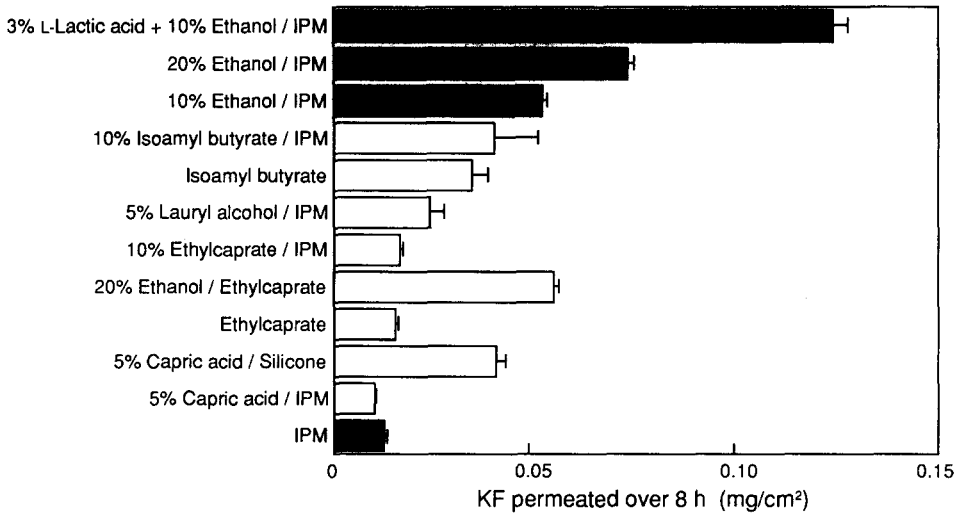

Fig. 2. Comparison of enhancing effect on the hairless rat skin permeability of KF among several lipophilic vehicles.

added after sampling to keep the cell volume constant. When a 2-chamber cell was used, the donor vehicle was changed periodically to avoid a marked fall of thermodynamic activity (Skelly et al., 1987) of KF and vehicle components in the system throughout the permeation experiment.

2.3. Analytical method

KF was determined by high performance liquid chromatography (HPLC). Sample containing KF was added to acetonitrile solution containing ethyl *p*-hydroxylbenzoate as an internal standard. After centrifugation of the mixed solution, the supernatant was injected to HPLC composed of a pump system (LC-6A, Shimadzu Seisakusho, Kyoto, Japan), a UV detector (SPD-6A, Shimadzu Seisakusho), Chromatopack (CR-3A, Shimadzu Seisakusho), and a reverse phase column (Ultron N-C_{18}; 150 mm × 4.6 mm i.d., Shinwa Kako, Kyoto). The elution phase was 0.1% phosphoric acid:acetonitrile (70:30), the flow rate was 0.8 ml/min, and detection was done at a UV wavelength of 295 nm. The retention times for KF and ethyl *p*-hydroxylbenzoate (internal standard) were about 4.7 and 11 min, respectively. The detection limit of KF was 0.1 μg/ml (CV, 2.2%).

Ethanol was measured by gas chromatography (GC) (GC-8A, Shimadzu Seisakusho). The conditions were: injection volume, 1 μl; column, Gaskuropack 54 (60/80 mesh, GL Science, Tokyo); column, injection and detection temperatures, 160, 200 and 200°C, respectively; carrier gas, N_2; carrier pressure, 1 kg/cm^2. A flame ionization detector (FID) was used, and the absolute calibration method was applied. The retention time of ethanol was 2.4 min. The detection limit was 0.1 mg/ml (CV, 3.5%).

L-Lactic acid was assayed by GC. The conditions were: column, Thermon-3000 (support Shincarbon A, 60/80 mesh, GL Science); column, injection and detection temperatures, 140, 180 and 180°C, respectively; carrier gas, N_2; carrier pressure, 0.5 kg/cm$_2$. FID and the absolute calibration method were used. The retention time and detection limit were 3.4 min and 0.1 mg/ml (CV, 11%), respectively.

3. Results and discussion

3.1. Preliminary screening

IPM was selected as a representative oleaginous component, because it is broadly used in pharma-

Table 1
Physicochemical properties of components

Component	Compound	Solubility parameter $(cal/cm^3)^{1/2}$	pK_a	Molecular weight
Oleaginous component	IPM	8.539	—	270.46
	Ethyl oleate	8.625	—	310.52
	Diispbutyl adipate	9.081	—	258.36
	Oleic acid	9.147	—	282.47
Alcohol	Methanol	13.767	—	32.04
	Ethanol	12.575	—	46.07
	n-Propanol	11.836	—	60.10
	n-Butanol	11.330	—	74.12
	n-Pentanol	10.962	—	88.15
	n-Hexanol	10.681	—	102.18
	Isopropanol	11.579	—	60.10
	Benzyl alcohol	12.782	—	108.14
Acid	L-Lactic acid	14.854	3.79	90.08
	Acetic acid	11.162	4.74	60.05
	Caproic acid	9.923	—	116.16
	Enanthic acid	9.778	4.85	130.19
	Benzoic acid	11.935	4.19	122.12
	Hydrochloric acid	—	—	36.46
	Phosphoric acid	—	2.15, 7.10, 12.32	98.00

ceutical and perfumery products and its skin tolerability is well established (Holzner, 1963; Campbell and Bruce, 1981). Fig. 2 shows the result of a preliminary screening using vertical-type diffusion cells. All the systems containing ethanol (10% or 20%) allowed higher KF flux than other systems without ethanol. Ethanol is a useful enhancer in lipophilic as well in hydrophilic vehicles (Ghanem et al., 1987; Morimoto et al., 1993; Okabe et al., 1989). Ethanol/IPM system (ethanol conc., 20%) showed a higher KF permeation than the corresponding ethanol/ethylcaprate system. Of all the systems used in this experiment, the system containing L-lactic acid, ethanol and IPM (3:10:97) exhibited the greatest enhancing effect on KF permeation through excised hairless rat skin. Thus, these three components were selected as fundamental additives for a multicomponent enhancer system.

The effect of each component in the system was investigated by comparing the enhancing effects among the various oleaginous components (1), the various alcohols (2), and the various acids (3). In this experiment, the 2-chamber diffusion cell and KF suspended vehicle were used to measure the maximum enhancing effect and the usefulness of the enhancer systems. Table 1 shows the physicochemical properties of the components used in this experiment. The solubility parameters for oleaginous components, alcohols, and organic acids were calculated by Fedors functional group method (Fedors, 1974) and the values ranged between 7.55 and 9.25 $(cal/cm^3)^{1/2}$, 10.68 and 13.77 $(cal/cm^3)^{1/2}$, and 9.78 and 14.85 $(cal/cm^3)^{1/2}$, respectively. Table 2 summarizes the solubilities of KF in the various vehicles used in this experiment.

In the ternary system of L-lactic acid/ethanol (10%)/IPM, no significant differences in KF permeation were obtained among 0.5, 1 and 3% L-lactic acid. L-Lactic acid is insoluble in IPM and only about 3% of the acid is soluble even in 10% ethanol in IPM. Concentrations of the acid and alcohol were set to 1 and 10%, respectively, taking into account the enhancing effect and physical stability of the system.

3.2. Effect of the oleaginous components

Fig. 3 shows the relationship between KF flux and the solubility parameters of applied systems containing an oleaginous vehicle as a main com-

Table 2
Solubility of KF in the various lipophilic vehicles at 37°C

Vehicle			Solubility (mg/ml)			
Oleaginous component	Alcohol	Acid	Neat solvent	1% L-lactic acid in oleaginous	10% ethanol in oleaginous	1% L-lactic acid–10% ethanol in oleaginous
IPM[a]	Ethanol	L-Lactic acid	0.10	0.11	0.85	2.22
Ethyl oleate			0.31	1.42	2.18	4.64
Diisobutyl adipate			0.26	1.17	1.52	3.47
Oleic acid			7.74	3.43	12.08	15.35
Oleaginous component	Alcohol	Acid			10% alcohol in IPM	1% L-lactic acid–10% alcohol in IPM
IPM[a]	Methanol	L-Lactic acid			0.80	3.26
	Ethanol				0.85	2.22
	n-Propanol				0.54	2.10
	n-Butanol				0.45	1.73
	n-Pentanol				0.22	0.65
	n-Hexanol				0.20	0.53
	Isopropanol				0.48	1.62
	BA[b]				0.27	0.66
Oleaginous component	Alcohol	Acid				1% acid–10% ethanol in IPM
IPM[a]	Ethanol	L-Lactic acid				2.22
		Acetic acid				1.06
		Caproic acid				0.84
		Enanthic acid				0.90
		Benzoic acid				0.71
		Hydrochloric acid				1.15
		Phosphoric acid				0.06

[a] Isopropyl myristate.
[b] Benzyl alcohol.

ponent. KF flux, when the oleaginous component alone or the oleaginous vehicle containing L-lactic acid (without alcohol) was applied, showed almost the same value regardless of kind of vehicle, with the exception of diisobutyl adipate with L-lactic acid. The KF solubility was decreased with the increase of the solubility parameter of the oleaginous component. However, KF flux was almost the same independently of the oleaginous component used, and this was due to the same thermodynamic activity of KF, the driving force of its permeation through skin, since it was applied in a suspension for all vehicles. In contrast, KF flux increased with the addition of ethanol at a concentration of 10%, both in cases with and without 1% L-lactic acid. These fluxes were dependent on the solubility parameter of vehicles, an index of their lipophilicity. In spite of the application of KF suspension, in an ethanol-containing system the flux was not constant and was regulated by the lipophilicity of the vehicle applied. This is explainable by the ethanol flux.

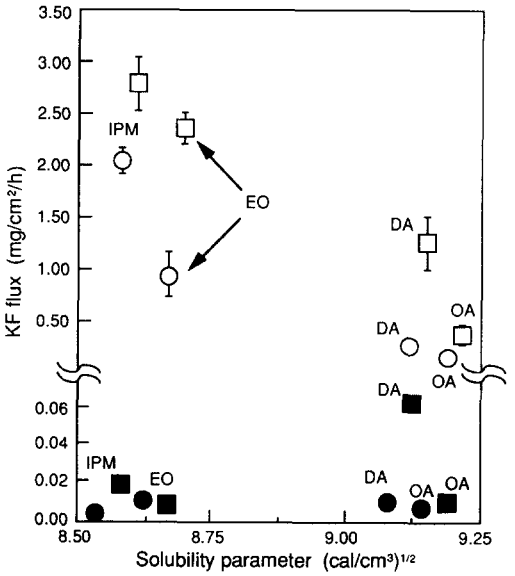

Fig. 3. Relationship between solubility parameter of oleaginous components and KF flux. (●), Not additive; (■), addition of 1% L-lactic acid; (○), addition of 10% ethanol; (□), addition of 1% L-lactic acid and 10% ethanol. Abbreviations: EO, ethyl oleate; DA, diisobutyl adipate; OA, oleic acid. Each value is the mean ± S.E. ($n = 3$–6).

Ethanol flux from ethanol/oleaginous component binary vehicle was then investigated. Fig. 4(a) shows the relationship between the ethanol flux and the solubility parameter of each vehicle. KF had a constant thermodynamic activity, since it was applied as a suspension, whereas ethanol had different activities depending on the vehicle, because we adjusted the ethanol concentration to 10%. The ethanol flux from ethanol/oleaginous vehicles with L-lactic acid was a little higher than from those without the acid. These ethanol fluxes decreased with increasing solubility parameter of the vehicles, which was similar to the KF permeation from oleaginous vehicles containing ethanol. The enhancing effect on the KF permeation by the system containing ethanol was thus strongly related to the ethanol permeability.

Fig. 4(b) shows the relation between the flux of L-lactic acid and the solubility parameter of vehicles. The permeation rate of L-lactic acid was independent of the solubility parameter of vehicles without ethanol. In vehicles with ethanol, however, L-lactic acid flux decreased with the solubility parameter of the vehicles. The acid flux was thus greatly influenced and enhanced by ethanol. This enhanced skin permeation of L-lactic acid by ethanol may be related to the enhanced KF flux.

Fig. 5 summarizes the good correlations between ethanol and KF fluxes; the enhanced KF permeation was greatly dependent on the ethanol permeation. Such a linear correlation between solvent and solute permeations was also exhibited for enhanced deliveries of nitroglycerin (Berner et al., 1989) and β-estradiol (Liu et al., 1991) from ethanol/water binary cosolvents. Ethanol in these systems showed an enhancing effect in response to an increase of drug concentration or acceleration of the drug diffusivity in the stratum corneum. Thus, the enhancing effect of ethanol on the KF flux is probably due to an increase of KF concentration in the skin. Since addition of ethanol to each vehicle brought about a 1.5–8-fold increase in KF solubility in the vehicle, KF solubility in skin may also be increased by the ethanol penetration into skin.

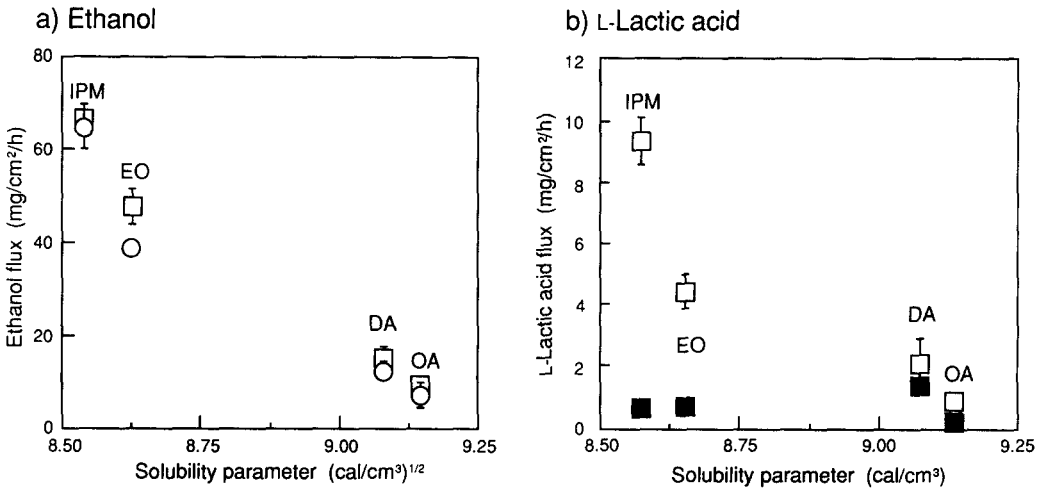

Fig. 4. Relationship between solubility parameter of oleaginous components and ethanol or L-lactic acid fluxes. (■), Addition of 1% L-lactic acid; (○), addition of 10% ethanol; (□), addition of 1% L-lactic acid and 10% ethanol. Abbreviations same as in Fig. 3. Each value is the mean ± S.E. ($n = 3$–6).

3.3. Effect of the alcohol component

Permeation of ethanol has an important function in enhancing KF permeation in an ethanol/oleaginous system. We therefore measured the influence of physicochemical properties of alcohol on KF flux when various IPM systems were applied which contained different alcohols with and without 1% L-lactic acid. Fig. 6 shows the permeation rate of alcohols from multicomponent systems against their solubility parameter (a) and molecular weight (b). The flux increased with the solubility parameter and decreased with the molecular weight. Benzyl alcohol showed a lower permeability than that predicted from its solubility parameter. The solubility parameter of benzyl alcohol estimated by Fedors functional group method was less compatible with its lipophilicity, compared with aliphatic alcohols used in this experiment. The low solubility of the highly lipophilic alcohols in the receiver solution or in the viable epidermis/dermis may result in the low alcohol flux. Fig. 7 shows the relationships between the KF permeation rate and the solubility parameter or molecular weight of alcohol, which were similar to those of the alcohol's own permeation rate. From these results, the KF flux was enhanced and influenced not only by ethanol permeation but also by other alcohol permeations. When a fixed oleaginous component was used, a more marked effect was found for alcohols with smaller molecular weight and lower lipophilicity. An isopropanol/IPM (1:1) mixture demonstrated the highest enhancement for estradiol flux among various alcohol/IPM cosolvents (Goldberg-Cettina et al., 1995). In a neat alcohol solvent,

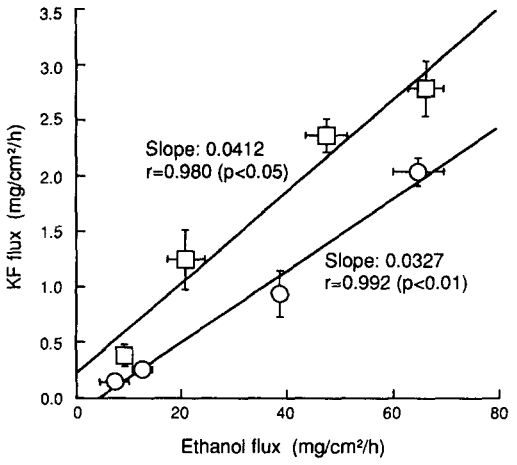

Fig. 5. Relationship between KF and ethanol fluxes. (○), Addition of 10% ethanol; (□), addition of 1% L-lactic acid and 10% ethanol. Each value is the mean ± S.E. ($n = 3$–6).

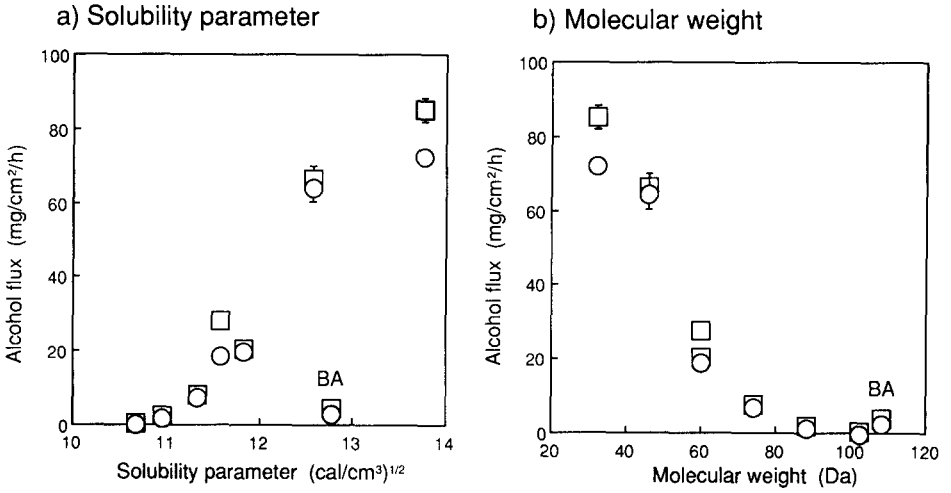

Fig. 6. Relationship between alcohol flux and solubility parameter (a) or molecular weight (b) of alcohol. (○), Addition of 10% alcohol; (□), addition of 1% L-lactic acid and 10% alcohol. BA, Benzyl alcohol. Each value is the mean ± S.E. ($n = 3$–6).

n-octanol showed the highest enhancing effect on the β-estradiol (Goldberg-Cettina et al., 1995). The highest levonorgestrel flux was obtained from *n*-butanol or isobutanol among a series of alcohols (Friend et al., 1988). In our case, however, ethanol had the greatest effect on KF permeation among the alcohols used in this experiment. This difference may be due to the drug lipophilicity and/or vehicle composition. Such enhanced skin delivery of many drugs can be accomplished by changing the kind and/or concentration of alcohol.

L-Lactic acid did not have a marked effect on the skin permeation of alcohol, whereas it did greatly enhance the skin permeation of KF, regardless of the kind of alcohol. If L-lactic acid has any effect on the barrier function in the stratum corneum or increases the penetration-enhancing effect of alcohol, it increases the alcohol flux as well as the KF flux; however, in own experiment there was no notable effect on the alcohol flux by the acid. L-Lactic acid may interact with KF in the vehicle or in the skin barrier. Ketotifen is a basic drug so it may form a complex with an acidic compound. Further studies are necessary to understand the mechanism and mode of action of the skin-penetration-enhancing effect of the acid.

3.4. Effect of the acid component

L-Lactic acid exhibited the enhancing effect on KF permeation from several vehicles. The permeation-enhancing ability of the organic acids; L-lactic acid, acetic acid, caproic acid, enanthic acid and benzoic acid, on the skin permeation of KF was examined and compared with that of the ethanol/IPM system without any acids. Table 3 summarizes the obtained fluxes and lag times of KF and ethanol from various acid systems consisting of one acid, ethanol and IPM. Systems containing L-lactic acid, acetic acid, caproic acid or benzoic acid showed a higher KF flux and a shorter lag time than that from the ethanol/IPM system (without any acids). In the straight chain acids, the KF flux was decreased with the carbon chain length of the acid. These acids decreased the lag time of ethanol permeation as KF did, but did not affect the permeation rate of ethanol. For all acid systems, the lag time of ethanol permeation was shorter than that of KF permeation. Berner et al. (1989) reported that ethanol significantly shortened the lag time of nitroglycerin without changing its diffusivity, when the nitroglycerin flux was enhanced by ethanol and the lag time of ethanol was shorter than nitroglycerin. In the present study, the same kind of results were ob-

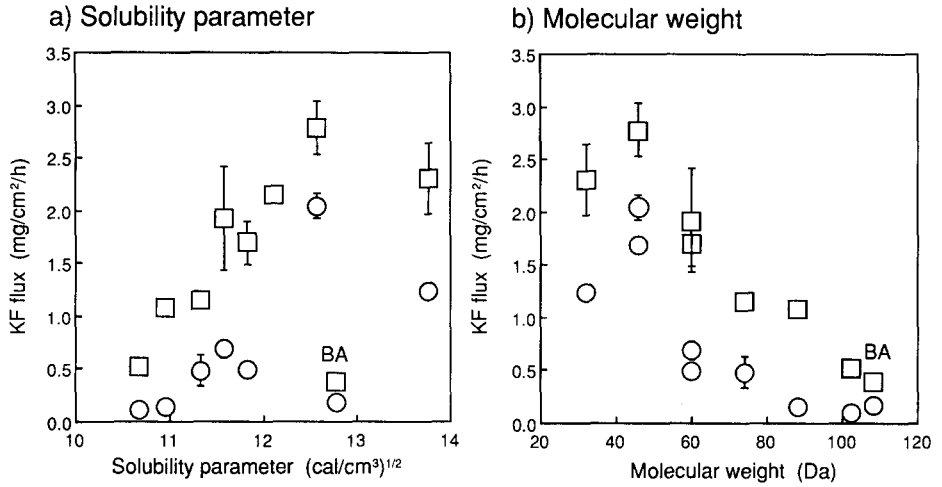

Fig. 7. Relationship between KF flux and solubility parameter (a) or molecular weight (b) of alcohol. (○), Addition of 10% alcohol; (□), addition of 1% L-lactic acid and 10% alcohol. BA, Benzyl alcohol. Each value is the mean ± S.E. ($n = 3$–6).

tained: the lag time of KF permeation was shortened by acids. This short lag time is suggested to be a result of the short lag time of ethanol permeation by an organic acid. L-Lactic acid showed the greatest enhancing effect among acids used in this experiment; it has a hydroxyl group and may show a high affinity with ethanol. The hydroxyl group played an important role in the enhancing effect of L-lactic acid, while benzoic acid increased the KF flux and prolonged the lag times of KF and ethanol. Benzoic acid may be bulkier than other acids due to its relatively high molecular weight and its aromatic ring. The prolonged lag times of KF and ethanol by benzoic acid were caused by slow migration of the acid. Different effects by organic acids can he explained by the acids having different functional groups.

The addition of acid may result in damage to the skin, so inorganic acids, hydrochloric acid or phosphoric acid were used as positive control of pH change. The addition of inorganic acid shortened the lag time of KF and ethanol permeation, but decreased the KF flux. The decreased lag time was found both with addition of inorganic and organic acid, but the increase of KF flux was found only with the addition of organic acids. Thus, the enhancing effect on KF flux is caused by other factors, without the pH change. Al-

Table 3
Comparison of flux (J) and lag time (T_L) in KF and ethanol permeation among several acid systems

Acid	J_{KF} (mg/cm² per h)	$T_{L\ KF}$ (h)	$J_{Ethanol}$ (mg/cm² per h)	$T_{L\ Ethanol}$ (h)
EI system	2.04 ± 0.122	2.24 ± 0.25	64.94 ± 4.80	1.40 ± 0.22
Enanthic acid	1.68 ± 0.068	2.35 ± 0.66	63.57 ± 5.09	1.71 ± 0.52
Caproic acid	2.20 ± 0.368	2.10 ± 0.40	64.91 ± 16.12	1.96 ± 0.31
Acetic acid	2.41 ± 0.368	1.73 ± 0.40	71.70 ± 3.40	1.32 ± 0.32
L-Lactic acid	2.79 ± 0.254	0.64 ± 0.13	66.41 ± 3.35	0.54 ± 0.15
Benzoic acid	2.45 ± 0.115	2.94 ± 0.03	64.50 ± 5.57	2.42 ± 0.28
Hydrochloric acid	1.09 ± 0.163	0.57 ± 0.47	45.01 ± 4.07	0.26 ± 0.05
Phosphoric acid	0.13 ± 0.027	1.30 ± 0.05	46.67 ± 1.33	0.76 ± 0.15

though the detailed mechanism(s) is/are not yet clear, L-lactic acid was most effective among the acids tested.

Alcohol was the most important component in the multicomponent lipophilic system composed of acid, alcohol and oleaginous component. Ethanol was reported to be a solvent-type enhancer which permeates through skin and increases partition of a drug to skin (Barry, 1991); short-chain alcohols may act in the same way. Since the alcohol flux affected the KF flux, alcohol can be used to regulate the rate and amount of drug delivery through skin. The oleaginous component had a 'push effect' on the ethanol (Kadir et al., 1987). The organic acids shortened the lag time of both ethanol and KF, and also increased KF flux, while the alcohols enhanced the KF permeation through an action related to their molecular weight and lipophilicity. Optimization of these components could achieve an ideal drug delivery.

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