Enhanced Skin Permeation of Salicylate by Ion-Pair Formation in Non-aqueous Vehicle and Further Enhancement by Ethanol and *l*-Menthol

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Enhancement of skin permeability of salicylate from non-aqueous vehicle by ion-pair formation with either alkylamines or benzylamine as model cationic ions was examined in excised guinea pig dorsal skin. Solubility of salicylate in isopropyl myristate (IPM) was increased by the addition of either alkylamines or benzylamine as counter ions. The increase was more significant in the presence of amines with longer alkyl chains. Flux of salicylate increased in the presence of these amines due to the increase in the solubility. Maximum flux was observed in the presence of *n*-hexylamine, which induced an 11-fold increase due to 137-fold increase in solubility. Flux and permeability coefficients of salicylate in the presence of *n*-butylamine, *n*-hexylamine, *iso*-octylamine and benzylamine as counter ions in IPM were larger than those of the non-ionic form of salicylic acid. Flux of 3-methylsalicylate (3-CH_3 substituent) and that of 5-hydroxysalicylate (5-OH substituent) were smaller than that of salicylate in the presence of *n*-hexylamine. After partition to the skin surface, the ion-pair is suggested to dissociate and permeate separately according to the study using lidocaine as the counter ion. Flux of salicylate increased in the presence of benzylamine as the counter ion by the addition of 15% ethanol and 15% ethanol plus 1% *l*-menthol due to further improvement in the solubility as well as an increase in the permeability coefficient.

Key words salicylate; skin permeability; ion-pair; alkylamines; benzylamine; enhancer

The lipid lamella of the stratum corneum of the skin acts as a barrier to the permeation of most drugs, especially as a hydrophobic barrier for hydrophilic drugs and ionized drugs. Most drugs are weakly acidic or weakly basic and their non-ionized forms seem to preferentially permeate through the skin. As reported for diclofenac and indomethacin,[2,3)] and as reported in our previous study for salicylate derivatives,[4)] the permeation of ionized drugs is not negligible in the pH regions in which drugs are present predominantly as ionized forms. However, their permeation is limited.

Ion-pairing has been widely used in analytical chemistry as an extraction method, in chromatography, and as a tool to study the penetration of ionized molecules through artificial and biological membranes.[5)] The use of ion-pairing has also been suggested as a possible means of facilitating the transdermal delivery of ionized drugs.[5—7)] The association with oppositely charged ions reduces or neutralizes the electrostatic charges. For salicylate, enhancement of skin permeation by ion-pair formation with alkylamines and quaternary ammonium ions in ethanol–pyopylene glycol (2 : 1 v/v)[6,7)] and in aqueous medium[8)] has been reported. Ion-pair absorption of lidocaine–*n*-alkanoate from ethanolic medium[9)] and ion-pair complex of cromolyn from isopropyl myristate (IPM) has also been reported.[10)]

In this study we examined the solubility increase of salicylate in non-aqueous vehicle by ion-pair formation with either alkylamines or benzylamine. We selected IPM as the non-aqueous vehicle, because it is often used as an organic solvent for topical use, and also because it is known to exhibit a high skin penetration-enhancing effect by itself.[11)] We observed the effects of ion-pair formation in IPM on skin permeation of salicylate and its substituents. We also compared the skin permeation of the ion-pairs with that of non-ionic salicylic acid. We furthermore examined the effects of combined use of ethanol and ethanol plus *l*-menthol, which have been used as chemical enhancers for skin permeation of various drugs.[12—14)]

Experimental

Materials Sodium salicylate, salicylic acid, hydrochloride salt of lidocaine and *l*-menthol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Hydrochloride salts of *n*-ethylamine, *n*-butylamine, *n*-hexylamine, *iso*-octylamine, benzylamine and 3-methylsalicylic acid (3-CH_3 derivative) sodium salt were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Isopropyl myristate (IPM) and 5-hydroxysalicylic acid (5-OH derivative) (gentisic acid) sodium salt were obtained from Nacalai Tesque (Kyoto, Japan). Ketotifen fumarate was from Sigma-Aldrich Japan Co. (Tokyo, Japan). Salicylate–benzylamine ion-pair complex was prepared by addition of hydrochloride salt of benzylamine (pK_a=9.4) to an equal concentration of sodium salicylate (pK_a=3.0) at pH 6.2 and cooling at 0 °C. Recrystalization was performed by dissolving the crystals in water and cooling at 0 °C.

Measurement of *in Vitro* Skin Permeation *In vitro* skin permeation of salicylate was examined as described previously.[15)] Full thickness dorsal skin was excised from male guinea pigs (Hartley strain, three weeks old) and subcutaneous fat and other extraneous tissues were trimmed. The skin was then mounted in a two-chamber diffusion cell with a water jacket (37 °C). The available diffusion area was approximately 0.65 cm^2, and each half-cell volume was approximately 5.4 ml. Donor cells were filled with IPM, and receiver cells with phosphate buffered saline (PBS) adjusted to pH 7.4, and pretreatment was carried out for 12 h with stirring at 450 rpm by a magnetic stirrer. After washing both cells, sodium salicylate (or sodium salts of its substituents) and hydrochloride salts of either alkylamines or benzylamine, which were suspended in IPM at final concentrations of salicylate and amines to be 20 mM, was added to the donor solution, and the permeation experiment was started. When benzylamine was used as the counter ion, recrystalized salicylate–benzylamine ion-pair complex was also used as a finally 20 mM suspension. One hundred and fifty microliters of sample was taken from the receiver cells periodically over a period of 10 to 30 h.

The concentration of salicylate was determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 detector (Hitachi) at 303 nm. Separation was achieved on a reversed-phase column (Mightysil RP-18 GP, 4.6 mm i.d., 150 mm) using a mobile phase consisting of methanol, water and phosphoric acid (750 : 1250 : 1) for the analysis of salicylate, at a flow rate of 0.7 ml/min. Ketotifen fumarate was used as an internal standard for the analysis. The concentrations of 3-CH_3 and 5-OH substituents of salicylate were determined at 308 nm and 331 nm, respectively, using the same mobile phase for salicylate analysis and a mobile phase consisting of methanol,

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water and phosphoric acid (100 : 100 : 1) for each substituent. The concentration of lidocaine was determined at 263 nm using a mobile phase consisting of methanol, water and phosphoric acid (30 : 170 : 1).

Apparent permeability coefficients (K_p) of salicylate derivatives were obtained according to Eq. 1 from the initial straight portion of the permeation curve dC_R/dt, although volume of skin and distribution rate of salicylate derivatives from donor compartment to skin and that from skin to receiver compartment should also be considered to obtain the precise values.

$$K_p = \frac{dC_R}{dt} \cdot \frac{V_R}{A} \cdot \frac{1}{C_D} \quad (1)$$

where C_R and V_R are the concentration of salicylate in the receiver compartment and compartment volume, respectively. A is the diffusion area and C_D is the concentration of salicylate in the donor compartment.

Statistical Analysis One way analysis of variance and Bonferroni's *post-hoc* test were used to analyze differences between the sets of data. A p-value less than 0.05 was considered significant.

Results

Increase in Solubility of Salicylate in IPM by Ion-Pair Formation with Alkylamines and Benzylamine The solubility of salicylate in IPM increased by ion-pair formation with alkylamines and benzylamine by mixing 20 mM sodium salicylate and hydrochloride salts of the amines. The maximum concentration of amines used was 20 mM, which was equal to the salicylate concentration. As shown in Fig. 1, solubility of salicylate markedly and linearly increased by the addition of amines as counter ions. The increase was more significant in the presence of amines with longer alkyl chains.

Effects of Ion-Pair Formation with Counter Ions on Skin Permeation of Salicylate Next the effects of ion-pair formation with these amines on the skin permeation of salicylate were examined by using suspension in IPM of the mixture of 20 mM sodium salicylate and 20 mM hydrochloride salts of either alkylamines or benzylamine. As shown in Table 1, 3.3-fold to 11.2-fold increases in flux of salicylate were observed by ion-pair formation with the amines due to the increase in solubility. The increases in the flux in the presence of *n*-butylamine, *n*-hexylamine and benzylamine were especially significant. The flux obtained by using recrystalized salicylate–benzylamine ion-pair complex was similar to that obtained by mixing 20 mM sodium salicylate and the same concentration of hydrochloride salt of benzylamine in IPM ($2.06 \pm 0.03\ \mu mol \cdot cm \cdot h^{-1}$).

Permeability coefficients decreased with increase of methylene groups in the alkylamines. The free energy of transfer of the methylene group of salicylate–alkylamine ion-pair from the IPM phase to skin, 230 J/mol, was obtained from the permeability data in Table 1 according to the following equation,[16]

$$\Delta(\Delta G) = -RT\Delta \ln(K_p) \quad (2)$$

where $\Delta(\Delta G)$ represents the free energy of transfer of methylene group from IPM to the skin, R is the gas constant, T is the absolute temperature and $\Delta \ln(K_p)$ is the slope of $\ln(K_p)$ *vs.* carbon number plot.

The permeation data of salicylate–amine ion-pairs were also compared with that of the 20 mM neutralized form of salicylic acid in IPM. As also shown in Table 1, the flux and permeability coefficients of salicylate in the presence of *n*-butylamine, *n*-hexylamine, *iso*-octylamine and benzylamine were larger than those of the non-ionic form of salicylic acid.

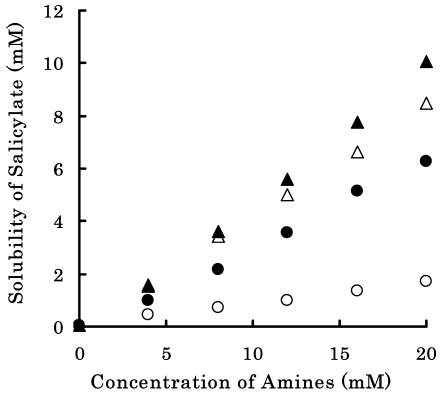

Fig. 1. Change in Solubility of Salicylate in IPM by Addition of Alkylamines

○, *n*-ethylamine; ●, *n*-butylamine; △, *n*-hexylamine; ▲, *iso*-octylamine. Data are means±S.D. of four experiments.

Table 1. Effects of Alkylamines and Benzylamine as Counter Ions on Solubility in IPM, Flux and Permeability Coefficients of Salicylate through Excised Guinea Pig Dorsal Skin

Amine	Solubility (mM)	Flux ($\mu mol \cdot cm \cdot h^{-1}$)	Flux ratio	K_p ($cm \cdot h^{-1}$)
None	0.062	0.193±0.049	—	3.11±0.79
n-Ethylamine	1.73	0.628±0.106	3.3	0.363±0.061***
n-Butylamine	6.28	2.04±0.44***	10.6	0.325±0.070***
n-Hexylamine	8.49	2.16±0.10***	11.2	0.254±0.012***
iso-Octylamine	10.1	1.50±0.17***	7.8	0.149±0.017***
Benzylamine[a]	8.61	2.07±0.41***	10.7	0.240±0.048***
Salicylic acid[b]	20	0.587±0.080	3.0	0.0294±0.0040

a) Recrystalized salicylate-benzylamine complex was used. *b*) 20 mM Salicylic acid dissolved in IPM was used without addition of amines. Data are means±S.D. of four experiments. *** $p<0.001$, compared with the value in the absence of amines and that of non-ionic salicylic acid.

The permeability coefficients of salicylate present as ion-pairs with amines were 5-times to 11-times larger than that of salicylic acid.

To see whether ion-pairs cross full-thickness skin as intact 1 : 1 ion-pairs, we examined the permeation of salicylate in the presence of lidocaine as a counter ion. Flux of salicylate increased 4.2-fold compared with that in the absence of counter ions. The results shown in Fig. 2 indicated that flux of salicylate was about twice as large as that of lidocaine. This finding was consistent with the previous finding for lidocaine–dodecanoate ion-pair, in which the flux of dodecanoate was larger than that of lidocaine.[9]

Difference of Effects of Ion-Pair Formation on Skin Permeation of Different Substituents of Salicylate To see the effects of hydrophobicity of drugs on the skin permeation due to the ion-pair formation in IPM, we next examined the difference of the effects of amines on skin permeation of salicylate substituents (3-CH_3 or 5-OH derivatives) with different hydrophobicities by using *n*-hexylamine as the counter ion. As shown in Table 2, in the absence of *n*-hexylamine, the solubility of the substituent with hydrophobic 3-CH_3 group was larger than that of salicylate. On the other hand, the solubility and flux of the substituent with hydrophilic 5-OH group were much smaller than those of salicylate.

As also shown in Table 2, in the presence of *n*-hexylamine as a counter ion, both the solubility and flux of 3-CH_3 sub-

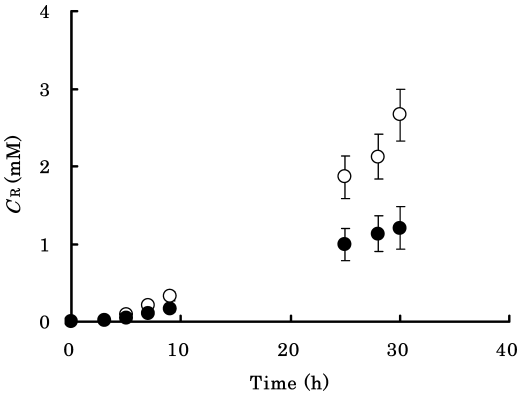

Fig. 2. Increase in Concentration of Salicylate and Lidocaine in Receiver Compartment, C_R, Due to Transfer of Salicylate–Lidocaine Ion-Pair in Donor Compartment

○, salicylate; ●, lidocaine. Data are means±S.D. of four experiments. Sodium salicylate and hydrochloride salt of lidocaine were suspended in IPM at final concentrations of salicylate and lidocaine to be 20 mM, respectively.

Table 2. Effects of *n*-Hexylamine as Counter Ion on Solubility in IPM, Flux, and Permeability Coefficients of Salicylate (None-substituent), 3-methylsalicylate (3-CH_3-Substituent), 5-Hydroxysalicylate (5-OH-Substituent) through Excised Guinea Pig Dorsal Skin

Substituent	Solubility (mM)	Flux (μmol·cm·h^{-1})	K_p (cm·h^{-1})
Without *n*-hexylamine			
None	0.062	0.193±0.049	3.11±0.79
3-CH_3	1.89	0.210±0.107	0.111±0.057***
5-OH	<0.01	0.030±0.018*	n.d.
With *n*-hexylamine			
None	8.49	2.16±0.10	0.254±0.012
3-CH_3	18.8	1.10±0.12***	0.059±0.006*
5-OH	2.12	0.894±0.434***	0.422±0.205

Data are means±S.D. of four experiments. n.d., not determined. *$p<0.05$, ***$p<0.001$, compared with the values of salicylate either in the presence or absence of *n*-hexylamine.

stituent increased, but the flux was smaller than that of the non-substituent (salicylate). Likewise, in the presence of the counter ion both solubility and flux of 5-OH substituent markedly increased, but solubility and flux were smaller than those of salicylate. Therefore, there seems to be an optimum hydrophobicity for the skin permeation of salicylate substituents in the presence of counter ions in non-aqueous vehicle, although molecular weight is also important for the permeation.[17]

Effects of Combined Use of Ethanol and *l*-Menthol as Enhancers with Ion-Pair Formation on Skin Permeation of Salicylate Although ion-pair formation with amines improved the solubility of salicylate in the non-aqueous vehicle, the permeability coefficient decreased due to ion-pair formation. For further improvement of skin permeation, an increase in the permeability coefficient is necessary. Since chemical enhancers are known to increase the permeability coefficient due to either an increase in the partition coefficient between vehicle and skin or an increase in the diffusion coefficient in skin,[18] we next examined the effects of chemical enhancers. We selected ethanol and ethanol plus *l*-menthol on skin permeation of salicylate by combination with counter ions for salicylate–benzylamine ion-pair. To avoid delipidization of the stratum corneum at a high percentage of

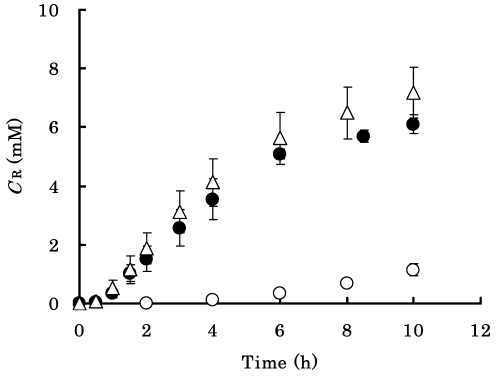

Fig. 3. Effects of 15% Ethanol and 15% Ethanol Plus 1% *l*-Menthol on Increase in Concentration of Salicylate in Receiver Compartment, C_R, Due to Transfer of Salicylate–Benzylamine Ion-Pair in IPM

○, control; ●, with 15% ethanol; △, with 15% ethanol plus 1% *l*-menthol. Data are means±S.D. of four experiments.

Table 3. Effects of Ethanol and Ethanol plus *l*-Menthol on Solubility in IPM, Flux, and Permeability Coefficients of Salicylate–Benzylamine Ion-Pair

Enhancer	Solubility (mM)	Flux (μmol·cm·h^{-1})	K_p (cm·h^{-1})
None	8.61	2.07±0.41	0.240±0.048
15% ethanol	20[a]	7.82±1.20**	0.391±0.060
15% ethanol+1% *l*-menthol	20[a]	9.86±1.63***	0.493±0.081**

Data are means±S.D. of four experiments. **$p<0.01$, ***$p<0.001$, compared with the value in the absence of the enhancers. *a*) 20 mM salicylate–benzylamine ion-pair dissolved in IPM in the presence of enhancers was used.

ethanol,[12] we examined the effect of 15% ethanol.

As shown in Fig. 3, permeation was markedly facilitated by 15% ethanol and 15% ethanol plus 1% *l*-menthol. Lag time was markedly shortened in the presence of these enhancers. The data shown in Table 3 clarified that addition of 15% ethanol induced a 3.8-fold increase in the flux of salicylate due to the increases in solubility. Addition of 1% *l*-menthol plus 15% ethanol induced a 4.8-fold increase in the flux due to the significant increase in the permeability coefficient as well as the increase in the solubility.

Discussion

The present study revealed that ion-pair formation in a non-aqueous vehicle like IPM improved the skin permeation of salicylate due to the marked increase in solubility by reducing or neutralizing electrostatic charge. These ion-pairs seem to be weakly associated as reported for lidocaine-alkanoate ion-pair.[9] They are still polar compared with non-ionic forms of the original drugs. Therefore, a relatively high partition coefficient between non-aqueous vehicle and skin can be expected. The large permeability coefficients of salicylate–amine ion-pairs compared with that of non-ionic salicylic acid observed in this study are probably due to differences in the partition coefficient. However, permeability coefficients decrease to some extent, accompanied by the ion-pair formation, compared with the original ionic drugs, due to the decrease in partition coefficient between vehicle and skin and due to the decrease in the diffusion coefficient accompanied by an increase in molecular weight. The combination of other chemical or physical enhancers is thus necessary for further

increase in drug flux by improving the permeability coefficients. *l*-Menthol and ethanol are useful for this purpose, as revealed in this study. The marked reduction of lag time in the presence of these enhancers indicates an increase in the diffusion coefficient,[19] by disruption of lipid lamella of stratum corneum.[14,20]

The ion-pair of drug and counter ion seems to distribute to the outermost layer stratum corneum of the skin. However, the mechanism of transdermal transport is unknown. The ion-pair seems to cross the lipophilic stratum corneum lipid lamella without dissociation. However, the ion-pair probably dissociates as soon as it reaches the high dielectric region of the skin as suggested by the present study on salicylate-lidocaine ion-pair and by a previous study on lidocaine-alkanoate ion-pair.[9]

Ion-pair formation could be a useful tool for ionic drugs to facilitate transdermal absorption. Combined use of ion-pairs with chemical enhancers as revealed in this study and other enhancement systems such as microemulsions[21] will develop into future clinical transdermal therapeutic systems for ionic drugs.

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