In Vivo Evaluation of Acyclovir Prodrug Penetration and Metabolism Through Rat Skin Using a Diffusion/Bioconversion Model

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Purpose. In order to evaluate the *in vivo* penetration of prodrugs which undergo metabolism in skin, we analyzed the *in vivo* penetration profiles of acyclovir prodrugs based on a two-layer skin diffusion model in consideration of metabolic process.

Methods. Acyclovir prodrugs (e.g., valerate, isovalerate and pivarate) were used as model prodrugs and the amounts excreted in urine were measured after percutaneous application. *In vivo* penetration profiles were then estimated by employing a deconvolution method and the penetration of acyclovir prodrugs was analyzed using a diffusion model. Subsequently, diffusion, partitioning and metabolic parameters were compared under *in vitro* and *in vivo* conditions.

Results. Although total penetration amounts at the end of the experiment were similar for the three prodrugs, the ratio of intact prodrug to total penetration amount differed significantly. Moreover, the excretion and absorption profiles were also very different for each prodrug. Enzymatic hydrolysis rate constants calculated under *in vivo* conditions were considerably larger than those obtained in the skin homogenate and *in vitro* penetration experiments.

Conclusions. The present skin diffusion/bioconversion model combined with computer analysis enables us to comprehensively account for diffusion, partitioning and metabolism during *in vivo* percutaneous absorption. Nevertheless, different enzymatic hydrolysis rate constants obtained under both *in vivo* and *in vitro* conditions demonstrate the difficulty of obtaining accurate values for *in vivo* enzymatic activity from related *in vitro* experiments.

KEY WORDS: *in vivo* skin penetration; acyclovir prodrug; diffusion/bioconversion model; deconvolution.

INTRODUCTION

In our series of investigations, a prodrug/enhancer combination offered an effective and rational way of increasing transdermal delivery of a wide range of drugs (1). In this approach, we showed that since skin possesses significant enzymatic activity, prodrugs applied topically are metabolized by the skin. Metabolic action occurring in underlying skin greatly influences the concentration of a drug in the skin, particularly those agents which exhibit local pharmacological effects. Thus, it is necessary to establish new concepts and to introduce accurate experimental approaches for evaluating skin metabolism after topical application of drugs. Indeed, there are already several ways to do this: through skin homogenate experiments (2), uptake-metabolism experiments (3), isolated perfused skin models (4), and model analysis of drug transport (5). However, since these experiments are conducted under *in vitro* conditions, certain questions remain as the anatomy and physiology of *in vivo* skin is complex. Actually, we were unable to assay *in vivo* cutaneous metabolism of metabolites produced by the skin before they entered the blood circulation.

In our previous study, we demonstrated quantitative *in vitro/in vivo* differences in skin penetration in terms of diffusion and partitioning based on a diffusion model (6). However, the differences in the metabolic process had yet to be elucidated at that time. Nevertheless, we did propose a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum, in relation to the metabolic process in the viable epidermis and dermis (7). This model could comprehensively account for *in vitro* skin permeation of acyclovir prodrugs and enabled us to discuss percutaneous drug absorption using parameters representing the diffusion process through each region (7).

The main objective of the present study is to evaluate *in vivo* penetration of prodrugs metabolized in skin. Initially, *in vivo* penetration profiles were determined using a deconvolution method which enabled us to estimate first-pass metabolism (8). Subsequently, in order to comprehensively investigate diffusion, partitioning and metabolism during *in vivo* percutaneous absorption, we analyzed the penetration of acyclovir prodrugs through rat skin using a diffusion model which included the metabolic process, while diffusion, partition and metabolic parameters were compared under both *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Materials

Acyclovir was kindly supplied by Nippon Wellcome K. K., Osaka, Japan. Esterification of acyclovir was carried out as described previously (1). In this study, we used three acyclovir prodrugs, all of which were structural isomers of acyclovir valerate. These prodrugs have a similar lipophilicity but a different enzyme affinity (7). Radiolabeled ^{3}H-acyclovir and ^{14}C-mannitol were obtained from Daiichi Pure Chemicals, Japan. Other materials were purchased from Nacalai Tesque Inc., Kyoto, Japan.

In Vivo Skin Penetration Experiment

The *in vivo* absorption experiment was performed as previously reported (6). Under anesthesia induced by intraperitoneal injection of 1 g/kg urethane, the abdominal hair of male Wistar rats 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 bare abdominal surface using cyanoacrylate adhesive (Aron Alpha A, Sankyo Co., Japan). A preapplication period of 6 hr was established to uniformly condition the skin, and then the urinary bladder was cannulated using 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 hr by injecting 0.2 ml 0.9% NaCl solution through the tubing twice

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before each sampling time. At the end of the experiment, drug remaining in the donor cell was recovered and the skin was excised. The stripped skin experiment using acyclovir was performed as previously reported (6).

Intravenous Injection Experiment

Intravenous injection was performed to investigate the disposition of the tested drugs and to estimate their absorption profiles by a deconvolution method (6,8). Under urethane anesthesia, 0.2 ml (0.5 mM) 0.9% NaCl solution containing drug was injected into the femoral vein. Urine was collected by the method described above.

Analysis of Radioactivity

Each urine sample was divided into two portions. One was used to measure the total penetrated amount and the other to obtain the ratio of acyclovir to total penetrated amount. After evaporation of the samples and resolubilization in methanol, they were analyzed by thin-layer chromatography (TLC) on silica-gel sheets (Silica gel $60F_{254}$, Merck Japan Ltd., Tokyo, Japan) using a solvent system of chloroform:methanol (3:1) and were visualized under ultraviolet (UV) light. Then, TLC fractions were directly mixed with a scintillation cocktail and the radioactivity was measured using a liquid scintillation counter (LSC-5000, Beckman, Japan). After solubilization of the urine samples to determine the total penetration amount and treating skin samples with Soluene-350 (Packard Instrument, IL), the radioactivity in each sample was determined.

Deconvolution Method

The *in vivo* fate of a prodrug undergoing first-pass metabolism in the skin after transdermal administration is schematized in Fig. 1. Prodrug in its intact form, after entering the systemic circulation from the absorption site, is either directly excreted in urine or further metabolized in the systemic circulation before being eliminated from the body. Similarly, metabolites formed during the absorption process undergo the same disposition fate as that of metabolites. By using Laplace transforms for input functions of prodrug and metabolized acyclovir ($\tilde{I}_p(s)$ and $\tilde{I}_d(s)$), the response function of metabolite ($\tilde{O}_d(s)$) can be given as follows:

$$\tilde{O}_d(s) = \{F_c \times g_p(s) \times \tilde{I}_p(s) + \tilde{I}_d(s)\} \times g_d(s)$$
$$= \tilde{I}_p(s) \times \{F_c \times g_p(s) \times g_d(s)\} + \tilde{I}_d(s) \times g_d(s) \quad (1)$$

where $g_p(s)$ and $g_d(s)$ are the transfer functions of total elimination of prodrug and metabolite from the body respectively, and F_c is the function of drug eliminated by metabolism. $F_c \times g_p(s) \times g_d(s)$ can be considered as a response function of metabolite after impulse input of prodrug.

Supposing that the characteristic response functions of a prodrug and its metabolite after impulse input of the prodrug are $G_p(t)$ and $G_{p \rightarrow d}(t)$ respectively, and that of the metabolite after impulse input of that same metabolite is $G_d(t)$, the response functions of the prodrug ($O_p(t)$) and its metabolite ($O_d(t)$) after transdermal administration of that prodrug are therefore as follows:

$$O_p(t) = \int_0^t I_p(\theta) G_p(t - \theta)\, d\theta \quad (2)$$

$$O_d(t) = \int_0^t I_p(\theta) G_{p \rightarrow d}(t - \theta)\, d\theta + \int_0^t I_d(\theta) G_d(t - \theta)\, d\theta \quad (3)$$

where $I_p(t)$ and $I_d(t)$ represent the rates at which the prodrug and its metabolite enter the systemic circulation. Hence, $I_p(t)$ and $I_d(t)$ can be estimated from the following successive calculations: (1) $I_p(t)$ is first estimated by a deconvolution calculation using $O_p(t)$ and $G_p(t)$; (2) The first term in Eq. (3) is obtained by convoluting $I_p(t)$ and $G_{p \rightarrow d}(t)$; (3) $I_d(t)$ is estimated by deconvoluting the term obtained by subtraction of the calculated first term from $O_d(t)$ together with $G_d(t)$. A series of calculations was performed based on the programs reported previously for oral administration (8). This analysis is not valid when the elimination of a drug from the body is a non-linear process, as described previously (8).

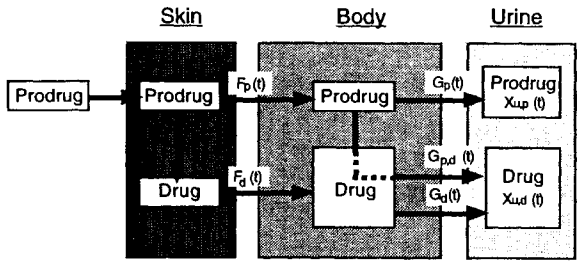

Fig. 1. The pharamacokinetic model to estimate prodrug penetration through the skin after *in vivo* percutaneous application using the deconvolution method. $F_p(t)$ and $F_d(t)$ denote the percutaneous absorption rate for prodrug and drug respectively; $G_p(t)$, $G_{p,d}(t)$, and $G_d(t)$ represent the urinary excretion rate for prodrug → prodrug, prodrug → drug, and drug → drug respectively; $X_{u,p}(t)$ and $X_{u,d}(t)$ indicate the amount excreted in urine for prodrug and drug respectively.

Data Analysis

In order to evaluate the *in vivo* penetration of acyclovir prodrugs that are metabolized in skin, the penetration profiles obtained *in vivo* were analyzed by a model which had been used for the analysis of skin penetration *in vitro* (7). *In vivo*, the equations were derived at finite donor conditions because the drugs applied were in solution (9). Fig. 2 (A) shows a diffusion model for prodrugs whereas (B) represents that of the regenerated parent drug. In the present context, skin is considered to be composed of two serial layers, i.e. stratum corneum and the underlying viable tissue. The former has parallel polar and nonpolar routes. The blood compartment is linked to the viable layer through the relationship of "clearance". Also, the vehicle as well as the blood compartment is assumed to exist under well-stirred conditions, and perfect sink conditions were assumed at the donor/skin boundary for the generated parent drug without considering the effect of diffusion boundary layer on the skin surface. Even when the resistance of the boundary layer was assumed to be infinitely large ($dC/dx = 0$ instead of $C = 0$), the penetrated amount of generated parent drug changed only by about 0.31%. Thus, it was confirmed by the simulation that the effect of the boundary conditions on drug penetration is minimal. In addition, the same assumptions

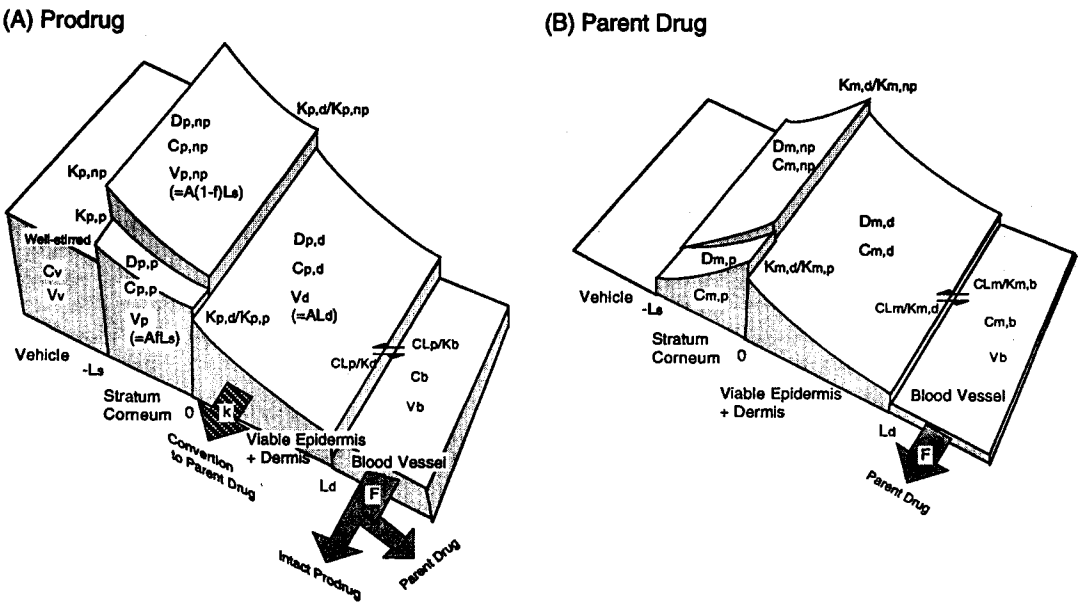

Fig. 2. The physiological skin diffusion/bioconversion model with polar and nonpolar routes in the stratum corneum combined with the blood compartment. (A) represents the diffusion model for prodrug, (B) shows the diffusion model for regenerated parent drug. Well-stirred and sink conditions are assumed in the vehicle and blood compartments. Note that each partition coefficient is defined against the vehicle. *K*, partition coefficient; *D*, diffusion coefficient; *V*, volume; *L*, distance; *f*, area fraction of the polar route; *v*, vehicle; *CL*, clearance through capillary wall; and *F*, blood flow. First subscripts *p* and *m* reveal prodrug and metabolized parent drug and second subscripts *p*, polar domain; *np*, nonpolar domain; *d*, viable epidermis and dermis, respectively.

as those previously reported were made (7). Based on this model, the Laplace transforms for the amount of prodrug and parent drug appearing on the receptors across the intact skin can be expressed as follows:

$$\tilde{Q}_{p,\mathrm{int}} = C_0 V_1 Z_{p,d}(Z_{p,p}\sinh d_{p,np} + Z_{p,np}\sinh d_{p,p})/s/k(s) \quad (4)$$

$$\tilde{Q}_{m,\mathrm{int}} = C_0 V_1 K_{p,d} V_d k(Z_{p,p}\sinh d_{p,np} + Z_{p,np}\sinh d_{p,p}) * (m(s)/I(s) - d_{p,d})/s/(d_{p,d}^2 - d_{m,d}^2)/k(s) \quad (5)$$

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

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

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

$$d_{p,d} = L_d\sqrt{(s + k)/D_{p,d}} \quad (8)$$

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

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

$$d_{m,d} = L_d\sqrt{s/D_{m,d}} \quad (11)$$

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

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

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

$$Z_{m,p} = D_{m,p}K_{m,p}V_p d_{m,p} \quad (15)$$

$$Z_{m,np} = D_{m,np}K_{m,np}V_{np}d_{m,np} \quad (16)$$

$$Z_{m,d} = D_{m,d}K_{m,d}V_d d_{m,d} \quad (17)$$

$$\begin{aligned} k(s) = V_v s(&Z_{p,p}\cosh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &+ Z_{p,np}\sinh d_{p,p}\cosh d_{p,np} * \sinh d_{p,d} \\ &+ Z_{p,d}\sinh d_{p,p}\sinh d_{p,np}\cosh d_{p,d}) \\ &+ Z_{p,p}\{Z_{p,p}\sinh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &+ Z_{p,np}\sinh d_{p,d} * (\cosh d_{p,p}\cosh d_{p,np} - 1) \\ &+ Z_{p,d}\cosh d_{p,p}\sinh d_{p,np}\cosh d_{p,d}\} \\ &+ Z_{p,np}\{Z_{p,np}\sinh d_{p,p}\sinh d_{p,np}\sinh d_{p,d} \\ &+ Z_{p,p}\sinh d_{p,d} * (\cosh d_{p,p}\cosh d_{p,np} - 1) \\ &+ Z_{p,d}\sinh d_{p,p}\cosh d_{p,np}\cosh d_{p,d}\} \end{aligned} \quad (18)$$

$$\begin{aligned} l(s) = &Z_{m,p}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{m,d} \\ &+ Z_{m,np}\sinh d_{m,p}\cosh d_{m,np} * \sinh d_{m,d} \\ &+ Z_{m,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{m,d} \end{aligned} \quad (19)$$

$$\begin{aligned} m(s) = &Z_{m,p}d_{m,d}\cosh d_{m,p}\sinh d_{m,np}\sinh d_{p,d} \\ &+ Z_{m,np}d_{m,d}\sinh d_{m,p}\cosh d_{m,np} * \sinh d_{p,d} \\ &+ K_{m,d}D_{m,d}d_{m,d}d_{p,d}\sinh d_{m,p}\sinh d_{m,np}\cosh d_{p,d} \end{aligned} \quad (20)$$

Note that the parameters used in the above equations are defined in the figure legends.

Despite the model shown in Fig. 1, *in vivo* absorption could be analyzed by these equations because the washout process via blood flow occurred quickly and could be neglected. Curve-fitting to *in vivo* data was carried out for the urinary

excretion profile in order to minimize any errors arising during numerical calculation. By expressing the excretion profile as a poly-exponential function ($dXu/dt = A*e^{-dt} + B*e^{-pt} + \cdots$), the Laplace transformed equation for the urinary excretion ($\tilde{X}u$) of a 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 the urinary excretion data after topical application. Curve-fitting of Eq. (4) and (5) to the data was conducted as previously reported (6,7,9). Due to the difficulty in determining the real diffusional pathlength, we modified D_j and K_j as follows:

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

$$K_j' = K_jV_j \qquad (j = p, np, \text{ or } d) \quad (23)$$

The seven hybrid parameters for each prodrug were determined according to the procedures reported previously (7). The $K_{p,d}'$ of all prodrugs was taken as being equal to the value for acyclovir and $D_{p,d}'$ was corrected for the molecular weight (10).

RESULTS

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 by poly-exponential functions as follows by a least squares regression analysis:

$$dXu/dt = 166*\exp(-2.61*t) + 17.4*\exp(-0.666*t) \quad \text{(for acyclovir)}$$

$$dXup/dt = 244*\exp(-15.1*t) \quad \text{(valerate} \rightarrow \text{valerate)}$$

$$dXu_m/dt = -180*\exp(-15.1*t) + 166*\exp(-2.61*t) + 15.0*\exp(-0.666*t) \quad \text{(valerate} \rightarrow \text{acyclovir)}$$

$$dXu_p/dt = 397*\exp(-9.08*t) \quad \text{(isovalerate} \rightarrow \text{isovalerate)}$$

$$dXu_m/dt = -147*\exp(-9.08*t) + 136*\exp(-2.61*t) + 11.0*\exp(-0.666*t) \quad \text{(isovalerate} \rightarrow \text{acyclovir)}$$

$$dXu_p/dt = 223*\exp(-5.72*t) \quad \text{(pivarate} \rightarrow \text{pivarate)}$$

$$dXu_m/dt = -185*\exp(-5.72*t) + 173*\exp(-2.61*t) + 11.2*\exp(-0.666*t) \quad \text{(pivarate} \rightarrow \text{ayclovir)}$$

where dXu/dt expresses the urinary excretion rate (% dose/hr), and subscripts p and m denote prodrug and metabolized acyclovir respectively. Since the standard deviation of each parameter was about 10% or less, errors arising during numerical calculations could be neglected (8).

Fig. 3 shows the excretion as well as the absorption profiles obtained by a deconvolution method for prodrugs applied *in vivo*. In the case of valerate, we evaluated the absorption profile by assuming that it was completely metabolized in the skin. Table I summarizes the drug amounts recovered in the donor, skin and urine, and the calculated amounts of systematically absorbed drugs at the end of the 4 hr absorption experiment. Although the total penetration amounts at the end of the experiment were almost the same for the three prodrugs, the ratio of intact prodrug to total penetration amount was significantly different. Valerate was completely excreted in urine as metabolized acyclovir, whereas 40% of pivarate remained intact. Furthermore, the excretion and absorption profiles of isovalerate as well as pivarate were very different; especially in the case of pivarate where 60% of the excreted amount appeared as metabolized acyclovir with hardly any metabolism in the skin. The enhancement ratio compared with acyclovir penetration by employing the prodrug approach was about two.

Analysis of Penetration Profiles Based on a Diffusion/Bioconversion Model with Polar and Nonpolar Routes in the Stratum Corneum

Table II summarizes the *in vivo* penetration parameters of acyclovir, isovalerate and pivarate for polar and nonpolar routes, and the lower viable layer. In order to determine the parameters for the nonpolar route, the predetermined D_p' and K_p' for mannitol, the D_d' and K_d' for each drug as well as the D_{np}' and K_{np}' for

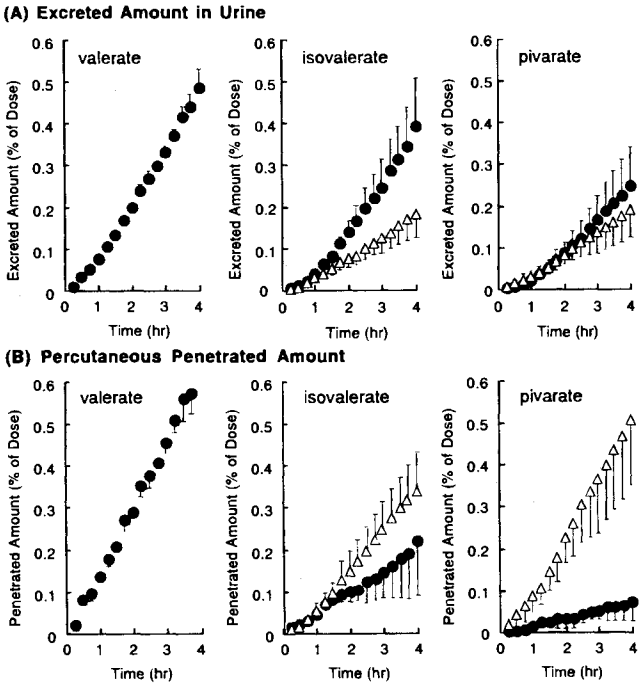

Fig. 3. (A) Time-courses of the amount excreted in urine of intact prodrug (△) and regenerated acyclovir (●) after percutaneous application of acyclovir prodrugs. (B) Time-courses of *in vivo* percutaneous absorbed amount of intact prodrug (△) and regenerated acyclovir (●) after percutaneous application of acyclovir prodrugs. The absorption profiles were evaluated from urinary excretion data using the deconvolution method. Each point represents the mean value of at least three experiments.

Table I. Amounts of Acyclovir and Acyclovir Prodrugs Recovered at the End of 4 hr *in Vivo* Absorption Experiments

		Acyclovir	Valerate	Isovalerate	Pivarate
Donor (% applied)		99.08 ± 1.14	98.09 ± 2.10	98.00 ± 1.37	97.68 ± 3.56
Skin (% applied)		0.32 ± 0.13	1.18 ± 0.33	0.94 ± 0.09	1.10 ± 0.39
Urine (% applied)	acyclovir	0.24 ± 0.13	0.47 ± 0.04	0.29 ± 0.11	0.25 ± 0.10
	prodrug	—	N.D.	0.14 ± 0.05	0.19 ± 0.06
Absorbed[a] (% applied)	acyclovir	0.30 ± 0.16	0.63 ± 0.08	0.21 ± 0.12	0.07 ± 0.05
	prodrug	—	N.D.	0.32 ± 0.09	0.51 ± 0.16
Total[b] (% applied)		99.70 ± 1.27	99.90 ± 1.94	99.48 ± 1.36	99.36 ± 3.68

[a] Values were calculated using a deconvolution method.
[b] Values are the sum of the amount of drugs in the donor, skin, and absorbed.

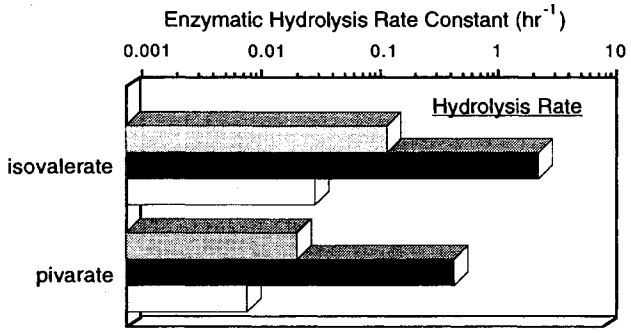

Fig. 4. Comparison of enzymatic hydrolysis rate constants obtained in *in vitro* (□), *in vivo* (■) and skin homogenate (□) experiments respectively. The *in vitro* experiments were referred from (7). In the homogenate experiments, evaluation was carried out with pseudo-first order rate constants in 3.3% (*w/v*) tissue homogenate (7).

acyclovir were employed in the calculations. The values of the D'_{np} and K'_{np} of isovalerate and pivarate were almost equal to those obtained from *in vitro* experiments (7).

Whereas it was possible to estimate from a mathematical analysis of the excretion profiles the *in vivo* enzymatic hydrolysis rate constants in the viable epidermis and dermis, this could not be done for valerate because it was excreted as metabolized acyclovir in urine. The enzymatic hydrolysis rate constant of pivarate was significantly smaller than that of isovalerate. In Fig. 4, the enzymatic hydrolysis rate constants estimated from skin homogenate, *in vitro* and *in vivo* penetration experiments, are compared for isovalerate and pivarate (7). The absolute values of the enzymatic hydrolysis rate constants differed among these experimental systems for both prodrugs, going in increasing rank order from skin homogenate, to *in vitro* and then *in vivo* penetration experiments.

DISCUSSION

After transdermal administration, drugs undergo first-pass metabolism through the skin before entering the systemic circulation. From a pharmaceutical viewpoint, it is important to have mach information as possible on the penetration of drugs and their metabolites in order to evaluate their therapeutic activity. So far, several pharmacokinetic models have been proposed to simulate the effect of skin metabolism on the percutaneous penetration of drugs (11) and to estimate their first-pass metabolism (12) under *in vivo* conditions. In most of these analyses, excluding simulations, drugs and their metabolites are usually assumed to be absorbed into the systemic circulation by a first-order process. On the other hand, the usefulness of a model-independent analysis has also been emphasized in many cases, for example a deconvolution method has been employed in the evaluation of drug absorption rates without the use of proper kinetic models. This method is applicable even when the input process cannot be approximated with simple terms (e.g. first- and zero-order input). In our previous reports, we proposed a deconvolution method for estimating the first-pass metabolism of orally administered drugs (8) and here we have applied this method to transdermal administration. Some studies have reported that acyclovir itself is metabolized in the body and undergoes two major metabolic reactions, i.e. oxidation of the acyclic moiety and 8-hydroxylation (13). Surprisingly, our experimental methods did not encounter any problems even although we measured only two forms in the urine, prodrug and acyclovir. This is because the biotransformation of acyclovir varies significantly among species and, in the case of rats, acyclovir does not undergo further metabolism (13).

Valerate was excreted as acyclovir in urine. Knowing that the degradation of valerate occurs quickly in skin homogenate, and that more than 92% of the drug appears in the receptor fluid under *in vitro* conditions in metabolized form (7), we managed to deconvolute valerate by assuming that it is completely metabolized in the skin. The branching of the side-chain of acyclovir pivarate resulted in much slower hydrolysis than that of valerate and isovalerate. This is attributed to steric hindrance of the substituent which inhibits drug binding to the active enzyme site. Similar results have been shown for acyclovir prodrugs in rat plasma (14), and in a *in vitro* penetration experiment (7).

In this study, we did not identify the specific type of enzyme involved in the metabolism of acyclovir prodrugs, but it has been reported to be carboxylesterase (14). Although the extent of enzymatic degradation differed among these prodrugs, total penetration amounts at the end of the experiment were almost the same and this phenomena was also seen under *in vitro* conditions (7). This is because the lipophilicity of these

Table II. Estimated Parameters for Drug Penetration Through the Skin Under *in Vivo* Conditions

	Stratum corneum				
	Polar route		Nonpolar route		Enzymatic hydrolysis rate constant
Drug	Dp'^a (hr^{-1})	Kp'^b ($\times 10^5 cm^3$)	Dnp'^c (hr^{-1})	Knp'^c (cm^3)	k^d (hr^{-1})
acyclovir	54.7	0.612	6.79	0.000126	—
	$Dd' = 2.84(hr^{-1})^e$		$Kd' = 0.084(cm^3)^f$		
valerate	49.2	0.612	—	—	—
	$Dd' = 2.56(hr^{-1})^e$		$Kd' = 0.084(cm^3)^f$		
isovalerate	49.2	0.612	5.84	0.000196	2.98
	$Dd' = 2.56(hr^{-1})^e$		$Kd' = 0.084(cm^3)^f$		
pivarate	49.2	0.612	5.20	0.000233	0.583
	$Dd' = 2.56(hr^{-1})^e$		$Kd' = 0.084(cm^3)^f$		

[a] Diffusion parameters for the polar route in the stratum corneum (Dp′) were calculated by correcting the corresponding values for mannitol for the molecular weight according to the following equation. $D_{drug} = D_{mannitol}\sqrt[3]{(MW_{mannitol})/(MW_{drug})}$

[b] Partition parameters for the polar route in the stratum corneum (Kp′) were considered to be the same as the corresponding values for mannitol.

[c] Parameters for the nonpolar route in the stratum corneum (Dnp′, Knp′).

[d] Enzymatic hydrolysis rate constant in the second viable layer (k).

[e] Parameters for the viable layer (Dd′) were calculated by correcting the corresponding values for acyclovir for the molecular weight.

[f] Partition parameters for the viable layer (Kd′) were considered to be the same as the corresponding values for acyclovir.

prodrugs is not very high and, consequently, penetration through the stratum corneum still acts as the rate-limiting step of total penetration. Therefore, metabolism in the viable epidermis and dermis essentially has no influence over the total penetration of these prodrugs.

Regarding the enhancement effect by a lipophilic prodrug, the total penetration amount of acyclovir at the end of the 4 hr experiment after administration of acyclovir prodrug was about twice that obtained after application of acyclovir, and this effect was more marked under *in vivo* than *in vitro* conditions (7). Due to the low lipophilicity of acyclovir prodrugs, the contribution of the polar route, where penetration is independent of lipophilicity (6,9), to the total penetration is considered to be large. It is also well-known that hydration of the stratum corneum is an important factor in skin penetration and this effect is more marked under *in vitro* conditions where the skin is immersed in saline (6). Hence, it can be concluded that the high enhancement effect observed under *in vivo* conditions for lipophilic prodrugs is due to the low contribution from the polar route to total skin penetration.

Using stripped skin to estimate penetration through the viable layer is a convenient approach. However, having realized that the physiological conditions relating to enzymatic activity may be altered through contact of the viable layer with donor solution, the penetration parameters of prodrugs in the viable layer were not estimated from stripped skin. Our previous studies reported that partitioning parameters of the viable layer did not depend on drug lipophilicity (7,9). We analyzed acyclovir penetration through stripped skin, and used the diffusion parameter of acyclovir which was corrected for molecular weight (10) and the same partitioning parameter of acyclovir instead of those of the prodrugs.

Based on the analysis using a diffusion/bioconversion model, the same one used for *in vitro* analysis, it was found that the values for the diffusion and partition parameters in the nonpolar route were similar to those obtained in *in vitro* experiments. Similar results have also been reported for nonmetabolized drugs (6). In addition, we managed to estimate the *in vivo* enzymatic hydrolysis rate constants and these were significantly larger than those obtained from other experiments (Fig. 4). This may be due to leakage of enzyme from the skin into receptor fluid (15), or a decline in enzymatic activity during the *in vitro* experiment (5). Since the *in vivo* experiment was conducted under near physiological conditions, the values for the hydrolysis rate constants are considered more reasonable.

In conclusion, we could intuitively estimate *in vivo* skin penetration profiles for both intact prodrug and metabolized acyclovir by means of this new deconvolution method. The present skin diffusion/bioconversion model combined with computer analysis enables us to comprehensively account for diffusion, partitioning and metabolism during *in vivo* percutaneous absorption. Nevertheless, the different enzymatic hydrolysis rate constants obtained under both *in vivo* and *in vitro* conditions show that it is difficult to obtain accurate values for *in vivo* hydrolysis activity from related *in vitro* experiments. However, this approach may be very effective in predicting the therapeutic usefulness of a transdermal drug delivery system, especially when design a prodrug.

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