Evaluation of Stereoselective Transdermal Transport and Concurrent Cutaneous Hydrolysis of Several Ester Prodrugs of Propranolol: Mechanism of Stereoselective Permeation

Shamim Ahmed,[1] Teruko Imai,[1,2] and Masaki Otagiri[1]

Received April 12, 1996; accepted July 25, 1996

Purpose. The purpose of this study was to evaluate the stereoselective permeation and concurrent cutaneous hydrolysis of a series of ester prodrugs of propranolol (PL).

Methods. In vitro studies were performed across full-thickness, stripped and diisopropylfluorophosphate (DFP) treated skins of hairless mouse with flow-through diffusion cells at 37°C.

Results. The permeability coefficients (K_p), which were dependent on partition coefficients (PC), of all the prodrugs were markedly increased compared to the parent drug. In full-thickness skin, the (*R*) caproyl-PL (CR-PL) showed the highest K_p, which was about 52-fold greater than that of PL. Most of the more lipophilic prodrugs showed stereoselectivity in K_p ($R > S$). All the prodrugs underwent stereoselective hydrolysis ($R > S$) during penetration. The prodrugs which showed stereoselectivity in permeation were comparatively lipophilic and showed great differences in hydrolysis percentages between the enantiomers. Permeation studies with stripped skin revealed that prodrugs were more permeable across stratum corneum compared to PL, whereas reverse was happened across viable skin. Although CR-PL showed high stereoselectivity in permeation across full-thickness skin and underwent higher percent of concurrent stereoselective cutaneous hydrolysis, the prodrug showed no stereoselectivity in permeation across DFP, an esterase inhibitor, treated skin and the concurrent cutaneous hydrolysis was also stopped.

Conclusions. Lipophilic prodrugs may readily pass the stratum corneum but may not be able to penetrate so easily through the deeper tissues. Unlike the (*S*) isomers, the (*R*) isomers of lipophilic prodrugs almost completely converted to propranolol in epidermis and can easily pass through the dermis layer, resulting in stereoselective penetration.

KEY WORDS: prodrug; penetration; stereoselectivity; hydrolysis.

INTRODUCTION

PL, a widely used β-adrenergic receptor blocker, has a very low and variable oral bioavailability due to extensive and variable hepatic first-pass metabolism. The transdermal route of administration has been demonstrated to be capable of avoiding hepatic first-pass effects, thus achieving higher systemic bioavailabilities of drugs (1). However, the relative impermeability of the stratum corneum, the outermost layer of skin, offers considerable resistance to the penetration of PL through the skin. The prodrug approach represents an alternative and promising method of enhancing the skin permeability of drugs by increasing their lipophilicity (2). In a previous report, using only two prodrugs [isovaleryl-(IV-) and cyclopropanoyl-(CP-) PL], we showed that ester prodrugs of propranolol could enhance the permeability coefficient and also underwent stereoselective hydrolysis in skin (3).

PL is clinically used as a racemic mixture of the pharmacologically active *S*(−) enantiomer and the inactive or less active *R*(+) enantiomer. Stereoselective penetration and hydrolysis may therefore play an important role in delivery of the active isomer of propranolol. Unfortunately, although transdermal delivery has attracted considerable attention during the last two decades, little attention has been paid to transfer characteristics of individual enantiomers of chiral species. In the present study we used ten ester prodrugs of PL with three different types of substitution in order to evaluate their stereoselective transport and concurrent cutaneous hydrolysis and to find out the relationships of stereoselective skin penetration with physicochemical and stereoselective hydrolytic properties during penetration. Furthermore, extensive research has been performed across full-thickness, stripped and esterase inhibitor treated skins of hairless mice to propose the possible mechanism of stereoselective penetration.

MATERIALS AND METHODS

Materials

Racemic PL hydrochloride and bovine serum albumin (fraction V) were purchased from Sigma Chemical Company (St. Louis, MO). DFP was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and reagents used were of analytical grade. The prodrugs were synthesized from racemic PL hydrochloride and fatty acid chlorides (Tokyo Kasei, Tokyo, Japan), according to the method described previously (4). In the present study, PL and its prodrugs were always used as the hydrochloride salt because the prodrug bases undergo intramolecular catalyzed hydrolysis and intramolecular O-N acyl transfer reaction (5).

Animal

Male hairless mice (Kyudo, Fukuoka, Japan), 8–9 weeks old, weighing 28–34 g, were used and the animals were maintained under normal housing conditions (25 ± 2°C, 60% relative humidity, and 12 h light/darkness cycle).

Skin Membrane Preparation

Hairless mice were sacrificed by snapping the spinal cord at the neck. Full-thickness skin was obtained from the dorsal surface. Adhering fat and other visceral debris were removed from the undersurface with tweezers. The excised skin was used immediately. Stripped skin was prepared from full-thickness skin. The stratum corneum was removed by 25 successive strippings using cellophane adhesive tape (Nichiban K. K., Tokyo, Japan), and used immediately. Esterase inhibitor treated skin was prepared by immersing the dermal side of full-thickness skin into 1 mM DFP for 4 h prior to use.

[1] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan.
[2] To whom correspondence should be addressed.

0724-8741/96/1000-1524$09.50/0

Permeation Studies

The in vitro permeation studies were performed using a flow-through diffusion cell according to the method described previously (3). Flow-through diffusion cell was used because it maintains skin viability for longer periods and mimic the in vivo situation well. Briefly, the receiving chamber had a volume of about 4 mL and the area available for diffusion was about 1 cm^2. The skin sample was mounted between the half-cells, with the dermis side in contact with the receptor fluid, 0.01 M acetate buffer (pH 4). In case of permeation study across DFP treated skin, the receptor fluid also contained 1 mM DFP. One milliliter of drug suspension (drug amount; 1.2 times of the amount required for saturation) in pH 4 acetate buffer was added to the donor half-cell. Both half-cells were maintained at 37°C and the receptor chamber was perfused with acetate buffer (pH 4) at a rate of 5 mL/h. Saturation concentration of the prodrugs was maintained in the donor compartment until the end of the penetration experiment. To minimize problems with skin, such as changes in its barrier function and enzyme activity, studies were conducted for only 10 h. The apparent permeation parameters were calculated from total drug (in case of prodrug, total drug means sum of intact prodrug and converted PL) penetrated into the receptor phase using the following equations:

$$J_S = (K_m \cdot D \cdot C)/\delta = K_p \cdot C \quad (1)$$

and

$$\tau = \delta^2/6D \quad (2)$$

where J_S is the steady state flux, K_m denotes the solvent membrane partition coefficient of drug, D is the diffusion coefficient, C is the drug concentration in the donor chamber, δ is the thickness of hairless mouse skin (0.07 cm), K_p denotes the permeability coefficient of drug, and τ represents the lag time.

Assay

PL and intact prodrug were simultaneously extracted from the collected samples and analyzed by HPLC according to the method described previously (3). Ovomucoid-conjugated column (Ultron ES/OVM; 150 × 4.6 mm i.d., Shinwa Chemical Industries, Japan) and Chiralcel OD column (250 × 4.6 mm i.d., Daicel Chemical Industries Ltd., Japan) were used for determination of prodrug and propranolol, respectively. The fluorescence spectrophotometer was used at excitation and emission wavelengths of 285 and 340 nm, respectively. The eluent used for different prodrugs determination consisted of 10% to 26% (v/v) acetonitrile in 20 mM KH_2PO_4, and the eluent used for PL determination consisted of *n*-hexane:ethanol:diethylamine [85:15:0.6 (v/v)].

Statistical Analysis

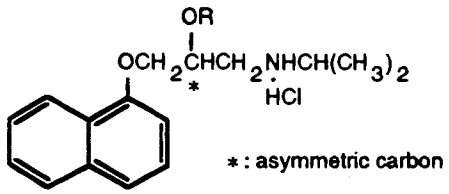
Data are expressed as means ±SD. Statistical analysis was performed with the *t*-test to detemine the significance of differences.

RESULTS AND DISCUSSION

Physicochemical Properties

Solubility, PC and dissociation constant (pKa) of 10 ester prodrugs of PL are listed in Table I. The solubility and PC of each isomer in the racemic prodrugs were determined in pH 4 buffer in which they proved to be most stable (4). Analysis by HPLC showed insignificant degradation (<0.5%) of the esters during the solubility and PC determination. There were no differences in the solubility and PC of (*R*) and (*S*) isomers in racemic compounds. As shown in Table I, the solubility of the esters decreased with increasing chain length of the promoiety of all the prodrugs, except butyryl-PL (BT-PL). The PC of the prodrugs in octanol-pH 4 buffer increased markedly compared with that of PL. The dissociation constant (pKa) of the prodrugs were slightly decreased compared with propranolol, and this type of decrease in pKa value was previously reported with other O-acyl prodrugs of propranolol (5). A small change in pKa values indicates slight effect on the dissociation of drugs in the acidic environment (~pH 5.5) of the skin because all compounds will be primarily in their ionized form due to their higher pKa values (8.59–9.44).

Table I. Structure, Solubility, Partition Coefficient (PC), and Dissociation Constant (pKa) of Propranolol and Its Prodrugs



Propranolol / Prodrug

Compound	R	Solubility[a] (μ mol/ml)	PC[b]	pKa[c]
Propranolol (PL)	H	391.72	2.40	9.44
O-Acetyl PL (AC-PL)	$COCH_3$	33.69	4.15	9.28
O-Propionyl PL (PP-PL)	$COCH_2CH_3$	31.21	11.36	—
O-Butyryl PL (BT-PL)	$CO(CH_2)_2CH_3$	162.73	34.60	9.02
O-Valeryl PL (VL-PL)	$CO(CH_2)_3CH_3$	17.16	97.35	—
O-Caproyl PL (CR-PL)	$CO(CH_2)_4CH_3$	6.40	197.00	—
O-Isobutyryl PL (IB-PL)	$COCH(CH_3)_2$	44.22	30.50	9.13
O-Isovaleryl PL (IV-PL)	$COCH_2CH(CH_3)_2$	13.56	88.90	8.59
O-Isocaproyl PL (IC-PL)	$CO(CH_2)_2CH(CH_3)_2$	7.69	99.10	—
O-Cyclopropanoyl PL (CP-PL)	$COcC_3H_5$	22.95	14.40	8.72
O-Cyclohexanoyl PL (CH-PL)	$COcC_6H_{11}$	3.60	112.00	—

[a] Solubility was determined in pH 4.0 acetate buffer at 37°C; the values represent the solubility of each isomer from racemate. The actual values of the solubility of racemic compounds are twice the values listed in the table.

[b] Partition experiments were performed in 1-octanol and pH 4.0 phosphate buffer at 25°C.

[c] pKa values were determined in deionized water at 25°C.

Permeation Studies

The best way to evaluate the penetration potential of a compound is to perform experiments using human skin, but unfortunately fresh human skin is not available.

On the other hand, hairless mouse skin has been widely used as a model for permeation experiments (6) as well as study to measure esterase activity in skin (7). Therefore, the in vitro stereoselective permeation of propranolol and its prodrugs was studied using hairless mouse skin.

Although all the prodrugs were more or less hydrolyzed during permeation and penetrated as both intact form and converted PL, to make the discussion simple only the total penetrated amount (sum of intact prodrug and converted PL) of each isomer of the prodrugs was considered. The cumulative penetrated amount (Q_{10}) of PL and its prodrugs after 10 h and the apparent permeation parameters are shown in Table II. The highest Q_{10} and flux (J_S) were obtained with BT-PL. The other prodrugs showed Q_{10} and J_S comparable or less to that of PL, although 2-110-fold lower concentration of prodrugs were used in the donor compartment. The K_P values of all the prodrugs were enhanced many folds compared to PL. K_P showed a linear positive correlation with PC in a double logarithmic scale (Figure not shown). CR-PL showed the highest K_P value, which was about 52- and 38- fold greater [(*R*) and (*S*) isomers, respectively] than that of PL. The higher K_P values of the prodrugs supports their superiority over PL for transdermal delivery. Thus, most of the prodrugs appear promising, especially with regard to greater permeation using lower doses of drug which is always preferable and is necessary to minimize skin irritation.

PL did not show any stereoselectivity in penetration across hairless mouse skin as reported by Heard et al. across human skin (8). However, most of the prodrugs of higher lipophilicity, e.g. BT-PL, valeryl PL (VL-PL), CR-PL, isocaproyl PL (IC-PL) and cyclohexanoyl PL (CH-PL), showed stereoselectivity in Q_{10}, J_S and K_P ($R > S$). The stereoselectivity of lag time of the isomers of these prodrugs was found opposite ($S > R$).

We investigated from the view point of the structure-activity relationship why the transdermal penetration of most of the lipophilic prodrugs were stereoselective. As expected from Table II, a very good linear correlation ($r = 0.98$) was found between the stereoselectivity of K_P and lag time of the prodrugs and the slope of the straight line was also found to be 0.93 (Figure not shown), indicating that the stereoselectivity could be due to the difference of diffusion coefficient between (*R*) and (*S*) enantiomers. As we have already mentioned that skin possesses stereoselective metabolic activity which is very high in its cytosol (3), the prodrugs were easily hydrolyzed during penetration resulting in stereoselectivity of intact prodrug as well as delivered propranolol concentration in the receptor fluid. Table II shows the hydrolyzed percentage (after 10 h) of the isomers of all the prodrugs; hydrolysis of (*R*) isomers of all the prodrugs was found to be remarkably greater than that of the correspondent (*S*) isomers. The *R/S* ratios of the hydrolyzed percentage of the isomers of the prodrugs at 10 h ranged from

Table II. Cumulative Penetrated Amount at 10 h (Q_{10}), Apparent Permeation Parameters, and Hydrolyzed Percentage (after 10 h) of the Isomers of Propranolol Prodrugs Through Full-thickness Skin of Hairless Mouse

Compound		Q_{10}(nmol/cm^2)	R/S	J_S(nmol/cm^2.h)	$K_p \times 10^4$(cm/h)	R/S	τ (h)	S/R	Hydrolyzed percentage	R/S
PL	(R)	164.0 ± 34.9	1.00	30.57 ± 2.74	0.78 ± 0.07	1.01	4.85 ± 0.44	0.99	—	—
	(S)	164.0 ± 33.2		30.22 ± 2.74	0.77 ± 0.07		4.79 ± 0.40			
AC-PL	(R)	169.4 ± 24.5	0.99	24.22 ± 3.00	7.19 ± 0.89	0.99	3.13 ± 0.16	1.01	6.8 ± 0.9*	2.83
	(S)	170.6 ± 24.3		24.53 ± 3.10	7.28 ± 0.92		3.17 ± 0.16		2.4 ± 0.4	
PP-PL	(R)	85.6 ± 10.9	0.93	19.48 ± 2.31	6.24 ± 0.74	0.87	6.12 ± 0.22	0.91	37.1 ± 5.2*	15.46
	(S)	91.7 ± 10.4		22.44 ± 2.56	7.19 ± 0.82		5.63 ± 0.18		2.4 ± 0.4	
BT-PL	(R)	253.8 ± 41.3*	1.24	56.96 ± 5.37*	3.49 ± 0.33*	1.09	6.08 ± 0.38*	1.13	64.8 ± 4.2**	4.95
	(S)	205.5 ± 28.3		52.23 ± 5.37	3.21 ± 0.33		6.87 ± 0.20		13.1 ± 5.1	
VL-PL	(R)	149.8 ± 1.1*	1.56	31.46 ± 4.26*	18.33 ± 2.48*	1.21	5.58 ± 0.70*	1.30	89.6 ± 1.6**	2.74
	(S)	95.9 ± 3.5		26.01 ± 2.45	15.14 ± 1.43		7.23 ± 0.89		32.7 ± 4.3	
CR-PL	(R)	181.0 ± 21.7*	1.74*	26.11 ± 1.98*	40.83 ± 3.10*	1.38	2.99 ± 0.31*	1.57	96.8 ± 3.0**	1.38
	(S)	104.5 ± 15.5		18.87 ± 2.18	29.49 ± 3.40		4.68 ± 0.89		69.9 ± 7.8	
IB-PL	(R)	120.2 ± 41.2	1.00	21.85 ± 3.67	4.94 ± 0.83	0.95	4.71 ± 0.68	1.07	18.2 ± 7.8*	4.79
	(S)	120.0 ± 41.3		23.06 ± 3.45	5.21 ± 0.78		5.05 ± 0.82		3.8 ± 1.6	
IV-PL	(R)	158.7 ± 20.3	1.00	32.62 ± 5.84	24.07 ± 4.31	1.01	5.48 ± 0.25	0.99	10.7 ± 2.6*	9.73
	(S)	158.7 ± 12.7		32.38 ± 6.85	23.89 ± 5.05		5.45 ± 0.56		1.1 ± 0.7	
IC-PL	(R)	123.9 ± 13.8*	2.47	19.75 ± 1.35*	25.67 ± 1.76*	1.54	3.81 ± 0.28*	1.55	95.6 ± 0.4**	2.07
	(S)	50.2 ± 8.3		12.63 ± 0.62	16.42 ± 0.80		5.90 ± 0.42		46.2 ± 8.4	
CP-PL	(R)	153.3 ± 57.1	0.91	29.15 ± 4.22	12.62 ± 1.83	0.95	4.69 ± 1.29	0.98	8.2 ± 2.4*	13.67
	(S)	167.8 ± 52.1		30.60 ± 5.99	13.33 ± 2.22		4.78 ± 1.06		0.6 ± 0.2	
CH-PL	(R)	49.3 ± 1.6*	4.81	7.77 ± 0.53*	21.59 ± 1.47*	2.14	3.75 ± 0.22*	2.25	96.9 ± 1.0**	2.84
	(S)	10.3 ± 1.6		3.63 ± 0.66	10.09 ± 1.83		8.42 ± 1.09		34.1 ± 5.4	

Note: Values are the mean ± S.D. (n = 4). * $p < 0.05$ vs. corresponding S- isomer; ** $p < 0.01$ vs. corresponding S- isomer.

1.4–15.5. Therefore, the time required for hydrolysis and the difference of diffusion coefficient between PL and prodrug might influence the stereoselective transport of prodrug. The chemical hydrolysis of the prodrugs in pH 4 buffer was found to be non stereoselective and insignificant (<1%) during the experimental period, indicating that the stereoselective hydrolysis of the prodrugs during penetration was enzymatic. Furthermore, the possibility of wash out of enzymes during penetration was investigated by separate experiment and no esterase leaching was found when the esterase activity of washed buffer was checked every 2 h up to 12 h (3). In all types of promoiety, the hydrolysis activity was generally increased with increases in carbon number, suggesting that hydrophobic interaction plays an integral part in the hydrolysis of propranolol prodrugs by skin esterase(s). However, it may be mentioned here that CP-PL and IV-PL showed lower cutaneous hydrolysis as compared with their lipophilicity which means that factors other than lipophilicity are also important, e.g., structure of prodrug moiety which may cause high steric hindrance (9).

Figure 1(A) revealed that when the difference of hydrolysis % between (*R*) and (*S*) isomers of any prodrug except CR-PL was less than 50%, it failed to show stereoselectivity in permeation. Especially, the prodrugs which are less susceptible to enzymatic hydrolysis [e.g., acetyl PL (AC-PL), IV-PL and CP-PL] showed similar permeation for both (*R*) and (*S*) isomers. The prodrugs which underwent greater percentages of hydrolysis with the (*R*) isomers showed stereoselective permeation. Figure 1(B) shows the relation of stereoselective permeation with the relative lipophilicity of the prodrugs, expressed as the ratio of log PC of prodrug and log PC of PL. Here, the more lipophilic prodrugs ($\log PC_{prodrugs}/\log PC_{propranolol} > 4.5$) showed stereoselective permeation. In the case of CR-PL, although the difference in the hydrolyzed percentages of its isomers was small, it did show stereoselective penetration. This is because CR-PL is the most lipophilic prodrug among those examined, so there was a large difference in diffusion between its intact form and converted propranolol across viable skin. On the other hand, although BT-PL showed large difference in the hydrolyzed percentage between its isomers, there was little stereoselectivity in permeation because the prodrug has low lipophilicity, which failed to result in big differences in diffusion between intact prodrug and converted propranolol. The prodrugs which showed stereoselectivity in permeation were more lipophilic and had greater stereoselective differences in hydrolyzed percentage of the isomers.

Moreover, to propose the possible mechanism of stereoselective permeation of propranolol prodrugs across hairless mouse skin, detailed permeation studies with stripped skin and enzymatically inactive skin were done. At first, in order to clarify the difference in barrier functions of stratum corneum and viable skin between hydrophilic PL and lipophilic prodrug (intact), the permeation of (*S*) PL and (*S*) IV-PL across full-thickness skin and stripped skin was compared (Figure 2). Here the (*S*) isomer of IV-PL was used as lipophilic prodrug because of its resistance to cutaneous hydrolysis. It is evident from the Figure that although the permeation of PL and prodrug is same from full-thickness skin, the permeability of PL tremendously increased across stripped skin, IV-PL failed to show such enhancement. Therefore, it can be concluded that stratum corneum is the main barrier for permeation of PL, while the drug can pass very easily across hydrophilic epidermis and dermis. Stratum corneum is also a barrier for lipophilic drug but the resistance is less as compared with PL. Moreover, the lipophilic prodrugs are less permeable across viable epidermis and dermis.

In order to get direct evidence that stereoselective hydrolysis has great influence on stereoselective permeation of the prodrugs, permeation experiments were performed with CR-PL across stripped skin and enzymatically inactive or less active full-thickness skin. Figure 3 indicates the stereoselective penetration of CR-PL across full-thickness, DFP treated and stripped skins. DFP has already been reported by many researchers to inhibit esterase activity of skin markedly (10). It was reported that DFP had less effect to the stratum corneum barrier properties and, hence, delivery because incubation of human stratum corneum in DFP resulted in no changes in lipid or protein thermal transition as measured by differential scanning calorimetry (11,12). It is evident from the Figure 3 that hydrolysis of CR-PL was completely stopped during permeation across DFP treated skin. There was no preferential formation of hydrophilic (*R*) PL resulting no stereoselectivity in permeation. We have already shown that IV-PL, a hydrolysis resistant lipophilic prodrug, did not show any stereoselectivity during permeation across enzymatically active full-thickness skin. The present result indicated that if the enzymatic activity of full-thickness skin could be destroyed at that time hydrolysis

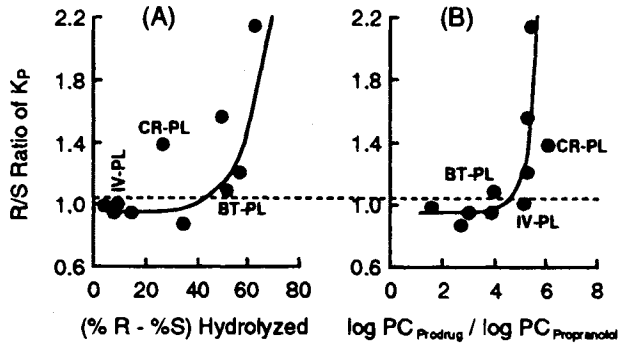


Fig. 1. Relations of stereoselective permeation of the prodrugs with differences in percentage of hydrolysis between their isomers (A) and their relative partition coefficients (as compared with propranolol) (B). The compounds above the dotted line showed statistically significant stereoselectivity in permeation.

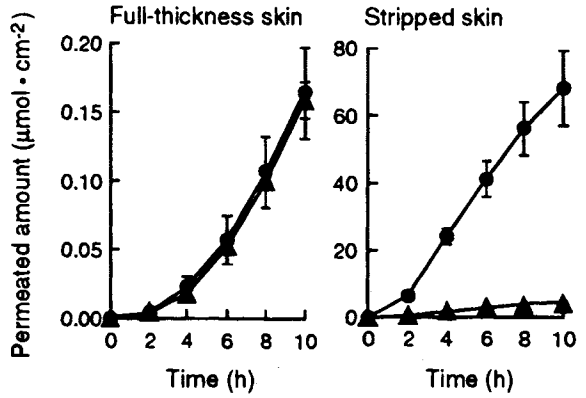


Fig. 2. Cumulative penetrated amount of (*S*) propranolol (circle) and (*S*) isovaleryl propranolol (triangle) across hairless mouse skin.

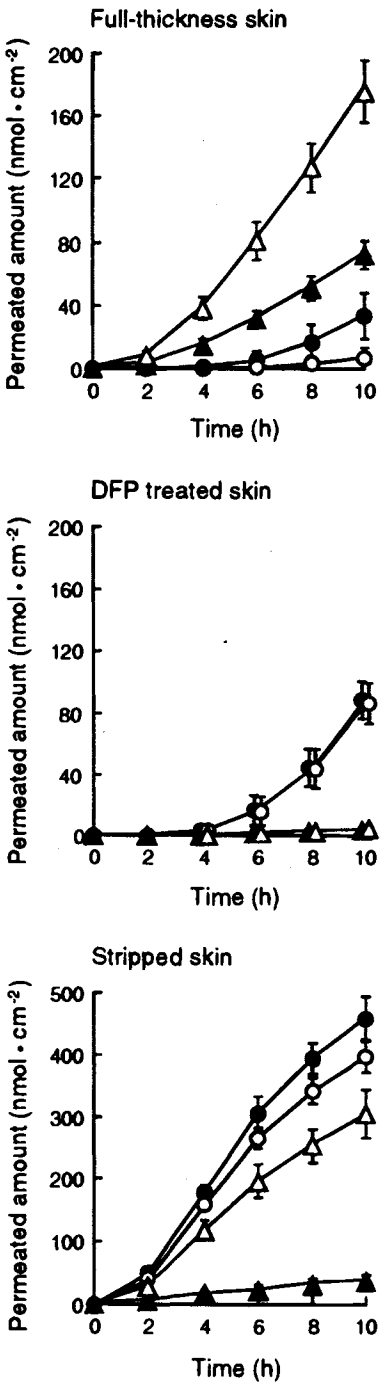


Fig. 3. Permeation profiles of caproyl propranolol across full-thickness, DFP treated and stripped skins of hairless mouse. Circles and triangles represent intact prodrug and converted propranolol, respectively. Open and closed symbols represent (*R*) and (*S*) isomers, respectively.

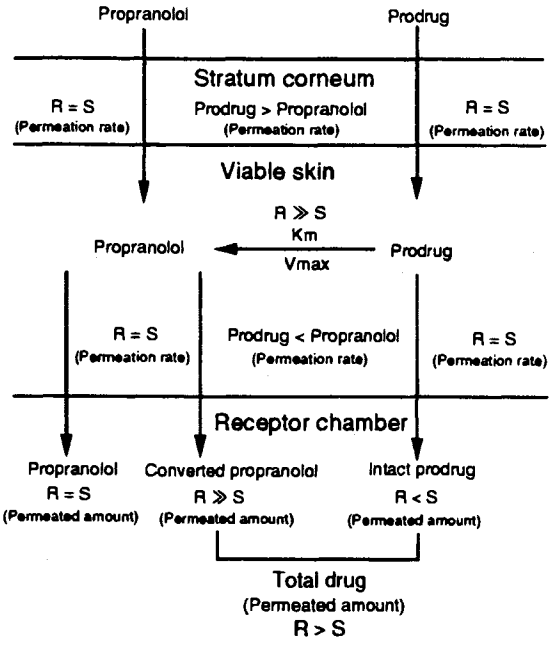


Fig. 4. Possible mechanism of stereoselective penetration of lipophilic propranolol prodrugs across hairless mouse skin.

sensitive prodrug also did not show enantiomeric differences in permeation. Furthermore, limited cutaneous hydrolysis was evident from the permeation of CR-PL across stripped skin. The permeation of intact CR-PL was greater than converted propranolol across stripped skin, although opposite result was obtained across full-thickness skin. But interestingly, the amount of converted (*R*)-PL across stripped skin was greater than that of across full-thickness skin, whereas the amount of converted (*S*)-PL was decreased in stripped skin, indicating that hydrolysis of (*S*) isomer was inhibited by (*R*) isomer. However, the decrease in hydrolysis percent in stripped skin might be because when the stratum corneum was removed from skin, the permeability of CR-PL was increased by many folds, and the amount of esterases became insufficient for hydrolysis of the prodrug, indicating enzyme saturation, i.e., non linear hydrolysis kinetics in stripped skin. This enzyme saturation was also supported by the previously reported results of CH-PL (13). When the hydrolysis rates of the isomers of CH-PL during penetration across both full-thickness and stripped skins were analyzed, it was observed that the hydrolysis rate was gradually increased with time due to increase concentration of prodrug in epidermis of full-thickness skin, whereas the hydrolysis rates become very fast and were decreased or remained constant for both (*R*) and (*S*) isomers during permeation across stripped skin.

Finally, on the basis of all the results, the mechanism of stereoselective permeation of lipophilic prodrug has been proposed and shown schematically by Figure 4. Stratum corneum is lipid-like in nature and favors the penetration of lipophilic drug, but the viable epidermis and dermis are hydrophilic and present a substantial barrier for lipophilic drug. Therefore, the prodrugs of higher lipophilicity readily enter and diffuse within the stratum corneum but be unable to penetrate so easily through the viable epidermis and dermis. The skin esterases are mainly present in viable epidermis (14) and the prodrugs were hydrolyzed here in a non linear hydrolysis kinetics. Since the hydrolysis of (*R*) isomers of most of the prodrugs of higher lipophilicity is very fast, the (*R*) isomers almost converted to propranolol, which is hydrophilic, and can easily passes the dermis layer. Similar results have been obtained with human and mouse skins in vitro, where metabolism of topically applied xenobiotics resulted in a polar compounds which were preferen-

tially removed to the aqueous buffer (10,15). However, the (*S*) isomer of the prodrugs mainly remained as intact form and faces difficulties in passing the dermal layer due to their higher lipophilicity. Therefore, it can be concluded that the stereoselective penetration was not due to stereoselective transport of PL or intact prodrug, but due to the great differences in hydrolysis percentages between the enantiomers and the great difference in lipophilicity between prodrug and PL.

REFERENCES

1. M. Corbo, J. C. Liu, and Y. Chien. *J. Pharm. Sci.*, **79**:584–587 (1990).
2. S. Y. Chan and A. L. W. Po. *Int. J. Pharm.*, **55**:1–16 (1989).
3. S. Ahmed, T. Imai, and M. Otagiri. *J. Pharm. Sci.*, **84**:877–883 (1995).
4. M. Shameem, T. Imai, M. Otagiri. *J. Pharm. Pharmacol.*, **45**:246–252 (1993).
5. A. Buur, H. Bundgaard, and V. H. L. Lee. *Int. J. Pharm.*, **42**:51–60 (1988).
6. R. J. Scheuplein and R. L. Bronaugh. In: L. A. Goldsmith (ed.), *Biochemistry and Physiology of the Skin*, Oxford University Press, New York, 1983, pp. 1255–1295.
7. C. D. Yu, N. A. Gordon, J. L. Fox, W. I. Higuchi, and N. F. H. Ho. *J. Pharm. Sci.*, **69**:775–780 (1980).
8. C. M. Heard, A. C. Watkinson, K. R. Brain, and J. Hadgraft. *Int. J. Pharm.*, **90**:R5–R8 (1993).
9. M. K. Ghosh and A. K. Mitro. *Pharm. Res.*, **7**:251–255 (1990).
10. R. O. Potts, S. C. McNeill, C. R. Desbonnet and E. Wakshull. *Pharm. Res.*, **6**:119–124 (1989).
11. M. Goodman and B. W. Barry. *Anal. Proc.*, **23**:397–398 (1986).
12. G. M. Golden, J. E. McKie and R. O. Potts. *J. Pharm. Sci.*, **76**:25–28 (1987).
13. S. Ahmed, T. Imai, and M. Otagiri. *Pharm. Sci.*, **1**:429–432 (1995).
14. R. C. Wester and P. K. Noonan. *Int. J. Pharm.*, **7**:99–110 (1980).
15. J. Kao and J. Hall. *J. Pharm. Exp. Ther.*, **241**:482–487 (1987).