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RESEARCH PAPER

Evaluation of Skin Permeation of β-Blockers for Topical Drug Delivery

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ABSTRACT

Purpose β-Blockers have recently become the main form of treatment of infantile hemangiomas. Due to the potential systemic adverse effects of β-blockers, topical skin treatment of the drugs is preferred. However, the effect and mechanism of dosage form pH upon skin permeation of these weak bases is not well understood. To develop an effective topical skin delivery system for the β-blockers, the present study evaluated skin permeation of β-blockers propranolol, betaxolol, timolol, and atenolol.

Methods Experiments were performed in side-by-side diffusion cells with human epidermal membrane (HEM) *in vitro* to determine the effect of donor solution pH upon the permeation of the β-blockers across HEM.

Results The apparent permeability coefficients of HEM for the β-blockers increased with their lipophilicity, suggesting the HEM lipoidal pathway as the main permeation mechanism of the β-blockers. The pH in the donor solution was a major factor influencing HEM permeation for the β-blockers with a 2- to 4-fold increase in the permeability coefficient per pH unit increase. This permeability *versus* pH relationship was found to deviate from theoretical predictions, possibly due to the effective stratum corneum pH being different from the pH in the donor solution.

Conclusions The present results suggest the possibility of topical treatment of hemangioma using β-blockers.

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INTRODUCTION

β-Adrenergic blockers are commonly used in the management of cardiovascular disorders, including hypertension, ischemic heart disease, congestive heart failure, and cardiac arrhythmias (1). Orally administered β-blockers appear to be rapidly absorbed from the gastrointestinal tract (2). β-Blockers are also used in the treatment of glaucoma by reducing aqueous humor production through the blockage of the beta receptors in the ciliary body (3,4). In the past few years, oral propranolol, a nonselective β-blocker, has been shown to be effective in the treatment of all types and locations of infantile hemangiomas (5–8). The mechanism of action of β-blockers in hemangioma treatment is believed to be related to β-blockers acting to decrease the vascular endothelial growth factor expression and basic fibroblast growth factor through the down-regulation of the RAF-miogen-activated protein kinase pathway (9,10), and triggering capillary endothelial cell apoptosis (11). Timolol is another nonselective β-blocker that has been studied for the treatment of infantile hemangiomas (12,13). These β-blocker treatments generally have fewer side effects than the use of corticosteroids. However, adverse effects commonly associated with systemic nonselective β-blockers (e.g., hypoglycemia, bradycardia, sweating and palpitations) remain a concern in infantile hemangioma therapy (14). Selective β_1-blockers such as atenolol have been investigated as an alternative to propranolol for hemangioma treatment (15).

Topical drug delivery is a common strategy for local therapy and to reduce systemic adverse effects in the treatment of skin diseases. In order to reach therapeutic drug

concentrations in the viable epidermal layer, effective drug permeation across the uppermost skin barrier, the stratum corneum (SC), is required. This process is affected by various factors such as the physicochemical properties of the drugs and the vehicles used for drug administration. Although local skin administration of β-blockers is preferred, commercial topical skin products of β-blockers are currently not available. Ophthalmic timolol (e.g., Timoptic) at 0.1–0.5% applied topically on skin has been studied as an alternative for the treatment of superficial infantile hemangiomas (16,17). Due to the lipophilic nature of the SC barrier, the degree of β-blocker ionization, a function of the drug pKa and dosage form pH, is an important factor in determining β-blocker permeation across SC. The pKa values of propranolol, betaxolol, timolol, and atenolol are 9.5 (18), 9.4 (19), 9.2 (18), and 8.6 (18), and their log octanol/water partition coefficients ($K_{O/W}$) are 3.27 (18), 3.5 (20), 2.1 (18), and 0.16 (20), respectively. The relationships between topical skin permeation of weak bases (positively charged drugs at physiological pH such as the β-blockers) and dosage form pH have been investigated (21–24), but the mechanism of the permeability *versus* pH relationships is not well understood. Formulation screenings are the primary method in the development of topical and transdermal drug delivery systems for this class of compounds.

The objectives of the present study were to (a) evaluate skin permeation of β-blockers propranolol, betaxolol, timolol, and atenolol for topical delivery, (b) study the effect of pH upon the permeation of these β-blockers across human epidermal membrane (HEM), (c) examine the relationship between the permeability coefficients of HEM for the β-blockers and their $K_{O/W}$, and (d) understand the role of drug ionization to examine whether the experimentally obtained permeability coefficients of β-blockers are consistent with theoretical prediction. HEM permeability experiments were conducted in side-by-side diffusion cells *in vitro*. The four β-blockers (propranolol hydrochloride, betaxolol hydrochloride, timolol maleate, and atenolol) selected in the present study were based on their physicochemical properties and clinical history. Table I summarizes the physicochemical properties of these β-blockers and their available commercial dosage forms. The present skin permeation results could provide important information for the development of an effective β-blocker topical formulation to improve the efficacy and safety of infantile hemangioma therapy.

MATERIALS AND METHODS

Materials

^{3}H-propranolol, hydrochloride [ring-^{3}H] (24 Ci/mmol) was purchased from Vitrax (Placentia, CA). ^{3}H-atenolol [ring-^{3}H] (5.1 Ci/mmol) was purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). Non-radiolabelled D,L-propranolol hydrochloride at purity≥ 98%, betaxolol hydrochloride and atenolol at purity≥99% were purchased from Sigma-Aldrich (St. Louis. MO). Timolol maleate was purchased from Letco Medical (Decatur, AL). Betaxolol hydrochloride ophthalmic solution 0.5% was purchased from Falcon Pharmaceuticals, Ltd. (Fort Worth, TX). Phosphate buffered saline tablets were purchased from MP Biomedicals, LLC (Solon, OH). Sodium chloride (NaCl), sodium azide (NaN_3), monobasic sodium phosphate monohydrate ($NaH_2PO_4.H_2O$), and dibasic sodium phosphate heptahydrate ($Na_2HPO_4.7H_2O$) were purchased from Acros Organics (Morris Plains, NJ). High-performance liquid chromatography (HPLC) grade methanol was purchased from Pharmaco-AAPER (Shelbyville, KY). HPLC grade glacial acetic acid and triethylamine were purchased from Fisher Scientific (Rochester, NY). Phosphate buffered saline (PBS) of pH 5.8 (consisting of 0.01 M phosphate buffer, 0.145 M NaCl), pH 6.6 (consisting of 0.01 M phosphate buffer, 0.144 M NaCl), pH 7.4 (consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M NaCl), and pH 8.0 (consisting of 0.01 M phosphate buffer, 0.141 M NaCl) were prepared and preserved with 0.02% (w/v) NaN_3. The pH of the solutions was checked with a pH meter (Oakton Instruments, Vernon Hills, IL). All buffer solutions were prepared to be isoosmotic to physiological fluids.

Preparation of HEM

Excised split thickness human cadaver skin from posterior torso of male aged between 20 and 64 years was obtained from the New York Firefighters Skin Bank (New York, NY). HEM, comprising the SC and viable epidermis, was prepared from the cadaver skin by heat separation (25). The cadaver skin was immersed in PBS pH 7.4 at 60°C for 1 min. After heat treatment, HEM was separated from the underlying dermis and soaked in PBS pH 7.4. The HEM sheet was then patted dry with Kimwipe, wrapped in aluminum foil, and subsequently stored in a freezer at −20°C for later use. Human stripped skin, comprising only the viable epidermis, was prepared by the removal of the SC from excised split thickness human cadaver skin via tape-stripping approximately 30 times using fresh 2-inch package sealing tape (3M, St. Paul, MN) for each stripping. After tape-stripping, the viable epidermis layer was separated from the underlying dermis by heat separation as described above. The viable epidermis was then patted dry with Kimwipe, wrapped in aluminum foil, and stored in a freezer at −20°C for later use. Tape-stripping was checked by trypsin digestion (0.0005% trypsin solution at 37 ± 1°C for 18 h) at the end of the permeability experiments to ensure at

Table I Physicochemical Properties of β-Blocking Agents

β-Blocking agents	Molecular weight (g/mol)	pKa	log $K_{O/W}$	Commercially available forms
Propranolol	259.3	9.5 ± 1.2[a]	3.27[a]	1 mg/mL propranolol hydrochloride intravenous solution
				20 mg/5 mL propranolol hydrochloride oral solution
				40 mg/5 mL propranolol hydrochloride oral solution
Betaxolol	307.4	9.4[b]	3.5[c]	5.6 mg/mL betaxolol hydrochloride eye drop
				2.8 mg/mL betaxolol hydrochloride eye drop
Timolol	316.4	9.2[a]	2.1[a]	5 mg/mL timolol maleate eye drop
Atenolol	266.3	8.6[a]	0.16[c]	5 mg/10 mL atenolol intravenous solution

[a] Values from the literature (18)

[b] Values from the literature (19)

[c] Values from the literature (20)

least 90% SC was removed in the tape-stripping procedure (26). The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, OH.

HEM Permeability Experiments

Prior to mounting the HEM samples in the side-by-side diffusion cells, the skin samples were allowed to thaw at room temperature and hydrate in PBS pH 7.4. Each HEM was mounted between the two diffusion half-cells of a side-by-side diffusion cell with a Millipore® filter (0.45 μm nitrocellulose) as a support placed between the viable epidermis side of the HEM sample and the receiver chamber. Each diffusion cell compartment had a 2-mL volume and an effective diffusional area of around 0.7 cm^2. Two milliliters of PBS were pipetted into both donor and receiver chambers. HEM was then equilibrated in the well-stirred diffusion cells in a circulating water-bath at 37 ± 1°C for approximately 12 h before the permeability experiments (27). The electrical resistance of HEM was used to prescreen the integrity of the membrane before each permeation experiment. To measure the electrical resistance of HEM, an electrical system was constructed using a 1.5-V battery and fixed resistors. The system applied a low voltage (<0.25 V) through two Ag/AgCl electrodes in the side-by-side diffusion cells across the HEM. The voltages across the HEM and the fixed resistor were determined by voltmeters, and the resulting electric current across HEM was calculated by the voltage across the fixed resistor and the resistance of the fixed resistor using Ohm's law. The electrical resistance of HEM was then calculated using the reading of the voltage across the HEM, the electric current, and Ohm's law. HEM with electrical resistance≥15–20 $k\Omega cm^2$ is indicative of good barrier integrity (28,29). Therefore, only HEM samples with initial resistance≥15 $k\Omega cm^2$ were used in the present study. The electrical resistance of HEM was also measured after each permeation experiment using the same method to check for membrane integrity in the experiment and evaluate the contribution of the HEM pore pathway to drug permeation (30). Briefly, the permeability coefficients of the HEM pore pathway in the present study were estimated using the HEM electrical resistance data and the correlation of "HEM pore pathway permeability" *versus* "HEM electrical resistance" as described previously.

Skin permeation experiments were conducted in the two-chamber side-by-side diffusion cells with HEM under either the symmetric (donor and receiver diffusion chambers had the same pH) or asymmetric (donor and receiver diffusion chambers had different pH and the receiver chamber pH was 7.4) conditions. Whereas the symmetric conditions provide the data for studying the transport mechanisms of β-blocker permeation across HEM, the asymmetric conditions provide data mimicking the situations in topical skin delivery in practice. The total duration of the skin permeation experiments from the assembly of HEM in the diffusion cells to the completion of the experiment was around 32 h. For the symmetric conditions, pH 5.8 and pH 7.4 conditions in both donor and receiver diffusion chambers were studied. For the asymmetric conditions, pH 5.8, pH 6.6, and pH 8.0 conditions in the donor chamber were studied. Three different drug concentration conditions in the donor chamber were used as described in the following sections. Table II summarizes the conditions of the permeation experiments investigated in the present study. During the permeation experiments, samples were withdrawn from the donor and receiver chambers at predetermined time intervals. Typically, 10-μL aliquots were taken from the donor chamber and 0.5 or 1-mL aliquots were withdrawn from the receiver chamber (0.5 mL in the non-radiolabelled drug experiments and 1 mL in the radiolabelled drug experiments). The

Table II Experimental Conditions Investigated in the Permeation Study

β-Blocker	Configuration/descriptions	Donor concentration (number of replicates)	Donor chamber pH	Receiver chamber pH
Propranolol hydrochloride	Asymmetric	Trace (n=3, except pH 5.8 with n=4)	5.8, 6.6, 8.0	7.4
		4 mg/mL (n=3, except pH 8.0 with n=4)	5.8, 6.6, 8.0	7.4
		Saturated (pH 7.0, n=3; pH 8.0, n=4)	7.0, 8.0	7.4
	Symmetric	Trace (n=4)	5.8, 7.4,	5.8, 7.4
		4 mg/mL (pH 5.8, n=4; pH 7.4, n=3)	5.8, 7.4	5.8, 7.4
	Asymmetric	Trace (n=3)	7.4	5.8
		4 mg/mL (n=4)	7.4	5.8
	Stripped skin, symmetric	4 mg/mL (n=4)	7.4	7.4
Betaxolol hydrochloride	Asymmetric	1 mg/mL (n=4, except pH 5.8 with n=3)	5.8, 6.6, 8.0	7.4
	Symmetric	1 mg/mL (n=4)	7.4	7.4
	Stripped skin, symmetric	1 mg/mL (n=3)	7.4	7.4
Timolol maleate	Asymmetric	10 mg/mL (n=3, except pH 5.8 with n=4)	5.8, 6.6, 8.0	7.4
	Symmetric	10 mg/mL (n=3)	7.4	7.4
	Stripped skin, symmetric	10 mg/mL (n=3)	7.4	7.4
Atenolol	Asymmetric	Trace (n=3, except pH 5.8 with n=5)	5.8, 6.6, 8.0	7.4
	Symmetric	Trace (n=4)	7.4	7.4
	Stripped skin, symmetric	Trace (n=3)	7.4	7.4

same volume of the fresh solution (same composition as the starting solution) was added back to the receiver chamber after each aliquot removal to maintain a constant volume. The samples taken from the donor chamber at the beginning and the end of the experiment were used to check for drug depletion in the donor solution during the experiment.

Experiments with Trace Amounts of Drugs in the Donor

In the permeation experiments with trace amounts of drugs, the PBS in the donor chambers were spiked with radiolabelled propranolol and atenolol (i.e., approximately 2 μCi ^{3}H-propranolol hydrochloride or ^{3}H-atenolol, respectively). During the experiments, the samples taken from the donor and receiver chambers were mixed with 10 mL of scintillation cocktail (UltimaGold, Packard Instrument, Meriden, CT) and analyzed by a liquid scintillation counter (Beckman Coulter LS 6500, Fullerton, CA).

Experiments with Non-radiolabelled Drugs at mg/mL Concentration Range in the Donor

In the permeation experiments using the concentration range similar to those of the β-blocker ophthalmic solutions, 0.1% to 1% propranolol hydrochloride, betaxolol hydrochloride, and timolol maleate in PBS were the donor solution. During the experiments, the samples taken from the donor and receiver chambers were diluted in the mobile phase and analyzed by HPLC.

Experiments with Non-radiolabelled Drug at Saturated Concentration in the Donor

For propranolol in the permeation experiments, saturated concentration of propranolol hydrochloride was also used in the donor. These experiments allowed the determination of the permeability coefficients of the lipoidal pathway to study the relationship between HEM permeability coefficient and pH and the estimation of the effective pH in SC for the permeation of the β-blockers. In the experiments, saturated solutions of propranolol hydrochloride in PBS of pH 7.0 and 8.0 were prepared by adding an excess amount of propranolol hydrochloride in PBS. After adding an excess amount of drug in PBS, the pH of the suspensions was adjusted with 0.1 N NaOH solution to obtain the desired pH. The drug suspension was equilibrated at 37 ± 1°C for 48 h, and the saturated solution was obtained by filtering the suspension with the Millipore filter. The solubilities of propranolol hydrochloride in the solutions were measured and the pH of the solutions was checked with the pH meter. During the permeation experiments, the samples taken from the donor and receiver chambers were diluted in the mobile phase and analyzed by HPLC.

HPLC Analysis

The HPLC system consisted of CBM-20A system controller, LC-20AT solvent delivery unit, SIL-20A autosampler, SPD-20A variable wavelength UV–Vis detector (Prominence, Shimadzu, Columbia, MD), and Microsorb-MV100-5 C18

column (15 cm × 4.6 mm, 4.6 μm, Varian, Lake Forest, CA). For the analyses of timolol, propranolol, and betaxolol samples, the mobile phase consisting of methanol/water/glacial acetic acid/triethylamine 500:500:3.5:1 by volume ratio was used. The detection wavelengths for timolol, propranolol, and betaxolol were 295, 295, and 273 nm, respectively. The flow rate was 1.0 mL/min, and the injection volume was 50 μL. HPLC was performed at room temperature and the retention times of timolol, propranolol, and betaxolol were 4.6, 12.7, and 10.9 min, respectively. The standard solutions used to construct the calibration curves were prepared in the mobile phase. The concentration was determined based on peak area measurement.

Data Analysis

The cumulative amount of drug permeated across the HEM (Q) was plotted against time (t). The steady-state flux of the drug ($\mathcal{J}$) was calculated from the slope of the linear regression of the linear region in the plot ($\mathrm{d}Q/\mathrm{d}t$) divided by the effective area (A).

$$\mathcal{J} = \frac{1}{A} \cdot \left(\frac{\mathrm{d}Q}{\mathrm{d}t} \right) \tag{1}$$

The apparent permeability coefficient (P or P_{app}) was calculated by dividing the flux by the concentration of the drug in the donor (C_D).

$$P_{app} = \left(\frac{1}{AC_D} \right) \cdot \left(\frac{\mathrm{d}Q}{\mathrm{d}t} \right) \tag{2}$$

In data analyses, the means ± standard deviations (SD) of the data are presented. Student's t-test and ANOVA were performed using GraphPad Prism (La Jolla, CA). Linest calculations were performed using Microsoft Excel (Redmond, WA). Statistical differences were considered to be significant at $p < 0.05$.

Theory and Model Analysis

The steady-state fluxes and permeability coefficients of HEM for the β-blockers are related to the permeability coefficients of the SC and viable epidermis and the pH in the donor and receiver chambers of the side-by-side diffusion cells. The analyses of the permeability coefficient results can be divided into three cases: (I) drug permeation across the HEM with considerable contribution from the SC pore pathway and the viable epidermis does not contribute as a barrier, (II) drug permeation across the SC when the contribution of the SC pore pathway is negligible compared to that of the lipoidal pathway and the viable epidermis is a barrier, and (III) drug permeation across the SC when the contributions from the viable epidermis and SC pore pathway are both negligible. For the β-blockers in the present study, Case I is applicable to the less lipophilic β-blockers under acidic donor pH conditions that the pore pathway becomes important when the fraction of ionized form is several orders of magnitude greater than that of the unionized form. Case II is applicable to the lipophilic β-blockers at donor pH close to the physiological range of pH 7.4. Case III can be used to describe the permeation of moderately lipophilic β-blockers across HEM at donor pH close to pH 7.4.

Case I

When the barrier contribution of viable epidermis is negligible compared to the SC, e.g., permeability coefficient of viable epidermis (P_{VE}) >> permeability coefficient of SC (P_{SC}), the permeability coefficient of the human epidermal membrane (P_{HEM}) approximately equals to P_{SC},

$$P_{HEM} = P_{SC} \tag{3}$$

The steady-state flux of a drug across the SC ($\mathcal{J}_{SC}$) can be described by the sum of the fluxes of the drug across the pore ($\mathcal{J}_P$) and lipoidal ($\mathcal{J}_L$) pathways of the SC:

$$\mathcal{J}_{SC} = \mathcal{J}_P + \mathcal{J}_L \tag{4}$$

Since only the unionized fraction of drug can permeate the lipoidal pathway, $\mathcal{J}_{SC}$ can be expressed as:

$$\mathcal{J}_{SC} = P_{SC} C_D = P_L f_{union} C_D + P_P C_D \tag{5}$$

where P_L and P_P are the permeability coefficients of the lipoidal pathway and pore pathway, respectively, and f_{union} is the fraction of unionized drug in the donor chamber:

$$f_{union} = \frac{1}{1 + 10^{pKa - pH}} \tag{6}$$

which is related to the pH of the donor solution (pH) and the pKa of the drug. Equation 5 assumes that the pH in the SC can be approximated by the pH in the donor. Substituting Eq. 6 into Eq. 5,

$$P_{SC} = \frac{P_L}{1 + 10^{pKa - pH}} + P_P \tag{7}$$

When pKa > pH in the donor that $10^{pKa - pH} >> 1$, Eq. 7 becomes

$$P_{SC} = 10^{pH - pKa} P_L + P_P \tag{8}$$

Case II

When the permeability coefficient of the viable epidermis is comparable to that of SC (e.g., $P_{VE} \approx P_{SC}$) and when the contribution of the SC pore pathway is negligible compared

to that of the lipoidal pathway (i.e., P_L:f_{union} >> P_P):

$$\mathcal{J}_{SC} = \mathcal{J}_L = P_L\left(f_{union}C_D - C_{union,I}\right) \quad (9)$$

and

$$\mathcal{J}_{VE} = P_{VE}C_{union,I}\left(1 + \frac{f'_{ion}}{f'_{union}}\right) \quad (10)$$

where $C_{union,I}$ and f'_{union} are the concentration and fraction of unionized drug at the interface between the SC and viable epidermis, respectively, and f'_{ion} is the fraction of ionized drug at this interface. The interface is assumed to have the same pH as the pH in the receiver chamber (pH'). In addition, Eq. 10 assumes the same permeability coefficient of the viable epidermis for the ionized and unionized forms of the drug.

At steady state, the flux of the drug across HEM equals to the flux of the drug across the SC and that across the viable epidermis. Equating and simplifying Eqs. 9 and 10, $C_{union,I}$ can be solved, and the apparent permeability coefficient of HEM for the drug can be expressed as:

$$P_{HEM} = \frac{f_{union}}{1/P_L + f'_{union}/P_{VE}} \quad (11)$$

In the present study, the pH in the receiver was equal to or less than 7.4 (i.e., pH'≤7.4), and hence $10^{pKa-pH'}$ >> 1. Thus, Eq. 11 can be written as:

$$P_{HEM} = \frac{1}{1 + 10^{pKa-pH}} \frac{P_L}{1 + (10^{pH'-pKa})P_L/P_{VE}} \quad (12)$$

When pKa > pH in the donor that 10^{pKa-pH} >> 1, Eq. 12 becomes

$$P_{HEM} = \frac{10^{pH-pKa}P_L}{1 + (10^{pH'-pKa})P_L/P_{VE}} \quad (13)$$

Taking the logarithm on both sides of Eq.13,

$$\log P_{HEM} = \log\frac{P_L}{1 + (10^{pH'-pKa})P_L/P_{VE}} - pKa + pH \quad (14)$$

Therefore, a plot of the logarithm of P_{HEM} *versus* donor pH would provide a straight line with a slope of unity when the receiver pH' is maintained constant under these conditions.

Case III

When the contribution of the SC pore pathway to drug permeation across SC is negligible compared to the lipoidal pathway and the viable epidermis is a negligible barrier compared to SC, the apparent permeability coefficient of HEM for the drug becomes the simplified form of Eqs. 8 and 14:

$$P_{SC} = 10^{pH-pKa}P_L \quad (15)$$

and the logarithmic form is:

$$\log P_{HEM} = \log P_L - pKa + pH \quad (16)$$

Again, a plot of the logarithm of P_{HEM} *versus* donor pH would provide a straight line with a slope of unity. Figure 1 illustrates the transport barriers of HEM for the β-blockers in Cases I, II, and III.

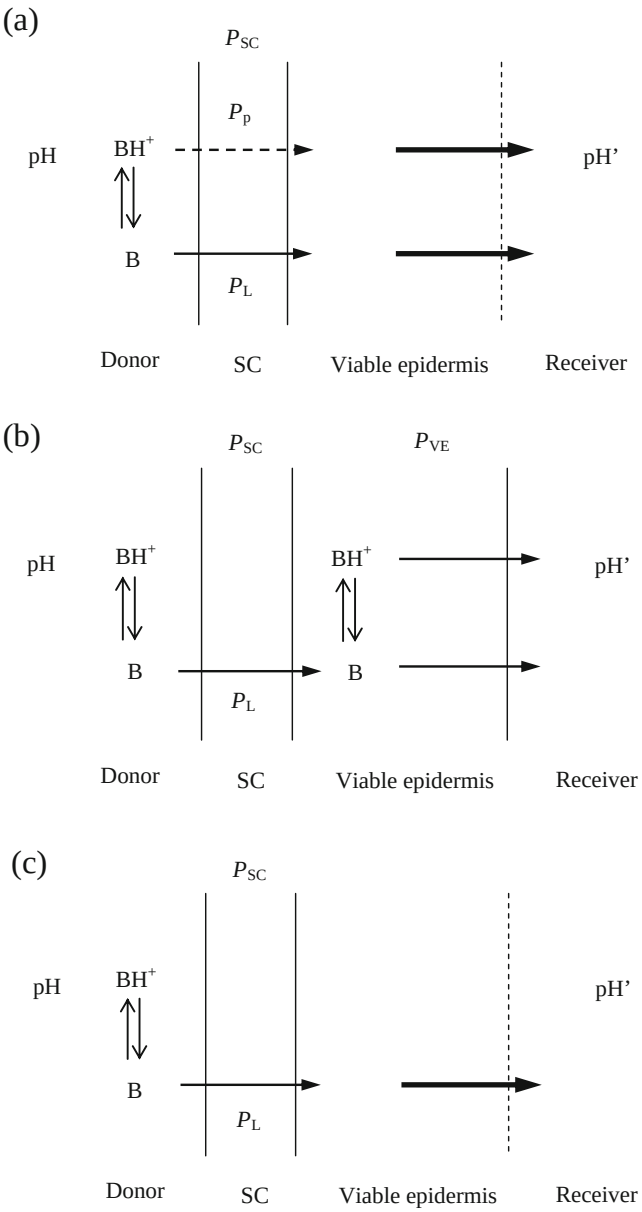

Fig. 1 Permeation of β-blockers in their ionized (BH^+) and unionized (B) forms across HEM under (**a**) Case I, (**b**) Case II, and (**c**) Case III.

Saturated Propranolol Hydrochloride Solution and Effective pH in SC

Under the condition of saturated drug solution in the donor chamber, the concentration of unionized drug equals the intrinsic solubility of the drug (C_S), i.e., f_{union} $C_D = C_S$. At relatively high pH where $P_L \cdot f_{union} >> P_P$, the flux of a β-blocker across the SC can be expressed as:

$$\mathcal{J}_{SC} = P_L\left(C_S - C_{union,I}\right) \tag{17}$$

Since $\mathcal{J}_{SC} = \mathcal{J}_{VE}$ at steady-state, equating Eqs. 10 and 17 and solving for $C_{union,I}$ lead to Eq. 18, which describes the apparent flux of the drug in the experiments with the saturated drug solution ($\mathcal{J}_{app}$):

$$\mathcal{J}_{app} = \frac{C_S P_L}{1 + P_L f_{union}/P_{VE}} \tag{18}$$

Rearranging Eq. 18, the permeability coefficient of the lipoidal pathway of the drug can be determined by the intrinsic drug solubility and the apparent flux $\mathcal{J}_{app}$ obtained in the experiments:

$$P_L = \frac{\mathcal{J}_{app}}{C_S - \mathcal{J}_{app}(10^{pH'-pKa})/P_{VE}} \tag{19}$$

From Eqs. 6 and 12, the fraction of unionized drug in the SC transport pathway $\left(f_{union}^{SC}\right)$ can be calculated using P_L, P_{VE}, and P_{HEM}:

$$f_{union}^{SC} = \frac{P_{HEM}\left(1 + 10^{pH'-pKa}P_L/P_{VE}\right)}{P_L} \tag{20}$$

The effective pH in the SC for drug permeation can be calculated using f_{union}^{SC}.

RESULTS AND DISCUSSION

Effect of β-Blocker Lipophilicity upon its Permeation Across SC

Figure 2 shows a plot of the logarithm of the apparent permeability coefficients of the β-blockers at pH 7.4 and their $K_{O/W}$. The $K_{O/W}$ values measure the lipophilicity of the β-blockers. The figure shows that the apparent permeability coefficients of the β-blockers increase with their lipophilicity. This suggests that the main transport pathway of the β-blockers across the SC is the SC lipids. Both lipophilic β-blockers propranolol and betaxolol have relatively high permeability coefficients among the β-blockers studied, suggesting they can

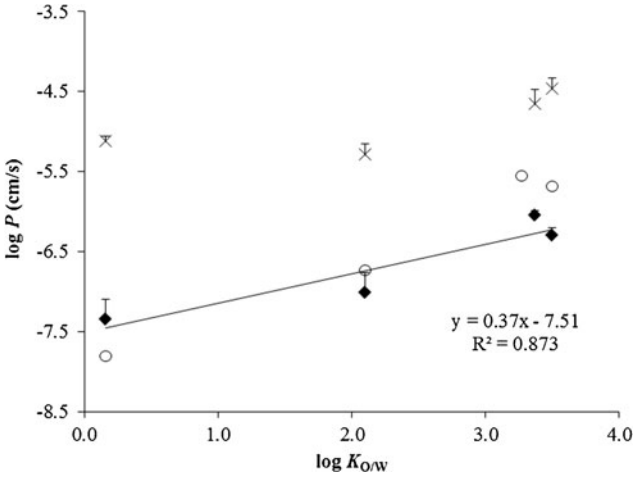

Fig. 2 Relationship between the logarithm of the permeability coefficients and octanol/water partition coefficients (lipophilicity) of the β-blockers across HEM and viable epidermis (heat separated stripped skin). Data obtained from experiments of pH 7.4 in the donor and receiver. The permeability coefficients of the viable epidermis are statistically different from those of the HEM for the β-blockers ($p < 0.05$, ANOVA). The permeability coefficients of HEM for propranolol and betaxolol are statistically different from those for timolol and atenolol ($p < 0.05$, ANOVA). Symbols: *diamonds*, HEM; *crosses*, viable epidermis; *circles*, predicted permeability coefficients from the Potts and Guy equation (31). Data of HEM and viable epidermis represent the mean ± SD ($n \geq 3$).

be effectively delivered through the topical route for percutaneous absorption. Data on steady-state human skin penetration of propranolol, timolol, and atenolol in previous studies are limited. In these previous studies, the permeability coefficients of propranolol, timolol, and atenolol at pH 7.4 were reported to be 5.0×10^{-7} cm/s (18), $0.1–1.1 \times 10^{-7}$ cm/s (32–35) and 0.14×10^{-7} cm/s (18), respectively, with large variation of the timolol data. In another study, a significantly high permeability coefficient of propranolol (2.0×10^{-5} cm/s) from 1 mg/mL propranolol hydrochloride donor solution was also reported (36). Aside from the result of this other study, the permeability coefficients of propranolol, timolol, and atenolol in the present study are in general agreement with (within the same order of magnitude as) those in the previous studies. For betaxolol, to our knowledge, the present study is the first on human skin permeability of this β-blocker (37).

For comparison, the permeability coefficients of the viable epidermis (stripped skin with heat separation) for the β-blockers are also plotted in the figure. The data show that the viable epidermis has permeability coefficients at least 10 times higher than those of HEM for the β-blockers. This suggests that the viable epidermal layer is not a major barrier for topical delivery of the β-blockers under the experimental conditions in the present study.

Figure 2 also presents the permeability coefficients predicted using the Potts and Guy model with the $K_{O/W}$ and molecular weights (MW) of the β-blockers (31):

$$\log P_{app} = \left(-6.3 + 0.71\left(\log K_{O/W}\right) - 0.0061(MW)\right) \quad (21)$$

Taking into the account of the fraction of unionized β-blockers at pH 7.4, the experimental apparent permeability coefficients in the present study are higher than those predicted from the model. However, considering the uncertainties in the correlation of skin permeability *versus* permeant log $K_{O/W}$ and MW in the Potts and Guy model, the experimental permeability coefficients of the β-blockers in the present study are reasonable.

The permeability coefficients of the HEM pore pathway were estimated using the electrical resistance of HEM (30). In general, the electrical resistance of HEM after the permeation experiments was not significantly different from those in the prescreening before the experiments. The electrical resistances of the HEM were within the range of 15–100 kΩcm^2. This corresponds to permeability coefficients of 0.2–3.0 × 10^{-8} cm/s for small polar permeants of molecular sizes similar to the β-blockers (i.e., mannitol) according to the correlation between the permeability coefficient of polar permeant across HEM and its electrical resistance (obtained previously under passive diffusion before and after iontophoresis (38)). These values were lower than the experimental apparent permeability coefficients of the β-blockers in the figure and particularly at least an order of magnitude below those of propranolol and betaxolol. Therefore, the pore pathway is not the major pathway for the permeation of the β-blockers across HEM at physiological pH. These results, together with the results of the viable epidermis and log P *versus* log $K_{O/W}$ correlation, support the lipoidal pathway being the main transport mechanism of the β-blockers across HEM.

Effect of pH upon β-Blocker Permeation Across SC

Figure 3 presents the relationship between the logarithm of the apparent permeability coefficients of the β-blockers and donor solution pH under the asymmetric conditions (pH 7.4 in the receiver). The apparent permeability coefficients of the β-blockers increased when the pH in the donor solution increased. This suggests that the donor pH was a major factor of skin permeation for the β-blockers when the pH in the receiver was maintained at pH 7.4. Although the trends of the relationship between permeability and solution pH in the figure are consistent with the effect of pH on the ionization of the β-blockers, the permeability *versus* pH relationship deviates from the slope of unity predicted from the theory (Eq. 14). Similar deviations of experimental results from the theory can be found in previous studies of weakly basic drugs

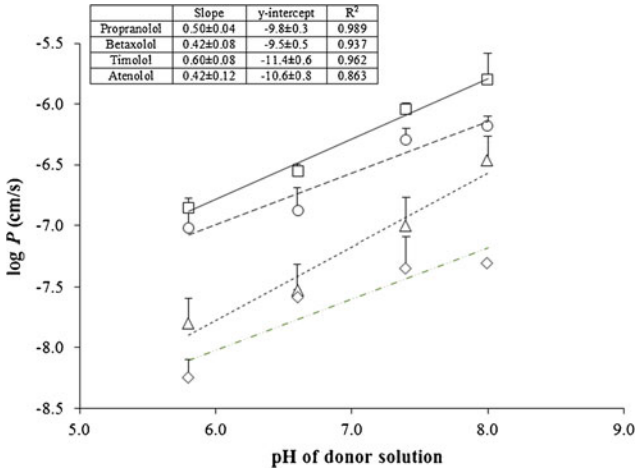

Fig. 3 Relationship between the logarithm of the permeability coefficients of HEM for the β-blockers and the pH of the donor solution. Receiver pH was constant at pH 7.4. The permeability coefficients of HEM at the higher pH are statistically different from those at the lower pH for the β-blockers (e.g., pH 8.0 *versus* pH 5.8, $p < 0.05$, ANOVA). Symbols: *squares*, propranolol; *circles*, betaxolol; *triangles*, timolol; *diamonds*, atenolol. The *lines* represent the linear least squares regressions for propranolol (*solid line*), betaxolol (*dashed line*), timolol (*dotted line*), and atenolol (*dashed-dotted line*). The slopes, y-intercepts, and R^2 of the linear least squares regressions are provided in the table insert (values ± standard errors). Data represent the mean ± SD ($n \geq 3$).

fentanyl, sufentanil, and butorphanol (22–24) but the observed deviations and the mechanism behind these deviations were not fully discussed. Possible explanations for the deviation in the present study include the asymmetric pH conditions in the donor and receiver chambers, breakdown of the assumption that $10^{pKa-pH} >> 1$ and the assumption of negligible contribution of the SC pore pathway to HEM permeation, possibility of specific transport pathway in the SC for the β-blockers, additional permeation mechanism related to ion-pair formation, and different drug pKa values in the SC from those in aqueous solution. The causes of the deviation are examined in the next section. In addition, the effective pH in the SC may be different from (e.g., higher than) the solution pH in the donor chamber. This can also explain the observed deviation and is discussed in the "SC Lipoidal Pathway and Effective pH of SC in β-Blocker Permeation" section.

Figure 4 shows the effects of receiver solution pH upon the permeation of propranolol across HEM. Higher apparent permeability coefficients of propranolol were observed when the pH of the receiver was pH 5.8 compared with those of pH 7.4. This observation is in contrast to the view that the pH in the receiver affects the pH in the SC and the ionization of the β-blocker during SC permeation. If the pH in the SC is influenced by receiver solution pH, the lower pH in the receiver (pH 5.8 *versus* pH 7.4) should increase the degree of β-blocker ionization in the SC and decrease the fluxes of the β-blocker across the SC lipoidal pathway, and hence decrease the apparent permeability coefficients of the β-blocker. This

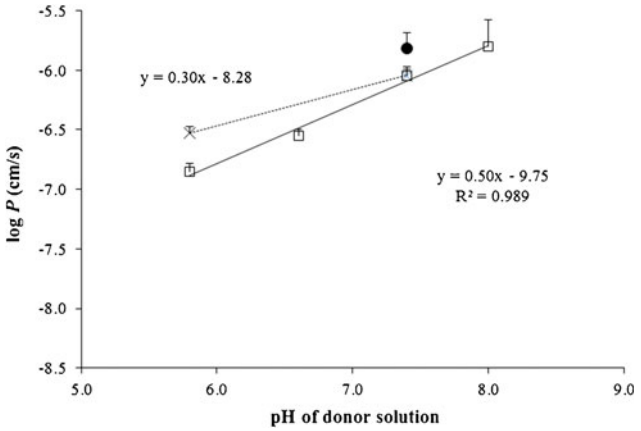

Fig. 4 Effect of receiver solution pH upon the permeability coefficients of HEM for propranolol and relationship between the logarithm of the permeability coefficients and the pH of the donor solution under the asymmetric and symmetric conditions. The permeability coefficients of HEM among the different donor pH conditions are statistically different ($p < 0.05$, ANOVA). Symbols: *squares*, asymmetric condition with varying donor pH at constant receiver pH of 7.4; *crosses*, symmetric condition of same pH in both donor and receiver; *circle*, donor pH of 7.4 and receiver pH of 5.8. The *lines* compare the slopes of the asymmetric (*solid line*) and symmetric conditions (*dotted line*). Data represent the mean ± SD ($n \geq 3$).

was not observed in the present study, thus supporting the donor pH as the dominant factor for the ionization and permeation of the β-blockers in the SC. On the other hand, the higher permeability coefficients at receiver pH of 5.8 (*versus* those at pH 7.4) suggest some barrier contribution from the viable epidermis for the lipophilic β-blocker investigated in the present study, which is consistent with the hypothesis made in the derivation of the equations in "Theory and Model Analysis" section Case II. When the viable epidermis contributes as a barrier and when the pH in the receiver decreases, the fluxes of propranolol across the viable epidermis would increase due to the ionization of the uncharged β-blocker at the SC-viable epidermis interface, resulting in the increase in the apparent permeability coefficients of the β-blocker across the HEM (Eq. 13). However, using the pKa, P_L, and P_{VE} of propranolol, the observed increase in experimental apparent permeability coefficients of propranolol due to receiver pH (pH 5.8) is larger than those calculated from Eq. 13 (2.1 and 1.7-fold *versus* 1.1-fold, respectively). This difference can be due to experimental variability and/or uncertainties of pKa, P_L, and P_{VE} values. Further investigation on this observation is needed.

In the experiments of ^{3}H-propranolol, no significant difference between the permeability coefficients of propranolol with trace amounts of ^{3}H-propranolol as the donor and propranolol hydrochloride at 4 mg/mL as the donor was observed ($p > 0.05$, ANOVA). This suggests that the β-blocker did not alter its permeation across the SC lipoidal pathway (i.e., the β-blocker was not a permeation enhancer) in the concentration range employed in the present study.

Deviation of the Permeability *Versus* pH Relationship from Theory

To investigate the effects of the asymmetric pH conditions upon the permeation of the β-blockers across the SC, the apparent permeability coefficients for propranolol under the symmetric conditions are compared with those under the asymmetric conditions. In Fig. 4, the slope of the plot of the logarithm of the permeability coefficients of propranolol against donor solution pH under the symmetric conditions (pH 5.8 or 7.4 in both donor and receiver) was 0.30 (*versus* slope = 0.50 under the asymmetric conditions). As the slopes of the logarithm of permeability coefficients *versus* donor pH under both symmetric and asymmetric pH conditions were less than 1.0 (the slope predicted from theory), the asymmetric pH conditions cannot explain the deviation of the experimental results from the theory.

Figure 5 presents the relationship between the percent of unionized β-blockers and pH to examine the assumption of $10^{pKa-pH} >> 1$ in the derivation of Eq. 14. As illustrated in the figure, there is a linear increase in the logarithm of the percent of unionized β-blockers with increasing pH from pH 5.8 to 8.0. This suggests that the assumption should hold and is not the cause for the deviation of the experimental results (the slope of logarithm of permeability coefficient *versus* pH) from the theory. In addition, the assumption of negligible contribution of the SC pore pathway to β-blocker permeation (i.e., $P_L f_{union} >> P_P$) is likely valid (for propranolol and betaxolol and to some extent timolol) and not the reason for the deviation according to the HEM electrical resistance data; the permeability coefficients of the pore pathway are in the order of 10^{-8}cm/s, significantly smaller than the apparent permeability coefficients of the β-blockers under the conditions in the present study.

It should be pointed out that the pKa of drugs in aqueous media may be different from those in the SC lipid microenvironment. Possible influences of microenvironment on the

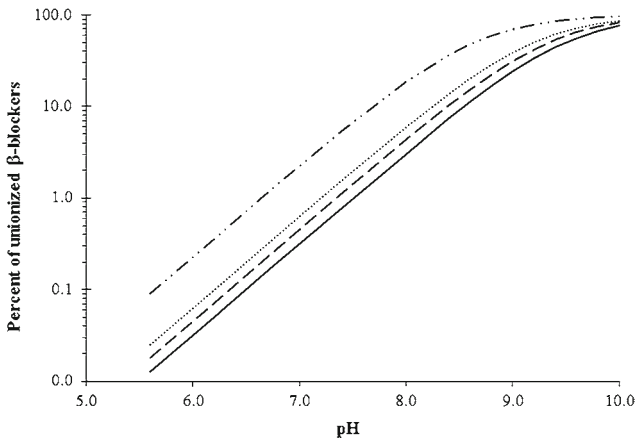

Fig. 5 Percent of unionized β-blockers as a function of pH calculated from Eq. 6. Symbols: propranolol (*solid line*); betaxolol (*dashed line*), timolol (*dotted line*), and atenolol (*dashed-dotted line*).

pKa values of molecule functional groups have been documented (39–41). Since the analyses of skin permeation in the present study relied on the (aqueous solution) pKa of the drugs obtained from the literature, this could introduce errors in the theoretical calculations. However, altering the pKa values would only affect the y-intercept of the linear correlation between the logarithm of permeability coefficients and pH rather than the slope (see Eq. 14) and could not explain the observed deviation of the experimental results from the theory. Similarly, the modification of the parallel transport pathway model in skin permeation by the incorporation of a new transport pathway or the involvement of a new permeation mechanism such as via ion-pair formation (21) for the ionized β-blockers affects only the minimum value or the y-intercept in the logarithm of permeability coefficient *versus* pH plot (instead of the slope). This should not result in the deviation observed between the experimental results and theory in the present study.

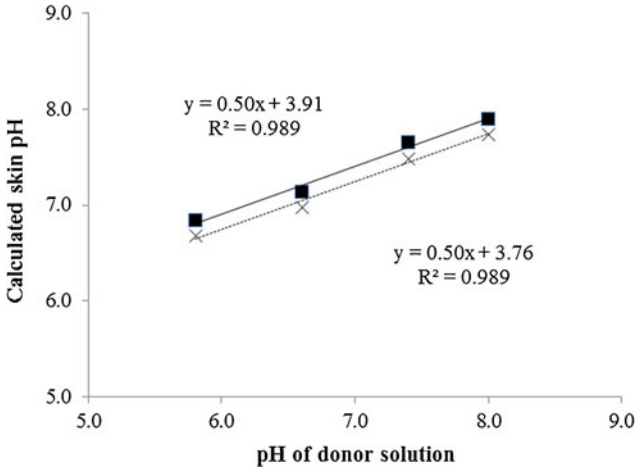

Fig. 6 Effective skin pH determined using the HEM permeation data of propranolol at varying donor solution pH under the asymmetric condition. Symbols: *squares*, pH calculated from saturated propranolol hydrochloride permeation data at pH.7.0; *crosses*, pH calculated from saturated propranolol hydrochloride permeation data at pH.8.0.

SC Lipoidal Pathway and Effective pH of SC in β-Blocker Permeation

Table III presents the results in the permeation experiments with saturated propranolol hydrochloride solutions in the donor at pH 7.0 and 8.0, and the total and intrinsic solubilities of propranolol determined under these conditions. The table also presents the permeability coefficients of the SC lipoidal pathway (P_L) for propranolol, which were determined using the apparent fluxes of propranolol under saturation, the intrinsic aqueous solubility of propranolol, and Eq. 19. These permeability values are within the same order of magnitude and larger than those of the permeability coefficients of the viable epidermis for propranolol, suggesting that the viable epidermis is a barrier to skin permeation of propranolol at the higher pH. This also suggests that the pH in the receiver chamber could affect skin permeation of the more lipophilic β-blockers such as propranolol (see Eq. 14).

The fraction of unionized propranolol in the SC for its permeation at each donor pH (pH 5.8, 6.6, 7.4, and 8.0) was calculated using the permeability data and Eq. 20. The effective pH for SC permeation was then determined by the fraction of unionized propranolol, its pKa, and Eq. 6. Figure 6 shows the effective pH in SC calculated using this method for propranolol SC permeation. It should be pointed out that the two P_L values obtained in the two sets of experiments at pH 7.0 and 8.0 provide two lines in the figure but these two lines almost overlap. In the figure, the calculated SC pH (or effective pH in SC) was approximately the same as the pH in the donor solution at pH 8 but started to deviate from the donor solution pH when the donor pH decreased and became more acidic. This may be related to the human skin buffering capacity reported in the literature (42,43), causing the less than expected effect of donor solution pH upon drug permeation across the skin. Studies are underway to further examine this phenomenon.

Topical β-Blockers for Infantile Hemangioma Treatment

Several recent studies of topical timolol treatment of skin hemangiomas have demonstrated the effectiveness of this strategy for local treatment of the disease (12,16,44). The present study has shown that propranolol and betaxolol are approximately ten times more permeable than timolol across HEM. This suggests that propranolol and betaxolol can be

Table III Total Solubility, Intrinsic Solubility, Flux, and Permeability Coefficient of the SC Lipoidal Pathway for Propranolol

pH in donor chamber	Total solubility of propranolol [a] (mg/mL)	Flux of propranolol (μg/s/cm^2)	Intrinsic solubility of propranolol [a] (mg/mL)	P_L for propranolol [a] ($\times 10^{-5}$) (cm/s)
7.0	58 ± 4	0.012 ± 0.001[a]	0.18 ± 0.02	6.6 ± 1.0
8.0	4.4 ± 0.5	0.013 ± 0.003[b]	0.14 ± 0.02	9.8 ± 2.8

[a] Mean ± SD (n=3)

[b] Mean ± SD (n=4)

better topical skin agents compared to timolol. For example, propranolol and betaxolol can be applied topically at lower concentration (than timolol) on the hemangioma skin to achieve the same drug concentration at the viable epidermal layer or hemangioma tissue (the target site of action). However, accurate comparison of the relative effectiveness of the β-blockers for topical skin application in the treatment of skin hemangiomas would require information on the relative pharmacological potencies and the mechanism of the β-blockers for the disease, which is currently not established (45–47). In addition, the use of steady-state permeability coefficients of HEM to assess the effectiveness of β-blockers in topical skin hemangioma treatment also requires the assumptions of (a) a correlation between the steady-state permeability coefficients (under infinite dose) and finite dose topical drug delivery after topical administration (48,49) and (b) the same β-blocker permeabilities across normal and hemangioma skin. For the development of topical β-blocker formulations, the permeability coefficient *versus* pH relationship illustrated in the present study suggests a less than expected effect of dosage form pH upon topical drug delivery of the β-blockers. As this relationship did not follow the theoretical prediction (i.e., the pH effect was less than the 10-fold increase per pH unit), alkaline topical dosage forms may not be required for effective local delivery of the β-blockers.

CONCLUSION

The present study investigated skin permeation of β-blockers under different pH conditions in the donor solution (pH 5.8, 6.6, 7.4 and 8.0). The results showed that the apparent permeability coefficients of the β-blockers increased with their lipophilicity. The estimated permeability coefficients of the HEM pore pathway were at least an order of magnitude lower than the apparent permeability coefficients of the lipophilic β-blockers, suggesting little contribution of the pore pathway to drug permeation. Together with the permeability *versus* lipophilicity relationship, these results indicate that the lipoidal pathway is the main transport pathway of the β-blockers in SC. While the lipoidal barrier of the SC is the major barrier determining skin permeation of the β-blockers, the viable epidermis could also contribute as a barrier in the permeation of the more lipophilic β-blockers, e.g., propranolol. The results in the present study indicated that the pH of donor solution was a major factor controlling skin permeation of β-blockers when the receiver pH was maintained constant at pH 7.4. However, such pH effect was found to be less than those according to theoretical prediction. A hypothesis was that the deviation from the slope of unity could be attributed to the difference between the pH in the donor solution and the effective pH in SC, probably related to the skin buffering capacity. For drug delivery in practice, the permeability results of the lipophilic β-blockers in the present study (e.g., propranolol and betaxolol) suggest the possibility of topical treatment of hemangioma using these β-blockers.

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