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Transdermal Permeation-enhancing Activities of some Inorganic Anions

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Effects of sodium salts of various monovalent inorganic anions on transdermal permeation of salicylic acid were investigated. In in-vitro experiment using a Franz-type diffusion cell and excised mouse skin, the permeation-enhancing activities of the sodium salts of inorganic anions were roughly proportional to lyotropic Hofmeister swelling abilities of the anions; $F^- < SO_4^{2-} < Cl^- < ClO_4^- < NO_3^- < SCN^- < Br^- < I^-$, i.e. I^-, Br^- and SCN^- increased the flux of drugs through the mouse skin, while F^-, SO_4^{2-}, Cl^-, ClO_4^- and NO_3^- decreased or did not affect the flux. In in-vivo experiment using the rabbit as the test animal, the plasma concentration of salicylic acid of the rabbit to which 10%-salicylic acid ointment containing 5%-NaI or NaBr was applied was significantly higher than that of the rabbit to which the ointment without the electrolytes was applied. The amounts of sterol leached out of stratum corneum sheet when the sheet was immersed in aqueous solutions of NaI, NaBr, or NaSCN were much more than that of stratum corneum immersed in aqueous solutions of the other inorganic anions. The FTIR/ATR spectroscopy showed that the peaks at 2853 cm^{-1} and 2924 cm^{-1} in the IR absorption spectrum of the stratum corneum sheet of the mouse were shifted to higher frequencies by the anions which enhanced the transdermal drug permeation, while not shifted by the anions which did not have any permeation-enhancing activities or have permeation-reducing activities. These results suggest that sodium salts of some anions such as iodide, bromide and thiocyanate enhance transdermal permeation of salicylic acid through swelling and perturbation of the skin structure by these anions.

Key words : Inorganic anions, Transdermal permeation, Salicylic acid, Lyotropic swelling

INTRODUCTION

Over the last decade there has been a dramatic increase in researches for transdermal drug delivery to produce systemic effects associated with topical drug applications (Barry, 1983; Barry, 1987; Chien, 1987). However, the concepts of using the skin as a delivery route came to limitations (Gardner, 1987). The main problems of drug delivery through this route are the poor permeability of most drugs through intact skin and the skin irritation or allergic responses that are induced by the drugs or vehicles (Kydonicus, 1987).

To enhance the permeability of drugs through the skin a number of transdermal permeation enhancers and devices have been developed (Barry, 1987b; Hadgraft, 1989; Mahjour, *et al.*, 1989; Sharad, 1988). Enhancers are compounds which would reversibly reduce the barrier function of the skin and thus allow the drugs to penetrate to the viable tissues and enter the systemic circulation. The transport mechanisms by which drugs cross the intact skin are still not elucidated completely despite intensive studies along this line, and it is, as yet, too early to assess the success of transdermal drug delivery.

The effect of inorganic anions on the transdermal permeation of drugs has not been investigated so far although these studies might give some help in elucidating the transdermal transport mechanisms of drugs. According to Elden (Elden, 1971), the capability of inorganic anions to swell skin collagen was consistent with the lyotropic Hofdmeister series of anions: $F^- < Cl^- < Br^- < SCN^-$. This suggests that even inorganic anions might have some effect on the transdermal flux of compounds through swelling of the skin. We tried to test the hypothesis that the lyotropic swelling of the skin by inorganic anions might induce the transdermal permeation enhancement of drugs, and that the agents which swell the skin enhance the transdermal permeation enhancement of drugs.

MATERIALS AND METHODS

Materials and animals

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Salicylic acid was purchased from Junsel Chemical Co., Japan, and trypsin from Sigma Chemical Co., USA. They were used without further purification. All other reagents and inorganic electrolytes were of reagent grades. ICR mice (2.5~3.0 kg, male) and rabbits (2.5~3.0 kg, male) were employed in *in-vitro* and *in-vivo* tests.

In vitro transdermal permeation test

Transdermal drug permeation was studied by the following method employing the mouse as the test animal. The mouse was sacrificed by cervical dislocation and the hair of the dorsal area was carefully removed with electrical razor and scissors. Full-thickness skin was seperated from the back side of the mouse and subcutaneous fats and other tissues were removed, and then rinsed several times with physiological saline. The skin was placed between a home-made Franz-type diffusion cell (diameter=1.8 cm). The receptor compartment of the diffusion cell contained 12ml of phosphate buffer solution (PBS), and the donor compartment was filled with 10 ml of 1 M salicylic acid aqueous solution which contains 30%-ethanol and the electrolytes. The pH of the donor compartment solution was adjusted to 2 with addition of trace amount of diluted HCl, and under these conditions, most of salicylic acid is in unionized form. The temperature around the diffusion cell was maintained at 36.7°C with water bath, and the receptor compartment was stirred with a magnetic stirrer through the diffusion experiment. 0.2 ml of sample was withdrawn from the receptor comparment at regular intervals, and the receptor compartment was refilled with fresh PBS. For measurement of the concentration of salicylic acid, the sample was diluted 20 times with water at pH 2 and the absorbance at 295 nm was measured with varian DMS 90 UV/visible spectrophotometer. The concentration was read from the previously made calibration curve.

In vivo transdermal permeation test

One day prior to the applications of ointment to the rabbit, the hair was carefully removed with a electrical clipper and razor from the skin of the dorsal area on both sides of the spine. Next day, the rabbit was immobilized with a rabbit holder and accurately weighed 8.0 g of the ointment was uniformly spread over the shaved skin of the animal (7×8 cm^2) and covered with a linear-low-density polythylene wrap film. The formula of the ointment was 10% salicylic acid vaseline ointment containing 5% inorganic electrolyte, which also contained 0.5 ml of DMSO and 2.0 ml of acetone per 10 g of the ointment for solubilizing the salicylic acid in the vehicle. The ointment was prepared with the fusion method. One-half ml of blood was withdrawn from the marginal ear vein of the rabbit 2 hours after the application of the oiment. Then, 0.1 ml of heparin sodium (20,000 IU/20 ml) was added to the blood sample. This mixture was centrifuged at 600 g for 10 minutes, and 0.5 ml of resulting plasm was taken into a test tube containing 1.0 ml of distilled water and 2.0 ml of Trinder's reagent (Trinder, 1954). Colorimetric analysis for salicylic acid was performed by reading the absorbance of the developed color at 540 nm. The concentration of the salicylic acid was calculated from the calibration curve.

Measurements of apparent solubility of salicylic acid

The apparent solubilities of salicylic acid in aqueous 30% alcohol solutions containing 1 M of electrolytes were determined by the general procedure for measuring the solubility.

Preparation of the stratum corneum sheet

Stratum corneum sheet was prepared by the method as follows. After seperating the epidermis from whole excised mouse skin by incubating the whole skin in warm distilled water (60°C) for 30 s, the resulting epidermis was placed with its epidermis side down on filter paper saturated with 0.5% trypsin in pH 7.4 PBS at 37°C for 2 hours. The stratum corneum was then carefully peeled from the epidermis with light vortexing in water and covered with the trypsin solution for 1 hour. Remaining nucleated cells were removed from the stratum corneum. Resulting stratum corneum sample was dried under reduced pressure between two filter papers and kept in a desicator until it is to be used. Prior to IR experiment the sample was then placed for 2 days in a chamber maintained at 95% relative humidity. Stratum corneum sample has been reported to be equilibrated to a water content of about 30% (w/w) under these conditions (Golden *et al.*, 1987).

Measurement of lipid leaching from the excised skin

The excised full-thickness mouse skin was set in a vertical diffusion cell. The effective diffusion area of the cell was calculated, and the volume of each chamber was estimated. Each electrolyte solution was added to the half cell in contact with the stratum corneum, and the same volume of distilled water was added to the other half cell in contact with the dermis. The experiment was performed at 37°C. For analysis of the total sterol leached out during 5 hours, sterol in 0.5 ml of the sample was extracted twice with 0.5 ml of chloroform : methanol (2 : 1) mixed solvent. After evaporating the organic phase, the amount of sterol in the residue was measured by $FeCl_3$-acetic

acid method. The residue was dissolved in glacial acitic acid, and the color developed on adding $FeCl_3$ reagent was read at 540 nm and compared with the calibration curve.

Measurement of FTIR/ATR spectra of stratum corneum sheet

Infrared spectrum of stratum corneum sheet was obtained using a Fourier transform infrared spectrometer (Bomem-100) equipped with nitrogen-cooled mercury-cadmium-telluride detector. All spectra represent an average of 100 scans obtained in about 8 minutes. During the data collection period the sample temperature was kept constant within $\pm 1^{\circ}C$.

RESULTS AND DISCUSSION

The effects of the presence of sodium salts of various anions in the donor solution of the diffusion cell on the amount of salicylic acid permeated through the excised mouse skin versus time in the *in-vitro* transdermal permeation experiment were measured, and the results were shown in Fig. 1~3. They show that permeated salicylic acid increased almost linearly with time during five-hour experimental period, and the lag times were insignificant. The permeability coefficients of salicylic acid in the presence of these inorganic electrolytes through the excised skin were calculated from these data employing the following equation;

$$1/A\ (dM/dt)=Js=Pe \times C$$

Here, dM/dt is the cumulative amount of drug permeated per unit time, A is the diffusion area, Js is the drug flux, and C is the concentration of the drug in the donor compartment. The permeability coefficients, Pe was obtained by dividing the fluxes by the concentration of the penentrant in the donor compartment. The calculated permeability coefficients were listed in Table I. In these experiments, the con-

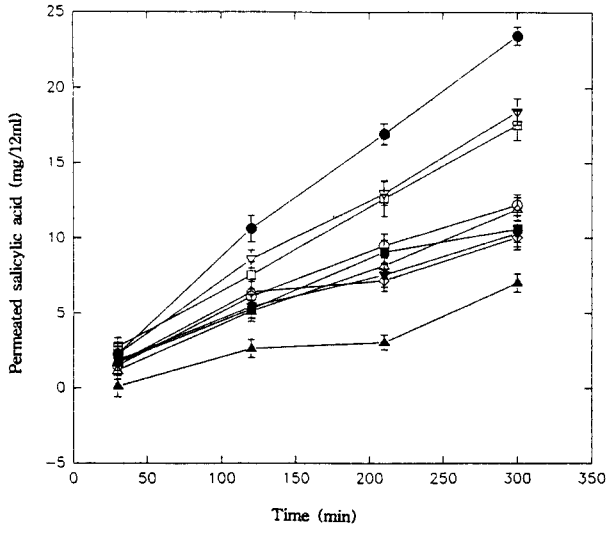

Fig. 1. Amounts of salicylic acid permeated through excised mouse skin in the Franz-type diffusion cell versus time in the presence of inorganic compounds at 37°C. The donor compartment contains 10 mM of 1 M salicylic acid aqueous solution which contains 30%-ethanol. The receptor compartment contains 12 ml of phosphate buffer solution. (○ : control, △ : addition of 1 M $NaNO_3$ to the donor solution, ■ : 1 M $NaClO_4$, □ : 1 M NaSCN, ▼: 1 M NaCl, ▽: NaBr, ● : 1 M NaI, ◇ : 0.5 M Na_2SO_4, ▲: 0.075 M NaF, n=5)

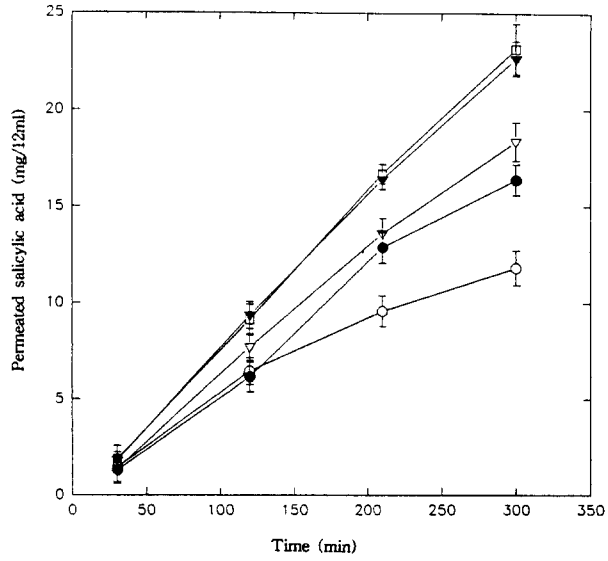

Fig. 2. Amounts of salicylic acid permeated through excised mouse skin in the Franz-type diffusion cell versus time in the presence of various concentration of NaI at 37°C in the donor solution. (○ : control, ● : addition of 0.5 M NaI, ▽: 1 M NaI, ▼: 1.5M NaI, □ : 2 M NaI, n=5)

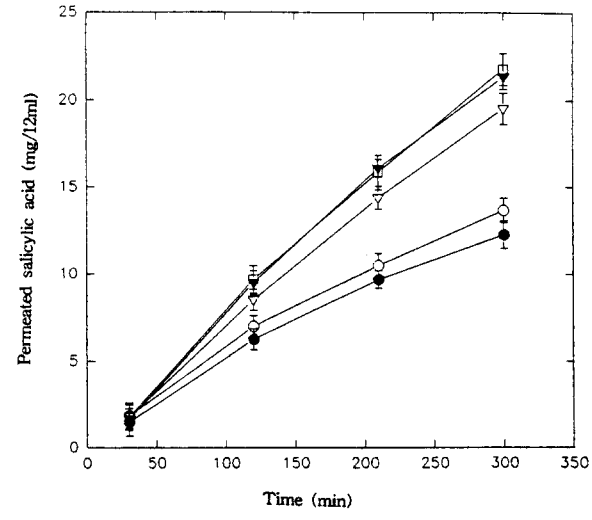

Fig. 3. Amounts of salicylic acid permeated through excised mouse skin in the Franz-type diffusion cell versus time in the presence of various concentration of NaBr at 37°C in the donor solution. (○ : control, ● : addition of 0.5 M NaBr, ▽: 1 M NaBr, ▼: 1.5 M NaBr, □ : 2 M NaBr, n=5)

Table I. Permeability coefficients of salicylic acid through the excised mouse skin in the Franz-type diffusion cell in the presence of inorganic compounds in the donor solution at 37°C (n=5)

Compound	Permeability coefficient (cm/h)	Enhancement ratio (-)
Control	6.71±0.42	1
0.075 M NaF	3.31±0.12	0.49
0.5 M Na_2SO_4	3.95±0.07	0.59
1 M NaCl	4.37±0.89	0.65
1 M $NaClO_4$	6.57±0.51	0.71
1 M $NaNO_3$	9.05±2.36	0.98
1 M NaSCN	5.62±2.22	1.33
0.5 M NaBr	9.26±2.08	0.84
1 M NaBr	10.2 ±1.44	1.38
2 M NaBr	10.3 ±1.28	1.53

centration of NaF was 0.075 M due to its limited solubility in the medium, and the reduced concentration of Na_2SO_4 was included for comparison considering the divalent character of the anion. The results show that the presence of 1 M sodium nitrate did not significantly affect permeability coefficient, and the presence of 1 M sodium salts of chloride, or perchlorate, 0.5 M sodium sulfate or 0.075 M sodium fluoride reduced the permeability coefficient of salicylic acid through the excised skin. Especially sodium fluoride was exceptionally effective in reducing the permeability coefficient of the drug. However, sodium salts of iodide, bromide, and thiocyanate significantly ($p<0.05$) increased the permeability coefficient, and the abilities of these anions to increase the flux of the drug were concentration-dependent. They also show that iodide and bromide anions increased the permeability coefficient up to 1 molar level, and then they showed saturation effect for higher concentrations. The relative strength of the anions to affect the flux of the drug through the excised mouse skin was in the increacing order; $F^- < SO_4^{2-} < Cl^- < ClO_4^- < NO_3^- < SCN^- < Br^- < I^-$. This order does roughly coincide with the lyotropic Hofmeister series which is also the order of the effectiveness of the anions to swell skin collagen (Elden, 1971). These results suggest that sodium salts of anions which have been reported to swell collagen effectively increased the flux of the drug, and skin swelling might play an important role in transdermal drug permeation. A significant discrepancy between two sets of data experimenting the effect of 1.0 M NaI on the permeation of salicylic acid was observed as shown in Fig. 1 and 2. This might be due to the variation of the skin condition of mouse along the season. The skin showed lower permeation in cold season.

In order to ascertain the permeation-enhancing activities of the anions such as iodide and bromide *in vivo*, the effects of the presence of these ions in the ointment on the permeation of salicylic acid through rabbit skin were investigated with the *in-vivo* transdermal permeation test. The results were listed in Table II. It shows that the addition of sodium iodide or sodium bromide to the vaseline ointment significantly ($p<0.05$) increased the transdermal permeation of salicylic acid through rabbit skin, and these ions, especially iodide was so effective that it might be employed as a transdermal permeation enhancer in topical formulations.

Table II. Blood concentration of salicylic acid at 2 hours after the application of the salicylic acid-vaseline ointment to rabbit skin (n=3)

Ointment	Concentration of salicylic acid in plasma
10% salicylic acid(control)	2.57±0.31 mM
10% salicylic acid containing 5%-NaI	4.53±0.43 mM
10% salicylic acid containing 5%-NaBr	3.33±0.47 mM

Table III. Thermodynamic activities of salicylic acid in the solution of the donor compartment containing electrolytes

Compounds	Apparent solubility (mM/ml)	Thermodynamic activities (-)
Control	1.547	0.646
Na_2SO_4	1.512	0.625
NaSCN	1.605	0.623
$NaClO_4$	1.621	0.617
NaCl	1.399	0.718
NaBr	1.632	0.613
NaI	1.544	0.648

*Apparent solubility means the solubility of salicylic acid in 30%-ethanol solution which contains 1 M of each inorganic compound.

In elucidating the permeation-enhancing mechanism of these anions, two factors must be considered; the first is thermodynamic factor, and the other kinetic factor. The apparent solubilities of salicylic acid in aqueous 30% alcohol solution containing 1 M of sodium salt of some of the anions were measured, and thermodynamic activities of the penetrant in the donor solution were calculated as the ratio of the concentration in the solution to the apparent solubility. These activities represent the escaping tendencies of salicylic acid in the donor solution (Zatz and Sarpotdar, 1987). The results were listed in Table III, which shows that these electrolytes did not significantly affect the escaping tendency of salicylic acid except sodium chloride, and there was no valid correlation between the permeability coefficients and the escaping tendencies. This means that the effects of these inorganic anions on the transdermal flux of salicylic acid are not due to the alteration of the thermodynamic activity of salicylic acid in the donor solution by these anions. During the previous experiments, it was observed that the excised skin of

Table IV. Absorbance of sterol leached from stratum corneum sheet by the aqueous solution of inorganic electrolytes during 5 hours (n=5)

Treatment solution	Absorbance at 460 nm[a]
H_2O	0.480±0.04
1 M NaBr	0.512±0.04
1 M NaI	0.638±0.05
1 M NaSCN	0.784±0.03
0.5 M Na_2SO_4	0.487±0.03
0.075 M NaF	0.464±0.05
1 M NaCl	0.430±0.03
1 M $NaClO_4$	0.475±0.02

[a]The amount of sterol was represented as the absorbance after $FeCl_3$-acetic acid treatment.

Table V. Frequency shifts of Fourier-transform infrared/ attenuated total reflection (FT-IR/ATR) spectra of stratum corneum sheet after treatment by 1 M solution of inorganic electrolytes (n=3)

Treatment	IR frequency (1/cm)	
H_2O	2853±0.2	2924±0.3
NaBr	2854±0.3	2926±0.3
NaI	2854±0.2	2927±0.2
NaSCN	2854±0.2	2926±0.3
NaCl	2853±0.3	2924±0.2
$NaClO_4$	2853±0.2	2923±0.2
Na_2SO_4	2853±0.4	2924±0.2
NaF	2853±0.3	2924±0.2
NaH_2PO_4	2853±0.2	2924±0.2
Na_2HPO_4	2853±0.2	2924±0.2
Na_2CO_3	2853±0.2	2924±0.2

the mouse became severely swollen when kept long in the aqueous solution of electrolytes of which anions enhanced the transdermal permeation of salicylic acid. In order to examine the abilities of the anions to leach out components of the excised skin due to swelling of the skin, the amounts of sterol leached out during 5 hours by 1 molar aqueous solution of the electrolytes were measured and the results were listed in Table IV. It shows that the aqueous solutions of sodium salts of the anions which did not enhance or reduce the transdermal permeation of salicylic acid did not significantly increase the amount of sterol leached out of the skin. Some of them rather decreased the lipid leaching. However, the aqueous solution of sodium salts of the anions which enhanced the transdermal permeation significantly ($p<0.005$) increased the amount of sterol leached out of the excised skin. This means that sodium salt of iodide, bromide or thiocyanate might enhance the transdermal permeation of salicylic acid by reduction of the barrier function of the skin through perturbing the skin components by swelling. It is expected that the structure of the lipid layers of stratum corneum might be perturbed so as to reduce the barrier function in the presence of these anions.

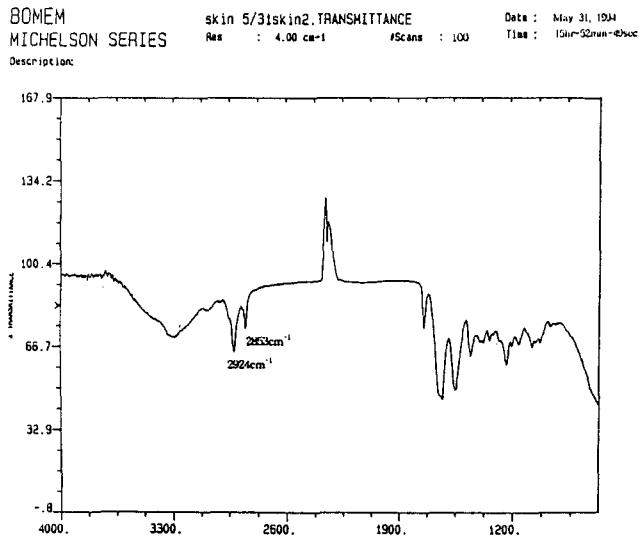

Fig. 4. IR-spectrum of the stratum corneum sheet of mouse

A typical IR spectrum of the stratum corneum sheet was measured, and represented in Fig 4. It shows that there are two peaks, 2853 and 2924 cm^{-1} in the hydrogen-stretching vibration region, which have been reported to be due to the symmetric and asymmeteic stretching vibrations of C-H bond of the acyl chains of phospholipids, respectively (Knutson *et al.*, 1987). These two peaks were significantly ($p<0.001$) shifted to higher frequencies by the treatment of sodium salts of iodide, bromide, or thiocyanate, which enhanced the transdermal permeation of salicylic acid, while the salts of other anions did not change the peaks. These results are similar with those reported with other transdermal permeatiom enhancers. Golden and his coworkers (Golden *et al.*, 1987) reported that there are some relations between shifts of the peak frequencies and percutaneous drug fluxes through the stratum corneum sheets. It has been suggested that the shift to higher frequency in the C-H stretching of lipid alkyl chains of stratun corneum results when methylene groups along the alkyl chain adopt an increased number of gauche (nonlinear) conformers, and the magnitude of the shift is directly related to the ratios of the number of gauche to trans conformers. This process is called a rotamer disordering. Thus, the frequency shift provides a measure of the lipid alkyl chain conformational disorder. This shift value and water permeability coefficient of stratum corneum have also been fairly correlated (Potts and Francoeur, 1993). Such frequency changes are possibly due to a disruption of lipid structures of stratum corneum associated with incorporation of penetrants into the lipid domain. This suggests that the permeation-enhancing anions might induce some alterations of the lipid layers of the skin structure, and enhance the transdermal drug permeation. It is interesting to note that the shift of the asymmetric

stretching frequency is more sensitive to the anions than the symmetric stretching frequency. This phenomenon has not been reported in other researches, and studies along this line would be rewarding.

It was found that the presence of sodium iodide, bromide or thiocyanate in the donor solution in the diffusion cell significantly increased the transdermal permeation of salicylic acid through the excised mouse skin, and incorporation of sodium iodide or sodium bromide in the salicylic acid ointment significantly increased the *in-vivo* transdermal permeation through rabbit skin. Their permeation-enhancing activities were ascribed to the reduction of the resistance property of the skin by swelling and perturbation of the skin structure by these anions.

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