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Analysis of in vitro skin penetration of acyclovir prodrugs based on a diffusion model with a metabolic process

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Abstract

The penetration of seven acyclovir prodrugs through the rat skin with or without an enhancer, 1-geranylazacycloheptan-2-one (GACH), was analyzed based on a newly developed two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum including metabolic process. The Laplace-transformed equations for prodrug and regenerated acyclovir were derived from Fick's second law assuming first-order hydrolysis and were fitted to the experimental data. Under the condition without GACH treatment, more lipophilic prodrugs gave higher partition parameters in the nonpolar route. The enzymatic hydrolysis rate constants estimated by the model analysis of the penetration experiment were basically similar in rank order to those obtained using skin homogenate. Concerning the effect of GACH, the estimated partition parameters of prodrugs in the nonpolar route increased with an increase in pretreatment dose of GACH, but their diffusivities were little affected being in good agreement with the theoretical prediction. In addition, GACH significantly decreased the enzymatic hydrolysis rate constants of all prodrugs in the skin.

Keywords: Percutaneous absorption; Acyclovir; Acyclovir prodrug; Enhancer treatment; Metabolism; Diffusion model

1. Introduction

In a series of our investigation, we have demonstrated that skin penetration of drug can be efficiently enhanced by a prodrug and enhancer combination (Bando et al., 1994). In addition, a two-layer diffusion model with polar and nonpolar routes in the stratum corneum enabled us to predict the enhancement effect by the combination approach (Bando et al., 1996). However, the comprehensive understanding of bioconversion process of prodrugs in the skin should also be needed to accomplish an improvement of drug penetration by this approach.

There are several methods for measuring the metabolic rate of drug in skin; that is, the usage of skin homogenate (Fort and Mitra, 1994; Ghosh and Mitra, 1990; Seki et al., 1990; Yano et al., 1991), uptake-metabolism experiment (Yu et

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al., 1979a; Valia et al., 1985), and model analysis of drug transport (Liu et al., 1990, 1991; Tojo et al., 1994; Yu et al., 1979b, 1980a,b). In vitro experiment using skin homogenate is convenient, but it is unclear whether the result reflects actual metabolism of drug in the process of penetration through the skin. The uptake-metabolism experiment is superior in this point of view, in which the skin structure is kept intact. However, this method is applicable only to in vitro condition because a drug is applied to dermal side. On the other hand, the analysis of transport and metabolite of a drug based on a skin diffusion model is free from these problems.

Evaluation of a metabolic rate by means of model analysis has been often done in the experiments using tape-stripped skin (Liu et al., 1990, 1991; Tojo et al., 1994; Yu et al., 1980a,b). By neglecting the penetration process through the stratum corneum, not only enzymatic degradation rate constants but also the distribution of skin enzymes could be more easily estimated and discussed at steady-state (Liu et al., 1990, 1991; Tojo et al., 1994; Yu et al., 1980a,b) or non-steady-state condition (Tojo et al., 1994). However, the metabolic activities of viable cells may be changed in this case because the cells are exposed to a vehicle and, in addition, we cannot clarify the effect of enhancers on metabolic activity of the skin.

On the other hand, we have developed an approach to analyzing whole skin permeation process by the use of a least-square regression program, MULTI(FILT), combined with a fast inverse Laplace transform (FILT) algorithm (Yamashita et al., 1993). This approach enables us to elucidate skin permeation of drugs in terms of diffusion and partitioning, even if a complex skin diffusion model, i.e. a two-layer model with parallel pathways, is assumed.

In this study, we derived Laplace-transformed equations for drug transport based on a diffusion model including metabolic process in the skin and evaluated each process of penetration, i.e. diffusion, partitioning and metabolism. The permeation of a series of prodrugs of acyclovir through the skin treated with or without an enhancer, 1-geranylazacycloheptan-2-one (GACH), was analyzed based on the present model.

2. Materials and methods

2.1. Materials

GACH was synthesized by Kuraray Co., Okayama, Japan. Acyclovir (I) was kindly supplied from Nippon Wellcome K.K., Osaka, Japan. Esterification of acyclovir was carried out as described previously (Bando et al., 1996). Chemical structures of acyclovir prodrugs tested (II–VIII) are in Fig. 1. Radiolabeled ^{14}C-mannitol (MT) was obtained from Daiichi Pure Chemicals, Japan. Other materials were obtained commercially from Nacalai Tesque Inc., Kyoto, Japan.

2.2. Hydrolysis of the prodrugs in skin homogenate

Hydrolysis of acyclovir prodrugs in the presence and absence of rat skin homogenate was measured. One gram of full-thickness abdominal skin was homogenized in 5 ml of phosphate buffered saline (PBS) (pH 7.4) at 4°C and the supernatant was obtained after centrifugation at 10000 g (SCR20B, Hitachi Koki Co., Tokyo, Japan). To the prodrug solution in 25 ml of PBS (pH 7.4), 5 ml of the supernatant was added to give an initial concentration of 0.005, 0.05 and 0.5 mM, and a 1-ml sample was periodically withdrawn for determination. The chemical stability was studied in PBS (pH 7.4). The rates of enzymatic hydrolysis were evaluated with pseudo-first-order rate constants in 3.3% (w/v) homogenates.

Fig. 1. Chemical structures of acyclovir prodrugs tested. (I) acyclovir: R = H; (II) acetate: R = CH_3CO; (III) propionate: R = CH_3CH_2CO; (IV) butyrate: R = $CH_3(CH_2)_2CO$; (V) valerate: R = $CH_3(CH_2)_3CO$; (VI) hexanoate: R = $CH_3(CH_2)_4CO$; (VII) isovalerate: R = $(CH_3)_2CHCH_2CO$; (VIII) pivarate: R = $(CH_3)_3CHCO$.

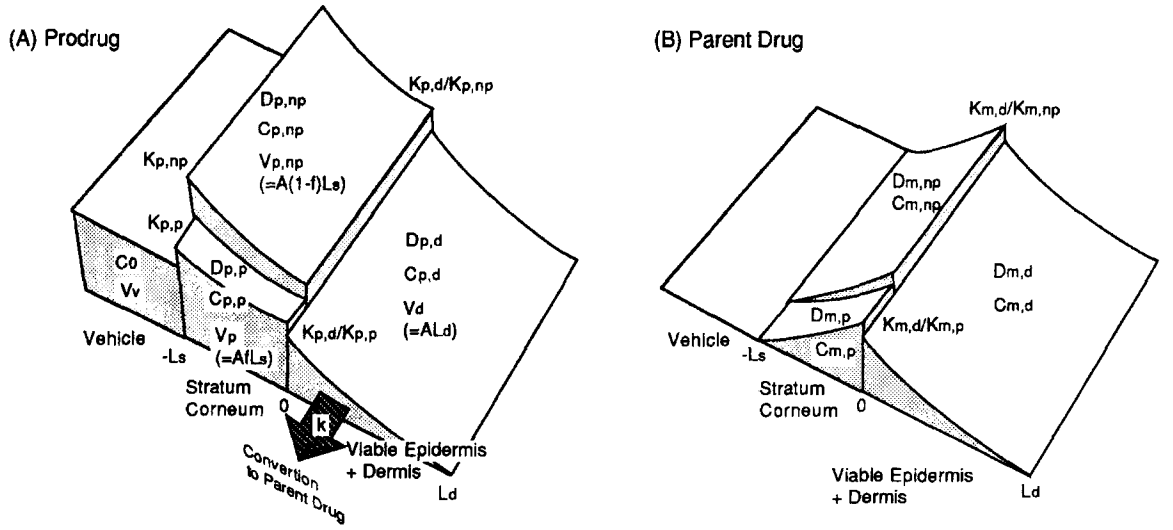

Fig. 2. Physiological skin diffusion/bioconversion model with polar and nonpolar routes in the stratum corneum. (A) diffusion model for a prodrug, (B) shows diffusion model for regenerated parent drug. Well-stirred and sink conditions are assumed in the vehicle and receptor, respectively. Note that each partition coefficient is defined against the vehicle. K, partition coefficient; D, diffusion coefficient; V, volume; L, distance; f, area fraction; v, vehicle; p, polar domain; np, nonpolar domain; d, viable epidermis and dermis: first subscripts p and m reveal prodrug and metabolized parent drug, respectively.

2.3. In vitro skin penetration experiment

The data of acyclovir and its prodrugs (II–VI) used for the present analysis were obtained previously with a full-thickness rat skin diffusion experiments (Bando et al., 1996). In this study, the same penetration experiments were additionally carried out for the structural isomers (VII, VIII) of V. In vitro penetration through tape-stripped skin was also studied to evaluate viable epidermis and dermis penetration. In brief, the rat abdominal skin was stripped 15 times with adhesive tape (Scotch tape, Sumitomo 3M Co., Japan), excised and punched out to yield a disk after removal of hair with a clipper. The skin sample was mounted on a diffusion cell and pretreated with saline for 6 h. Saline was then removed by blotting with tissue paper, and the drug was applied in a PBS suspension to reduce osmotic convective flow. The dermal side of the skin was continuously washed with the same PBS containing 30% (v/v) ethanol. The sample was analyzed by HPLC after adequate dilution by distilled water. The rest of the experimental procedures were the same as for intact skin reported previously (Bando et al., 1994).

The polar route penetration was estimated with diffusion experiment using ^{14}C-MT as described previously (Yamashita et al., 1994). The receptor fluid flowed at a rate of 5 ml/h and collected every 60 min for 12 h. The radioactivities of ^{14}C-MT in the receptor fluid were determined by liquid scintillation counter (Aloka Lsc-900, Japan).

2.4. Data analysis

Fig. 2 shows the diffusion model employed in the present analysis. (A) shows a diffusion model for prodrugs and (B) shows that of regenerated parent drug. The skin is considered to be composed of two serial layers, stratum corneum and the underlying viable tissue, with parallel polar and nonpolar routes in the stratum corneum. The polar route in the stratum corneum is assumed to be filled with water (Ackermann and Flynn, 1987; Ackermann et al., 1987). A well-stirred condition in the donor solution and sink condition in the receptor phase is also assumed. In addition, the following assumptions are made: (a) The hydrolytic enzyme, non-specific esterase, is homogeneously distributed in the secondly layer. (b) The

hydrolysis follows first-order reaction kinetics. (c) The stratum corneum essentially serves as a diffusion barrier. Thus, prodrugs which penetrate through the stratum corneum are metabolized to the parent drug in the viable epidermis and dermis, and then the parent drug diffused in the skin according to the drug concentration gradient. Based on this model, the Laplace transform for the amount of prodrug and parent drug appearing in the receptor across the intact and stripped skin are expressed as follows;

$$\tilde{Q}_{p,int} = C_0 Z_{p,d}(s+k)^*(Z_{p,p}\sinh d_{p,np} + Z_{p,np}\sinh d_{p,p}/s^2/k(s) \quad (1)$$

$$\tilde{Q}_{p,st} = C_0 Z_{p,d}(s+k)/s^2/\sinh d_{p,d} \quad (2)$$

$$\tilde{Q}_{m,int} = C_0 V_d K(Z_{p,p}\sinh d_{p,np} + Z_{p,np}\sinh d_{p,p}) * (m(s)/l(s) - d_{p,d})/s^2/(d_{p,d}^2 - d_{m,d}^2)/k(s) \quad (3)$$

$$\tilde{Q}_{m,st} = C_0 Z_{p,d} k d_{p,d}(n(s)/o(s) - d_{p,d})/(d_{p,d}^2 - d_{m,d}^2)/s^2/\sinh d_{p,d} \quad (4)$$

Where s is the Laplace operator with respect to time and C_0 is the concentration in the donor.

$$d_{p,p} = L_s\sqrt{s/D_{p,p}} \quad (5)$$

$$d_{p,np} = L_s\sqrt{s/D_{p,np}} \quad (6)$$

$$d_{p,d} = L_d\sqrt{(s+k)/D_{p,d}} \quad (7)$$

$$d_{m,p} = L_s\sqrt{s/D_{m,p}} \quad (8)$$

$$d_{m,np} = L_s\sqrt{s/D_{m,np}} \quad (9)$$

$$d_{m,d} = L_d\sqrt{s/D_{m,d}} \quad (10)$$

$$Z_{p,p} = K_{p,p}V_p/d_{p,p} \quad (11)$$

$$Z_{p,np} = K_{p,np}V_{np}/d_{p,np} \quad (12)$$

$$Z_{p,d} = K_{p,d}V_d/d_{p,d} \quad (13)$$

$$Z_{m,p} = K_{m,p}V_p/d_{m,p} \quad (14)$$

$$Z_{m,np} = K_{m,np}V_{np}/d_{m,np} \quad (15)$$

$$Z_{m,d} = K_{m,d}V_d/d_{m,d} \quad (16)$$

$$k(s) = Z_{p,p}\cosh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} + Z_{p,np}\sinh d_{p,p}\cosh d_{p,np} * \sinh d_{p,d} + Z_{p,d}\sinh d_{p,p}\sinh d_{p,np}\cosh d_{p,d} \quad (17)$$

$$l(s) = Z_{m,p}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{m,d} + Z_{m,np}\sinh d_{m,p}\cosh d_{m,np} * \sinh d_{m,d} + Z_{m,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{m,d} \quad (18)$$

$$m(s) = Z_{m,p}d_{m,d}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{p,d} + Z_{m,np}d_{m,d}\sinh d_{m,p}\cosh d_{m,np} * \sinh d_{p,d} + K_{m,d}D_{m,d}d_{m,d}d_{p,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{p,d} \quad (19)$$

$$n(s) = V_v d_{m,d}\sinh d_{p,d} + Z_{m,d}d_{p,d}\cosh d_{p,d} \quad (20)$$

$$o(s) = V_v\sinh d_{m,d} + Z_{m,d}\cosh d_{m,d} \quad (21)$$

where V_v is the volume of vehicle; $D_{i,j}$, $K_{i,j}$ and $V_{i,j}$ (i = p, m and j = p, np, d) are the diffusion coefficient in the j domain, the partition coefficient between the j domain and vehicle, and the effective volume of the j domain for diffusion, respectively; and the first subscripts p and m reveal prodrug and metabolized acyclovir, respectively, and the second subscripts s, p, np and d denote the stratum corneum, the polar route, and the nonpolar route and the second layer, respectively. V_j is obtained from the area (A), area fraction of the polar route (f) and diffusional pathlength (L_j) as,

$$V_p = AfL_s \quad (22)$$

$$V_{np} = A(1-f)L_s \quad (23)$$

$$V_d = AL_d \quad (24)$$

The penetration profiles were analyzed based on this model. Curve-fitting of Eq. (1), (2), (3) and (4) to penetration data was conducted using the nonlinear regression program MULTI(FILT) (Yano et al., 1989) on the main frame computer M-382 of the Kyoto University Data Processing Center. Because of the difficulty in determining the real diffusion pathlength, we involving diffusional pathlength as follows:

$$D'_j = D_j/L_j^2 \quad (25)$$

$$K'_j = K_j V_j \ (j = p, np \text{ or } d) \quad (26)$$

The aimed seven hybrid parameters for each prodrug were determined according to the following procedures: At first, $D'_{i,d}$ and $K'_{i,d}$ values were obtained from fitting Eq. (2) to a penetration profile through the tape-stripped skin under the assumption that all of the drugs penetrated through the skin without degradation (k = 0) because enzymatic activity was removed by 30% (v/v) ethanol in the receptor fluid. Secondly, the penetration profiles of MT through the intact skin were analyzed using Eq. (1) under the assumptions that MT, a highly hydrophilic compound, only penetrates the polar domain in the stratum corneum ($K_{np} = 0$) and that MT is not metabolized in the skin (k = 0). Since the partitioning from the aqueous vehicle to the polar route (water channel) is considered to be unity for all penetrants (Ackermann et al., 1987), the obtained parameters were treated as the common ones for all tested drugs. On the other hand, diffusion parameters in the polar route of the tested drugs were corrected based on their molecular weight (Lambart et al., 1989). Thirdly, the parameters corresponding to the nonpolar route for acyclovir ($D'_{m,np}$ and $K'_{m,np}$)were estimated by fitting Eq. (1) to the penetration profile of acyclovir with four pre-determined parameters ($D'_{m,d}$, $D'_{m,p}$, $K'_{m,d}$ and $K'_{m,p}$) under k = 0 as acyclovir did not metabolize through the skin. Finally, the parameters corresponding to the nonpolar route for acyclovir prodrugs ($D'_{p,np}$ and $K'_{p,np}$) and enzymatic hydrolysis rate constant (k) were estimated by simultaneous fitting of Eq. (1) and Eq. (3) to the penetration profiles of prodrugs and the regenerated acyclovir. Ten pre-determined parameters ($D'_{m,d}$, $D'_{m,p}$, $D'_{m,np}$, $K'_{m,d}$, $K'_{m,p}$, $K'_{m,np}$, $D'_{p,d}$, $D'_{p,p}$, $K'_{p,d}$ and $K'_{p,p}$) were utilized in the calculation.

3. Results

3.1. Hydrolysis of the prodrugs in skin homogenate

All prodrugs were very stable in PBS (pH 7.4) and hydrolysis rate constants were in the range from 1.01×10^{-3} (V) to $1.8\ 3 \times 10^{-3}$ h^{-1} (II). Hydrolysis of the prodrugs in PBS with rat skin homogenate was examined (Fig. 3). Hydrolysis rate constants of prodrugs at 0.5 mM in skin homogenate were $5.77 \pm 0.16 \times 10^{-2}$ (II), $5.57 \pm 0.11 \times 10^{-2}$ (III), $2.13 \pm 0.03 \times 10^{-1}$ (IV), $4.84 \pm 0.49 \times 10^{-1}$ (V), 1.37 ± 0.06 (VI) $3.79 \pm 0.27 \times 10^{-2}$ (VII), and $1.03 \pm 0.01 \times 10^{-2}$ (VIII) h^{-1}, respectively. Saturation of enzymatic degradation was not observed within a range of the tested concentration (data not shown). Being corresponded to the amount of the acyclovir prodrug which disappeared from the incubation medium, regeneration of acyclovir was confirmed.

3.2. In vitro skin penetration experiment

The penetration profiles of acyclovir and its prodrugs (II–VI) were previously reported. The penetration profiles of structural isomers (VII,

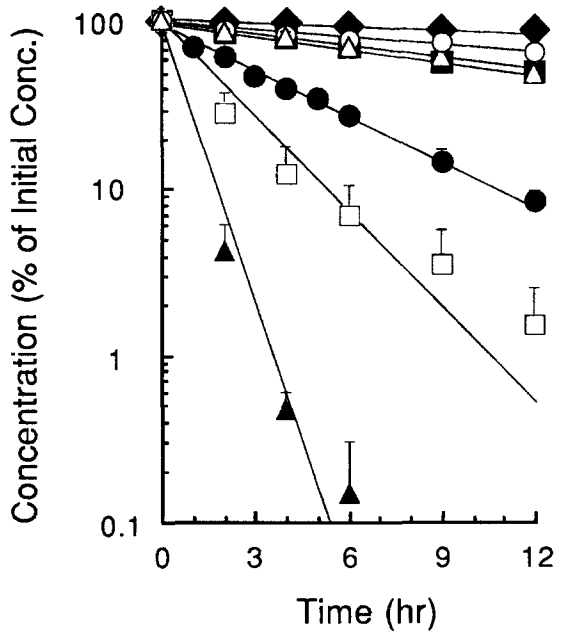

Fig. 3. Hydrolysis of acyclovir prodrugs in phosphate buffered saline (pH 7.4) with rat skin homogenate at 37°C. Initial concentration was 0.5 mM. II: (■); III: (△); IV: (●); V: (□); VI: (▲); VII: (○); VIII: (◆). Each point represents the mean ± S.D. value of at least three experiments.

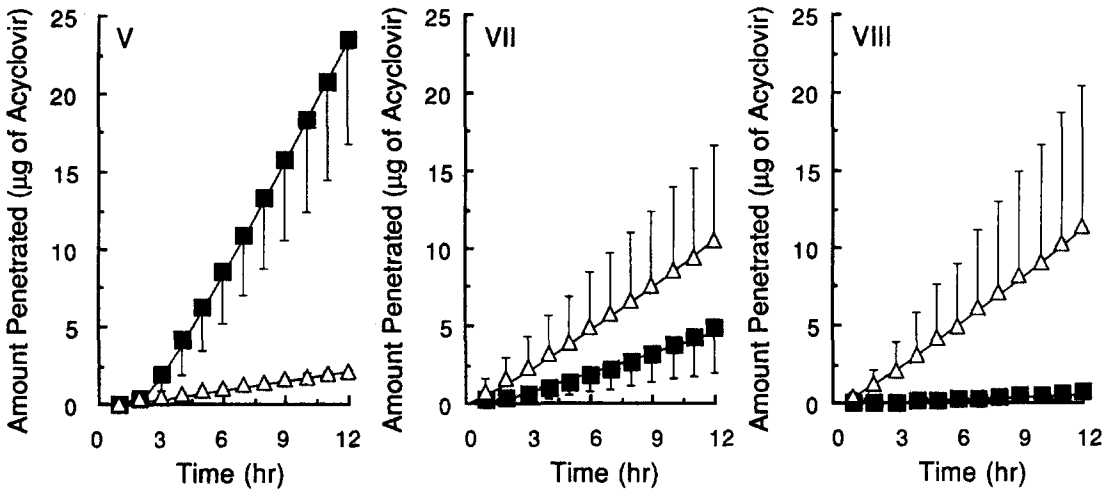

Fig. 4. Time courses of penetrated amount of prodrug (△) and regenerated acyclovir (■) through the rat skin without GACH treatment. V, VII and VIII were applied in forms of suspension. Each point represents the mean ± S.D. value of least three experiments.

VIII) of V through the rat skin were shown in Fig. 4 without GACH treatment and in Fig. 5 with it. In Fig. 4, although almost all V appeared in the receptor fluid as a metabolized form, i.e. acyclovir, VIII showed little metabolism through permeation of the skin. VII shows an intermediate metabolism rate between V and VIII.

3.3. Analysis of penetration profiles based on a diffusion/bioconversion model with polar and nonpolar route in the stratum corneum

We analyzed all permeation profiles of drugs based on a diffusion/bioconversion model. Table 1 summarizes penetration parameters of I and its prodrugs (II–VIII) for the polar route, nonpolar route and the lower viable layer. The diffusion and partition parameters in the lower dermis were relatively similar among tested drugs.

Parameters for the polar route were obtained by analyzing the penetration profiles of MT assuming that it penetrates only through there due to its high hydrophilicity (Ackermann et al., 1987). As shown in Table 1, the K'_p value increased up to two times with an increase in the dose of GACH. This would be explained by a rise in V_p value or effective area of the polar route since K'_p is a product of $K_p(=1)$ and V_p. The D'_p value, on the contrary, changed only by a factor of 1.04 as compared with that of control.

In order to determine the parameters for the nonpolar route, predetermined D'_p and K'_p for MT, and D'_d and K'_d for each drug and D'_{np} and K'_{np} for acyclovir were employed in the calculations. Under the condition without GACH treatment, significant relationships were obtained between D'_{np} and K'_{np} values and acyl chain length of prodrugs, i.e. D'_{np} gradually decreased and K'_{np} significantly increased as acyl chain length increased (Table 1). As regards the effects of GACH on the nonpolar route, K'_{np} increased extensively in all drugs with an increase in GACH pretreatment dose, while the D'_{np} value did not change so much.

With respect to enzymatic hydrolysis rate constants in the viable epidermis and dermis without GACH pretreatment, II and III showed approximately equal levels and the rate constant increased as acyl chain length prolonged. In three structural isomers, the order of hydrolysis rate constant was V > VII > VIII. In Fig. 6, the enzymatic hydrolysis rate constants estimated from two different experiments are compared against acyl chain length of the promoieties (a) or between structural isomers (b). While absolute values of enzymatic hydrolysis rate constant were

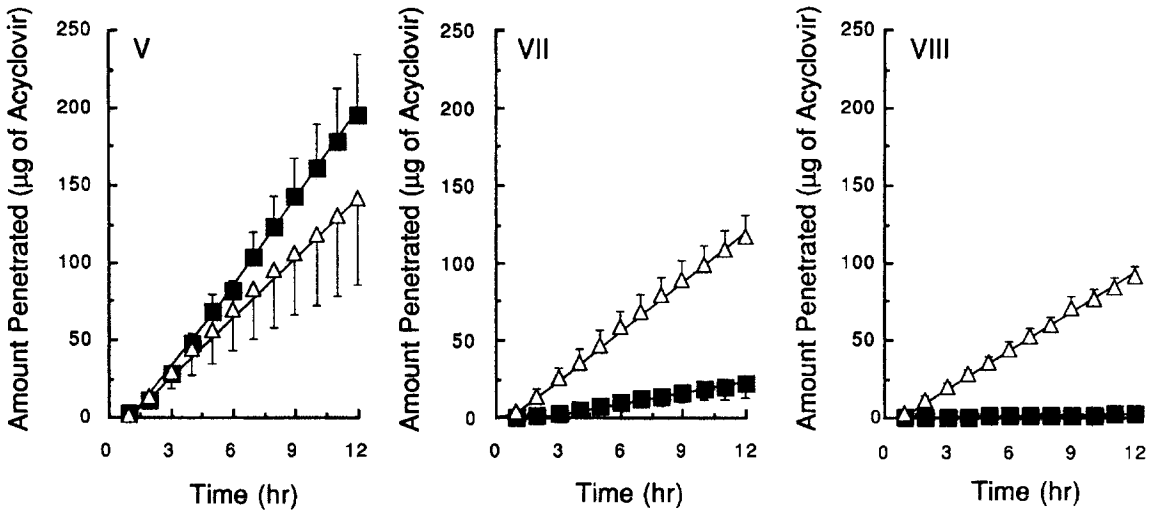

Fig. 5. Time courses of penetrated amount of prodrug (△) and regenerated acyclovir (■) through the rat skin pretreated with GACH (25.5 μmol). V, VII and VIII were applied in forms of suspension. Each point represents the mean ± S.D. value of least three experiments.

different, the rank order was almost similar between these experimental systems. As shown in Table 1, GACH significantly decreased the enzymatic activity for all prodrugs by a factor of 0.54 (II)–0.23 (V) as compared with that of control.

4. Discussion

In the previously reported diffusion/metabolism models, non-uniform localization of enzyme degrading drugs in skin was assumed (Liu et al., 1990, 1991, 1994; Tojo et al., 1994). They may be more accurate because the localization of various enzymes was observed (Martin et al., 1987). However, it requires further information such as drug transport data in the diffusion experiment using the reversed skin and then the model is not usually applicable to in vivo absorption. In contrast, a model assuming homogenous distribution of enzymes in the viable layer is more easily applicable to describe penetration profiles of the drug and its metabolite. In this analysis, each penetration parameter was successively determined by analyzing penetration profiles of acyclovir and its prodrug under different conditions. The rationality of this approach was discussed in a previous report (Yamashita et al., 1993). To estimate diffusion and partition parameters in a viable layer, diffusion experiments with stripped skin were done. For prodrug application, 30% ethanol was added to a receptor solution to inhibit metabolic activities of the skin. It was reported that 30% ethanol solution completely inhibited the metabolism of β-estradiol without any change in its permeability coefficient (Liu et al., 1991). Our preliminary study suggested that the penetration of acyclovir, which is not metabolized, through stripped skin did not significantly change by adding ethanol in a receptor solution. This method can be used to estimate the bioconversion rate constants of prodrugs under more physiological condition, since the contact of a viable layer with a donor solution is avoided by the existence of the stratum corneum.

Many reports demonstrated the presence of esterases in the skin and Ghosh et al. have recently shown the features of cutaneous esterase in hairless mice and its inhibition by various inhibitors (Ghosh and Mitra, 1990). In this study, while all acyclovir prodrugs were very stable in PBS (pH 7.4), they rapidly converted to parent drug in rat skin homogenate (Fig. 3). These findings suggest that the hydrolysis of all the prodrugs proceeds enzymatically in the skin.

Table 1
Estimated penetration parameters for drug penetration through the skin pretreated with various doses of GACH

Drug	GACH dose (μmol)	Stratum corneum				Enzymatic hydrolysis rate constant k (h^{-1})
		Polar route		Nonpolar route		
		D'_p[a] (h^{-1})	K'_p[b] ($\times 10^5$ cm^3)	D'_{np}[c] (h^{-1})	K'_{np}[c] (cm^3)	
(I)	0	84.7	2.05	10.4	0.000102	–
	6.4	85.5	3.86	10.7	0.000261	–
	25.5	88.5	4.34	10.6	0.000690	–
	Stripping	$D'_d = 0.365$ (h^{-1})[d]		$K'_d = 0.177$ (cm^3)[d]		
(II)	0	80.0	2.05	8.35	0.000102	1.10
	6.4	80.8	3.86	8.91	0.000487	0.590
	25.5	83.6	4.34	9.03	0.00639	0.734
	Stripping	$D'_d = 0.437$ (h^{-1})[d]		$K'_d = 0.182$ (cm^3)[d]		
(III)	0	78.7	2.05	8.12	0.000138	0.900
	6.4	79.4	3.86	8.01	0.000516	0.569
	25.5	82.2	4.34	8.43	0.00778	0.298
	Stripping	$D'_d = 0.256$ h^{-1})[d]		$K'_d = 0.259$ (cm^3)[d]		
(IV)	0	77.4	2.05	9.16	0.000273	2.44
	6.4	78.2	3.86	8.40	0.00111	1.20
	25.5	80.8	4.34	8.34	0.0109	0.700
	Stripping	$D'_d = 0.200$ (h^{-1})[d]		$K'_d = 0.485$ (cm^3)[d]		
(V)	0	76.2	2.05	7.22	0.000378	3.28
	6.4	77.0	3.86	7.49	0.00130	0.992
	25.5	79.6	4.34	7.67	0.0170	0.756
	Stripping	$D'_d = 0.314$ (h^{-1})[d]		$K'_d = 0.216$ (cm^3)		
(VI)	0	75.1	2.05	7.24	0.000663	4.04
	6.4	75.8	3.86	7.64	0.00391	1.51
	25.5	78.4	4.34	7.20	0.0417	1.14
	Stripping	$D'_d = 0.314$ (h^{-1})[d]		$K'_d = 0.247$ (cm^3)[d]		
(VII)	0	76.2	2.05	7.91	0.000373	0.156
	25.5	79.6	4.34	7.22	0.0107	0.0685
	Stripping	$D'_d = 0.173$ (h^{-1})[d]		$K'_d = 0.371$ (cm^3)[d]		
(VIII)	0	76.2	2.05	8.48	0.000446	0.0269
	25.5	79.6	4.34	7.62	0.0164	0.0111
	Stripping	$D'_d = 0.207$ (h^{-1})[d]		$K'_d = 0.223$ (cm^3)[d]		
Mannitol	0	90.9	2.05	–	–	–
	6.4	91.8	3.86	–	–	–
	25.5	95.0	4.34	–	–	–
	Stripping	$D'_d = 0.287$ (h^{-1})[d]		$K'_d = 0.216$ (cm^3)[d]		

[a]Diffusion parameters for polar route in the stratum corneum (D'_p). The value for each drug was calculated from the corresponding values of mannitol by correcting with molecular weight.
[b]Partition parameters for polar route in the stratum corneum (K'_p). The value for each drug was the same as the corresponding value of mannitol.
[c]Parameters for the nonpolar route in the stratum corneum (D'_{np}, K'_{np}).
[d]Parameters for the second viable layer (D'_d, K'_d). These values were considered to be common to each drug regardless of GACH dose.

In regard to the susceptibility of prodrugs to esterase, many researchers reported similar types of ester prodrugs (Seki et al., 1990; Shao et al., 1994a). In a series of zidovudine esters, caprate or caprylate showed the highest reactivity in the presence of rat skin homogenate and either the

decrease or the increase in the acyl chain length resulted in the decrease of the reactivity to enzymes (Seki et al., 1990). As shown in Fig. 3, the effect of acyl chain length to the reactivity of cutaneous esterase is almost the same as that of zidovudine, and similar results had been shown about acyclovir prodrugs in rat plasma (Shao et al., 1994a) and in rat nasal homogenate (Shao et al., 1994b).

The ratio of intact prodrug's flux to total prodrug plus acyclovir flux was increased by GACH in our previous report (Bando et al., 1994). In the present analysis, hydrolysis rate constants of all prodrugs were also shown to be decreased by the pretreatment with GACH (Table 1). Similar phenomenon was reported for other enhancers, e.g. Azone in propylene glycol was decreased metabolism of nitroglycerin (Higo et al., 1992) and l-menthol and dl-camphor also inhibited the hydrolysis of methyl salicylate in hairless mouse skin (Yano et al., 1991). One of the possible mechanism is saturation of metabolic activity due to increased flux of the prodrugs by GACH. However, pretreatment with oleic acid in ethanol gave similar enhancement of total flux to that of GACH without changing metabolism (data not shown). Further, GACH also inhibited the metabolism of II, its total penetration was not so much increased. These results suggest little effect of saturation mechanism in metabolism and GACH is concluded to decrease metabolic activity in the skin.

As same as our previous findings (Yamashita et al., 1993), this analysis demonstrated that GACH mainly affected the nonpolar route in the stratum corneum. Recently, Azone was reported to increase the electric resistance of the stratum corneum, deducing that drug penetration enhancement by enhancer might take place due to increased drug partitioning (Kontturi et al., 1990). Previously, our analysis based on a linear free-energy relationship revealed that the change of drug partitioning by GACH is caused by increasing the polarity of the nonpolar route (Yamashita et al., 1993). The present result suggests that even if metabolic process is included, this model analysis gives reasonable values of diffusion and partition.

In conclusion, the present skin model and computer analysis enable us to comprehensively discuss the mechanism of skin penetration of drug which is metabolized in there and the action of enhancers on it. It may be also useful in predicting therapeutic utilities of the transdermal drug delivery system, such as prodrug derivation and application of penetration enhancers. Furthermore, since this model has some generality in physiology and anatomy of the skin, it could also be expanded to an in vivo model by introducing some additional physiological factors such as blood flow.

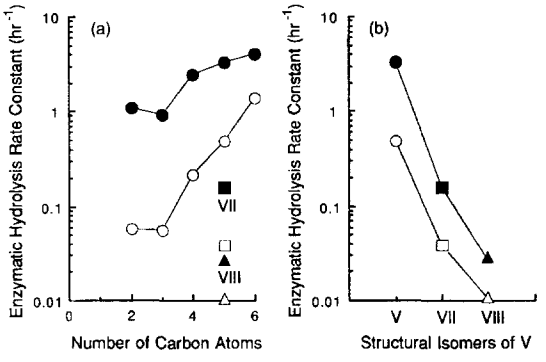

Fig. 6. Effect of substituent of prodrugs on enzymatic hydrolysis rate constant in the viable epidermis and dermis. (a) Prodrugs with different acyl chain lengths. (b) Prodrugs of structural isomers. Enzymatic hydrolysis rate constants determined from skin penetration experiment (closed symbol) and from homogenate experiment (opened symbol) are compared.

Appendix

The Fick's second law of diffusion for each domain about prodrug is expressed as follows;

$$\partial C_{p,p}/\partial t = D_{p,p}(\partial^2 C_{p,p}/\partial x^2) \quad \text{(A1)}$$

$$\partial C_{p,np}/\partial t = D_{p,np}(\partial^2 C_{p,np}/\partial x^2) \quad \text{(A2)}$$

$$\partial C_{p,d}/\partial t = D_{p,d}(\partial^2 C_{p,d}/\partial x^2) - kC_{p,d} \quad \text{(A3)}$$

Assuming that the donor is under a well-stirred infinite-dose condition and the receptor is under perfect sink condition, respectively, the boundary conditions are given as follows;

$$K_{p,p}C_v = C_{p,p} \quad (x = -L_s) \quad \text{(A4)}$$

$$K_{p,np}C_v = C_{p,np} \quad (x = -L_s) \quad \text{(A5)}$$

$$K_{p,d}C_{p,p}/K_{p,p} = C_{p,d} \quad (x = 0) \tag{A6}$$

$$K_{p,d}C_{p,np}/K_{p,np} = C_{p,d} \quad (x = 0) \tag{A7}$$

$$D_{p,p}Af(\partial C_{p,p}/\partial x) + D_{p,np}A(1-f)(\partial C_{p,np}/\partial x) = D_{p,d}A(\partial C_{p,d}/\partial x) \quad (x = 0) \tag{A8}$$

$$C_{p,d} = 0 \quad (x = Ld) \tag{A9}$$

where C_v is the drug concentration in the vehicle. The initial conditions are as follows;

$$C_v = C_0 \tag{A10}$$

$$C_{p,p} = C_{p,np} = C_{p,d} = 0 \tag{A11}$$

where C_0 is the initial concentration, solubility of each drug in the vehicle. Eqs. (A1–A3) have the general Laplace transforms, respectively:

$$\tilde{C}_{p,p} = \gamma_{p,p} \sinh (s/D_{p,p})^{1/2}x + \delta_{p,p} \cosh (s/D_{p,p})^{1/2}x \tag{A12}$$

$$\tilde{C}_{p,np} = \gamma_{p,np} \sinh (s/D_{p,np})^{1/2}x + \delta_{p,np} \cosh (s/D_{p,np})^{1/2}x \tag{A13}$$

$$C_{p,d} = \gamma_{p,d} \sinh \{(s+k)/D_{p,d}\}^{1/2}x + \delta_{p,d} \cosh \{(s+k)/D_{p,d}\}^{1/2}x \tag{A14}$$

From Eqs (A4–A9), $\gamma_{p,p}$, $\delta_{p,p}$, $\gamma_{p,np}$, $\delta_{p,np}$, $\gamma_{p,d}$ and $\delta_{p,d}$ are determined as;

$$\gamma_{p,p} = K_{p,p}C_0 \{\cosh d_{p,p} \sinh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/k(s) - 1\}/s/\sinh d_{p,p} \tag{A15}$$

$$\delta_{p,p} = K_{p,p}C_0 \sinh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/s/k(s) \tag{A16}$$

$$\gamma_{p,np} = K_{p,np}C_0 \{\cosh d_{p,np} \sinh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/k(s) - 1\}/s/\sinh d_{p,np} \tag{A17}$$

$$\delta_{p,np} = K_{p,np}C_0 \sinh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/s/k(s) \tag{A18}$$

$$\gamma_{p,d} = -K_{p,d}C_0 \cosh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/s/k(s) \tag{A19}$$

$$\delta_{p,d} = -K_{p,d}C_0 \sinh d_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})/s/k(s) \tag{A20}$$

The Laplace-transformed equation for the cumulative amount of a prodrug penetrating the skin ($\tilde{Q}_{p,int}$) is caluculated by,

$$\tilde{Q}_{p,int} = -D_{p,d}A/s * (\partial C_{p,d}/\partial x)_{x = L_d} \tag{A21}$$

$$= -D_{p,d}A/s * \{(s+k)/D_{p,d}\}^{1/2} \times (\gamma_{p,d} \cosh d_{p,d} - \delta_{p,d} \sinh d_{p,d}) \tag{A22}$$

Likewise, the Fick's second law of diffusion about metabolized parent drug is expressed as follows;

$$\partial C_{m,p}/\partial t = D_{m,p}(\partial^2 C_{m,p}/\partial x^2) \tag{A23}$$

$$\partial C_{m,np}/\partial t = D_{m,np}(\partial^2 C_{m,np}/\partial x^2) \tag{A24}$$

$$\partial C_{m,d}/\partial t = D_{m,d}(\partial^2 C_{m,d}/\partial x^2) + kC_{p,d} \tag{A25}$$

Assuming that the donor and receptor is under perfect sink condition, the boundary conditions are given as follows;

$$K_{m,p}C_v = C_{m,p} \quad (x = -L_s) \tag{A26}$$

$$K_{m,np}C_v = C_{m,np} \quad (x = -L_s) \tag{A27}$$

$$K_{m,d}C_{m,p}/K_{m,p} = C_{m,d} \quad (x = 0) \tag{A28}$$

$$K_{m,d}C_{m,np}/K_{m,np} = C_{m,d} \quad (x = 0) \tag{A29}$$

$$D_{m,p}Af(\partial C_{m,p}/\partial x) + D_{m,np}A(1-f)(\partial C_{m,np}/\partial x) = D_{m,d}A(\partial C_{m,d}/\partial x) \quad (x = 0) \tag{A30}$$

$$C_{m,d} = 0 \quad (x = L_d) \tag{A31}$$

where C_v is the drug concentration in the vehicle. The initial conditions are as follows;

$$C_{m,v} = 0 \tag{A32}$$

$$C_{m,p} = C_{m,np} = C_{m,d} = 0 \tag{A33}$$

Eqs. (A23–25) have the general Laplace transforms respectively:

$$\tilde{C}_{m,p} = \gamma_{m,p} \sinh (s/D_{m,p})^{1/2}x + \delta_{m,p} \cosh (s/D_{m,p})^{1/2}x \tag{A34}$$

$$\tilde{C}_{m,np} = \gamma_{m,np} \sinh (s/D_{m,np})^{1/2}x + \delta_{m,np} \cosh (s/D_{m,np})^{1/2}x \quad \text{(A35)}$$

$$\tilde{C}_{m,d} = \gamma_{m,d} \sinh (s/D_{m,d})^{1/2}x + \delta_{m,d} \cosh (s/D_{m,d})^{1/2}x + k\tilde{C}_{p,d}/D_{m,d}/\{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A36)}$$

From Eqs. (A26–31), $\gamma_{m,p}$, $\delta_{m,p}$, $\gamma_{m,np}$, $\delta_{m,np}$, $\gamma_{m,d}$ and $\delta_{m,d}$ are determined as;

$$\gamma_{m,p} = k \cosh d_{m,p}K_{m,p}K_{p,d}C_0 \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} * \{d_{p,d} - m(s)/l(s)\} /\sinh d_{m,p}/K_{m,d}/D_{m,d}/\{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A37)}$$

$$\delta_{m,p} = kK_{m,p}K_{p,d}C_0 \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} * \{d_{p,d} - m(s)/l(s)\} /K_{m,d}/D_{m,d}/\{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A38)}$$

$$\gamma_{m,p} = k \cosh d_{m,p}K_{m,p}K_{p,d}C_0 \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} * \{d_{p,d} - m(s)/l(s)\} /\sinh d_{m,np}/K_{m,d}/D_{m,d}/\{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A39)}$$

$$\delta_{m,np} = kK_{m,np}K_{p,d}C_0 \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} * \{d_{p,d} - m(s)/l(s)\} /K_{m,d}/D_{m,d}/\{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A40)}$$

$$\gamma_{m,d} = -kK_{m,p}K_{p,d}C_0 \cosh d_{m,p} \{m(s)/l(s)/d_{m,d}\} * \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} /\sinh d_{m,d}/D_{m,d} \{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A41)}$$

$$\delta_{m,d} = -kK_{m,p}K_{p,d}C_0 \{m(s)/l(s)/d_{m,d}\} * \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} /D_{m,d} \{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A42)}$$

The Laplace-transformed equation for the cumulative amount of a regenerated parent drug ($\tilde{Q}_{m,int}$) is,

$$\tilde{Q}_{m,int} = -D_{m,d}A/s * (\partial C_{m,d}/\partial x)_{x=L_d} \quad \text{(A43)}$$

$$= -D_{m,d}A/s * \{(s/D_{m,d})^{1/2} \times (\gamma_{m,d} \cosh d_{m,d} - \delta_{m,d} \sinh d_{m,d})\} - \{(s+k)/D_{m,d}\}^{1/2}/kK_{p,d}C_0 * \{Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}/s/k(s)\} /D_{m,d} \{s/D_{m,d} - (s+k)/D_{p,d}\} \quad \text{(A44)}$$

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