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Effect of pH on the skin permeability of a zwitterionic drug, cephalexin

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Abstract

Skin permeability of the zwitterionic drug, cephalexin, was measured at various pH. A U-shaped curve was obtained for the relationship between the permeability coefficient and pH. Although cephalexin degraded dependent on the pH, the concentration in the suspended donor solution was maintained constant by the high dissolution rate. The barrier function of skin, which was assessed by the permeation of the nonelectrolytes, cortisone and D-mannitol, did not change over the range from pH 3.0 to 7.0. The permeability coefficient of cephalexin decreased with increase in zwitterion fraction and decrease in fractions of cation and anion, suggesting that each ionic species has different skin permeability. The permeability coefficient of zwitterion, estimated on this assumption of each ionic species having different permeability, was about 10% that of cation and anion. The octanol/buffer distribution coefficient and diffusion coefficient were also lower for zwitterion than for cation and anion. This suggests that the pH dependency in skin permeability of cephalexin may reflect the permselective property of skin dependent on the lipophilicity and/or diffusivity of ionic species.

Keywords: Zwitterionic drug; Cephalexin; Skin permeability; pH; Ionic species

1. Introduction

Current significant therapeutic agents contain a number of zwitterionic drugs such as peptides, angiotensin-converting enzyme inhibitors and β-lactam antibiotics, and these drugs are of increasing interest for administration via the transdermal route. Zwitterions are compounds having a state with zero net charge due to multiple oppositely charged groups but possessing large intramolecular multipole moments. The relatively high melting point and then low solubility in lipophilic media result in low permeability through skin and other biological membranes. Therefore, it is believed that transdermal administration of these drugs is impossible without the use of skin permeation enhancers or iontophoretic treatment. Although systemic studies on the passive transport across skin are very

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important to achieve optimal enhancement, limited information is available (Ruland and Kreuter, 1991; Mazzenga et al., 1992; Sznitowska et al., 1993). In most reports on skin permeation of zwitterionic drugs, passive transport was treated only as a reference (Wearley et al., 1990; Green et al., 1991).

The objective of the present study was to analyze the skin permeation behavior of zwitterionic drugs. Cephalexin, a β-lactam antibiotic with an amino and a carboxyl group, was selected as a model of these drugs, skin permeability was measured at various pH, and the pH dependency was discussed in relation to each type of permeation behavior of the ionic species.

2. Materials and methods

2.1. Materials

Cephalexin was a gift from Toyo Pharmar Co. (Toyama, Japan). Cortisone and D-mannitol were obtained from Nacalai Tesque Co. (Kyoto, Japan). D-[1-^{3}H(N)]Mannitol was purchased from NEN Research Products (Boston, USA). Other chemicals and solvents were of reagent grade and used as received.

2.2. Skin permeation experiment

Rat skin was freshly excised from the abdomen of male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), aged 7 weeks, after being shaved carefully. The skin sample was mounted between two diffusion half-cells with a water jacket connected to a water bath at 37°C, each having 3.0 ml volume and 0.966 cm^2 effective diffusion area. The receiver compartment was filled with a pH 7.0, 0.1 M phosphate buffer solution and the donor compartment with an appropriate phosphate buffer solution (pH 2.0–8.0, 0.1 M), and the receiver and donor solutions were then stirred at 1440 rpm with a star-head bar driven by a synchronous motor. A 14 h period of equilibration was allowed and then the receiver solution was replaced with fresh buffer solution and the donor solution with cephalexin or cortisone suspension in the respective buffer solution, which make the drug concentration in receiver solution detectable throughout experiments. D-Mannitol was applied to skin in a 1 mg/ml solution containing 1.3 μCi/ml radiolabelled D-mannitol. At specified time intervals, 200 μl of samples was withdrawn from the receiver compartment and the same volume of fresh buffer solution was added to keep the volume constant. Permeant concentration in the samples was analyzed, and the pseudo steady state permeation rate and permeability coefficient were calculated.

2.3. Determination of ionization constants

The apparent ionization constants, pK_{a1} and pK_{a2} for the dissociation of carboxylic acid and the ammonium group of cephalexin were determined by the potentiometric titration of 0.01 M aqueous solution at an ionic strength of 0.5 and 37°C (Albert and Serjeant, 1971). The isoelectric pH was calculated from the resulting pK_{a1} and pK_{a2} values.

2.4. Degradation experiments

The kinetics of degradation of cephalexin was assessed in 0.1 M phosphate buffer solution at various pH (pH 2.0–8.0). Cephalexin was dissolved in each solution to make a 100 μg/ml solution, and this was stored in a screw-capped glass tube at 37°C. Samples were taken at appropriate time points and assayed immediately.

The degradation of cephalexin in skin homogenates was also evaluated in order to check the possibility of skin metabolism. Skin homogenates (2.5%) were made with full-thickness rat skin and phosphate buffer solution (pH 7.0, 0.1 M) using a glass Potter-Elvehjem tissue grinder. The homogenates were centrifuged for 10 min at 9000 $\times g$ and 4°C, and the supernatant liquid was used immediately for degradation experiments. After mixing 1 ml of homogenate medium with 4 ml of cephalexin solution (100 μl/ml) in pH 7.0, 0.1 M phosphate buffer solution, the degradation experiment described above was again performed.

2.5. Dissolution experiment

The solubility of cephalexin and cortisone in 0.1 M phosphate buffer solution at various pH was determined as follows. An excess amount of a drug was added to a test buffer solution, and the suspension was stirred and kept at 37°C. After equilibrium was achieved, a part of the suspension was withdrawn and filtered quickly through a membrane filter (DISMIC-13cp, Advantec Toyo Co., Tokyo, Japan). The filtrate was diluted with the corresponding buffer solution, and drug concentration was then determined.

The dissolution rate of cephalexin into 0.1 M phosphate buffer solution at various pH values was estimated from the dissolution profiles of a cephalexin tablet. Cephalexin powder was directly compressed by a rotary tabletting machine (HT-P18, Hata Iron Works Co., Kyoto) to make a tablet 11 mm in diameter and 3.67 mm thick. The tablet was mounted between two half diffusion cells as described above together with a membrane filter (C045A025A, Advantec Toyo Co.). Only the half-cell of the filter side was charged with a test buffer solution and stirred at 37°C. Samples (200 μl) were withdrawn from the half-cell for assay, and 200 μl of fresh buffer solution was added to keep the volume constant. The dissolution profiles were fitted to the Noyes-Whitney's equation (Eq. 1) to obtain the apparent dissolution rate constant, k_{app}:

$$\frac{dC}{dt} = k_{app}S(C_s - C) \quad (1)$$

where S is the surface area of cell, C_s denotes the solubility of cephalexin in a test buffer solution and C is the concentration in buffer solution at time t. The true dissolution rate constant, k_{dis}, was obtained based on the following equation:

$$\frac{1}{k_{app}} = \frac{1}{k_{dis}} + \frac{C_s V}{J_m} \quad (2)$$

where V is the cell volume and J_m represents the permeation rate across the membrane filter which was separately obtained from the permeation experiment. The product of the true dissolution rate constant and the surface area of cephalexin powder in donor solutions, $k_{dis}S'$, was calculated using the amount of powder and specific surface of cephalexin.

2.6. Determination of octanol / buffer distribution coefficient

Distribution coefficients of cephalexin between octanol and buffer solutions were determined by a shake-flask method. Octanol and 0.1 M phosphate buffer solution (pH 3.0–7.0) were previously saturated with each solvent. A precisely weighed quantity of cephalexin was dissolved in each buffer solution to give a final concentration of 100 μg/ml. Exactly 4 ml of octanol and cephalexin-buffer solution was put into a screw-capped glass tube and shaken at 200 strokes/min for 90 min at 25°C. After equilibrium was achieved, the aqueous phase was centrifuged at 3000 rpm for 10 min at 25°C, and then the cephalexin concentration was determined. The octanol/ buffer distribution coefficient, K, was calculated from:

$$K = \frac{C_0 - C}{C} \quad (3)$$

where C is the concentration of cephalexin in buffer solution after distribution and C_0 denotes the initial concentration which was determined by a simultaneous shaking of cephalexin in the same buffer solution for the same period as used in the distribution experiment.

2.7. Determination of apparent diffusion coefficient in buffer solution

The apparent diffusion coefficients of cephalexin in 0.1 M phosphate buffer solution at various pH (pH 3.0–7.0) were determined by following the capillary-cell method of Saraf et al. (1963). A microliter syringe (710RNCH, Hamilton Co., Reno, USA) was used as a capillary. 1 l of a buffer solution was placed in a beaker, and then prewarmed in a water bath at 37°C. The capillary was filled with 70 μl of cephalexin solution, which was prepared with the same buffer solution to give a concentration of 200 μg/ml, and was carefully immersed in the bulk buffer

solution in the beaker. The bulk buffer solution was stirred at 120 rpm with a stirrer bar driven by a synchronous motor. After 30 min, the capillary was removed from the bulk buffer solution, and the amount of cephalexin remaining was measured immediately. The apparent diffusion coefficient, D was calculated from an approximate equation of diffusion:

$$D = \frac{4l^2}{\pi^2 t} \ln\left(\frac{8C_0}{\pi^2 C}\right) \quad (4)$$

where l is the length of capillary, C is the concentration of cephalexin at time, t and C_0 is the initial concentration determined by simultaneously keeping cephalexin solution alone in another sealed capillary.

2.8. Analytical methods

Cephalexin was assayed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (LC-6A, Shimadzu, Kyoto), a 4.6 mm × 150 mm stainless-steel column packed with Nucleosil® 5C18 (Macherey Nagel, Germany), in a column oven (CTO-6A, Shimadzu) set at 40°C, an ultraviolet detector (SPD-6A, Shimadzu) and an integrator (C-R6A, Shimadzu). Analytical conditions were: mobile phase, methanol/50 mM sodium dihydrogen phosphate (20:80); flow rate, 1.0 ml/min; detector, UV 250 nm; internal standard, phenol.

Cortisone concentrations were also determined by HPLC. Analytical conditions were: mobile phase, acetonitrile: 0.05% phosphoric acid (35:65); flow rate, 0.8 ml/min; detector, UV 243 nm; internal standard, dexamethasone.

D-Mannitol content was measured by mixing 200 μl of a sample with 4 ml of a scintillation cocktail (Atomlight®, NEN Research Products) and counting with a liquid scintillation counter (LS3801, Beckman, Munich, Germany).

2.9. Data analysis

Data analysis was carried out by a non-linear least-squares regression program, MULTI (Yamaoka et al., 1981), which was run on a personal computer (PC-9801DA, NEC, Tokyo).

3. Results and discussion

3.1. pH dependency in skin permeation of cephalexin

Permeability of cephalexin through rat skin was measured at various pH values, and the

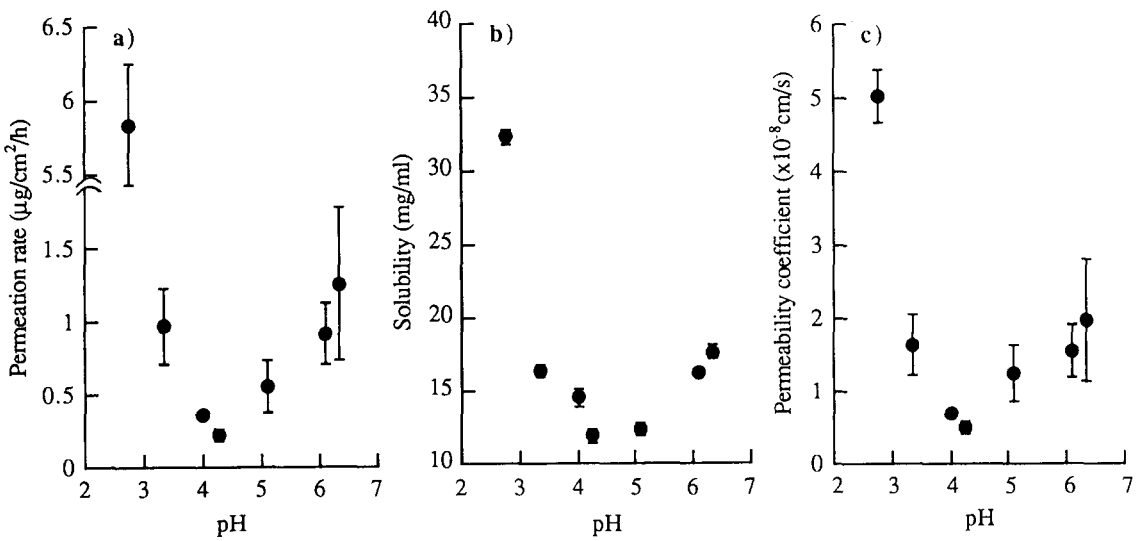

Fig. 1. Skin permeation rate (a), aqueous solubility (b) and permeability coefficient (C) of cephalexin at various pH values. Each value represents the mean ± S.E. of 3–7 experiments.

pseudo steady state permeation rate was plotted against pH as shown in Fig. 1a. The plot shows a U-shaped curve with 25-fold difference between the maximum rate at pH 2.75 and the minimum at 4.26. Because the aqueous solubility of cephalexin was dependent on pH (Fig. 1b), the permeability coefficient was calculated by dividing the permeation rate by the solubility at each pH. The U-shaped curve was also observed for the permeability coefficient although the difference was smaller than that in the permeation rate (Fig. 1c). Therefore, the pH dependency in the skin permeation of cephalexin must be caused by something other than the pH-dependent solubility.

3.2. *Influence of degradation of cephalexin on its skin permeability*

It has been reported that cephalexin degrades in an aqueous solution following pseudo first-order kinetics (Yamana and Tsuji, 1976). This suggests that the cephalexin concentration in donor solution and thus the skin permeability may change depending on pH. There is also a possibility that cephalexin is metabolized by some skin enzymes, whose activity generally depends on pH (Wester and Noonan, 1980). The degradation of cephalexin was thus evaluated in 0.1 M phosphate buffer solution at various pH and skin homogenates. Fig. 2 shows the time courses, expressed as percent of the initial concentration, of the disappearance of cephalexin in buffer solutions. The semilogarithmic plots over the entire pH range were reasonably linear, indicating that the drug's degradation follows pseudo first-order kinetics. The degradation profile in skin homogenate was almost the same as that in pH 7.0, 0.1 M phosphate buffer solution (data not shown). There seems to be little enzymatic degradation of cephalexin in skin. The pseudo first-order rate constants, k_{deg} were calculated from profiles in Fig. 2 and plotted against pH in Fig. 3. The rate constant increased with increase in pH, however, the tendency did not correspond to that of the skin permeability. The rate constants were slightly higher than those reported Yamana and Tsuji (1976), especially at high pH. This may be caused

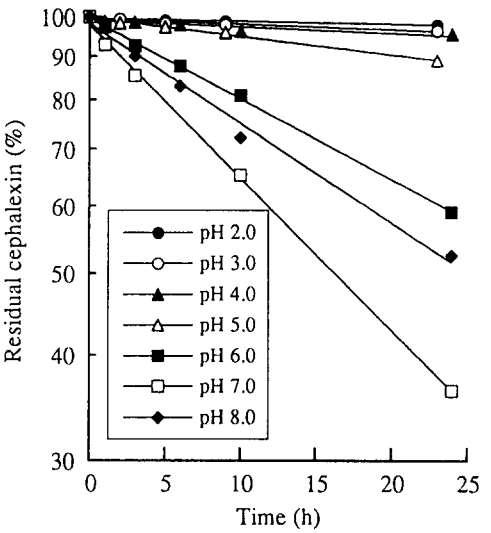

Fig. 2. Apparent first-order plots for the degradation of cephalexin at various pH values. Each value represents the mean of 3 experiments. Each S.E. is contained in each symbol.

by differences in buffer system and temperature. Phosphate buffer is known to have a catalytic effect on cephalexin degradation (Rattie et al., 1978).

If the dissolution rate of a drug is faster than the degradation rate, the application of suspen-

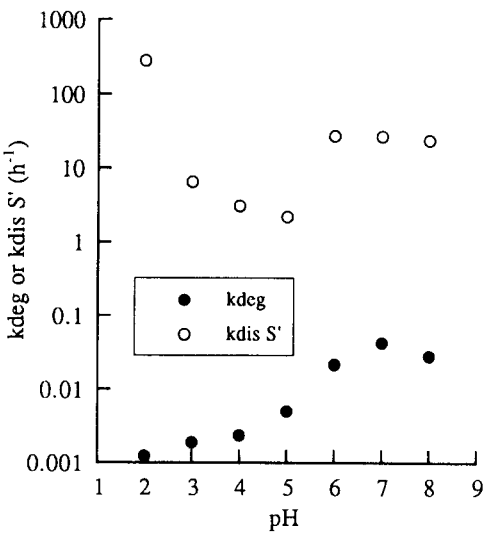

Fig. 3. Comparison of degradation rate with dissolution rate of cephalexin at various pH values.

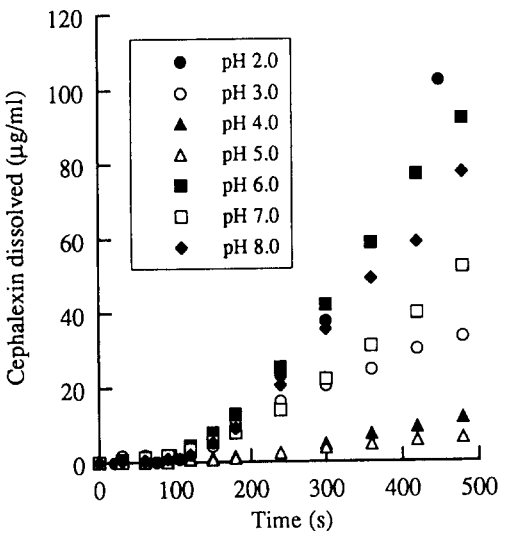

Fig. 4. Dissolution profiles of cephalexin from the tablet into phosphate buffer at various pH values.

sion to skin will make the concentration in donor solution constant. Thus, the dissolution of cephalexin was also examined in the buffer solutions. The dissolution profiles of cephalexin from tablet form into 0.1 M phosphate buffer solutions at various pH values are shown in Fig. 4. From these profiles, the product of true dissolution rate constant and surface area of powder in donor solution, $k_{dis}S'$ was calculated according to the procedure in section 2. To compare the dissolution rate with the degradation rate, the $k_{dis}S'$ obtained, which has the same dimension as the degradation rate constant, k_{deg}, is plotted in Fig. 3 together with k_{deg}. The $k_{dis}S'$ values were higher than those of k_{deg} throughout the pH range examined; the minimum difference between the two values was about 500-fold at pH 5.0. Therefore, the concentration of cephalexin in donor solution was equal to the solubility, so that overestimation of the donor concentration was not the cause of pH-dependent skin permeation of cephalexin.

3.3. Influence of pH on skin barrier

In order to determine the change in barrier property of skin due to the pH, the skin permeabilities of nonelectrolytes were measured at various pH values. Cortisone and D-mannitol were selected as model permeants for lipophilic and hydrophilic nonelectrolytes, respectively. Fig. 5 shows the relationships between permeability coefficient of cortisone or D-mannitol and pH of their donor solutions. The permeability coefficient of cortisone at pH 2.0 was higher than that at other pH values, whereas D-mannitol was more permeable at pH 2.0 and 8.0. This high perme-

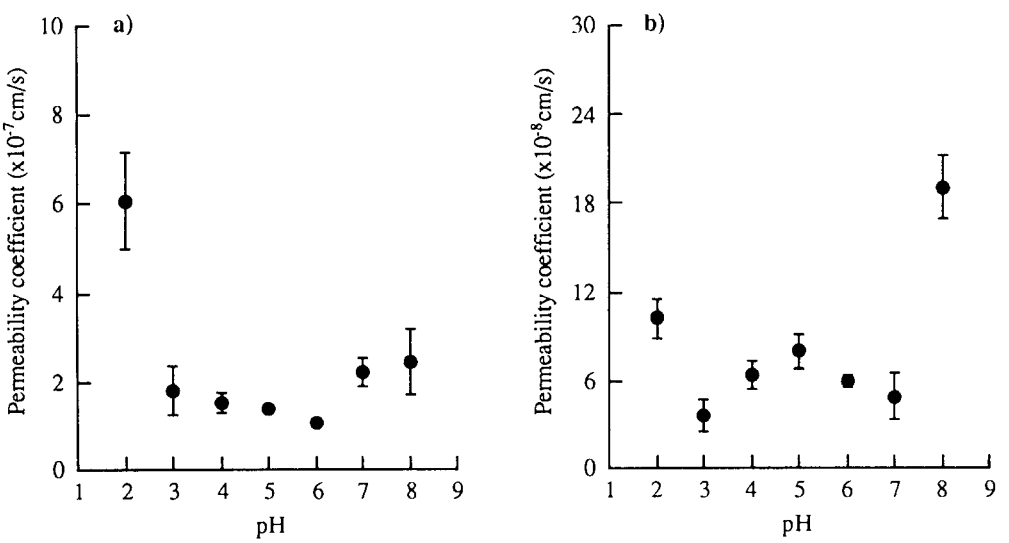

Fig. 5. Permeability coefficient of cortisone (a) and D-mannitol (b) at various pH values. Each value represents the mean ± S.E. of 3–6 experiments.

ability indicates there is some damage to the skin barrier. A similar phenomenon was seen for corrosive chemicals and protein denaturants such as acids and alkalies (Flynn, 1979). However, the permeability of both permeants was almost constant at all pH values except for pH 2.0 and 8.0, suggesting no change in barrier function of skin.

3.4. Relationship between skin permeability and physicochemical property of cephalexin ions

Cephalexin is always ionized in an aqueous solution, but the fractions of ionic species, cation, zwitterion and anion vary depending on pH of the solution. Next, the relationship between permeability coefficient and fraction of ionic species for cephalexin was investigated. The fraction of ionic species at various pH values was calculated using the dissociation constants, k_1 and k_2 (1.95×10^{-3} and 2.40×10^{-7}). The data at pH 2.75 was omitted because of the possibility of skin barrier damage. As shown in Fig. 6, the permeability coefficient decreased as the zwitterion fraction increased and the fraction of cation or anion decreased, and the minimum value was found around the isoelectric pH (pH 4.67). This suggests that each ionic species has its own skin permeability.

If this assumption is true, the total permeability coefficient of cephalexin, P_{tot} can be represented by:

$$P_{tot} = \frac{C_{cat}}{C_{tot}} P_{cat} + \frac{C_{zwi}}{C_{tot}} P_{zwi} + \frac{C_{ani}}{C_{tot}} P_{ani} \qquad (5)$$

where P and C are the permeability coefficient and concentration, and subscripts tot, cat, zwi and ani indicate total, cation, zwitterion and anion, respectively. Introducing the activity of hydrogen ion, a_H^+ and dissociation constants, k_1 and k_2 into Eq. 5, P_{tot} is given by:

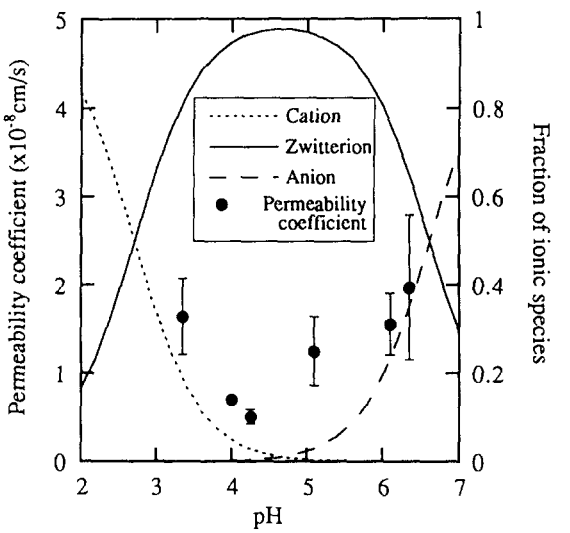

Fig. 6. Relationship between the permeability coefficient and fraction of ionic species of cephalexin.

$$P_{tot} = \frac{a_{H^+}^2 P_{cat} + K_1 a_{H^+} P_{zwi} + K_1 K_2 P_{ani}}{a_{H^+}^2 + K_1 a_{H^+} + K_1 K_2} \qquad (6)$$

By computer-fitting the permeation data at various pH values to Eq. 6, the permeability coefficients of ionic species listed in Table 1 were obtained. The best-fit curve is also shown in Fig. 7 together with the observed data. The permeability coefficients of cation and anion were almost the same and about 10-times that of zwitterion. This permselectivity of skin may be the cause of the pH-dependent skin permeability of cephalexin.

To further understand the pH dependency in skin permeation of cephalexin, two physicochemi-

Table 1
Permeability coefficient, distribution coefficient and diffusion coefficient of each ionic species of cephalexin [a]

Ionic species	P (cm/s) [b]	K [c]	D (cm^2/s) [d]
Cation	$5.39 \times 10^{-8} \pm 2.25 \times 10^{-8}$	$7.38 \times 10^{-2} \pm 2.31 \times 10^{-2}$	$1.41 \times 10^{-4} \pm 3.75 \times 10^{-5}$
Zwitterion	$6.71 \times 10^{-9} \pm 2.39 \times 10^{-9}$	$1.81 \times 10^{-2} \pm 4.77 \times 10^{-3}$	$2.33 \times 10^{-5} \pm 7.74 \times 10^{-6}$
Anion	$4.49 \times 10^{-8} \pm 9.97 \times 10^{-8}$	$8.03 \times 10^{-2} \pm 1.00 \times 10^{-2}$	$3.65 \times 10^{-5} \pm 1.63 \times 10^{-5}$

[a] Mean ± S.D.
[b] Permeability coefficient through rat skin at 37°C.
[c] Octanol/phosphate buffer distribution coefficient at 25°C.
[d] Diffusion coefficient in phosphate buffer at 37°C.

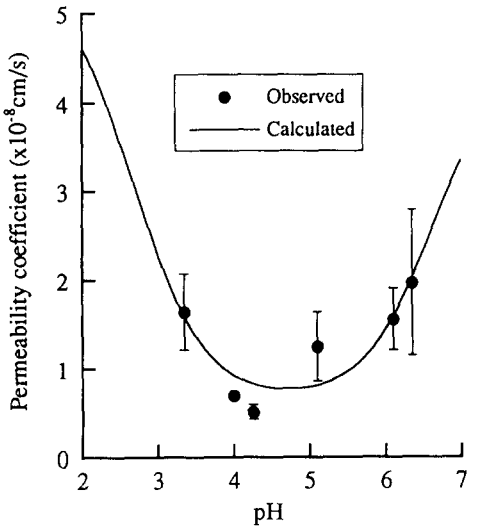

Fig. 7. Permeability coefficient of cephalexin at various pH values.

cal parameters influencing the permeability, partition coefficient and diffusion coefficient, were assessed at various pH values. Fig. 8 shows the octanol/buffer distribution coefficient and diffusion coefficient in buffer as functions of pH. The plots of both parameters were U-shaped with minimum at around isoelectric pH, similar to the permeability coefficient in Fig. 7. The distribution coefficient and diffusion coefficient of each ionic species were then estimated in the same way as for the permeability coefficient using the following equations:

$$K_{tot} = \frac{a_{H^+}^2 K_{cat} + K_1 a_{H^+} K_{zwi} + K_1 K_2 K_{ani}}{a_{H^+}^2 + K_1 a_{H^+} + K_1 K_2} \quad (7)$$

$$D_{tot} = \frac{a_{H^+}^2 D_{cat} + K_1 a_{H^+} D_{zwi} + K_1 K_2 D_{ani}}{a_{H^+}^2 + K_1 a_{H^+} + K_1 K_2} \quad (8)$$

where K and D are the octanol/buffer distribution coefficient and diffusion coefficient in buffer, respectively. The parameters obtained are listed in Table 1 and the fitting curves are shown in Fig. 8. Both parameters of zwitterion were significantly low compared with the corresponding parameters of cation and anion. The pH-dependent skin permeation of cephalexin may reflect the permselective property of skin due to the different lipophilicity and diffusivity of each ionic species.

The permselectivity of skin to cation transport has been clearly demonstrated in iontophoretic transport (Burnette and Ongpipattanakul, 1987). This was thought to be the result of electroosmotic flow due to the negative charge of skin (Pikal, 1990). In the present study, however, no difference in permeability between cation and anion was found, and only the permeability of zwitterion was lower. The driving force under

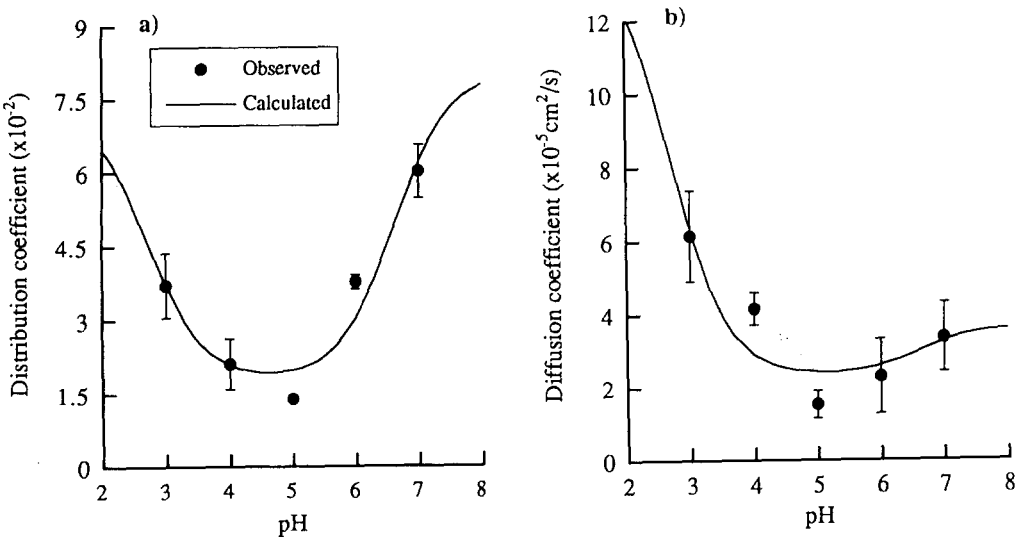

Fig. 8. Distribution coefficient (a) and diffusion coefficient (b) of cephalexin at various pH values. Each value represents the mean ± S.E. of 3–8 experiments.

iontophoresis is not only the concentration gradient but also the electrical potential difference. On the other hand, the concentration gradient was the main driving force for the passive transport in this study, so that the permeability is affected strongly by the partition coefficient and diffusion coefficient of the permeant. Low water solubility and low partition coefficient of zwitterion has been reported for cephalexin and other β-lactam antibiotics (Purich et al., 1973; Tsuji et al., 1977; Irwin et al., 1988), which is in agreement with our data. This seems due to the reflection of greater crystal lattice energy than heat of fusion in water and of less solvation tendency against the dipolar ion of lipophilic media than that of water. If cephalexin ions pass through skin via the lipophilic domain, the permeability of zwitterion would be lowered because of its low lipophilicity. The ammonium and carboxylic ions in a cephalexin molecule can be hydrated in aqueous solution, so that the zwitterion may be larger that cation and anion and have low diffusivity in aqueous medium (Kiso, 1972). If the skin permeation pathway of cephalexin is in the aqueous domain, the low diffusivity of zwitterion would make the permeability low. Actually, the permeability coefficients of cation and anion were almost the same as those of D-mannitol, which is considered to be transported via pores in skin (Peck et al., 1994). Then, the main permeation pathway of cephalexin may be in the aqueous domain.

The present study has demonstrated that the pH-dependent skin permeation of cephalexin may reflect the permselective property of skin due to the different lipophilicity and/or diffusivity of each ionic species. However, because cephalexin is not the only model zwitterionic drug, no definitive conclusion can be reached at this time. Further studies are required to determine in detail the skin permeation behavior of zwitterionic drugs.

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