Calculation of Skin Permeability Coefficient for Ionized and Unionized Species of Indomethacin

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The contribution of ionized and unionized species to the overall permeation of weak electrolytes through the skin was investigated to determine the effect of pH in the vehicle on the permeability of indomethacin (IDM), as a model drug, through hairless rat skin. The permeability of IDM through polydimethylsiloxane (silicone) and poly(2-hydroxyethyl methacrylate) (pHEMA) membranes which may reflect lipid and aqueous pathway, respectively, was also measured for comparison. As the pH in the vehicle increased, there was an exponential increase in the skin permeation rate of IDM. The permeation rate of IDM through the silicone membrane was constant independent of pH, whereas that through the pHEMA membrane increased with increasing pH, similar to the skin permeation. The permeability coefficients of ionized and unionized species through the skin estimated using the skin permeation rates and solubilities of IDM at various pHs were 1.50×10^{-7} and 2.79×10^{-5} cm/s, respectively. These results indicated that the permeation of ionized species greatly contributed to the total permeation of IDM at higher pH, and that the total permeation rate of IDM was determined by the permeation of unionized species at lower pH. These contributions depend on the pH and pK_a values and the ratio of permeability coefficient of each species. It was also confirmed that the skin has at least two kinds of permeation pathways and these two species permeate through a different pathway.

Keywords indomethacin; weak electrolyte; permeability coefficient; permeation pathway; hairless rat skin

Primary resistance to percutaneous absorption is in the stratum corneum, which is morphologically and physiologically different from the epithelium of intestinal membranes. The skin has been treated as a homogeneous lipoidal membrane in the study of fundamental mechanisms and kinetics for percutaneous absorption. Therefore, the results of skin permeation experiments often conflict with the theoretical predictions. We have suggested the existence of lipid and aqueous pathways in the stratum corneum.[1] The existence of these parallel pathways has been recognized in terms of both the skin permeation properties of drugs[2,3] and skin (stratum corneum) morphology.[4,5] The permeation properties of ionized and unionized species are probably different from each other, and hence, the contribution of each species to the two permeation pathways may be different.

The objective of the present study was to clarify the skin permeation properties of a model weak electrolyte, indomethacin (IDM). In this paper, the effect of pH in the vehicle (donor solvent) on the permeability of IDM through the hairless rat skin was measured and the contribution of each species to the steady-state skin permeation rate was estimated by determining the permeability coefficient of each species. In addition, the effect of pH on the membrane permeation rate of two typical artificial membranes, polydimethylsiloxane (silicone) and poly(2-hydroxyethyl methacrylate) (pHEMA) membranes,[1] was compared with that on skin permeation to confirm the permeation mechanism of weak electrolytes.

Materials and Methods

Materials IDM and isosorbide dinitrate (ISDN) were supplied by Toko Pharmaceutical Co. (Tokyo, Japan). ^{14}C-IDM (specific activity 740 MBq—1.48 GBq) was purchased from Dupont-New England Nuclear (Boston, MA, U.S.A.). Other chemicals and solvents were of reagent grade and obtained commercially.

Animals Male hairless rats (WBN/ILA-Ht strain) weighing about 150—170 g (6—7 weeks old), supplied by Saitama Laboratory Animals (Saitama, Japan) were used in all animal experiments.

Membrane Preparation For the skin permeation studies, a 5.0 cm^2 section of full-thickness abdominal skin of hairless rat was excised before the skin permeation experiment.

A silicone rubber and pHEMA membrane were used as artificial membranes. A polydimethylsiloxane sheeting (Silastic®, 0.005 inch thickness, Dow Corning, Midland, MI, U.S.A.) was used as a silicone membrane, and the pHEMA membrane used (0.05 cm thickness) was synthesized by a radical polymerization of 2-hydroxyethyl methacrylate. Detailed methods were shown previously.[1]

Permeation Study The pH control of a series of phosphate buffer was accomplished by mixing appropriate ratios of 1/30 M solution of H_3PO_4 : NaH_2PO_4 (pH 2—4), or NaH_2PO_4 : Na_2HPO_4 (pH 4—7.4).

A diffusion cell consisting of two half cells was used in this study. Membrane was mounted between the two half cells, each having 2.5 ml of volume and 0.95 cm^2 of effective diffusion area. The receiver compartment was filled with 2.5 ml of pH 7.4 phosphate buffer and the donor compartment was filled with 2.5 ml of drug suspension (IDM and ISDN) or solution (KCl, 3.35 mM). The concentrations of IDM and ISDN were about double that of each solubility. The permeation studies were conducted at 37 °C. At appropriate times, samples were withdrawn from the receiver compartment for analysis. The same volume of fresh buffer was added to the receiver compartment to keep the volume constant. The pH value in the donor cell was almost constant throughout the experiments. For the permeation study using ^{14}C-IDM, the donor compartment was filled with 2.5 ml of pH 2, 4 and 6 phosphate buffer containing 15 μl (11.1 kBq) of ^{14}C-IDM ethanol solution. After complete mixing (about 2 min later), 10 μl of sample was withdrawn from the donor compartment to determine initial concentration of the drug. At appropriate times, 10 μl and 1 ml of samples were withdrawn from the donor and receiver compartments, respectively. These samples were transferred to a vial with 10 ml of scintillation cocktail (composition : 4 g 2,5-diphenyloxazole, 0.4 g 1,4-bis-2-(5-phenyl-2-oxazolyl)benzene, 1.0 l toluene, 0.333 l Triton X-100). Calculation of permeability coefficient was done by the method previously reported.[1]

Determination of Solubility Excess IDM was added to 1/30 M phosphate buffer solution (pH 2—7) in a screw-capped vial and stirred at 37 °C in a water bath. After equilibration, a part of the suspension was withdrawn and quickly filtered through a cellulose acetate membrane (0.2 μm pore size, Advantec Toyo, Tokyo). After measuring their pH, the filtrates were adequately diluted with 1/30 M phosphate buffer solution (pH 7.4) to determine the solubility.

Analysis The concentrations of IDM and ISDN were analyzed by a HPLC system equipped with a pump (LC-6A, Shimadzu, Kyoto), an ultraviolet spectrophotometric detector (SPD-6A, Shimadzu), a 4.6 mm × 250 mm stainless steel column packed with Nucleosil 5C_{18} (Macherey Nagel, Germany) and an integrator (C-R6A, Shimadzu). Details were given previously.[1] Radioactivities were assayed on an Aloka scintillation counter (LSC 700, Tokyo). The concentration of potassium ion was determined using a clinical ion meter (CIM-104A, Shimadzu).

Calculation Method The permeability coefficients of ionized and unionized species of IDM were determined to assess the contribution of each species to the total skin permeation rate. The solubility of weak acidic

electrolytes such as IDM can be described by:

$$C_s = C_{HA} + C_{A^-} = C_{HA}(1 + 10^{pH - pK_a}) \quad (1)$$

where C_s, C_{HA} and C_{A^-} are the solubilities of total acid, ionized and unionized species, respectively. If the concentration of penetrant in the donor compartment is maintained and a sink condition in the receiver compartment can be achieved, the steady-state skin permeation rate per unit area of IDM, J, can be given as:

$$J = C_v \cdot P \quad (2)$$

where C_v is the concentration of penetrant in a vehicle (donor solution) and P is the permeability coefficient of the penetrant. Assuming that each permeability coefficient for ionized or unionized species is constant irregardless of pH, the total permeation rate of the drug, J_{total} is the sum of the permeation rate of each species:

$$J_{total} = P_{HA} C_{HA} + P_{A^-} C_{A^-} \quad (3)$$

where P_{HA} and P_{A^-} are the permeability coefficients of the ionized and unionized species, respectively.

Equations 2 and 3 can be combined to give:

$$J_{total} = C_{HA}(P_{HA} + P_{A^-} 10^{pH - pK_a}) \quad (4)$$

With the observed solubilities and steady-state permeation rates of IDM as a function of pH, the values of pK_a, C_{HA}, P_{HA} and P_{A^-} can be estimated by curve fitting using Eqs. 1 and 4. In this study, these 4 parameters were estimated by a non-linear least squares program (MULTI).[7]

Results and Discussion

Figure 1 shows the time course of the cumulative amount of IDM permeated through the hairless rat skin from its suspension at various pH values. The amount of IDM linearly increased after a short lag time at any pH; however, the steady-state skin permeation rate varied with pH. As the pH increased, an exponential increase in the skin permeation rate was observed (Fig. 2). Generally, the thermodynamic activity of a drug in suspension is equal to that of the solid drug and independent of vehicles.[8] In addition, on the assumption that the permeation resistance in the skin is not altered by a vehicle, the diffusion and activity coefficients of a drug in the membrane and the membrane thickness would be constant. Therefore, it is expected that the steady-state permeation rate of IDM at various pHs should be identical. However, the result cannot be explained by this theory alone. Figure 3 displays the solubility of IDM in phosphate buffer solution at a specific pH. The solubility of IDM also exponentially increased with increasing pH. IDM has a pK_a of 4.5 (at 25 °C)[9] and the ionized species were dominant in the solubility of IDM at the pH above the pK_a value. Thus, it is suggested that the ionized form of IDM is permeable through the skin.

The contribution of ionized and unionized forms of IDM to the total skin permeation was then estimated by the obtained results. The permeability coefficient of each species was used as a parameter of skin permeation, because the apparent permeation rate was difficult to adopt to the theoretical consideration using Eqs. 3 and 4. The calculated values of pK_a, C_{HA}, P_{HA} and P_{A^-} by the curve fittings of the solubility (Fig. 3) and permeation rate (Fig. 2) of IDM to Eqs. 2 and 4 are shown in Table I. Considering the pK_a at 25 °C (4.5), the calculated value at 37 °C (4.12) is acceptable. The C_{HA} value almost corresponds to the solubility at pH 2, where IDM exists as an unionized species. The permeability coefficient of unionized species (2.79×10^{-5} cm/s) was about 200 times higher than that of ionized form (1.5×10^{-7} cm/s). The values of P_{HA} and P_{A^-} obtained

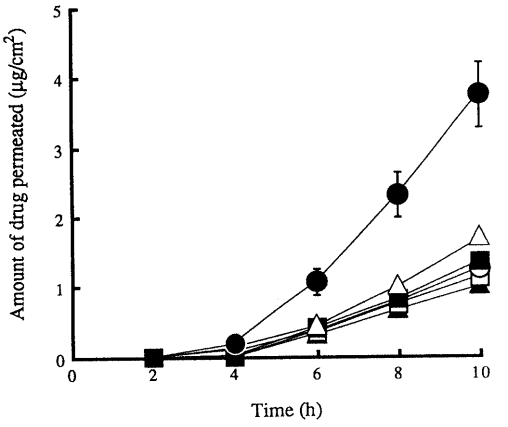

Fig. 1. Permeation Profiles of IDM through the Excised Hairless Rat Skin

□, pH 1.85; ▲, pH 2.90; ○, pH 4.23; ■, pH 5.25; △, pH 6.10; ●, pH 6.8. Each point represents the mean ± S.E. of 3—6 experiments.

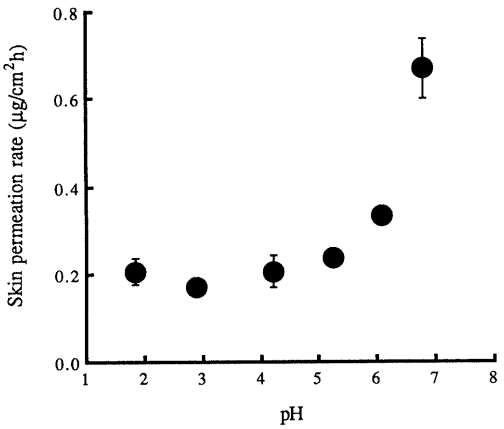

Fig. 2. Relationship between the Skin Permeation Rate of IDM and Donor pH

Each point represents the mean ± S.E. of 3—6 experiments.

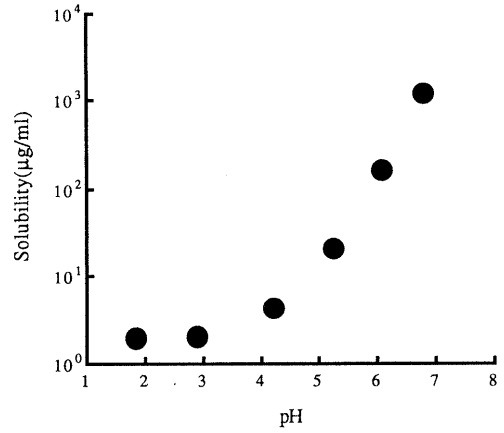

Fig. 3. Solubility of IDM at Several pHs

Each point represents the mean of 3 measurements. Each circle contains the S.E.

TABLE I. pK_a of IDM, Solubility of Its Unionized Form and Permeability Coefficient of Its Unionized and Ionized Form Obtained by Curve Fitting

pK_a	4.12
C_{HA} (μg/ml)	1.95
P_{HA} ($\times 10^{-5}$ cm/s)	2.79
P_{A^-} ($\times 10^{-7}$ cm/s)	1.50

here can be used to estimate the contribution of each species to the permeation rate of IDM. In weak acidic drugs, the effect of permeation of ionized species on the total drug permeability is small for the acidic pH range, but increases for the alkalic pH range due to the predominant concentration of ionized species. This contribution of ionized species depends on the pK_a value and the ratio of the two permeability coefficients (P_{HA}/P_{A^-}) as follows:

$$\frac{J_{A^-}}{J_{total}} = \frac{1}{\frac{C_{H^+}}{K_a} \cdot \frac{P_{HA}}{P_{A^-}} + 1} \quad (5)$$

As the pH value increases beyond the sum of pK_a and logarithm of P_{HA}/P_{A^-} value($pH > pK_a + \log(P_{A^-}/P_{HA})$), the contribution of ionized species to the total permeation rate of drugs is more than 50%. In IDM, this value is about 6.4, therefore, the permeation rate of IDM is expected to be double, because the rate is governed by the permeation of unionized species having larger permeability coefficient. The experimental results shown in Fig. 2 confirm this theory. In small P_{HA}/P_{A^-} and small pK_a values of the drug, the drug permeation could also be affected by the permeation of ionized species.

To confirm the mechanism for the permeation of weak electrolytes, the effects of pH on the permeability of IDM through typical artificial membranes of silicone and pHEMA were investigated. Those membranes can be treated as a solution-diffusion and a porous membrane, respectively.[1,10] The steady-state permeation rates through the silicone and pHEMA membranes *vs.* pH profiles are shown in Fig. 4. The permeation rate through the silicone membrane was almost constant at any pH. For the pHEMA membrane, in contrast, the permeation rate of IDM increased with increasing pH similar to that of skin. Some researchers have indicated more than two permeation pathways in the stratum corneum.[11,12] We previously reported that the permeation pathway for lipophilic drugs was different from that for hydrophilic drugs: the former was the lipid pathway, while the latter was the aqueous pathway.[1] If the skin can be treated as a lipoidal homogeneous membrane, the skin permeation behavior of IDM should be similar to the silicone membrane permeation. However, the skin permeation profile was totally different from the silicone membrane permeation. The permeation rate of IDM through the pHEMA membrane depended on the pH as shown in Fig. 4. As pH increased, the solubility of IDM also increased. The increase in solubility is the main reason for the increase of pHEMA membrane permeability.

It was also confirmed by the results with both artificial membranes that the skin is not a homogeneous membrane and has at least two permeation pathways, and that these lipid and aqueous pathways serve, respectively, as main pathways for unionized species and ionized species in the overall permeation of IDM.

The same permeation experiment was carried out at a trace level of IDM, and the permeability coefficient of IDM was compared with that from suspension at various pHs (Table II). The permeability coefficient from the dilute solution was slightly lower than that from suspension. Although IDM may have some penetration enhancing effect

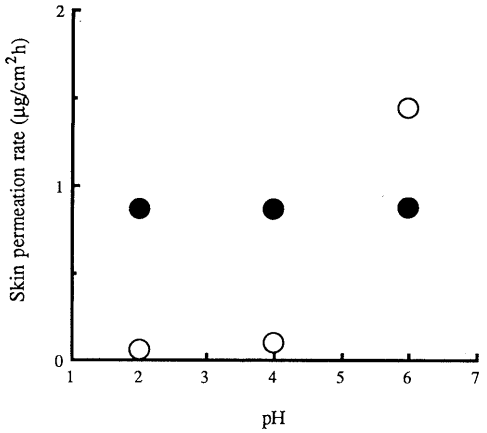

Fig. 4. Effect of pH on the Permeation Rate of IDM through the Silicone and pHEMA Membrane

●, silicone membrane; ○, pHEMA membrane. Each value represents the mean of 3—4 experiments. Each symbol contains the S.E.

TABLE II. Comparison of Permeability Coefficient of IDM from Suspension and Dilute Solution

pH	Permeability coefficient ($\times 10^{-5}$ cm/s)	
	Suspension	Dilute solution
2	3.03±0.459	2.03±0.408
4	1.41±0.261	0.735±0.185
6	0.0588±0.00277	0.0427±0.00497

Each value represents the mean±S.E. of 3—6 experiments.

like salicylic acid which can be used as a kelatolytic,[13] this difference in permeability was small enough to be neglected.

Since the permeation study was performed under different pH conditions, compensation was necessary for the skin barrier function against acidic and alkaline pHs. We selected potassium ion, which is expected to permeate mainly through the aqueous pathway,[14] and ISDN, which is expected to transport through the lipid pathway,[1,10] as model compounds for this objective. The permeation rate of ISDN was almost the same value at any pHs (15.4±1.4, 14.6±1.8 and 15.5±1.4 μg/cm²/h for pH 2, 4 and 6, respectively). In contrast, the permeation rate of potassium ions (61.7±12.1 μg/cm²/h) at pH 6 was about 1.6 times higher than that (37.6±6.1 μg/cm²/h) at pH 2, suggesting that the aqueous pathway was slightly affected by the buffer pH.

As previously reported,[1] hydrophilic drugs have almost constant permeability coefficient (8.33×10^{-8} cm/s). This is supported by the permeability coefficients of ionized species of IDM (1.5×10^{-7} cm/s) and potassium ion (1.28×10^{-7} cm/s at pH6). In contrast, the permeability coefficients of lipophilic drugs, which can diffuse *via* the lipid pathway, can be viewed as a function of their partition coefficients. Thus, the contribution from ionized species may be determined by the partition coefficient of unionized species. Hence, the contribution of ionized species can be predicted at each pH formulation.

This study indicates the importance of determining the permeability coefficient of ionized and unionized species of weak electrolytes. It is important to understand the

contribution of each species to the total drug permeation, especially when a chemical and/or physical enhancing system is used in the formulations.

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