Permeability of Ionized Salicylate Derivatives through Guinea Pig Dorsal Skin

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pH-dependency of skin permeability to salicylic acid was examined in excised guinea pig dorsal skin. Permeation followed the pH-partition theory at acidic pH. However, above pH 5.0 the observed permeability coefficients were larger than the estimated values obtained from the ratio of the undissociated forms. These findings are quite different from those obtained using the same drug and a silicone rubber membrane, in which permeability coefficients were consistent with the pH-partition theory. The findings suggested that permeation of salicylate as anions occurred at a neutral skin pH. The permeability coefficient of the ionized form was estimated to be about 1.6% of the nonionized form. We also examined the skin permeability of salicylate and its five 5-substituents and two 3-substituents at pH 7.4. We investigated the relationship between their permeability coefficients and the physico-chemical properties of the substituents. Multi regression analysis on the permeability coefficients showed a parabolic relationship between the values of the hydrophobic parameter (π) and the logarithms of the permeability coefficients. These findings suggested that the ionic permeation pathway of salicylate derivatives is controlled by hydrophobic as well as hydrophilic properties.

Key words salicylate; skin permeability; hydrophobicity; multi-regression analysis

The lipid lamella of the stratum corneum of the skin acts as a barrier for the permeation of most drugs, especially as a hydrophobic barrier for hydrophilic drugs and ionized drugs. Most of the drugs used are weakly acidic or weakly basic and their nonionized forms seem to preferentially permeate through the skin. Although a number of routes have been suggested for skin permeation by drugs,[1,2] it has been reported that the permeabilities of weakly acidic and basic drugs basically follow the pH-partition theory.[3] However, as reported for diclofenac[4] and indomethacin,[5] the permeation of ionized drugs is not negligible in the pH regions in which drugs are present predominantly as ionized forms. Ionized drugs may penetrate the skin through the hydrated keratin matrix or aqueous regions.[6,7] Therefore, in this study we examined the pH-dependency of the permeability coefficient of salicylic acid in excised guinea pig dorsal skin, and investigated the contribution of ionized forms on permeation. We compared the results with those using a silicone rubber membrane, which is an artificial hydrophobic membrane that only nonionized drugs can permeate through. Since the physicochemical properties of the ionic drugs which control the permeability were unknown, we examined the ionic permeability of salicylate and its seven mono-substituents in the skin and tried to clarify them.

Experimental

Materials Salicylic acid derivatives were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Tokyo Kasei Kogyo Co. (Tokyo, Japan). Ketotifen fumarate was from Sigma-Aldrich Japan Co. (Tokyo, Japan). Silicone rubber membranes were obtained from Wakomudenso Co. (Tokyo, Japan).

Measurement of *in Vitro* Skin Permeation *In vitro* skin permeation of drugs was examined as described previously.[8] Full thickness dorsal skin was excised from male guinea pigs 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 either saline, whose pH was adjusted with 10 mM citrate buffer (at pH 4.0, 5.0), or 10 mM phosphate buffer (at other pHs), and receiver cells with phosphate buffered saline (PBS pH 7.4), and pretreatment was carried out for 12 h with stirring at 450 rpm by a magnetic stirrer. After washing both cells, 20 mM solution of salicylate or its derivatives in saline (pH adjusted with either phosphate buffer or citrate buffer) was added to the donor solution, and the permeation experiment was started. The concentration of the donor solution was low enough to avoid the changes in pH and osmotic pressure during the experiments. The decreases in the concentration of the donor solution were less than 1%. One hundred and fifty microliters of sample was taken from the receiver cells periodically over a maximum period of 29 h. The change in pH in the donor solution was less than 0.2 during the experiment.

The concentrations of salicylate derivatives were determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 detector (Hitachi) at 303 nm for salicylate and 5-CH_3 derivative, 331 nm for 5-OH derivative, 313 nm for 5-Cl derivative, 311 nm for 5-NO_2 derivative, 308 nm for 3-CH_3 derivative, 315 nm for 3-Br derivative and 316 nm for 3-C_6H_5 derivative. 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 and its 5-CH_3, 5-OH, 5-Cl and 5-NO_2 derivatives, a mobile phase of methanol, water and phosphoric acid (100 : 100 : 1) for 3-CH_3 and 3-Br derivatives, and a mobile phase of methanol, water and phosphoric acid (1250 : 750 : 1) for 3-C_6H_5 derivative, at a flow rate of 0.7 ml/min. Ketotifen fumarate was used as an internal standard for the analysis of salicylate and its 5-CH_3 and 5-NO_2, derivatives, and salycilate was used for all other derivatives.

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.

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

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

Measurement of Silicone Rubber Membrane Permeation Silicone rubber membrane (TSE221-5U, 0.5 mm thickness) was cut from the sheet and placed in the two-chamber diffusion cells. Permeation of salicylate through the silicone rubber membrane was examined in the same way as described above at 37 °C. In addition to phosphate buffer and citrate buffer, 50 mM HCl was used to adjust the pH of the donor solution to 1.6.

Measurement of pK_a of Salicylate This was obtained by measuring the ratio of UV absorption at 296 nm (λ_{max} for ionized form) and 303 nm (λ_{max} for nonionized form) at 37 °C for 0.2 mM salicylate solution in 150 mM NaCl containing phosphate buffer or phosphoric acid.

Statistical Analysis Multi regression analysis was conducted using StatView (SAS Institute Inc. (Cary, NC, U.S.A.)).

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Results and Discussion

pH-Dependency of Skin Permeability to Salicylic Acid At acidic pHs, the permeability coefficient of salicylic acid decreased with an increase in the ratio of the ionized form, as shown in Fig. 1. Therefore, permeation of salicylic acid basically followed the pH-partition theory. However, as shown in the same figure, above pH 5.0 the permeability coefficients were not consistent with the estimated values obtained from the ratios of the undissociated forms of salicylic acid, which were calculated from the pK_a value (2.9). The permeability coefficient at pH 7.4 was similar to that at pH 6.0. These findings were quite different from the permeability coefficients of the same drug through the silicone rubber membrane. Permeability coefficients in the membrane followed the pH-partition theory since the experimental values and the estimated values obtained from the ratios of the undissociated forms were consistent with each other, as shown in Fig. 2. Permeation was scarcely observed at neutral pH. This agreed with the findings of Lee *et al.*, that the ionic species of salicylate in aqueous media did not permeate through the hydrophobic membrane.[9]

These findings suggested that the permeation of salicylate as anions occurred during weakly acidic skin conditions (pH 4.5—6.5[10]). According to the present findings, the permeability coefficient of the ionized form ($0.00164\,cm\cdot h^{-1}$) was estimated to be about 1.4% of the nonionized form ($0.118\,cm\cdot h^{-1}$) under the assumption that the total flux was the sum of the individual fluxes of the nonionized and ionized forms.[4,5,11] Although the value for the ionized form was much smaller than that for the nonionized form, ionic permeation does not seem to be negligible at weakly acidic skin pHs. These findings are consistent with previous findings on diclofenac by Obata *et al.* in hairless rats,[4] and on indomethacin in hairless rats.[5] The findings were also consistent with those on 4-oxo-4*H*-1-benzopyran-2-carboxylic acids in human skin,[11] although the relative contribution of ionic permeation was smaller than that observed in this study.

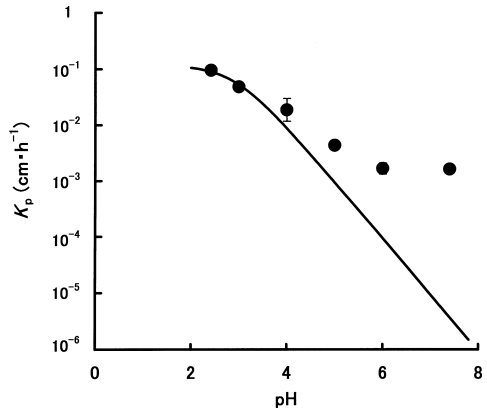

Fig. 1. pH-Dependency of Permeability Coefficients, K_p, of Salicylic Acid through Guinea Pig Dorsal Skin

Solid line represents the estimated values from the permeability coefficients of the nonionized form, which were calculated from the permeability coefficients and the ratio of the nonionized form at pHs 2.4 and 3.0. Data are means±S.D. of four experiments.

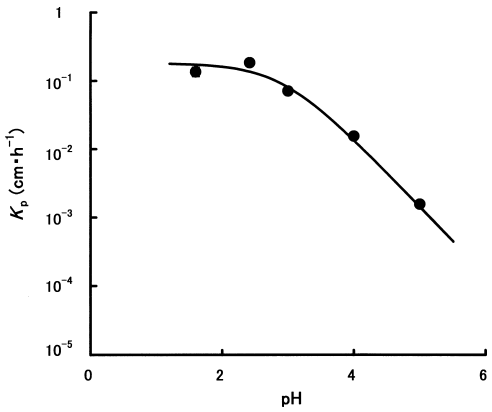

Fig. 2. pH-Dependency of Permeability Coefficients, K_p, of Salicylic Acid through the Silicone Rubber Membrane

Solid line represents the estimated values from the permeability coefficients of the nonionized form, which were calculated from the permeability coefficients and the ratio of the nonionized form at pHs 1.6 and 2.4. Data are means±S.D. of four experiments.

Skin Permeability of Salicylate Derivatives The above findings revealed that the permeation of salicylate through guinea pig skin occurred as anions at weakly acidic or neutral pHs. Therefore, we examined the skin permeability of mono-substituents of salicylate at pH 7.4. We investigated the relationship between their permeability coefficients and the physico-chemical properties of the substituents for salicylate and its five 5-substituents and two 3-substituents, as shown in Table 1. Permeability coefficients of the relatively hydrophilic 5-OH and 5-NO_2 substituents, especially that of 5-OH substituent, were smaller than those of salicylate and all other substituents.

Multi regression analysis on the permeability coefficients indicated that hydrophobicity is a very important factor in the skin permeability of salicylate derivatives. The analysis showed a parabolic relationship between the values of the hydrophobic parameter (π) and logarithms of the permeability coefficients, which has often been reported for the percutaneous absorption of various compounds,[12—15] as shown in Fig. 3 and Eq. 3.

$$\log K_p = 0.47\pi - 3.37 \quad (n=8,\ r=0.575) \tag{2}$$

$$\log K_p = 1.16\pi - 0.55\pi^2 - 3.26 \quad (n=8,\ r=0.853) \tag{3}$$

Better correlations were found for the seven substituents except the 3-C_6H_5-substituent

$$\log K_p = 1.34\pi - 1.84\pi^2 - 2.88 \quad (n=7,\ r=0.947) \tag{4}$$

Potts and Guy suggested the skin permeability of drugs could be predicted by a simple model based upon a permeant size [molecular volume (MV) or molecular weight (MW)]

Table 1. Hydrophobic Parameters, π, and Permeability Coefficients, K_p, of Salicylate Substituents at pH 7.4

Substituent	$\pi^{a)}$	K_p ($\times 10^{-3}\,cm\cdot h^{-1}$)$^{b)}$
H	0.00	1.62±0.24
5-CH_3	0.56	1.02±0.40
5-OH	−0.67	0.017±0.003
5-Cl	0.71	1.46±0.45
5-Br	0.86	1.85±0.75
5-NO_2	−0.28	0.85±0.13
3-CH_3	0.56	1.24±0.18
3-C_6H_5	1.96	0.95±0.34

a) π values, which were calculated from the changes in the logarithm values of the *n*-octanol/H_2O partition coefficient of benzene with the addition of each substituent, were cited from ref. 17. *b*) Data are means±S.D. of four experiments.

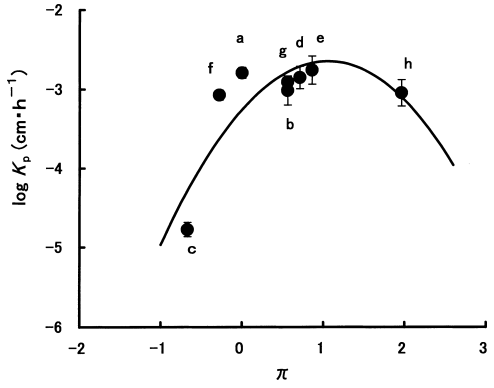

Fig. 3. Relation between Logarithm Values of Permeability Coefficients, K_p, and Hydrophobic Parameters, π, of Salicylate Substituents at pH 7.4

Solid line represents the estimated values from Eq. 3. Data are means±S.D. of four experiments. a, nonsubstituent; b, 5-CH_3-; c, 5-OH-; d, 5-Cl-; e, 5-Br-; f, 5-NO_2-; g, 3-CH_3-; h, 3-C_6H_5-substituent.

and the octanol/water partition coefficient.[16] However, improvement of the correlation was not observed by the addition of the MW term for the ionic permeability of salicylate derivatives, as shown in Eq. 5.

$$\log K_p = 0.58\pi - 0.005\mathrm{MW} - 2.61 \quad (n=8,\ r=0.582) \tag{5}$$

The analysis shown here suggested that the optimum ionic permeation of salicylate derivatives occurs at an optimum hydrophobicity. Therefore, hydrophobic interactions seem to be involved in the transport process of salicylate derivatives and their ionic pathways seem to be controlled by hydrophobic properties as well as hydrophillic properties.

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