l-Menthol, Oleic Acid and Lauricidin in Absorption Enhancement of Free and Sodium Salt of Diclofenac Using Ethanol Treated Silicone Membrane as Model for Skin

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The mechanism of *l*-menthol, oleic acid and lauricidin as enhancers on percutaneous absorption was examined using diclofenac (DH) as a hydrophobic drug and sodium diclofenac (DNa) as a hydrophilic drug in *in vitro* diffusion experiments with two kinds of membranes: ethanol-treated and untreated silicone membranes; these were models for the lipid and pore pathways of skin. A 20% w/w ethanol–aqueous solution decreased the flux of DH but increased the flux of DNa significantly across the treated membrane compared with those fluxes across the untreated membrane, suggesting that DH penetrated by the lipid pathway and DNa by the pore pathway. The permeability of DNa through the pore pathway decreased significantly across the treated membrane with the addition of oleic acid and lauricidin. *l*-Menthol increased the permeability coefficients of DH and DNa more in a treated membrane than in an untreated one, showing the same tendency as in rat skin. Thus, while oleic acid and lauricidin did not increase the permeation of DNa by the pore pathway, *l*-menthol appeared to enhance the permeation of the drug by both the lipid and pore pathways.

Key words *l*-menthol; oleic acid; lauricidin; diclofenac; silicone membrane; ethanol

One of the tactics for designing transdermal drug delivery systems involves the combined effects of enhancers and a cosolvent of an ethanol–aqueous solution to increase permeation of a drug.[1-3] *l*-Menthol,[4] oleic acid[5] and lauricidin have often been used as effective enhancers but their mechanism is not still clear. The mode of action of these enhancers was examined using a free diclofenac (acid, DH) as a hydrophobic drug model and sodium diclofenac (DNa) as a hydrophilic one. Since skin is a heterogeneous membrane with both lipid and pore pathways,[6] a lipophilic membrane such as an ethanol-treated silicone membrane containing a porous part was used as a model membrane for skin to examine which pathway these enhancers might affect.

We have already reported the effect of ethanol on silicone membranes[7] and the permeation through a silicone membrane of diclofenac salts in water.[8] We also reported the experimental and calculated partition coefficients[9,10] and the ionic behavior of DH and DNa.[11]

In the present study, the mechanisms of the three absorption enhancers, *l*-menthol, oleic acid and lauricidin, were examined using model drugs, DH and DNa, in water and 20% ethanol–aqueous solution (20% ethanol), and ethanol-treated and untreated silicone membranes.

Experimental

Materials DH was obtained by recrystallization twice from an ethanol–aqueous solution in an acidic state by addition of hydrochloric acid to a DNa solution (Sigma Chemical Co., St. Louis, MO). *l*-Menthol and oleic acid were purchased from Tokyo Kasei Kogo Co., Ltd. (Tokyo, Japan). Lauricidin®, glyceryl monolaurate was kindly supplied by Lauricidin Inc. (Galena, IL). Water used throughout the experiments was membrane-filtered (Milli-Q, Nihon Millipore Kogyo, K.K., Tokyo, Japan). Ethanol was of guaranteed reagent grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All other chemicals were of analytical reagent grade. Nonreinforced polydimethylsiloxane sheeting (Dow Corning, Midland, MI), Silastic® 500-1 (0.0127 cm thick), was used as a diffusive barrier. Silastic® 500-5 (0.0508 cm thick), which is the same quality as Silastic® 500-1, was used for membrane solubility studies of the drug. The membranes were presoaked in water for 24 h at 25 °C to remove extractables.

Calculation of the Nonionized Fraction The pK_a values of DH, 4.16 in water and 4.69 in 20% ethanol, were determined by the titration method at 25 °C.[9] The nonionized fractions (F_u) of DH and DNa were calculated from each pK_a value of DH and the pH of the drug suspension in 20% ethanol using the Henderson–Hasselbalch equation: $F_u = 1/[1 + \text{antilog}(pH - pK_a)]$.

Vehicle Solubility (C_w), Membrane Solubility (C_p) and Partition Coefficient (P_m) The saturation concentration of each solute (C_w) and membrane solubility (C_p) in the applied vehicle solution were determined, respectively, by suspending an excess of the solute in the solvent and allowing the mixture to stand for one week at 25±0.05 °C as previously reported.[7] The apparent partition coefficient (P_{app}) was defined as the ratio of C_p to C_w. The true partition coefficient of the nonionized form of DH and of the ionized form of DNa between the membrane and solvent (P_m) was expressed as $P_m = P_{app}/F_u$ and $P_m = P_{app}$, respectively, since P_{app} for DNa is equal to P_m when F_u is near zero ($P_m = P_{app}/(1 - F_u)$), and the ionized form of DNa forms an ion-pair which distributes into the membrane.

Ethanol-Treated Membrane Previously weighed membranes were equilibrated in 99.5% ethanol at 30 °C for 7 d. From the change in weight of the membrane after solvent uptake, the porous part may be defined as the porosity (ε) as previously described.[7] The ε was calculated to be 0.066 after treatment by ethanol.

Permeation Studies Horizontal diffusion cells, maintained at 25 °C, were used to perform the permeation studies for 8 h as described in a previous study.[7] The receptor solvent was 6 ml of water or 20% ethanol, the same as the donor solvent. A suspension of DH and DNa in 20% ethanol with or without 3% *l*-menthol, 1% oleic acid and 3% lauricidin was used as the donor solution. Two kinds of membranes were used, ethanol-treated and untreated. The available permeation area was 0.968 cm^2. The concentration of solute in the sample was analyzed by HPLC[7] and *l*-menthol by a gas chromatographic (GC) assay.[4] Five μl of the sample was injected in a GC system (GC-7A, GC-F1D, Shimadzu Seisakusho, Kyoto, Japan). Conditions were: column, Chromosorb WHP; column and injection temperatures, 200 and 250 °C, respectively; carrier gas N_2; flow rate, 50 ml/min. These studies were generally performed in quadruplicate.

Calculation of the Permeability Coefficient The flux (J) through the membrane was evaluated as a pseudo-steady-state slope of the plot of the cumulative amount of drug per unit surface area *vs.* time at 6 points for 8 h. The apparent permeability coefficient (Kp_{app}) was calculated from $Kp_{app} = J/C_w$ where C_w is the drug solubility in the donor solution.

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Statistical Analysis Statistical analysis was performed using ANOVA; system (1) consisted of two membranes and two vehicles for each drug, and system (2) consisted of two membranes and three enhancers for each drug. A value of $p<0.05$ was considered statistically significant.

Results

Table 1 shows the C_w, C_p, P_{app} and $\log P_{app}$ values of DH and DNa in water and in 20% ethanol with or without enhancers. C_w of DH increased in 20% ethanol with enhancers compared to that without enhancers, but C_w of DNa decreased with added enhancers compared to that without enhancers. C_p of DH and DNa increased with *l*-menthol and oleic acid. The three enhancers decreased the log P_{app} of DH whereas they increased the log P_{app} of DNa, especially oleic acid. This corresponds to the fact that *l*-menthol and oleic acid increase the uptake of drug into the membrane (C_p). Lauricidin increased log P_{app} of DNa while decreasing the solubility of DNa in water.

$Kp_{\cdot app}$ depends on the permeability coefficients for the nonionized form ($Kp_{\cdot n}$) and the ionized form ($Kp_{\cdot i}$) of the drug, and on the fraction of the drug in each state according to Eq. 1:

$$Kp_{\cdot app}=Kp_{\cdot n}\cdot F_u+Kp_{\cdot i}\cdot(1-F_u) \quad (1)$$

A DH suspension in water and in 20% ethanol is not totally ionized, and the nonionized form of DH may penetrate through the membrane. The DNa suspension in 20% ethanol is ionized by more than 99.8% as shown in Table 2. Because the ionized fraction ($1-F_u$) values of DNa are almost unity in 20% ethanol, the term ($Kp_{\cdot n}\cdot F_u$) in Eq. 1 is negligible. The $Kp_{\cdot app}$ value of DNa is therefore $Kp_{\cdot app}=Kp_{\cdot i}$ for the ionized forms of DNa. Suppose that DH and DNa penetrate across the membrane by the lipid pathway, as indicated in the Discussion, $Kp_{\cdot n}$ of DH and $Kp_{\cdot i}$ of DNa for lipid pathway ($Kp_{\cdot ip}$) are expressed as:

$$Kp_{\cdot n}=Kp_{\cdot ip}=(1-\varepsilon)P_m\cdot D_m/h \quad (2)$$

where D_m is the diffusion coefficient of the nonionized form of DH and ionized form of DNa in the membrane, h is the thickness of the membrane, and ε is zero or

Table 1. Physicochemical Properties of Diclofenac and Its Salt in Water and in 20% Ethanol, with and without Enhancers, across Silicone Membrane

	EtOH (w/w %)	Enhancer (w/w %)	C_w (g/l)	C_p (mg/g·silicone)	P_{app} [a]	log P_{app}
DH	0	—	0.001	1.25±0.11	1250	3.10
	20	—	0.003	1.38±0.14	460	2.66
		3% *l*-Menthol	0.048	2.88±0.78	60.0	1.78
		1% Oleic acid	0.039	2.88±0.40	73.8	1.87
		3% Lauricidin	0.013	0.32±0.07	24.6	1.39
DNa	0	—	13.202	0.38±0.08	0.029	−1.54
	20	—	33.404	0.14±0.02	0.004	−2.38
		3% *l*-Menthol	19.247	0.68±0.09	0.035	−1.45
		1% Oleic acid	10.148	2.82±0.50	0.278	−0.56
		3% Lauricidin	10.5	0.13±0.00	0.012	−1.91

a) $P_{app}=C_p/C_w$. Each value represents the mean or mean±S.D. of four experiments.

Table 2. Influence of Various Enhancers on the Permeation of DH and DNa in Water and in 20% Ethanol across Untreated and Treated Silicone Membranes

	EtOH (w/w %)	Enhancer (w/w %)	Treatment [a]	pH [b]	F_u [c]	J (μg/s cm²)	$Kp_{\cdot app}$ [d] (cm/s)	log $Kp_{\cdot app}$ (cm/s)
DH	0	—	u	5.21	0.10	3.81±0.45	9.37×10^{-4}	−3.03
			t			3.22±0.18	7.93	−3.10
	20	—	u	5.23	0.22	17.13±0.78	18.4	−2.74
			t			12.32±0.28	13.3	−2.88
		l-Menthol	u	4.69	0.50	19.64±1.16	1.14	−3.94
			t			22.61±2.25	1.31	−3.88
		Oleic acid	u	4.06	0.81	7.40±0.34	0.53	−4.28
			t			7.34±0.21	0.52	−4.28
		Lauricidin	u	4.75	0.47	7.33±0.31	1.57	−3.80
			t			6.25±0.21	1.34	−3.87
DNa	0	—	u	7.60	0.00	9.42±2.39	20.0×10^{-8}	−6.70
			t			4.64±0.49	9.8	−7.01
	20	—	u	7.82	0.00	3.14±0.13	2.6	−7.59
			t			5.80±0.88	4.8	−7.32
		l-Menthol	u	8.07	0.00	5.47±1.96	7.9	−7.10
			t			6.70±0.67	9.7	−7.01
		Oleic acid	u	7.33	0.00	11.94±0.58	33.3	−6.48
			t			7.44±3.56	20.0	−6.70
		Lauricidin	u	8.10	0.00	9.87±0.73	24.0	−6.62
			t			2.41±0.18	2.3	−7.64

a) u: untreated, t: treated membrane. b) pH of DH and DNa suspensions. c) F_u was calculated using the pK_a values, 4.16 in water and 4.69 in 20% ethanol. d) $Kp_{\cdot app}=J\cdot10^{-3}/(3600\cdot C_w)$.

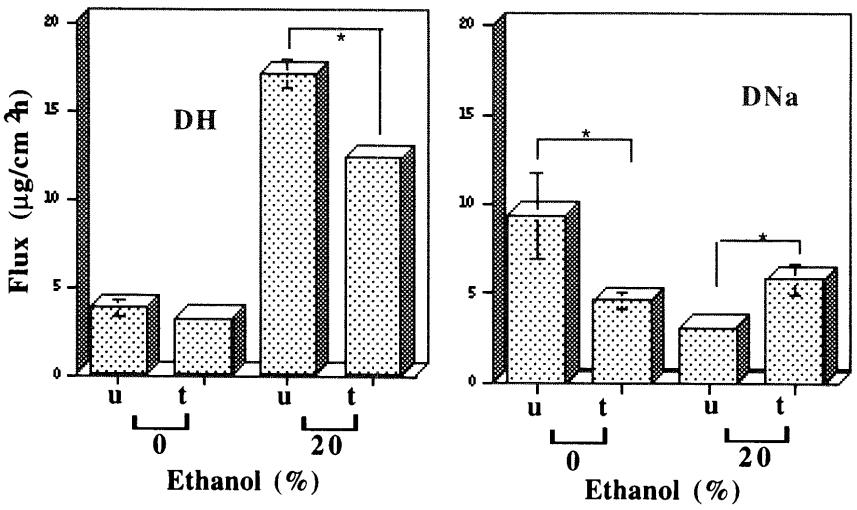

Fig. 1. Effect of Ethanol Treatment on the Permeation of DH and DNa Suspensions across Treated (t) and Untreated (u) Silicone Membrane in Water (0) and in 20% Ethanol (20)

$*p<0.05$. Each value represents the mean ± S.D. of 3—4 experiments.

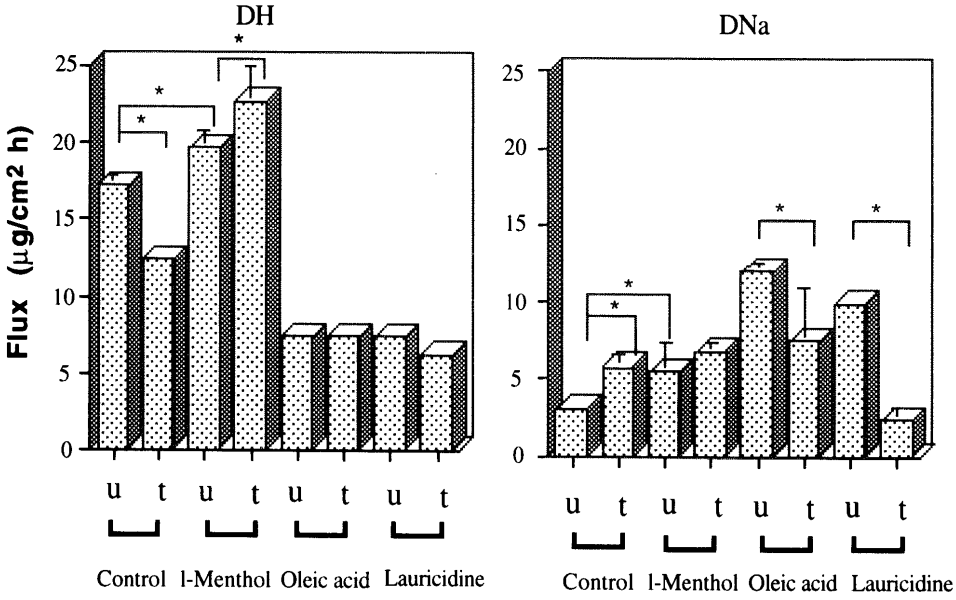

Fig. 2. Influence of Enhancer and Ethanol Treatment of Membrane on the Flux of DH and DNa Suspensions in 20% Ethanol across Treated (t) and Untreated (u) Silicone Membrane

$*p<0.05$. Each value represents the mean ± S.D. of 3—4 experiments.

0.066 in the untreated or treated membrane, respectively.

The drug may penetrate across the treated membrane having a porous part by the lipid pathway and/or by the pore pathway. If DNa penetrates across the treated membrane by the pore pathway predominantly, $Kp_{\cdot i}$ of DNa is expressed as:

$$Kp_{\cdot i} = \varepsilon \cdot D'_m / h \quad (3)$$

where D'_m is the diffusion coefficient of DNa by pore pathway and the h value is assumed to be maintained constant.

Figure 1 shows the effect of ethanol treatment of the membrane on the fluxes of DH and DNa in water and in 20% ethanol. Flux of DH across the treated membrane decreased significantly in 20% ethanol compared to the untreated membrane. The flux of DNa across the treated membrane decreased significantly in water but increased significantly in 20% ethanol compared to the untreated membrane.

Figure 2 shows the influence of enhancer on the flux of DH and DNa in 20% ethanol across the treated and untreated membranes. *l*-Menthol increased significantly the fluxes of DH and DNa across the untreated membrane compared to the control without enhancer and also the flux of DH across the treated membrane compared to the untreated one. On the other hand, oleic acid and lauricidin did not greatly change the flux of DH and decreased significantly the flux of DNa across the treated membrane compared with the untreated one.

The permeation profiles of *l*-menthol in DH suspensions in 20% ethanol across the untreated and treated membrane were very similar (Fig. 3), while the profile of *l*-menthol in DNa appears different since it reached a plateau during 4—8 h in the treated membrane (Fig. 3). The permeating

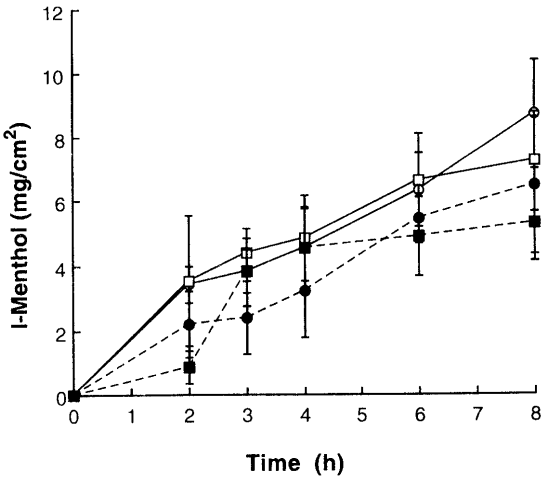

Fig. 3. Permeation Profiles of *l*-Menthol in DH or DNa in 20% Ethanol across Treated (t) and Untreated (u) Silicone Membrane

DH (—○—) and DNa (—□—) across the untreated membrane, DH (- -●- -) and DNa (- -■- -) across the treated one. Each value represents the mean ± S.E. of 3—4 experiments.

amounts of *l*-menthol appear to be higher with both DH and DNa through the untreated membrane than those through the treated one.

Figure 4 shows the log $Kp._{app}$ of DH and DNa in 20% ethanol with and without enhancers, across treated and untreated membranes. log $Kp._{app}$ of DH in the treated membrane decreased with oleic acid and lauricidin. *l*-Menthol increased log $Kp._{app}$ of DNa in the treated membrane but oleic acid and lauricidin decreased it significantly compared with that in the untreated.

Table 2 summarizes the influence of various enhancers on the permeation of DH and DNa in water and in 20% ethanol across the treated and untreated membranes in Figs. 1, 2 and 4.

Table 3 summarizes the log $Kn._p$ of DH and log $Kn._{ip}$ of DNa across the treated membrane in water and in 20% ethanol with and without *l*-menthol. It shows the influence of the three enhancers on the diffusion coefficients of DH and DNa across the two types of membrane. D_m and D'_m were calculated from $Kp._{ip}$ and $Kp._i$ using Eqs. 2 and 3,

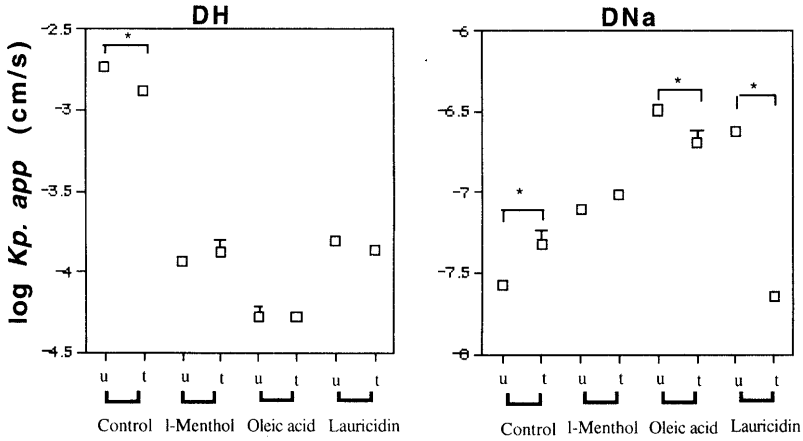

Fig. 4. log $Kp._{app}$ of DH and DNa with and without Enhancers across the Treated (t) and Untreated (u) Membranes in 20% Ethanol

$*p<0.05$. Each value represents the mean ± S.D. of 3—4 experiments.

Table 3. Influence of Various Enhancers on the Diffusion Coefficients of DH and DNa in 20% Ethanol across Untreated and Treated Silicone Membranes

	Enhancer	M[a]	log P_m[b]	Lipid pathway log $Kp._n$[c] (DH) log $Kp._{ip}$ (DNa)	Lipid pathway log D_m[d] (cm^2/s)	Pore pathway log $D_{m'}$[e] (cm^2/s)
DH	—	u	3.38	−2.09	−7.37	
		t		−2.23	−7.48	
	l-Menthol	u	2.08	−3.64	−7.62	
		t		−3.58	−7.53	
	Oleic acid	u	1.96	−4.19	−8.05	
		t		−4.19	−8.02	
	Lauricidin	u	1.72	−3.47	−7.09	
		t		−3.54	−7.13	
DNa	—	u	−2.38	−7.59	−7.11	−9.49
		t		−7.32	−6.81	−8.04
	l-Menthol	u	−1.45	−7.10	−7.55	−9.00
		t		−7.01	−7.43	−7.73
	Oleic acid	u	−0.56	−6.48	−7.79	−8.38
		t		−6.70	−8.01	−7.42
	Lauricidin	u	−1.91	−6.62	−6.58	−8.52
		t		−7.64	−7.60	−8.36

a) u: untreated membrane, t: treated membrane. *b*) $\log P_m = \log(P_{app}/F_u)$ for DH or $\log(P_{app}/(1-F_u))$ for DNa. *c*) $Kp._n = Kp._{app}/F_u$, $Kp._{ip} = Kp._{app}$. *d*) For DH, $\log D_m = \log[Kp._n \cdot h/\{P_m(1-\varepsilon)\}]$, untreated; $\log D_m = \log Kp._n - \log P_m - 1.896$, treated; $\log D_m = \log Kp._n - \log P_m - 1.866$ ($\varepsilon = 0.066$, $h = 0.0127$ cm). *e*) Untreated; $\log D_{m'} = \log(Kp._i \cdot h) = \log Kp._i - 1.896$, treated; $\log D_{m'} = \log(Kp._i \cdot h/\varepsilon) = \log Kp._i - 0.716$.

respectively. The D_m of the lipid pathway is higher than the D'_m of the pore pathway except oleic acid.

Discussion

DNa is reported to be a hydrophilic drug but penetrate across the rat skin[4,11,12] and silicone membrane.[7] The untreated silicone membrane has no aqueous pores; therefore, it is reasonable to assume that the ionized form of DNa forms an ion-pair that, as the nonionized form of DH, may penetrate the membrane by means of a lipid pathway.[7,8]

In vivo the rate determining step for drug permeation is generally the passage across the stratum corneum. For very lipophilic molecules the partitioning from the stratum corneum into the epidermis is important, whereas for hydrophilic drugs the permeation into the stratum corneum is the crucial step. In this study we used DH and DNa, the partition coefficients of which in isopropyl myristate–water are 2.66 and −1.63, respectively.[7] In this sense, it may be enough to consider the stratum corneum as a barrier for permeation, and therefore the silicone membrane is an appropriate model membrane for skin penetration.[13,14]

Two types of pathways have been postulated for drugs to permeate the skin: a lipid and a pore pathway, since the stratum corneum consists of a lipid layer and a porous part.[15] The ethanol treatment induces a porous part in the membrane[7] and therefore the ethanol-treated silicone membrane more suitably mimics skin.

We examined the following four points in this system: i) the enhancement effect of *l*-menthol across the treated silicone membrane compared with rat skin, ii) the pathway of drug across the treated membrane, iii) the mechanism of effect of the three enhancers, and iv) permeation of *l*-menthol with drug.

Comparison of the Silicone Membrane with Rat Skin First, the effects of enhancers on the permeabilities of DH and DNa across the ethanol-treated membrane were compared with those through skin. In rat skin, 2% *l*-menthol in 40% ethanol enhanced log $Kp._n$ of DH and log $Kp._{ip}$ of DNa almost 10 and 100 fold, respectively, compared to the control.[4] The experimental conditions in rat skin are different from those in the silicone membrane: rat skin was pretreated by 2% *l*-menthol in 40% ethanol, the drug suspension was applied and then a permeation study was started; the donor and receiver contained only buffer solution. However, when the concentration of *l*-menthol was higher than its solubility in the ethanol–aqueous solution, *i.e.*, was saturated, the enhancement effect of *l*-menthol was the same.[4,16] The 3% *l*-menthol in 20% ethanol and 2% *l*-menthol in 40% ethanol solutions also were saturated with respect to *l*-menthol; therefore, they were expected to show almost the same enhancement effect.

l-Menthol in the treated membrane increased log $Kp._n$ of DH, and log $Kp._{ip}$ of DNa slightly (Table 3) showing the same tendency as in rat skin[4]; however, this enhancement ratio did not correspond with the results with rat skin. This difference observed between the effect of *l*-menthol on the permeation of drug through the silicone membrane and rat skin may reflect that the latter is more hydrophilic than the former[7] and/or that the effects of ethanol on them may be different.[15]

l-Menthol appears to show the enhancement of permeation of the drug across the treated membrane as in rat skin differently from the enhancement ratio.

The Pathway of Drug across the Treated Membrane We previously reported that DH and DNa penetrated across the untreated membrane by a lipid pathway from ethanol–aqueous solution containing up to 60% ethanol.[7] After ethanol treatment, the membrane contains a porous part, and thus the structure of the membrane may be changed. Two extreme situations are possible: DH and DNa may penetrate the treated membrane predominantly by the pore pathway or they may penetrate across the $(1-\varepsilon)$ part of the membrane by the lipid pathway as before treatment.

Comparing Eqs. 2 and 3, $Kp._i$, *i.e.*, $Kp._{app}$, may increase by the pore pathway (Eq. 3) when ε increases in the treated membrane. However, permeation of the drug by the lipid pathway may decrease in the treated membrane (Eq. 2) assuming that h remains constant, since the membrane is stretched in two dimensions. The lipid pathway may be predominant in the treated membrane as in the untreated membrane when the $Kp._{ip}$ value decreases. This corresponds with the finding that the flux and $Kp._{app}$ of DH in water and 20% ethanol and of DNa in water, decreased across the treated membrane (Figs. 1, 4). The pore pathway may predominate, however when the flux and $Kp._{app}$ of DNa in 20% ethanol across the treated membrane increased compared with the untreated membrane (Fig. 4). Twenty percent ethanol might affect the membrane and/or change the permeation of DNa from the lipid pathway to the pore pathway since DNa is a hydrophilic drug.

In the treated membrane, flux and permeability coefficient of DH decreased and those of DNa in 20% ethanol increased, suggesting that DH penetrated by a lipid pathway and DNa by a pore pathway.

Mechanism of Effect of the Three Enhancers In the untreated silicone membrane, oleic acid and lauricidin increase significantly the $Kp._{app}$ of DNa but do not increase the $Kp._{app}$ of DH since P_{app} of DH decreases with enhancers (Table 1). Oleic acid and lauricidin significantly decreased $Kp._{ip}$ of DNa in the treated membrane compared with that in untreated one, but did not change $Kp._n$ for DH (Fig. 5, Table 3). These findings suggest that oleic acid and lauricidin did not affect the pore pathway, and corresponds well with the fact that oleic acid increased drug penetration by modification of the nonpolar route, *i.e.*, the lipid pathway, in the stratum corneum.[5] $Kp._{ip}$ of DNa in treated membrane with lauricidin was less compared with control since P_{app} decreased.

However, the effect of *l*-menthol is different from that of the other two enhancers, *i.e.*, *l*-menthol increases P_{app} (Table 1) and $Kp._{app}$ of DNa in a treated membrane (Fig. 4). With DH, *l*-menthol caused $Kp._n$ to increase slightly in the treated membrane compared with the untreated membrane.

Ethanol also increases significantly the $Kp._{app}$ of DNa across the treated membrane compared with the untreated in control (Fig. 4). Ethanol decreases the cohesion

parameter of the vehicle, resulting in a decrease of P_{app}.[10] An increase of P_{app} by addition of *l*-menthol may be caused by changing the cohesion parameters of the skin and/or the vehicle. *l*-Menthol may be adsorbed at the surface of the membrane and change its hydrophobicity. In addition, 20% ethanol may favor ion-pair formation[11] and make DNa penetration easier; therefore, the $Kp._{ip}$ values might increase. *l*-Menthol appears to affect the pore pathway of the hydrophilic drug. This is consistent with the finding that the addition of ethanol and 5% *l*-menthol to water increased the contribution of the pore pathway to skin permeation.[17] It is possible that *l*-menthol changes the pore pathway of DNa that penetrates across the treated membrane in 20% ethanol.

These findings suggest that oleic acid and lauricidin did not affect the pore pathway.

Permeation of *l*-Menthol with Drug The enhancement produced by *l*-menthol has been interpreted to be due to the combined effect of this compound and ethanol; *l*-menthol penetrates with ethanol and ethanol penetrates with *l*-menthol.[18] In the present system, the driving force for permeation of a drug across the membrane is the concentration gradients of the drug and of the enhancer in the donor, whereas the concentration of ethanol is the same on both sides and, hence, there is no ethanol flux. However, *l*-menthol resulted in enhancing the flux of the drug (Fig. 2).

Permeability coefficients of *l*-menthol, either with DH or DNa, (3.38×10^{-4}, 2.39×10^{-4} cm/s, respectively) were higher across the untreated membrane than the treated (2.95×10^{-4}, 2.24×10^{-4} cm/s, respectively)(Fig. 3). In contrast, the penetration of DNa across the treated membrane was higher than that across the untreated, but the permeation of DH in 20% ethanol across the treated membrane was lower than that across the untreated (Table 1). No correlation between DNa and *l*-menthol permeation was found, but DH and *l*-menthol, hydrophobic substances preferred to penetrate across the untreated, hydrophobic membrane.

Permeation studies using ethanol-treated membrane may be useful to examine the mechanism of enhancers. However, further work is required to elucidate the relationship between permeation through skin and silicone membrane.

Conclusions

DH and DNa, either in water or in 20% ethanol, penetrated silicone membrane by the lipid pathway. In ethanol treated membrane, flux and permeability coefficient of DH decreased and those of DNa in 20% ethanol increased, suggesting that DH penetrated by a lipid pathway and DNa by a pore pathway.

Permeability coefficients of DNa decreased significantly when the membrane was treated with oleic acid or lauricidin, suggesting that these compounds did not affect the pore pathway. *l*-Menthol, on the other hand, increased the permeability coefficients of DH and DNa in a treated membrane compared with those in untreated, showing the same tendency as in rat skin. *l*-Menthol appears to affect the lipid pathway for hydrophobic drugs and the pore pathway for hydrophilic drugs, but the enhancement ratios of permeability coefficients of DH and DNa by *l*-menthol were different in the silicone membrane from those in rat skin.

Therefore, the ethanol-treated silicone membrane employed as a model for skin in this study is not a reliable model for assessing the enhancement ratio by enhancers on human percutaneous absorption, but it may be useful in the evaluation of enhancers.

References and Notes

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