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RESEARCH PAPER

Influence of pH on the Permeability of *p*-Toluidine and Aminopyrine Through Shed Snake Skin as a Model Membrane

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ABSTRACT

The influence of pH on the permeability of p-toluidine (pKa, 5.3) and aminopyrine (pKa, 5.0) through shed snake skin as a model membrane was studied. The pH was adjusted to several values, and the solubility of the drugs in each donor was measured. Flux rates and permeability coefficients were calculated from the steady-state penetration portions. The flux rates of p-toluidine decreased as the pH value in the donor solution increased. On the other hand, the flux rates of aminopyrine were constant at any pH value. The permeability coefficients of each drug increased as the pH value in the donor solution increased. The partition coefficients (octanol/buffer) of each drug were dependent on the molecular fraction of un-ionized species. From these results, it is suggested that ionized species of p-toluidine transports through shed snake skin, but the ionized species of aminopyrine does not.

KEY WORDS: Permeability; Shed snake skin; *p*-Toluidine; Aminopyrine; Ionized species; Nonionized species.

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INTRODUCTION

Stratum corneum, the outermost layer of mammalian epidermis, is generally regarded to be the rate-limiting barrier of pharmaceutical interest for transport of most solutes across the skin. The stratum corneum consists of a mosaic of cornified cells containing cross-linked keratin filaments and intercellular lipid-containing regions (1–3). From the heterogeneous nature of the stratum corneum, it has been suggested that several distinct domains exist in the stratum corneum (4–6). The findings that the permeability of a permeant increases as the lipophilicity of the permeant increases (7–10) and that enhancers increase the fluidity of the intercellular lipid of stratum corneum (11–13) support the idea that the lipid domain plays an important role in drug permeation.

Weak acids and bases are subject to pH-dependent ionization. It is presumed that ionized species penetrate poorly through the skin compared with non-ionized species. Some reports suggest that the contribution of the ion species to the total flux is very small (14–17). However, other reports suggest that the penetration of the ionized species of some weak acids and bases may contribute significantly to the total flux of the compound (18–21).

Shed snake skin of Elaphe obsoleta (back rat snake) is similar to human stratum corneum in terms of composition and permeability characteristics (22,23), and thus shed snake skin may be a good model membrane. In a previous report (24), we suggested that the permeabilities of cationic compounds may be controlled by the lipophilicity of a drug and its molecular size. Itoh et al. (25) also reported similar results using anionic and neutral compounds. It has been suggested that lipophilicity may affect the partition of the drug into the skin and that the molecular size may affect the diffusion of the drug in the skin.

In this study, the permeabilities of two compounds at several pH values through shed snake skin as a model membrane were measured to compare the permeabilities of ionized and nonionized species. Aminopyrine and *p*-toluidine were selected as model compounds. These were chosen because the pKa values of these compounds are approximately the same (aminopyrine, 5.0 (26); and *p*-toludine, 5.3 (27)), but the molecular weight and partition coefficient between buffer (pH 7.0) and octanol are different.

METHODS

Materials

p-Toluidine and aminopyrine were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Other reagents used were of analytical grade and were used without additional purification.

Solubility Measurement

The solubilities of *p*-toluidine and aminopyrine were determined at several pH values: 4.0, 4.5, 5.0, and 7.0. Excess of *p*-toluidine or aminopyrine was added to 10 ml of phosphate buffer at each pH level. The samples were stirred in a water bath at 32°C for 24 h. The pH of the samples was checked, adjusting with 1 mol/l hydrochloric acid as necessary. The suspensions were filtered using a cellulose acetate-membrane filter (0.45-μm pore size, Toyo Roshi, Tokyo, Japan). The concentration of *p*-toluidine or aminopyrine in the filtrate was determined spectrophotometrically.

Determination of Partition Coefficient

Octanol and buffer solution were saturated with each other before use. Octanol (5 mL) was shaken with 5 mL of buffer solution with *p*-toluidine or aminopyrine (66 m*M*) for 8 h at 32°C. The mixture was then centrifuged, and the concentration in each phase was determined spectrophotometrically. The pH of the water phase was also measured after incubation.

In Vitro Penetration Study

Details of the experimental set-up have been published previously (24). Briefly, shed snake skin of Elaphe obsoleta (black rat snake) was mounted in Franz-type diffusion cells with an available surface area for diffusion of 1.77 cm^2. A suspension of *p*-toluidine or aminopyrine was placed on the donor side, and the receptor solution (0.1 *M* phosphate buffer, pH 7.2) was kept at 32°C and stirred with a magnetic stirrer.

The permeability rates and permeability coefficients were calculated according to Equation (1) from the initial linear portion of the penetration curve.

$$dQ/dt = J = \mathrm{Pe} \cdot A \cdot C_d \quad (1)$$

where $dQ/dt = J$ is the slope of the straight portion of the penetration curve, Pe is the permeability coefficient, A is the surface area (1.77 cm^2 for the diffusion cells used in this study), and C_d is the drug solubility in the donor phase.

Analysis

p-Toluidine and aminopyrine were analyzed with HPLC method as described previously (24).

RESULTS AND DISCUSSION

The penetration profiles of *p*-toluidine from aqueous suspensions having different pH values are shown in Figure 1. The permeated amount increased linearly after 2 h at each pH value. The flux rate and permeability coefficients are listed in Table 1. The flux rate decreased as the pH values of the donor solution increased, and the total flux at pH 4.0 was approximately two times higher than that at pH 7.0. The permeability coefficients increased as the pH values increased because the solubility of *p*-toluidine in the donor solution decreased with increasing pH. The pH values selected for investigation are not expected to alter the barrier characteristics of the shed snake skin. We used an aqueous suspension as the donor solution; thus, the thermodynamic activity of a drug is equal to that of the solid state and is independent of the vehicle (28). In addition, the hydration and swelling of shed snake skin are not expected to change between pH 4.0 and 7.0. Thus, any changes in *p*-toluidine flux should result from changes in the relative amounts of ionized and non-ionized species present in solution. This suggests that the ionized species of *p*-toluidine penetrates through shed snake skin.

For an ionogenic compound at various pH values, there will be the simultaneous presence of both ionized and non-ionized species in solution. If each species moves through the skin by diffusion down its own concentration gradient across the skin membrane, the total penetration flux is the sum of the flux contribution from the two species.

$$J_{\text{total}} = J_{\text{B}} + J_{\text{BH+}} = \text{Pe}_{\text{B}}[\text{B}] + \text{Pe}_{\text{BH+}}[\text{BH}^+] \quad (2)$$

where J_{total} is the total flux, J_{B} and Pe_{B} are the flux and permeability coefficient of the nonionized species, and $J_{\text{BH+}}$ and $\text{Pe}_{\text{BH+}}$ are the flux and permeability coefficient of the ionized species, respectively. The concentrations of ionized and non-ionized species were calculated from the pK_a value (5.3) of *p*-toluidine and the pH value in the donor solution. A linear relationship was obtained between the total flux rate and the calculated concentration of ionized species (Fig. 2). Pe_{B} and $\text{Pe}_{\text{BH+}}$ were calculated from a nonlinear least-squares program (MULTI) (29). The permeability coefficient of the nonionized species ($3.23 \times$

Table 1.

Permeation Parameters and Solubility of p-Toluidine

pH	Solubility[a] (m*M*)	J^b (μmol/cm^2/h)	Pe$^b \times 10^3$ (cm/h)
4.0	2.52×10^3	6.41 ± 0.47	2.55 ± 0.19
4.5	7.12×10^2	4.62 ± 0.62	6.48 ± 0.86
5.0	2.96×10^2	3.25 ± 0.29	10.96 ± 0.98
7.0	8.70×10	2.90 ± 0.15	33.33 ± 1.77

[a] Solubility was measured at 32°C.

[b] The values of steady state flux (J) and permeability coefficient (Pe) were calculated from the stright line in Figure 1 and solubility. Each value represents the mean $\pm$ SD ($n = 3$–5).

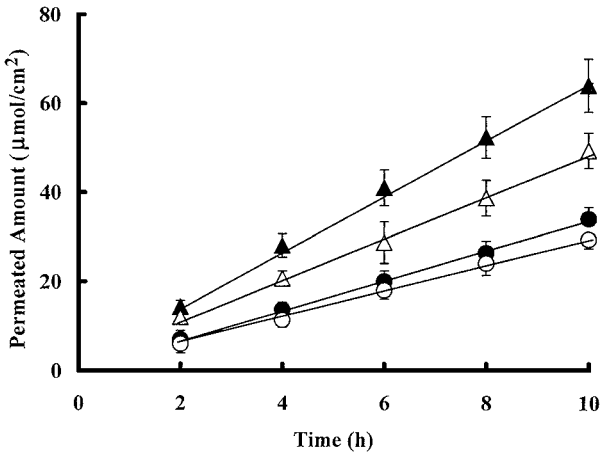

Figure 1. Permeation profiles of *p*-toluidine through shed snake skin. Key: (▲) pH 4.0; (△) pH 4.5; (●) pH 5.0; (○) pH 7.0. Each point represents the mean $\pm$ SD of three to five experiments.

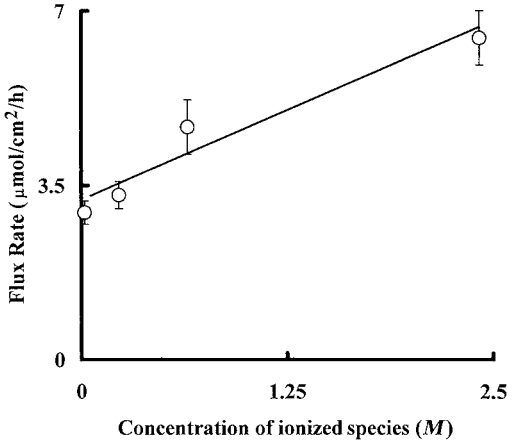

Figure 2. Relationship between the permeation rate of *p*-toluidine and the concentration of ionized species. Each point represents the mean $\pm$ SD, and the line is calculated with a nonlinear least-squares method.

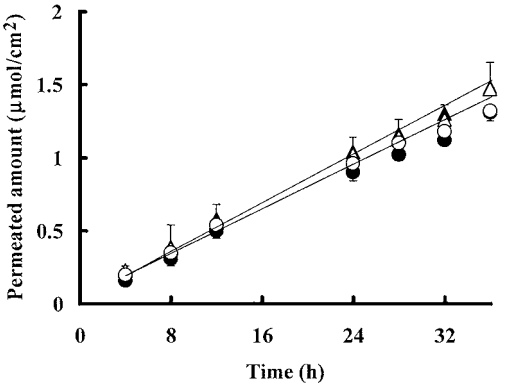

Figure 3. Permeation profiles of aminopyrine through shed snake skin. Key: (▲) pH 4.0; (△) pH 4.5; (●) pH 5.0; (○) pH 7.0. Each point represents the mean ± SD of three to five experiments.

10^{-2} cm/h) was about 30 times higher than that of the ionized species (1.06×10^{-3} cm/h).

Scheuplein and Bronagh (3) have suggested that shunt diffusion through skin appendages may play a significant role for ionic permeants. Other researchers also have demonstrated the penetration of ionized species of several drugs using rat, mouse, or human skin (14–21). A shed snake skin is a nonliving pure stratum corneum with no hair follicles (22). In this study, ionized species of *p*-toluidine was found to penetrate through stratum corneum. Fleeker et al. (16) also reported that the ionized species of clonidine and indomethacin penetrated through shed snake skin.

The penetration profiles of aminopyrine in suspension formulations of different pH values are shown in Figure 3. The flux rate and permeability coefficient are calculated and listed in Table 2. No significant difference was observed among the total flux of different pH values. The permeability coefficient increased as the pH value increased because the solubilities of aminopyrine decreased as the pH value increased. These results suggested that the ionized species of aminopyrine did not penetrate through shed snake skin.

Table 2.

Permeation Parameters and Solubility of Aminopyrine

pH	Solubility[a] (m*M*)	$J^b \times 10^2$ (μmol/cm^2/h)	$Pe^b \times 10^5$ (cm/h)
4.0	2.57×10^3	3.57 ± 0.44	1.37 ± 0.17
4.5	1.37×10^3	3.89 ± 0.35	2.90 ± 0.25
5.0	4.85×10^2	3.40 ± 0.11	7.00 ± 0.21
7.0	2.89×10^2	3.42 ± 0.13	11.80 ± 0.43

[a] Solubility was measured at 32°C.
[b] The values of steady state flux (*J*) and permeability coefficient (Pe) were calculated from the stright line in Figure 3 and solubility. Each value represents the mean ± SD ($n = 3$–5).

It is possible that the ionized species of *p*-toluidine interacts with an anionic species in the buffer solution to make a lipophilic complex. To test this possibility, partition coefficients between buffer and octanol were measured. Figure 4 shows the relationship between the pH value of the buffer and the partition coefficient or fraction of nonionized species. A good relation was observed between the partition coefficient and fraction of nonionized species. From these results, the probability of ionized species penetrating the skin may be negligible.

The stratum corneum may be represented by a lipoidal pathway in parallel with a pore pathway. It has often been suggested that lipophilic compounds primarily penetrate through a lipoidal pathway and that very hydrophilic compounds penetrate through a pore pathway in the stratum corneum. Thus, nonionized species may be expected to penetrate through the lipoidal pathway and ionized species may penetrate through the pore pathway. In this study, the ionized species of *p*-toluidine penetrated through shed snake skin, but that of aminopyrine did not. These data may suggest that ionized species of *p*-toluidine can penetrate through the pore pathway but that that of aminopyrine cannot.

The relative values of the permeability coefficients of *p*-toluidine species are similar to the results reported previously for other compounds. The ratio of the permeability coefficients (nonionized/ionized) of oxycodone through mouse, rat, and rabbit was 4:20 (19), 10 for clonidine through shed snake skin (16), 50 for lidocaine through human skin (20), and 20 for ephedrine and scopolamine through human skin (14). However, the penetrations of ionized species of chlorpheniramine, indomethacin, and a series of chromone acids were restricted, and the ratios were 200 [for chlorpheniramine through human skin (15)], 100 [for indomethacin through shed snake skin (16) and hairless rat skin (17)], and 10,000 [for a series of chromone acids through human skin (14)]. Hatanaka et al. (30) suggested that the pore size of rat skin is 0.38–1.58 nm. Peck et al. (31) also reported that the effective pore radius of human skin is 1.5–2.5 nm. The mechanism of penetration through the pore pathway and the pore size of shed snake skin are not clear. Additional investigations are needed to clarify the reason that the ionized species of *p*-toluidine penetrated through shed snake skin and that of aminopyrine did not. However, based on the hypothesis that the pore pathway is restricted by the molecular weight

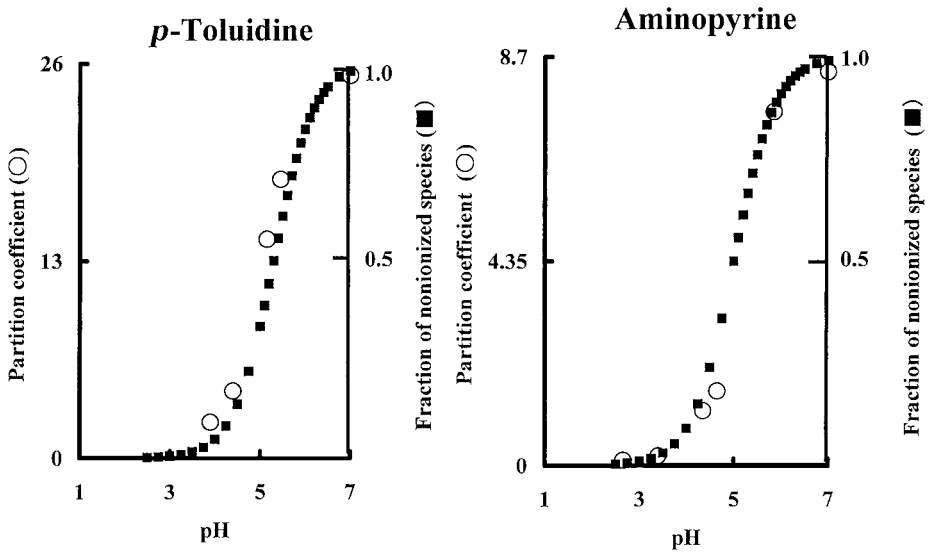

Figure 4. Relationship between the fraction of the nonionized species and the partition coefficient (*n*-octanol/buffer).

or molecular size of permeant, one can explain the results of this study.

CONCLUSION

The results of this study indicate that the ionized species of p-toluidine penetrated through shed snake skin, but that the ionized species of aminopyirne did not. This might be attributed to the pore pathway of shed snake skin restricted by molecular weight or molecular size of permeant.

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