Keratinized Epithelial Transport of β-Blocking Agents. I. Relationship between Physicochemical Properties of Drugs and the Flux across Rat Skin and Hamster Cheek Pouch

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The maximum fluxes (J_{max}) of β-blockers through keratinized membranes were determined *in vitro* and compared with their physicochemical parameters such as lipophilicity ($\log k'_0$) and melting point (mp). Rat abdominal skin and hamster cheek pouch mucosa were used as the model membranes. Propranolol, metoprolol, timolol, pindolol, nadolol and agenolol were used as β-blockers with a variety of physicochemical characters. Linear relations of J_{max} with either $\log k'_0$ or mp were observed both in intact rat skin and in intact hamser cheek pouch, suggesting that the lipophilicity and thermodynamic activity of a drug in the crystal state primarily affect the drug's permeation through these membranes. However, the slope, $dJ_{max}/d(\log k'_0)$, for cheek pouch mucosa was greater than that for rat skin, corresponding to the lack of appendageal shunt pathways in cheek pouch. Penetration studies using the delipidized membranes and the isolated stratum corneum sheet of hamster cheek pouch mocosa clarified that the primary rate-limiting barrier function might exist in the lipid layer of the stratum corneum. J_{max} values for the tape-stripped and delipidized skins correlated with both the solubilities of drugs in the vehicle and with the mp, suggesting the polar porous characteristics of both model membranes. However, a theoretical approach confirmed that the contribution of an intracellular or aqueous pore route in the intact membrane to the permeation of drugs with positive lipophilic indexes is negligible.

Keywords β-blocker; percutaneous absorption; physicochemical property; intercellular lipid; rat skin; hamster cheek pouch; isolated stratum corneum sheet

Introduction

The transdermal or transmucosal application of drugs is an alternative delivery route with some biopharmaceutical benefits, such as bypassing hepatic first-pass elimination and improving compliance. But drugs are not always permeable enough to reach therapeutic plasma levels in these application sites. The stratum corneum layer is known to be a major barrier to drug permeation in the keratinized epithelia. Drug permeation through biological layers, including the stratum corneum layer, might be subject to physicochemical properties of the drug such as lipophilicity, pK_a, molecular weight and the melting point.

A positive relationship between the partition coefficients and drug permeation through the skin or the mucosa has been reported for a variety of drugs such as β-adrenergic blockers,[1-3] steroids,[4,5] *n*-alkyl-*p*-aminobenzoate esters,[6] progesterone[7] and aliphatic alcohols.[8,9] Some favorable physicochemical properties, including adequate lipophilicity, should be required in designing or in developing a transdermal or a transmucosal therapeutic system.

The purposes of this study are to clarify the relationships between the physicochemical parameters and the *in vitro* permeation characteristics of six β-adrenergic blockers, propranolol, metoprolol, timolol, pindolol, nadolol and atenolol, using rat abdominal skin and hamster cheek pouch mucosa as models of the keratinized epithelia, and to discuss the differences in drug permeation mechanisms between the skin and the keratinized oral mucosa.

Materials and Methods

Materials Propranolol hydrochloride was purchased from Nacalai Tesque Co. (Kyoto). Metoprolol tartrate and pindolol were obtained from Sigma Chemical Co. (St. Louis, MO). Timolol maleate, nadolol and atenolol were kindly supplied by Banyu Pharmaceutical Co. (Tokyo), Dainippon Pharmaceutical Co. (Osaka) and I.C.I. Pharma Co. (Osaka), respectively. Other chemicals, obtained commercially, were of a reagent grade. All these materials were used without further purification.

Isolation of Rat Abdominal Skin Shaved abdominal skin without the adipose tissue was carefully excised from male Wistar rats (200—250 g) as described previously.[10]

Isolation of Hamster Cheek Pouch The cheek pouch was excised from the male golden hamster (180—200 g) and rinsed with saline. The tissue was cleaned from extraneous tissue by careful dissection.

Preparation of Isolated Stratum Corneum Sheet of Hamster Cheek Pouch The stratum corneum sheet was isolated from the cheek pouch tissue using the trypsin-treatment method reported previously.[11]

Preparation of Stripped Skin Membrane The stratum corneum layer of the shaved abdominal skin of anesthetized rat was stripped with cellophane tape (30 times) prior to the excision of the tissue. The adipose tissue was removed in the same manner used for the intact skin.

Preparation of Delipidized Membrane The delipidization of membranes was carried out according to the methods of Scheuplein *et al.*[12] Either the rat abdominal skin or the hamster cheek pouch was mounted in a Franz-type diffusion cell (see the drug permeation experiments) with the stratum corneum side up. The level of the receiver solution (pH 7.4) was kept about 10 mm below the membrane so as not to bathe the lamina propria. This procedure enables the membrane to maintain moderate hydration. A mixture of chloroform–methanol (2 : 1 by volume) was poured into the donor compartment to expose the stratum corneum. After 1 h of treatment, the organic solvent was removed completely. The drug permeation study was then started immediately.

Drug Permeation Experiments A Franz-type diffusion cell having an available diffusion area of 3.14 cm^2 was employed.[10] The receptor compartment was filled with 18 ml of an isotonic phosphate buffer solution (pH 7.4). The tissue preparation was mounted in the cell with the stratum corneum facing upwards. In the case of the isolated stratum corneum sheet of the cheek pouch, a glass filter (GC 50, Advantec Toyo Co., Tokyo) was clamped under the stratum corneum sheet as a support. Drug permeation through the glass filter is fast enough not to interfere with the permeability of the stratum corneum sheet. Drug solutions were prepared with isotonic buffer solutions (pH 3.0, 7.4 and 9.0). In the case of pindolol, the drug concentrations were 2 and 0.2 mM at pH 7.4 and 9.0, respectively, because of pindolol's limited solubility. The other drug solutions were prepared to 5 mM. Two ml of each drug solution was applied to the donor compartment and the donor chamber was closed from environmental influences. The diffusion cell was thermoregulated with a water jacket at 37 °C and the receiver compartment was stirred continuously at 600 rpm with a magnetic stirrer. At appropriate times, an aliquot of the receiver fluid (0.2 ml) was withdrawn and the same volume of the fresh buffer solution was applied to the receiver compartment. Sample solution was filtered through a 0.45 μm pore-size filter (Nihon Millipore Kogyo, Yonezawa) for the analysis.

Analytical Methods Drug concentration was determined by high performance liquid chromatography (HPLC). A HPLC pump (LC-5A, Shimadzu, Kyoto) was equipped with a UV detector (SPD-2A, Shimadzu) or a fluorescence detector (RF-530, Shimadzu). The system was used in a

reversed phase with an Inertsil ODS column (4.6 mm i.d. × 150 mm, GL Sciences Inc., Tokyo). A mixture of methanol and 10% acetic acid was used as the mobile phase at a flow rate of 1.0 ml/min. The analytical conditions are listed in Table I.

Calculation of Drug Maximum Flux (J_{max}) The cumulative amount of a drug which permeated the membrane, Q, was plotted against the time of sampling,[11] and the permeation rate of the drug, dQ/dt, was estimated from the slope of the steady-state linear portion. Assuming that the drug permeation mechanism is a passive diffusion and that the receiver compartment is kept in a sink condition, the permeation rate can be expressed as the following equation:

$$dQ/dt = K_m \cdot D \cdot C_v/h = K_p \cdot C_v \quad (1)$$

where K_m, D and C_v are the partition coefficient of the drug to the membrane, the diffusion coefficient of the drug molecule in the membrane and the drug concentration in the vehicle, respectively, and h and K_p are the membrane thickness and the apparent permeability coefficient of the drug, respectively.

The maximum flux (J_{max}; observed flux/thermodynamic activity) has been used to describe the intrinsic permeability of the membrane to a specific penetrant.[13] Since thermodynamic activity can be approximated by the ratio of the drug concentration to its saturated solubility in the vehicle,[14] J_{max} can be calculated by the following equation.

$$J_{max} = K_p \cdot C_v \cdot C_s/C_v = K_p \cdot C_s \quad (2)$$

where C_s is the drug solubility in the vehicle. In the present study, the ability of a drug to permeate through the membrane can be discussed by using the J_{max} value as its intrinsic parameter. In the case of metoprolol, the solubility in the vehicle was more than 2 M under each pH condition. J_{max} for metoprolol was calculated using the C_s value as 2 M.

Measurement of Lipophilic Index The lipophilic index ($\log k'_0$), a parameter of the lipophilicity of a drug, was determined by reversed-phase HPLC measurement according to the method of Yamana *et al.*[15] The mobile phases used were mixtures of methanol and aqueous buffer solutions (1/10 isotonic; pH 3.0, 7.4 and 9.0). Methanol concentration of the mobile phase was varied to obtain a peak with adequate retention time for each drug. When $\log k'$, defined as follows:

$$\log k' = \log((t_R - t_0)/t_0) \quad (3)$$

where t_R and t_0 are the retention times of a drug and an unretained substance, respectively, was plotted against the methanol concentration, the extrapolation of the resulting linear relationship ($r < -0.999$) to 0% methanol gives a lipophilic index, $\log k'_0$. Formamide was used as the unretained substance.

Measurement of Solubility An excess amount of each drug was added to an aliquot of an isotonic buffer solution (pH 3.0, 7.4 or 9.0) and was agitated at 37 °C for 12 h in a shaking water bath. The resulting suspension was centrifuged and the supernatant was filtered through a 0.45 μm pore-size filter. The diluted filtrate was applied to HPLC to determine the drug concentration.

Measurement of Melting Point The salt-form drugs, propranolol, metoprolol and timolol were dissolved in an alkaline solution and extracted with chloroform in order to obtain the free drug bases. After drying, the crystals of a drug were transferred to a glass capillary and the melting point was measured according to JP XI. For the other drugs, pindolol, nadolol and atenolol, the melting points were obtained from the reference (Merck Index X).

Determination of Tissue Partition Coefficient A 0.5 ml of a buffer solution (pH 7.4) containing an adequate amount of a drug and accurately weighed rat skin (or hamster cheek pouch) tissue were placed in a glass tube. The tube was covered with Parafilm® and placed in a water bath at 37 °C for 12 h with gentle agitation. The hydrated skin or the cheek pouch tissue was then removed and the drug concentration in the bathing solution after the equilibrium was then measured by HPLC. The partition coefficient (K_m) was calculated by the following equation.

$$K_m = (C_0 - C') \cdot W_s/C' \cdot W_m \quad (4)$$

where C_0 and C' are the drug concentration of the bathing solution before and after the incubation, respectively. W_s and W_m are the weight (g) of the drug solution and tissue, respectively. In the case of atenolol, since the concentration change of the bathing solution was too small for exact determination, the partition coefficient was calculated by the direct measurement of drug amounts in the tissue after the extraction.

TABLE I. HPLC Conditions for Drug Concentration Measurements

Drug	UV wavelength (nm)	Fluorescence wavelength		Mobile phase MeOH : 10% AcOH
		Ex (nm)	Em (nm)	
Propranolol	290	295	340	45 : 55[a]
Metoprolol	273	272	308	30 : 70
Timolol	290	—	—	30 : 70
Pindolol	263	250	302	20 : 80
Nadolol	269	269	303	20 : 80
Atenolol	274	270	305	3 : 97

a) By volume.

Results and Discussion

Physicochemical Properties of Drugs The physicochemical properties of six β-adrenergic blockers, molecular weight (Mw), pK_a,[2,16] melting point (mp), lipophilic index and solubility are summarized in Table II. These six drugs are similar with respect to pK_a and molecular weight, but differ in the lipophilicity and the melting point of their free base form. In order to clarify the factors affecting the skin or the mucosal permeability to drugs, the correlation of the permeation rate with these two different properties, the lipophilicity and the melting point, was investigated in this study.

Relationship between Maximum Flux and Lipophilicity The permeability of rat abdominal skin and hamster cheek pouch to six different β-adrenergic blockers was investigated. Figure 1 shows a relationship between drug lipophilicity and the J_{max} at pH 7.4 and 9.0. Drug flux at pH 3.0 was too small to evaluate J_{max}. J_{max} values seemed to depend on the drug lipophilicity both in the skin and in the cheek pouch. As shown in Fig. 1, the slope of the regression line for the cheek pouch was greater than that for the skin. This might be attributed to histological differences such as membrane lipophilicity and the existence

TABLE II. List of Physicochemical Characteristics of β-Adrenergic Antagonists Used in This Study

Drug	Mw	mp (°C)	pK_a	Lipophilic index			Solubility (mM)		
				pH 3.0	pH 7.4	pH 9.0	pH 3.0	pH 7.4	pH 9.0
Propranolol	259.3	96	9.23[a]	3.135	3.788	3.851	484.2	454.6	314.5
Metoprolol	267.4	35	9.24[a]	1.911	2.651	3.207	2000<	2000<	2000<
Timolol	316.4	72	9.21[a]	1.702	2.225	2.926	199.6	252.3	326.4
Pindolol	248.3	172	8.80[b]	1.394	1.620	2.312	329.0	21.2	1.1
Nadolol	309.4	130	9.39[a]	1.293	1.326	2.368	372.3	48.9	72.5
Atenolol	266.3	147	9.32[a]	0.304	0.645	1.817	455.1	84.2	115.9

a) From ref. 2. b) From ref. 16.

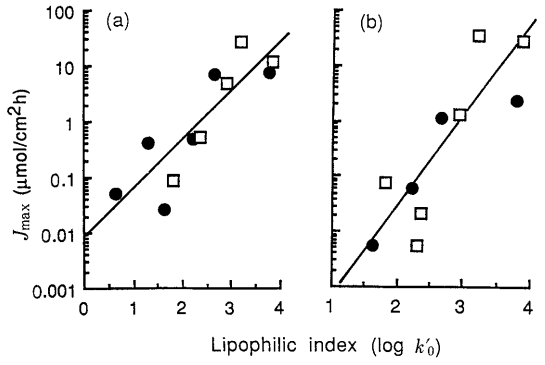

Fig. 1. Relationship between Lipophilicities and Maximum Flux Values (J_{max}) through Rat Skin (a) and Hamster Cheek Pouch (b)

●, pH 7.4; □, pH 9.0. Each J_{max} value represents the mean of 6 experiments. (a) $r=0.867$; (b) $r=0.870$.

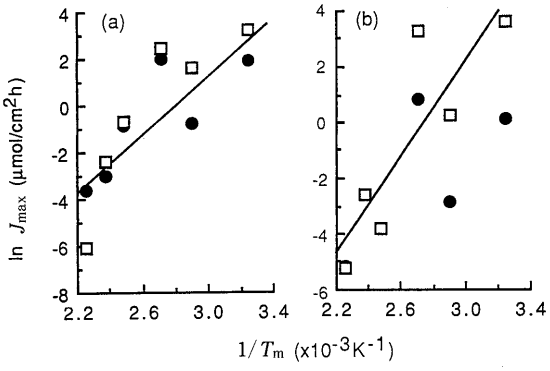

Fig. 3. Relationship between Drug Melting Points and Maximum Flux (J_{max}) through Rat Skin (a) and Hamster Cheek Pouch (b)

●, pH 7.4; □, pH 9.0. Each value of the melting point and the maximum flux represents the mean of 2 and 6 experiments, respectively. (a) $r=0.832$; (b) $r=0.738$.

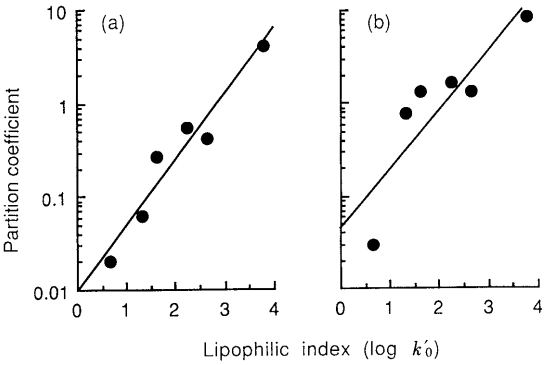

Fig. 2. Relationship between Drug Lipophilicities and Actual Partition Coefficients from Vehicle to Rat Skin (a) and Hamster Cheek Pouch (b) at pH 7.4

Each value represents the mean of 3 experiments. (a) $r=0.966$; (b) $r=0.870$.

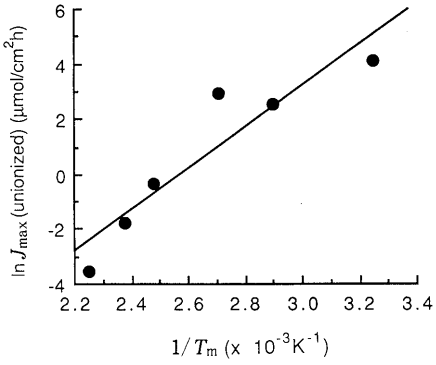

Fig. 4. Relationship between Maximum Flux Values (J_{max}) of Unionized Drugs through Rat Skin and Melting Points

J_{max} values were calculated from the permeation rates at pH 7.4 and 9.0 using each pK_a value. Each value of the melting point and the maximum flux represents the mean of 2 and 6 experiments, respectively. $r=0.930$.

of a pore route. However, high lipophilic drugs had almost the same J_{max} value in each membrane, whereas low lipophilic drugs had a relatively high J_{max} value only in rat skin. In Fig. 1a, the intercept on the ordinate axis tended to deviate upward. This suggests the existence of a different permeation route for drugs having low lipophilic indexes in the skin. The increment of J_{max} in drugs with higher lipophilicity could be accounted for by the increments of both K_m and D. The actual partition coefficients of drugs to the membranes were verified by the partition experiments. Figure 2 shows the relationships between the lipophilic index and the partition coefficient for the skin and the cheek pouch. The partition coefficient to the membrane showed a linear correlation with drug lipophilicity, suggesting that drug permeation through these biological membranes is governed mainly by the process of drug partition to the membranes.

Relationship between Maximum Flux and Thermodynamic Activity In general, the rate of drug permeation through the membrane is in proportion to the thermodynamic activity of the drug in the vehicle, as shown in Eq. 5.[17]

$$dQ/dt=(a_v \cdot D)/(\gamma_m \cdot h) \quad (5)$$

where a_v and γ_m are the drug activity in the vehicle and the activity coefficient in the membrane, respectively. This thermodynamic activity is also proportional to the drug concentration. On the saturated vehicle solution, the higher the drug thermodynamic activity in the solid state, the greater the drug permeation. The solid state thermodynamic activity of a drug relates with the melting point as follows[18]:

$$\ln a=\Delta H(1/T_m-1/T)/\boldsymbol{R} \quad (6)$$

where a, ΔH and T_m are the activity in the solid state, the mole heat of fusion and the melting point of a drug, respectively, and $\boldsymbol{R}$ is the gas constant. Thus, the relationship between the melting point and the permeation rate of drugs was examined. As shown in Fig. 3, linear correlations between the inverse of melting points and the permeation rates were confirmed in both membranes. This supports the relevancy of the thermodynamic activity of a drug in the solid state and the maximum flux. Further, the relationship between the permeation rates of the free base form of drug molecules (J_{max} (unionized)) and the melting points was investigated. J_{max} (unionized) was calculated by the following equation:

$$J_{max}=f \cdot J_{max}\,(\text{unionized})+(1-f) \cdot J_{max}\,(\text{ion}) \quad (7)$$

where f is the fraction of an unionized molecule, and J_{max} (unionized) and J_{max} (ion) are the J_{max} for unionized and ionized molecules, respectively. Figure 4 shows a good correlation of the permeation rate of the free base in rat skin with the melting point. This result suggests that the permeation of the free base form of a drug molecule is well controlled by its thermodynamic activity.

pH-Partition and Transappendageal Route It is well known that drug absorption through biological membranes follows the pH-partition hypothesis.[19] For instance, the absorption of drugs from the oral cavity is governed by the pH-partition hypothesis, which is well explained by the passive diffusion mechanism.[1,11,20-23] The effect of pH on a drug's permeation rate through membranes was investigated. As shown in Figs. 5 and 6, lowering the pH of a drug solution resulted in poor permeation through the skin (Fig. 5) and the cheek pouch (Fig. 6) because of the increase in ionic form molecules. These results suggest that the drug permeations through rat skin and hamster cheek pouch follow the pH-partition hypothesis. In addition, J_{max} values through the hamster cheek pouch for poorly lipophilic drugs or under highly ionized conditions were smaller than those through rat skin. There are morphological differences between hamster cheek pouch mucosa and rat skin; for example, the existence of hair follicles and sweat ducts in the skin. In rat skin, poorly lipophilic drugs or ionized drugs permeate through these transappendageal routes to a certain extent, while the lack of transappendageal routes in the cheek pouch obliges those drugs to permeate through the stratum corneum layer, which has a much higher barrier function.[11,24] In *in vitro* permeation measurement, these transappendageal routes such as hair follicles in rat skin might be highly hydrated and thus, the drug permeation through transappendageal routes might be overestimated. Further *in vivo* experiments may solve the details.

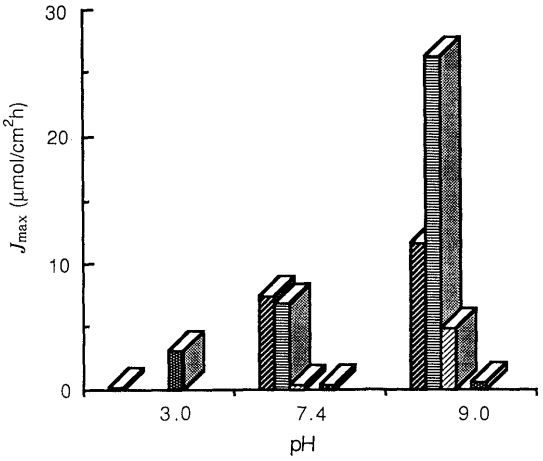

Fig. 5. Effect of pH of Vehicle Solution on Drug Maximum Flux Values (J_{max}) through Rat Skin

The vehicles used were isotonic buffer solutions of pH 3.0, 7.4 and 9.0. ▨, propranolol; ▤, metoprolol; ▨, timolol; □, pindolol; ■, nadolol; ■, atenolol.

Effect of Delipidization and Tape-Stripping The changes in the barrier properties of the stratum corneum following the delipidization and the tape-stripping were investigated. Figure 7 shows the effects of these treatments on drug permeation. The permeability enhancement indexes for the stripped skin (J_{max} in stripped skin/J_{max} in intact skin) were higher in drugs with low lipophilic indexes (Fig. 7a). This suggests that the contribution of the stratum corneum layer to maintaining the barrier function against the permeation of xenobiotics is much more pronounced for the hydrophilic drugs. Similar results were obtained in the delipidized membranes (Fig. 7b, c). Further, the drug permeability of delipidized skin was almost similar to that of stripped skin. These findings point out that the main barrier function of the stratum corneum to hydrophilic drugs exists in the intercellular lipids.

Many investigators have reported on the partition coefficients of drugs in relation to percutaneous absorption. Stoughton *et al.* investigated the effect of the partition coefficient on the absorption of nicotinic acid and its derivatives.[25] In general, percutaneous absorption of drugs with either high hydrophilicity or high lipophilicity is poor, while drugs having intermediate partition coefficients are absorbed rapidly. Tavakoli-Saberi and Audus reported of β-blockers that the permeability to a relatively lipophilic drug is attributed to the cellular retention of the drug, and that a sigmoidal relationship between lipophilicity and permeability could be observed.[26] But as shown in Fig. 1, the drug retention in the cells was not observed, even in propranolol, the most lipophilic drug used in the present study. Figure 8 shows the pH-dependency of J_{max} in stripped rat skin. J_{max} values of propranolol, metoprolol and timolol

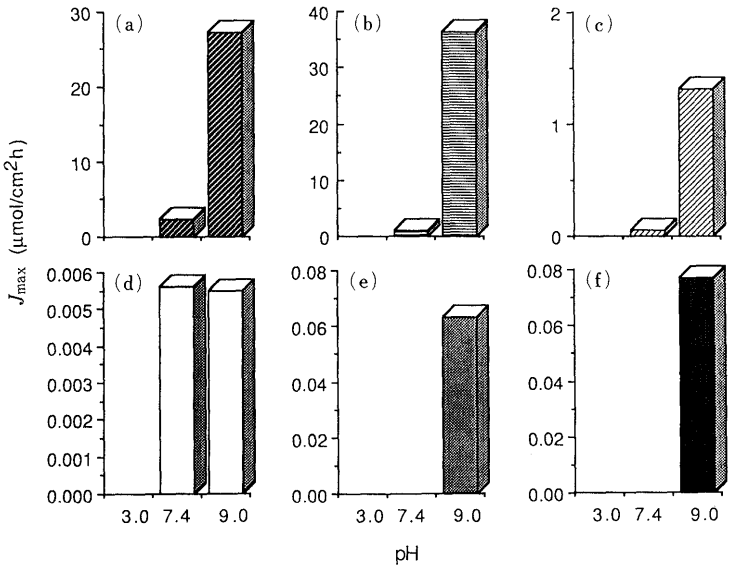

Fig. 6. Effect of pH of Vehicle Solution on Drug Maximum Flux Values (J_{max}) through Hamster Cheek Pouch

The vehicles used were isotonic buffer solutions of pH 3.0, 7.4 and 9.0. a, propranolol; b, metoprolol; c, timolol; d, pindolol; e, nadolol; f, atenolol.

through stripped skin depended on the fraction of the ionized form, but J_{max} of less lipophilic drugs, pindolol, nadolol and atenolol, inversely depended on their ionization. From these results, it is suggested that the epidermis underlying the stratum corneum has relatively high hydrophilic property, as well as lipophilic properties, and the diffusion through this hydrophilic layer in the drug permeation process should play a considerable role in regulating percutaneous permeation.

Permeation through Isolated Stratum Corneum Sheet The barrier function of the stratum corneum layer on drug permeation was investigated using the isolated stratum corneum sheets of hamster cheek pouch. Figure 9 shows the relationship between drug lipophilicity and J_{max} through the isolated stratum corneum sheet. J_{max} values determined in the isolated stratum corneum sheet are similar to those determined in intact (full-thickness) cheek pouch tissue (see Fig. 1). This result suggests that drug permeation through the hamster cheek pouch is also governed by the barrier function of the stratum corneum layer, similar to the case of rat skin. Thus, the relationship between J_{max} and either the lipophilicity or the melting point of a drug was clarified in both the skin and the keratinized mucosa. On the other hand, no significant relation was observed between the lipophilicity and J_{max} through either the delipidized membranes or the stripped skin.

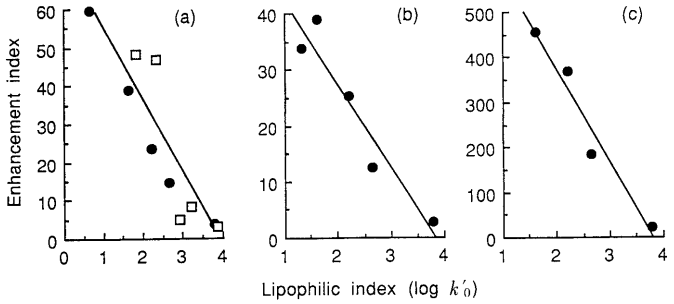

Fig. 7. Effects of Delipidization and Tape-Stripping of Stratum Corneum on Membrane Permeability

(a) rat stripped skin; (b) rat delipidized skin; (c) hamster delipidized cheek pouch. ●, pH 7.4; □, pH 9.0. Enhancement indexes were calculated from the ratio of maximum flux values with and without these treatments. The delipidization was carried out using chloroform–methanol (2 : 1 by volume), and the tape-stripping was carried out using a cellophane tape (30 times stripping). Each value represents the mean of 6 experiments.

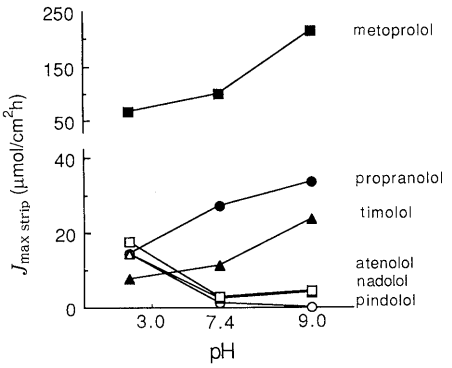

Fig. 8. Effect of pH of Vehicle Solution on Drug Maximum Flux Values (J_{max}) through Stripped Skin at pH 3.0, 7.4 and 9.0

Each value represents the mean of 6 experiments.

Effect of Solubility in a Vehicle To investigate how drug solubility in a vehicle contributes to permeation, the J_{max} values of a drug through stripped rat skin, delipidized skin and delipidized cheek pouch mucosa were plotted against the solubility in the vehicle (Fig. 10). As is evident from Fig. 10a, J_{max} values of relatively highly lipophilic drugs, propranolol, metoprolol and timolol, did not show any relation to their solubilities, but only to their lipophilicities. So, the J_{max} of such a drug could be explained by the drug partition to the skin. However, for other drugs, J_{max} values correlated well with their solubilities. Further, as shown in Figs. 10b, c, the J_{max} values of all drugs in delipidized tissues correlated well with the solubility of drugs in the vehicle. These results suggest that stripped or delipidized skin and delipidized cheek pouch share the physical property of polar porous membranes.

Theoretical Approach by Considering Aqueous Pore

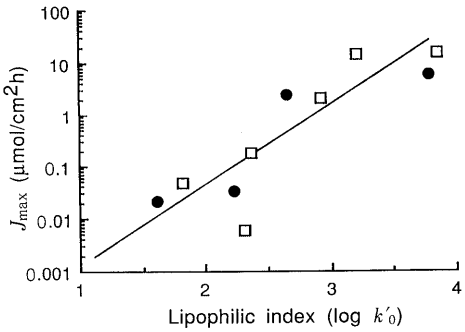

Fig. 9. Relationship between Drug Lipophilicities and Maximum Flux Values (J_{max}) through Isolated Stratum Corneum Sheet of Hamster Cheek Pouch

●, pH 7.4; □, pH 9.0. Each value of the J_{max} represents the mean of 6 experiments. $r=0.865$.

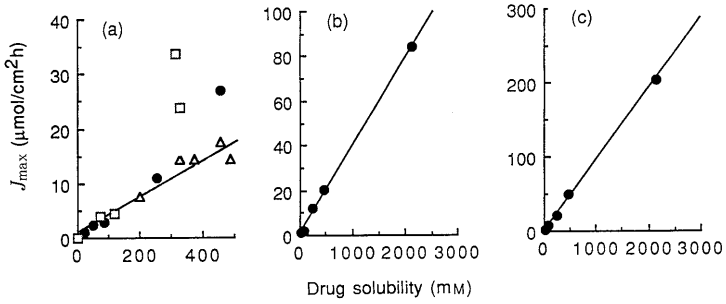

Fig. 10. Relationship between Drug Solubilities in Vehicles and Maximum Flux Values (J_{max}) through Stripped and Delipidized Rat Skin (a, b) and Hamster Cheek Pouch (c)

The vehicles used were isotonic buffer solutions. △, pH, 3.0; ●, pH 7.4; □, pH 9.0. Each value of the J_{max} represents the mean of 6 experiments. (a) $r=0.98$; (b) $r=0.99$; (c) $r=0.99$.

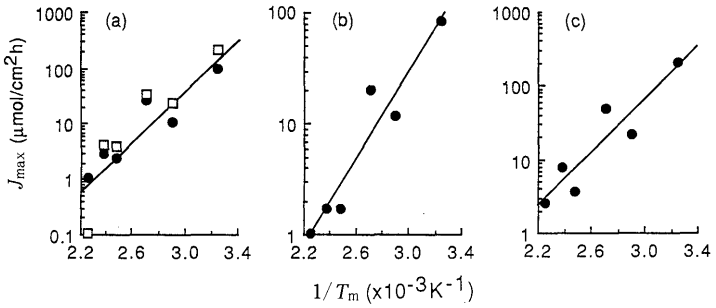

Fig. 11. Relationship between Drug Melting Point and Maximum Flux (J_{max}) through Stripped and Delipidized Rat Skin (a, b) and Hamster Cheek Pouch (c)

●, pH 7.4; □, pH 9.0. Each value of J_{max} and the melting point represents the mean of 6 and 2 experiments, respectively. (a) $r=0.90$; (b) $r=0.95$; (c) $r=0.92$.

Route In general, the drug permeation rate (dQ/dt) through pores in a porous membrane can be evaluated from the following equation:

$$dQ/dt = K_{vp} \cdot D_v \cdot \varepsilon \cdot C_v/\tau \cdot h = a_v \cdot D_v \cdot \varepsilon/\gamma_v \cdot \tau \cdot h \quad (8)$$

where K_{vp} is the partition coefficient of a drug between the bulk solvent and the solvent in the membrane pore (assumed to be 1), D_v is the diffusion coefficient in the membrane pore filled with the solvent, ε and τ are the porosity and the tortuosity of the membrane, respectively, a_v and γ_v are the activity and the activity coefficient of the drug in the solvent, respectively, and h is the thickness of the porous route in the membrane. It is evident from this pore theory that the permeation rate of a drug is directly proportional to the drug activity in the vehicle solvent. To investigate this relationship in stripped or delipidized membranes, the J_{max} values of drugs are plotted against their melting points, and the results are shown in Fig. 11. A linear correlation between J_{max} and the reciprocal of melting point could be observed in all three membranes. Thus, it is suggested that the J_{max} values of the drugs through these treated membranes correlate with their activities in the solid state.

Permeation through the Stratum Corneum Layer Since the main barrier function of a keratinized membrane to drug permeation is assumed to exist in its stratum corneum layer, investigations focused on permeation routes (or mechanisms) through the stratum corneum layer must be required.

Three permeation routes in the stratum corneum with different permeation mechanisms can be assumed. The first is the intercellular lipid pathway, the main permeation route especially for lipophilic drugs, the second is the transcellular pathway, and the third is the aqueous pore pathway. So, the drug permeation rate through the stratum corneum layer can be expressed by the following equation derived from the combination of the solution–diffusion theory and the pore theory[18]:

$$dQ/dt = (1-\varepsilon_1-\varepsilon_2)D_L \cdot K_{VL} \cdot C_V/h_1 + \varepsilon_1 \cdot D_C \cdot K_{VC} \cdot C_V/h_2 + \varepsilon_2 \cdot D_V \cdot K_{VP} \cdot C_V/h_3 \quad (9)$$

where dQ/dt is the drug permeation rate, ε_1 and ε_2 are the porosity of the transcellular route and the pore route, respectively, D and K are the diffusion coefficient and the partition coefficient, respectively, with the subscripts, L, V and C, which stand for the parameters in the intercellular stratum corneum lipids, in the vehicle and in the keratinocytes, respectively, and h_1, h_2 and h_3 are the effective thickness of the intercellular lipid route, the transcellular route and the pore route, respectively. Biomembrane selectivities (K) can be described by Eq. 10 using a linear free-energy relationship.[27]

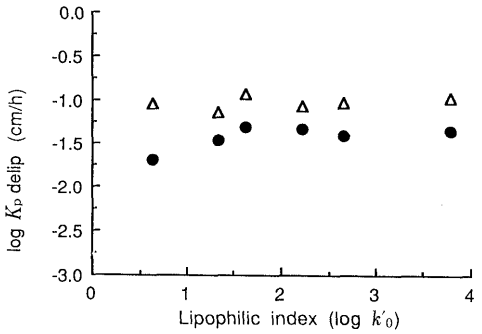

Fig. 12. Plots of Permeability Coefficients (K_p) of Drugs through Delipidized Rat Skin (●) and Hamster Cheek Pouch (△) against Lipophilicities at pH 7.4

Each value represents the mean of 6 experiments.

$$\log K = a \cdot \log K_{o/w} + b \quad (10)$$

In the present study, the lipophilic index ($\log k'_0$), obtained by HPLC measurement, was used instead of the logarithm of the octanol/water partition coefficient ($\log K_{o/w}$). Using this parameter, Eq. 10 can be transformed to the following equations:

$$K_{VL} = \alpha \cdot k'^{\beta}_0 \quad (11a)$$

$$K_{VC} = \gamma \cdot k'^{\delta}_0 \quad (11b)$$

In the present study, the permeation of drugs with similar molecular weights across similar membranes (stratum corneum) was examined. Therefore, h, ε_1 and ε_2 in Eq. 9 could be assumed to be constant, and K_{VP} is equal to 1. Further, we assumed that the diffusion constants, D_L, D_C and D_V, are constant because the permeation of drugs with similar molecular weights is controlled mainly by the drug partition to the membrane. Finally, the permeability constant, K_p, can be given by the following equation:

$$K_p = A \cdot k'^{\beta}_0 + B \cdot k'^{\delta}_0 + C \quad (12)$$

where A, B and C are the intrinsic constants for the membrane. In the case of the delipidized membrane, the intercellular lipid pathway can be replaced by the aqueous pore pathway to a certain extent. So, the permeability constant for delipidized stratum corneum, $K_{p\,delip}$, can be described by Eq. 13:

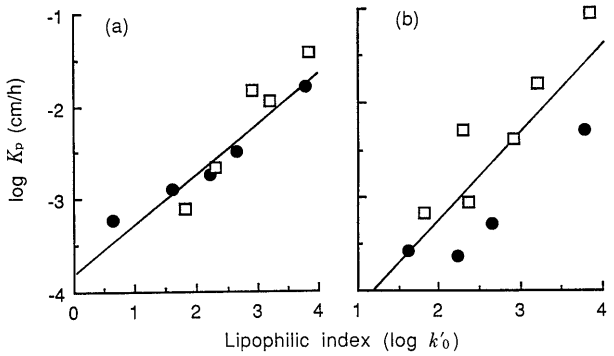

Fig. 13. Relationship between Drug Lipophilicities and Permeability Coefficients (K_p) through Rat Skin (a) and Hamster Cheek Pouch (b)
●, pH 7.4; □, pH 9.0. Each value represents the mean of 6 experiments.

$$K_{p\,delip}=\varepsilon_1\cdot D_C\cdot K_{VC}/h_2+[\chi(1-\varepsilon_1-\varepsilon_2)+\varepsilon_2]D_V/h_1+(1-\chi)A\cdot k'^{\beta}_0+C$$
$$=B\cdot k'^{\beta}_0+E+(1-\chi)A\cdot k'^{\beta}_0 \quad (0<\chi\leq 1) \tag{13}$$

where E and χ are the intrinsic constant and the replacement fraction of the lipid pathway, respectively. Since the $K_{p\,delip}$ values in *in vitro* experiments were almost the same as those obtained in the stripped skin and were almost constant in all drugs (Fig. 12), the barrier function of delipidized stratum corneum layer was assumed to be negligible. Therefore, the terms, $B\cdot k'^{\beta}_0$ and $(1-\chi)A\cdot k'^{\beta}_0$, expressing the contribution of the transcellular route and the intercellular lipid route, respectively, can be neglected. This means that the transcellular route plays a fairly insignificant role. Hence, the Eq. 12 can be simplified to Eq. 14:

$$K_p=A\cdot k'^{\beta}_0+F \tag{14}$$

where F is the intrinsic constant for the membrane. As for the drugs used in this study, having positive $\log k'_0$, a linear correlation could be observed between $\log k'_0$ and $\log K_p$ in each membrane as shown in Fig. 13, the F value for each membrane was negligible. So, the membrane permeabilities for these drugs can be evaluated only by drug lipophilicities. A and β were calculated from the Y-intercept and the slope in Fig. 13, respectively. The final equation can be obtained as follows:

$$K_{p(rat)}=1.334\times 10^{-4}k'^{0.584}_0 \quad \text{for rat skin} \tag{15}$$
$$K_{p(ham)}=9.262\times 10^{-6}k'^{0.888}_0 \quad \text{for hamster cheek pouch mucosa} \tag{16}$$

From these equations, it is suggested that the apparent membrane lipophilicity of hamster cheek pouch ($\beta=0.888$) is greater than that of rat abdominal skin ($\beta=0.584$).

Elias reported that the intercellular spaces of the stratum corneum, filled with hydrophobic substances, appear to be the main pathway of the percutaneous penetration of both hydrophilic and hydrophobic types of drugs.[28] Though the total volume of the intercellular space has been estimated to be 10—30% of the whole volume of the stratum corneum, in comparison to other tissues, where the intercellular volume fraction is 0.5—1.5%,[29] the drugs would have to penetrate rapidly through the relatively small intercellular space if this pathway contributed predominantly to the steady-state flux.

Conclusions

In the present study, the relationships between the physicochemical properties of drugs and the penetration kinetics through rat skin or hamster cheek pouch were investigated. Drug permeation rates linearly correlated with drug lipophilicities or the inverse of melting points. We assumed three permeation routes in the stratum corneum with different permeation mechanisms: (a) intercellular lipid pathway, (b) transcellular pathway, and (c) pore pathway. In general, there is a concept involving at least two pathways, one lipoidal and the other watery, and these concepts seems to be secured with hard data. What and exactly where these pathways lie within the stratum corneum or whether they are external to it by way of shunts are still subject to guess and argument. But from the permeation data, it could be suggested that for the drugs used in this study, having positive lipophilic indexes, the permeabilities of the skin and the cheek pouch could be evaluated by drug lipophilicities and that primary permeation route might be an intercellular lipid pathway.

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