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In vivo skin penetration enhancement of acyclovir by theoretical design of prodrug-enhancer combination

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Abstract

The effectiveness of prodrug-enhancer combination under in vivo skin penetration enhancement was studied using acyclovir and its lipophilic prodrugs, i.e. acyclovir valerate, isovalerate and pivarate together with an enhancer, 1-geranylazacycloheptan-2-one (GACH). Under in vivo penetration experiment with rat skin, we estimated absorption amounts of both prodrug and metabolized acyclovir respectively from the excretion amount by employing a deconvolution method. In the absence of GACH, the total amount of acyclovir absorbed at the end of 4 h experiment, after application of prodrug in the form of aqueous solution, was about twice than that obtained after administration of acyclovir itself. On the other hand, GACH showed slight absorption enhancement for acyclovir but demonstrated drastic enhancement effect on its prodrugs, especially valerate. Also, this enhancement effect was more remarkable under in vivo condition than in vitro one. Indeed, to elucidate the differences in enhancement effect among three prodrugs, we carried out some simulations to clarify the relationship among enhancement effect, lipophilicity of prodrugs and enzymatic hydrolysis rate constants. From the results of simulations, it was obviously noticed that metabolism only exerted an important effect on skin penetration of these prodrugs when applied with GACH simultaneously. Furthermore, according to this model analysis under in vivo condition, we came to understand that GACH significantly decreased the enzymatic activity in skin. Copyright © 1996 Elsevier Science B.V.

Keywords: Acyclovir; Acyclovir prodrugs; In vivo percutaneous absorption; Penetration enhancement; Prodrug-enhancer combination; 1-Geranylazacycloheptan-2-one

1. Introduction

The mechanisms about skin penetration of drug and/or its enhancement have been examined from

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various points of view (Hsieh, 1994). Nevertheless, there have been few reports on the optimization of drug absorption achieved rationally based on these mechanisms. In a series of our investigations, we discovered that prodrug-enhancer combination has offered an effective and rational way to increase transdermal delivery of a wide range of drugs (Bando et al., 1994, 1996a). This methodology could in fact be theoretically designed based on an absorption enhancement mechanism by the enhancer 1-geranylazacycloheptan-2-one (GACH) which has already been revealed by a diffusion model analysis (Yamashita et al., 1993). The experiments were studied under in vitro condition and since the ultimate purpose of in vitro studies is to practically predict the behavior of in vivo absorption, it is important to clarify the differences between in vivo and in vitro system accurately. In our previous reports, differences concerning percutaneous penetration between them were demonstrated quantitatively as follows: (1) the diffusion length of the viable layer was shorter under in vivo; (2) the effective area of the polar route in the stratum corneum was larger under in vitro; and (3) enzymatic activity was higher under in vivo, by means of a diffusion model analysis (Yamashita et al., 1994; Bando et al., 1996).

In the case of this combination approach, since prodrugs applied topically were metabolized to hydrophilic drug through the skin, the difference between in vivo and in vitro, i.e. enzymatic activity and contribution of polar route on total penetration, would influence its enhancement effect. In the present study, in order to achieve larger percutaneous absorption enhancement of acyclovir under in vivo condition, the possibility of prodrug-enhancer combination which was theoretically designed based on the action mechanism of GACH under in vitro condition (Bando et al., 1996a; Yamashita et al., 1993) was investigated.

Initially, after application of acyclovir prodrug, in vivo absorption profiles for both intact prodrug and metabolized acyclovir were evaluated using a deconvolution method which enabled us to estimate the first-pass metabolism (Yamashita et al., 1995). In the meantime, enhancement ratio by this combination method was also compared between in vivo and in vitro condition. Subsequently, we analyzed the penetration of acyclovir prodrugs through rat skin which was pretreated with and without GACH based on a diffusion model in consideration of metabolic process (Bando et al., 1996). Meanwhile, the effect of metabolism in skin on percutaneous penetration of drugs was taken into account by means of simulations in order to clarify the relationship among enhancement ratio, lipophilicity of prodrugs and enzymatic hydrolysis rate constants.

2. Materials and methods

2.1. Materials

GACH was synthesized by Kuraray Co., Okayama, Japan. Acyclovir was kindly supplied by Nippon Wellcome K.K., Osaka, Japan. Esterification of acyclovir was carried out as described previously (Bando et al., 1996a,b). In this study, we used three acyclovir prodrugs which were the structural isomers of acyclovir valerate. Radiolabeled ^{3}H-acyclovir were obtained from Daiichi Pure Chemicals, Japan. Other materials were purchased from Nacalai Tesque, Kyoto, Japan.

2.2. Determination of solubilities

Solubilities of acyclovir and its prodrugs in water and octanol were determined at 37°C as previously reported (Bando et al., 1994, 1996a). After suspending excess compounds in the solvents (3 ml) for 24 h with agitation, the mixtures were centrifuged at 5000 rpm (RL-100, Tomy Seiko, Tokyo, Japan), filtered using a Cosmonice-filter with 0.45 μm pore diameter (Nacalai Tesque, Kyoto, Japan) before diluting with corresponding solvents for determination. The ratio of solubility of octanol to water is considered to be equal to the partition coefficient between them ($PC_{oct/w}$)

2.3. In vivo skin penetration experiment

In vivo absorption experiment was performed as previously reported (Bando et al., 1996; Ya-

mashita et al., 1994). Under anesthesia induced by intraperitoneal injection of 1 g/kg urethane, abdominal hair of male Wistar strain rat weighing about 200 g was removed with an electric hair clipper and a glass half-chamber (effective area, 3.14 cm^2) was attached to the abdominal surface using cyanoacrylate adhesive (Aron Alpha A, Sankyo, Tokyo, Japan). The skin surface was pretreated with 0.2 ml of ethanolic solution containing 0 and 25.5 μmol of GACH and 6 h later ethanol remaining in the donor cell was evaporated with hair dryers. The urinary bladder was cannulated with vinyl tubing (i.d. 0.50 mm, o.d. 0.90 mm, Dural Plastics and Engineering, Australia) before placing a 1 ml aliquot of drug solution in the glass chamber. Urine was collected every 15 min for 4 h by injecting 0.2 ml of 0.9% NaCl solution through the tubing twice before each sampling time. At the end of experiment, drug remaining in the donor cell was recovered and skin was excised.

2.4. Intravenous injection experiment

Intravenous injection experiment was performed in order to elucidate disposition behavior of the tested drugs and to estimate their absorption profiles by a deconvolution method (Yamashita et al., 1995; Bando et al., 1996). Under urethane anesthesia, 0.2 ml (0.5 mM) of 0.9% NaCl solution of drugs was injected into the femoral vein. Urine was collected by the same method as described above. This deconvolution method is not applicable when the elimination of a drug from the body is a non-linear process, as described previously (Yamashita et al., 1995).

2.5. Analysis of radioactivity

Each urine sample was divided into two samples. One was measured for the total penetrated amount and the other was determined for the ratio of acyclovir to total penetrated amount.

After evaporation of the latter samples and resolubilization in methanol, they were analyzed by thin-layer chromatography (TLC) on silica gel sheets (Silica gel $60F_{254}$, Merck Japan, Tokyo, Japan) with a solvent system of chloroform:methanol (3:1) and were visualized under ultraviolet (UV). Then, TLC fractions were directly mixed with scintillation cocktail and the radioactivities were measured using a liquid scintillation counter (LSC-5000, Beckman, Japan). After solubilization of the urine samples for determining total penetration amount and skin samples with Soluene-350 (Packard Instrument, IL), these radioactivities were evaluated.

2.6. Data analysis

The penetration profiles for both intact prodrugs and metabolized acyclovir obtained under in vivo study were analyzed based on a model in which a receptor phase under perfect sink conditions was connected with a viable layer (Bando et al., 1996). In addition, the following assumptions are made: (a) the hydrolytic enzyme, especially non-specific esterase, is homogeneously distributed in the second layer; (b) the hydrolysis undergoes first-order reaction kinetics; and (c) the stratum corneum essentially serves as a diffusion barrier. Thus, part of the prodrug that successfully penetrated this layer is metabolized to its parent drug in the viable epidermis and dermis before reaching the blood compartment and being transferred to the systemic circulation via a diffusion along drug concentration gradient in the skin. Based on this model, the Laplace transforms for the amount of prodrug and parent drug appearing in the receptor across the intact skin are expressed as follows:

$$\tilde{Q}_{p,int} = C_0 V_1 Z_{p,d}(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p}) \times /s/k(s) \quad (1)$$

$$\tilde{Q}_{m,int} = C_0 V_1 K_{p,d} V_d k(Z_{p,p} \sinh d_{p,np} + Z_{p,np} \sinh d_{p,p})(m(s)/I(s) - d_{p,d}) \times /s/(d_{p,d}^2 - d_{m,d}^2)/k(s) \quad (2)$$

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

$$d_{p,p} = L_s\sqrt{s/D_{p,p}} \quad (3)$$

$$d_{p,np} = L_s\sqrt{s/D_{p,np}} \quad (4)$$

$$d_{p,d} = L_d\sqrt{(s+k)/D_{p,d}} \quad (5)$$

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

$$d_{m,np} = L_s\sqrt{s/D_{m,np}} \quad (7)$$

$$d_{m,d} = L_d\sqrt{s/K_{m,d}} \quad (8)$$

$$Z_{p,p} = D_{p,p}K_{p,p}V_p d_{p,p} \quad (9)$$

$$Z_{p,np} = D_{p,np}K_{p,np}V_{np}d_{p,np} \quad (10)$$

$$Z_{p,d} = D_{p,d}K_{p,d}V_d d_{p,d} \quad (11)$$

$$Z_{m,p} = D_{m,p}K_{m,p}V_p d_{m,p} \quad (12)$$

$$Z_{m,np} = D_{m,n}K_{m,np}V_{np}d_{m,np} \quad (13)$$

$$Z_{m,d} = D_{m,d}K_{m,d}V_d d_{m,d} \quad (14)$$

$$\begin{aligned} k(s) &= V_v s(Z_{p,p}\cosh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &\quad + Z_{p,np}\sinh d_{p,p}\cosh d_{p,np}\sinh d_{p,d} \\ &\quad + Z_{p,d}\sinh d_{p,p}\sinh d_{p,np}\cosh d_{p,d}) \\ &\quad + Z_{p,p}\{Z_{p,p}\sinh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &\quad + Z_{p,np}\sinh d_{p,d}(\cosh d_{p,p}\cosh d_{p,np} - 1) \\ &\quad + Z_{p,d}\cosh d_{p,p}\sinh d_{p,np}\cosh d_{p,d}\} \\ &\quad + Z_{p,np}\{Z_{p,np}\sinh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &\quad + Z_{p,p}\sinh d_{p,d}(\cosh d_{p,p}\cosh d_{p,np} - 1) \\ &\quad + Z_{p,d}\sinh d_{p,p}\cosh d_{p,np}\cosh d_{p,d}\} \end{aligned} \quad (15)$$

$$\begin{aligned} l(s) &= Z_{m,p}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{m,d} \\ &\quad + Z_{m,np}\sinh d_{m,p}\cosh d_{m,np}\sinh d_{m,d} \\ &\quad + Z_{m,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{m,d} \end{aligned} \quad (16)$$

$$\begin{aligned} m(s) &= Z_{m,p}d_{m,d}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{p,d} \\ &\quad + Z_{m,np}d_{m,d}\sinh d_{m,p}\cosh d_{m,np}\sinh d_{p,d} \\ &\quad + K_{m,d}D_{m,d}d_{m,d}d_{p,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{p,d} \end{aligned} \quad (17)$$

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; whereas 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, polar route, 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,

$$V_p = AfL_s \quad (18)$$

$$V_{np} = A(1-f)L_s \quad (19)$$

$$V_d = AL_d \quad (20)$$

Curve-fitting to in vivo data was carried out for a urinary excretion profile in order to minimize the error arose during numerical calculations (Yamashita et al., 1992). By expressing the excretion profile after intravenous application as a poly-exponential function ($dXu/dt = Ae^{-\alpha t} + Be^{-\beta t} + \cdots$), the Laplace transformed equation for urinary excretion ($\tilde{X}u$) of topically applied drug may be written as follows:

$$\tilde{X}u = \tilde{Q}*\{A/(s+\alpha) + B/(s+\beta) + \cdots\} \quad (21)$$

This equation was fitted to urinary excretion data after topical application of prodrugs. Curve-fitting to data was conducted using the nonlinear regression program MULTI(FILT) (Yano et al., 1989) on the M-382 mainframe computer of the Kyoto University Data Processing Center. Due to the difficulty in determining the real diffusional pathlength, we defined two parameters for drug diffusion and partitioning involving the diffusion length as follows (Yamashita et al., 1993, 1994; Bando et al., 1996b, 1996):

$$D'_j = D_j/L_j^2 \quad (22)$$

$$K'_j = K_j V_j \quad (j = p, np, \text{or } d) \quad (23)$$

The seven targeted hybrid parameters for each prodrug were determined according to the following procedures reported previously (Bando et al., 1996b). $K_{p,d}$ of all prodrugs were substituted with the value of acyclovir obtained and $D_{p,d}$ were corrected based on their molecular weight (Lambart et al., 1989). In order to determine the parameters for nonpolar route, predetermined D_p and K_p for mannitol, D_d and K_d for each drug as well as D_{np} and K_{np} for acyclovir were employed

in the calculations (Bando et al., 1996). The parameters of mannitol were calculated from in vivo penetration profiles as previously reported (Yamashita et al., 1994). Its diffusion and partition parameters in the stratum corneum at control condition were 58.7 (h^{-1}) and 0.612 ($\times 10^5$ cm^3) respectively and those at GACH treatment were 60.2 (h^{-1}) and 14.3 ($\times 10^5$ cm^3) respectively. In this analysis, three assumptions were introduced: (1) the polar route is filled with water and partition coefficient between polar route and aqueous vehicle is unity; (2) diffusion in polar route obeys Einstein–Stokes equation; and (3) GACH does not affect the penetration of viable layer concerning partitioning and diffusivity (Yamashita et al., 1993).

2.7. Simulation for effect of metabolism on skin penetration

The penetration amounts of prodrug and metabolized acyclovir at any time were calculated from Eqs. (1) and (2) respectively using a fast inverse Laplace transform FILT algorithm (Yano et al., 1989) on the main frame computer M-382 of the Kyoto University Data Processing Center. In this calculation, partition coefficient for the nonpolar route of prodrug ($K_{p,np}$) was related to the octanol–water partition-coefficient ($PC_{oct/w}$) based on a linear free-energy relationship (Collander, 1951; Leo et al., 1971).

$$\log K_{np} = \alpha \log K_{oct/w} + \beta \quad (24)$$

where the values of α and β are 1.3 and -3.0 for control, 1.1 and -0.9 for treatment with 25.5 μmol of GACH, respectively (Bando et al., 1996a; Yamashita et al., 1993). The partition coefficients for polar route ($K_{p,p}$, $K_{m,p}$) in the stratum corneum were assumed to be unity as we used water for the vehicle (Ackermann and Flynn, 1987a, Ackermann et al., 1987b) Since there is little difference in the permeation of drugs with diverse lipophilicities through the tape stripped skin (Yamashita et al., 1993; Bando et al., 1996b), we assume that the partition coefficients ($K_{p,d}$, $K_{m,d}$) between vehicle and viable skin layer are one regardless the lipophilicities of drugs. Diffusion parameter in each route was independent of the species of drugs. Each parameter was referred from our previous reports on the analysis of skin penetration of drugs and acyclovir prodrugs through rat skin under in vitro condition with different lipophilicities (Bando et al., 1996b; Yamashita et al., 1994). Fig. 2(a) and (b) show the relationship among enhancement ratio, $PC_{oct/w}$ of prodrugs and hydrolysis rate constants pretreated without and with GACH respectively. In these figures, the enhancement ratio revealed the ratio of total penetration amount after application of prodrug to penetration amount after application of acyclovir calculated at 12 h.

3. Results

3.1. Solubilities and $PC_{oct/w}$ of acyclovir and its prodrugs

Water solubilities of acyclovir, valerate, isovalerate and pivarate were 11.9, 2.50, 1.70 and 1.18 mM respectively and their $PC_{oct/w}$ values were 0.0123, 0.702, 1.64 and 1.53, respectively.

3.2. In vivo skin penetration experiments

After intravenous injection of prodrugs, both intact prodrug and metabolized acyclovir were excreted in urine. Urinary excretion profiles of drugs were approximated with poly-exponential functions as follows by a least square regression analysis:

$$dXu/dt = 166 \exp(-2.61t) + 17.4 \exp(-0.666t)$$

(for acyclovir)

$$dXu_p/dt = 244 \exp(-15.1t)$$

(valerate → valerate)

$$dXu_m/dt = -180 \exp(-15.1t) + 166 \exp(2.61t) + 15.0 \exp(0.666t)$$

(valerate → acyclovir)

$$dXu_p/dt = 397 \exp(-9.08t)$$

(isovalerate → isovalerate)

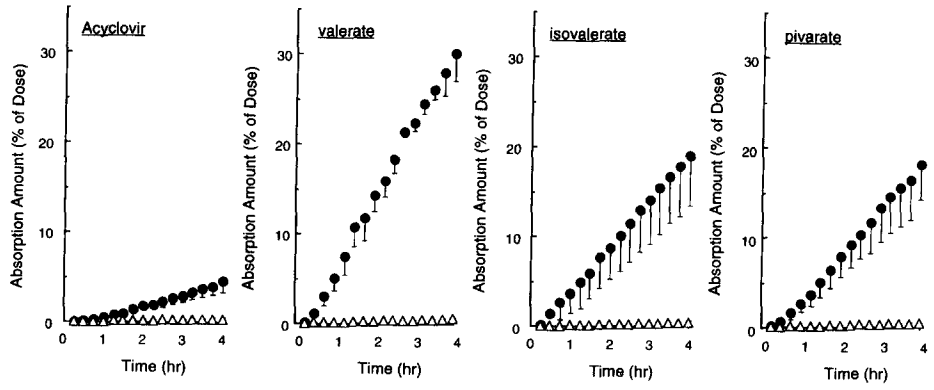

Fig. 1. Time courses of total acyclovir amount absorbed through rat skin pretreated with ethanolic solution of 0 (△) and 25.5 (●) μmol of GACH. In the case of prodrug application, the sum of acyclovir and its prodrug is shown. In vivo absorption profiles were obtained from urinary excretion data using a deconvolution method. Each point represents the mean ± S.D. value of at least three experiments.

$$dXu_m/dt = -147\exp(-9.08t) + 136\exp(2.61t) + 11.0\exp(0.666t)$$

(isovalerate → acyclovir)

$$dXu_p/dt = 223\exp(-5.72t)$$

(pivarate → pivarate)

$$dXu_m/dt = -185\exp(-5.72t) + 173\exp(2.61t) + 11.2\exp(0.666t)$$

(pivarate → acyclovir)

where dXu/dt expresses urinary excretion rate (% of dose/h), and subscripts p and m denote prodrug and metabolized acyclovir respectively. Since the standard deviation of each parameter was about 10% or less, errors arose during numerical calculations could be neglected (Yamashita et al., 1995).

Fig. 1 shows the in vivo absorption profiles of tested drugs obtained by a deconvolution method (Yamashita et al., 1995; Bando et al., 1996). Although we could estimate absorption profiles concerning both prodrug and metabolized acyclovir, they are expressed as total acyclovir amount in the cases of prodrug application in these figures. Without GACH, total absorption amounts at the end of experiment were almost the same among three prodrugs and these were about twice of that obtained after administration of acyclovir (Table 1). As was expected from previous in vitro results (Bando et al., 1994, 1996a), GACH remarkably enhanced total absorption of acyclovir in the case of prodrug application, whereas it shows slight improvement in the absorption amount of acyclovir after application of acyclovir itself. Particularly, it showed much larger enhancement effect on valerate where 60% was metabolized in the skin than that on pivarate where 95% was penetrated as intact prodrug form. On the other hand, the ratio of the absorbed amount of acyclovir as a prodrug form to total absorbed amount was significantly increased by GACH treatment. Table 1 summarizes drug amount recovered in the donor, skin and urine as well as systematically absorbed drug amount calculated by a deconvolution method at the end of 4 h absorption experiment.

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

Table 2 summarizes in vivo penetration parameters of acyclovir and its prodrugs for polar, nonpolar route and lower viable layer. Regarding the effects of GACH on nonpolar route, K_{np} was increased extensively in all prodrugs, but D_{np} was not altered much by GACH treatment.

With respect to enzymatic hydrolysis rate constants in the viable epidermis and dermis without

Table 1
Amounts of acyclovir and acyclovir prodrugs recovered at the end of 4 h under in vivo absorption experiments

	Acyclovir		AC Valerate		AC Isovalerate		AC Pivarate	
	Control	GACH (25.5 μmol)	Control	GACH (25.5 μmol)	Control	GACH (25.5 μmol)	Control	GACH (25.5 μmol)
Donor (% applied)	99.08 ± 1.14	80.18 ± 3.26	98.09 ± 2.10	57.93 ± 9.84	98.00 ± 1.37	70.84 ± 9.09	97.68 ± 3.56	67.38 ± 3.29
Skin (% applied)	0.32 ± 0.13	1.18 ± 0.98	1.18 ± 0.33	3.91 ± 1.25	0.94 ± 0.09	3.97 ± 0.61	1.10 ± 0.39	4.16 ± 1.47
Urine (% applied) Acyclovir	0.24 ± 0.13	3.32 ± 0.84	0.47 ± 1.99	21.32 ± 1.99	0.29 ± 0.11	9.51 ± 3.19	0.25 ± 0.10	7.30 ± 1.57
Prodrug	—	—	N.D.	1.97 ± 0.86	0.14 ± 0.05	6.68 ± 1.71	0.19 ± 0.06	6.63 ± 1.60
Absorbed[a] (% applied) Acyclovir	0.30 ± 0.16	4.56 ± 1.28	0.63 ± 0.08	18.06 ± 7.22	0.21 ± 0.12	3.66 ± 1.90	0.07 ± 0.05	0.89 ± 0.33
Prodrug	—	—	N.D.	12.18 ± 5.32	0.32 ± 0.09	15.46 ± 3.92	0.51 ± 0.16	17.47 ± 4.00
Total[b] (% applied)	99.70 ± 1.27	85.93 ± 4.53	99.90 ± 1.94	92.08 ± 5.61	99.48 ± 1.36	93.93 ± 3.40	99.36 ± 3.68	90.14 ± 1.86
Enhancement ratio[c]	—	15.2	—	48.0	—	36.1	—	31.7
Prodrug/total absorbed amount ratio[d]	—	—	0.0	0.40	0.60	0.81	0.88	0.95

[a] Values were calculated using a deconvolution method.
[b] Values are the sum of amounts of drugs in the donor, skin, and absorbed.
[c] Values were calculated by dividing the absorbed amounts under GACH treatment condition by that under control condition.
[d] Values were calculated by dividing the absorbed amounts of intact prodrug by the sum of absorbed amounts of intact prodrug and metabolized acyclovir at the end of 4 h experiments.

Table 2
Estimated penetration parameters for drug penetration through rat skin pretreated with GACH under in vivo condition

Drug	GACH dose (μmol)	Stratum corneum				Enzymatic hydrolysis rate constant
		Polar route		Nonpolar route		k^{d} (h^{-1})
		$D'_{p}{}^{a}$ (h^{-1})	$K'_{p}{}^{b}$ (10^{5} cm^{3})	$D'_{np}{}^{c}$ (h^{-1})	$K'_{np}{}^{c}$ (cm^{3})	
Acyclovir (AC)	0	54.7	0.612	6.79	0.000126	—
	25.5	56.1	14.3	6.01	0.00187	—
	Stripping	$D'_{d} = 2.84$ $(h^{-1})^{e}$		$K'_{d} = 0.084$ $(cm^{3})^{f}$		—
AC valerate	0	49.2	0.612	N.E.[g]	N.E.[g]	N.E.[g]
	25.5	50.7	14.3	6.39	0.0185	4.11
	Stripping	$D'_{d} = 2.56$ $(h^{-1})^{e}$		$K'_{d} = 0.084$ $(cm^{3})^{f}$		—
AC isovalerate	0	49.2	0.612	5.84	0.000196	2.98
	25.5	50.7	14.3	6.03	0.0124	1.30
	Stripping	$D'_{d} = 2.56$ $(h^{-1})^{e}$		$K'_{d} = 0.084$ $(cm^{3})^{f}$		—
AC pivarate	0	49.2	0.612	5.20	0.000233	0.583
	25.5	50.7	14.3	5.80	0.0119	0.177
	Stripping	$D'_{d} = 2.56$ $(hr^{-1})^{e}$		$K'_{d} = 0.084$ $(cm^{3})^{f}$		—

[a] Diffusion parameters for polar route in the stratum corneum (D'_{p}) were calculated by correcting the corresponding values for mannitol based on molecular weight.
[b] Partition parameters for polar route in the stratum corneum (K'_{p}) were considered to be the same as the corresponding values for mannitol.
[c] Parameters for the nonpolar route in the stratum corneum (D'_{np}, K'_{np})
[d] Enzymatic hydrolysis rate constant in the second viable layer (k).
[e] Parameters for the viable layer (D'_{d}) were calculated by correcting the corresponding values for acyclovir based on molecular weight.
[f] Partition parameters for the viable layer (K'_{d}) were considered to be the same as the corresponding values for acyclovir.
[g] Parameters of valerate without GACH could not be calculated, because it was excreted as metabolized acyclovir in urine.

GACH pretreatment, isovalerate exhibited greater value than pivarate. As shown in Table 2, GACH significantly decreased the enzymatic activity for prodrugs by a factor of 0.43 for isovalerate and 0.30 for pivarate as compared with that of control under in vivo condition.

4. Discussion

Although $PC_{oct/w}$ of prodrugs are about 57–133 times greater than that of acyclovir, total absorption amount of acyclovir at the end of 4 h experiment was only about twice than that obtained after application of acyclovir itself. In our model analysis, this is theoretically confirmed (Bando et al., 1996a). Because lipophilicities of acyclovir prodrugs are not very high, contribution of the polar route, where penetration is independent of the lipophilicity of drug (Ackermann and Flynn, 1987a, Ackermann et al., 1987b), on total penetration process is still considered large. On the other hand, the enhancement effect of prodrugs was more remarkable under in vivo than in vitro conditions (Bando et al., 1996b). This observation was also theoretically confirmed based on our model analysis. We reported previously that the larger partition parameter for polar route under in vitro condition should be explained by the volume enlargement of the polar route (Yamashita et al., 1994). Also, it is well-known that hydration of the stratum corneum is an important factor in skin penetration (Idson, 1974) and this effect is more obvious under in vitro study where the skin is immersed in saline. Thus, the contribution of polar route towards total penetration under in vivo was smaller than that observed under in vitro condition. Therefore, the difference of

enhancement effect between in vivo and in vitro was explained by the difference of the contribution of polar route.

GACH greatly enhanced acyclovir up to 15.2-fold under in vivo condition, contrary to the results reported previously under in vitro condition (3.37-fold) (Bando et al., 1994, 1996a). This difference was also attributed to the low contribution of polar route to total penetration under in vivo system. GACH showed much larger enhancing effect for acyclovir prodrugs (Fig. 1) than that for acyclovir, corresponding to the previously presented theory as well as experimental results obtained under in vitro condition (Bando et al., 1994, 1996a). Moreover, enhancement effect induced by prodrug-enhancer combination was more remarkable under in vivo condition (maximum 48-fold for valerate). Hence, it can be concluded that higher enhancement effect observed under in vivo condition by lipophilic prodrugs as well as by prodrug-enhancer combination was due to the low contribution of polar route on total skin penetration.

Using stripped skin to estimate penetration through the viable layer was a convenient approach. However, having realized that the physiological conditions relating to enzymatic activity may be altered through the contact of the viable layer with donor solution, penetration parameters of prodrugs in the viable layer were not estimated from stripped skin. Our previous studies obviously reported that partition parameters of the viable layer did not depend on the lipophilicity of drugs (Bando et al., 1996; Yamashita et al., 1993). We analyzed acyclovir penetration through stripped skin, used the diffusion parameter of acyclovir which was corrected based on molecular weight (Lambart et al., 1989) and the same partition parameter of acyclovir instead of those of prodrugs.

Similar to our previous findings using the non-metabolized drugs (Yamashita et al., 1994), this model analysis demonstrated that drug partitioning (K'_{np}) of the nonpolar route was increased extensively in all prodrugs, but diffusivity (D'_{np}) was not much altered by GACH treatment, and this effect of GACH was almost the same between in vivo and in vitro condition (Bando et al., 1996b). 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 an increase in the polarity of the nonpolar route (Yamashita et al., 1993). The present results suggest that even if metabolic process is included under in vivo condition, this model analysis gives reasonable values of partitioning and diffusivity.

From our previous study, we had concluded that GACH decreased the enzymatic activity of skin under in vitro condition (Bando et al., 1996b). Similar phenomena were reported for other enhancers, e.g. Azone dissolved in propylene glycol decreased metabolism of nitroglycerin (Higo et al., 1992) and L-menthol as well as DL-camphor inhibited the hydrolysis of methyl salicylate in hairless mouse skin (Yano et al., 1991). According to this model analysis under in vivo condition, hydrolysis rate constant of isovalerate as well as pivarate was also shown to be decreased by the pretreatment with GACH (Table 2).

As shown in Table 1, absorption amounts of three prodrugs were almost the same under control condition, but for skin that was pretreated with GACH, the amounts were significantly different. The same tendency was also observed under in vitro condition (Bando et al., 1996b). From our previous reports (Bando et al., 1996a), we had expected similar enhancement effect on prodrugs which had the same lipophilicities. In our results, GACH showed greater enhancement effect on valerate than on the other two prodrugs. We failed to explain this phenomenon based on a diffusion model without considering the contribution of metabolism in the skin. In regard to the effect of metabolism on skin penetration, some reports theoretically revealed that lag time became shorter when a drug was metabolized in the skin (Tojo et al., 1985) and metabolic activity did change the ratio of drug and metabolize concentrations both appearing in the receptor fluid and distributing in the skin. (Tojo, 1987). In our previ-

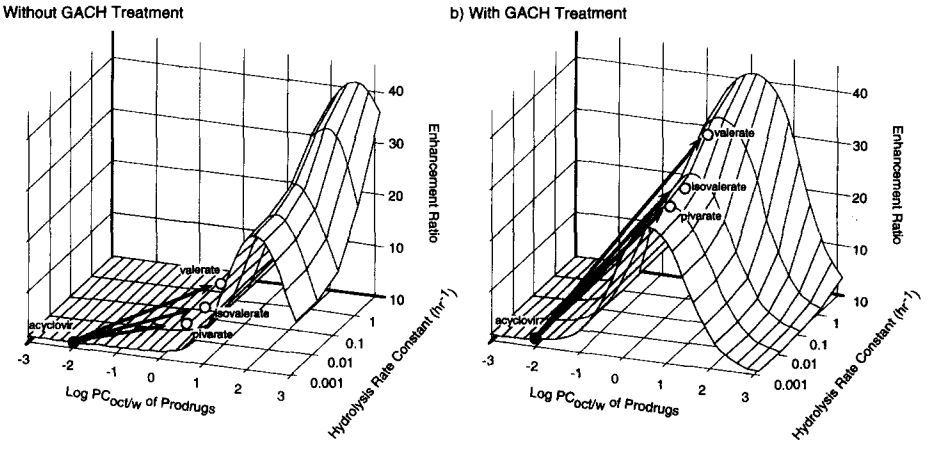

Fig. 2. Relationship among octanol/water partition coefficients of prodrugs, hydrolysis rate constants and enhancement ratio (a) without and (b) with GACH treatment calculated using penetration parameters reported previously (Yamashita et al., 1994). Enhancement ratio is defined as the total penetration amount of acyclovir within 12 h pretreated with and without GACH after application of prodrugs divided by the penetration amount of acyclovir pretreated without GACH after the application of acyclovir itself. The following parameters were used in this simulation: $D_{p,p}/L_s^2 = 50$ (h^{-1}); $D_{m,p}/L_s^2 = 60$ (h^{-1}); $D_{p,np}/L_s^2 = 1.3$ (h^{-1}), $D_{m,np}/L_s^2 = 1.5$ (h^{-1}); $D_{p,d}/L_d^2 = 0.07$ (h^{-1}); $D_{m,d}/L_d^2 = 0.08$ (h^{-1}) $K_{p,p}V_p = 0.00007$ (cm^3); $K_{m,p}V_p = 0.00007$ (cm^3); $K_{m,np}Y_{np} = 0.0001$ (cm^3) $K_{p,d}V_d = 3.5$ (cm^3); $K_{m,d}V_d = 3.5$ (cm^3); $\log K_{p,np}V_{np}$ (cm^3) $= 1.3 \log PC_{oct/w} - 3.0$ (without GACH); $\log K_{p,np}V_{np}$ (cm^3) $= 1.1 \log PC_{oct/w} - 0.9$ (with 25.5 μmol of GACH).

ous reports, skin diffusion/bioconversion model enabled us to comprehensively discuss percutaneous penetration concerning not only diffusivity and partitioning but also metabolism in the skin (Bando et al., 1996b). We carried out simulations to clarify the effect of lipophilicities of prodrugs and hydrolysis rate constants on the enhancement ratio based on this model. In this simulation, the enhancement ratio was determined from the ratio of total penetration amount after application of prodrugs to penetration amount after application of acyclovir ($\log PC_{oct/w} = -1.9$). From the results of simulation, it suggested that under control condition shown in Fig. 2(a), hydrolysis rate constants did not show any effect on the enhancement ratio of our prodrugs ($\log PC_{oct/w} \leq 0$), but only had an effect on the more lipophilic prodrugs ($\log PC_{oct/w} > 2$). Recent reports theoretically revealed that by using SM-10902 which possesses $\log PC_{oct/w}$ of 4.5, the higher metabolic rate constants were, the more skin penetration amount would increase (Sato and Mine, 1996). This phenomenon was explained based on a two-layer skin diffusion model without considering polar and nonpolar route in the stratum corneum. However, the model failed to explain the effect of metabolism on skin penetration of the more hydrophilic drugs where the contribution of polar route was still considered large. GACH increases partitioning of most drugs into nonpolar route, and enhances drug permeation by shifting the relationship between penetration amount and $PC_{oct/w}$ to the left. As a result, hydrolysis rate constants show a significant effect on the enhancement ratio of our prodrugs, i.e. higher hydrolysis rate constant indicates higher total drug penetration (Fig. 2(b)). These experimental and theoretical results suggested that it is important to consider metabolic process in order to accurately estimate skin penetration of drug which is metabolized through the skin, especially in the cases where high lipophilic prodrug or prodrug-enhancer combination is used.

In conclusion, prodrug-enhancer combination based on theoretical considerations also revealed great enhancement effect on the skin penetration of acyclovir under in vivo condition. Base on a series of our experimental and theoretical investigations, an effective approach to increasing drug absorption can be proposed. By synthesis of a

prodrug with $\log PC_{oct/w}$ of around -0.3 and with higher degradation rate constant, skin permeation of a drug can be markedly enhanced by GACH. This combined approach would be applicable to a wide range of drugs, since extreme alteration of the physicochemical properties of drugs is not necessary as in single prodrug application. In addition, the amount of an enhancer necessary to improve drug absorption might be reduced by optimizing physicochemical properties of prodrugs based on theoretical design.

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