Theoretical Design of Prodrug-Enhancer Combination Based on a Skin Diffusion Model: Prediction of Permeation of Acyclovir Prodrugs Treated with 1-Geranylazacycloheptan-2-one

Hiroto Bando,[1] Toshihide Takagi,[1] Fumiyoshi Yamashita,[1] Yoshinobu Takakura,[1] and Mitsuru Hashida[1,2]

Received May 22, 1995; accepted November 14, 1995

Purpose. A theoretical design of percutaneous penetration enhancement in which prodrug derivation and enhancer application are combined is proposed based on the skin diffusion model and it is experimentally verified.

Methods. Employing acyclovir as a model drug, the hypothesis was tested by synthesis of its prodrugs and evaluation of their in vitro permeation in the rat skin, with or without a penetration enhancer, 1-geranylazacycloheptan-2-one(GACH).

Results. Among five acyclovir prodrugs, those with higher lipophilicities (propionate, butyrate, valerate, and hexanoate prodrugs) showed greater skin penetration than those of hydrophilic prodrugs (acetate), when administered in combination with GACH. Furthermore, the observed enhancement ratios were in good agreement with those predicted by theoretical consideration.

Conclusions. Thus, skin permeation of prodrugs applied with an enhancer can be predicted and optimized by model analysis.

KEY WORDS: prodrug-enhancer combination; percutaneous absorption; acyclovir; 1-geranylazacycloheptan-2-one.

INTRODUCTION

Recently, some methodologies for improving skin permeation of poorly absorbed drugs, such as application of penetration enhancers (1), prodrugs (2), and iontophoresis (3) have been established. In addition, two or more of these approaches have been combined to increase drug absorption more efficiently (4). Although some approaches have helped improve skin permeation of drugs, these delivery systems have been developed only through trial and error. The mechanisms of skin permeation of drugs and/or its enhancement have been examined from various points of view. Nevertheless, there have been few reports on optimization of drug absorption rationally achieved based on these mechanisms.

For rational design of drug absorption enhancement, a pharmacokinetic model which enables us to analyze skin permeation of drugs quantitatively and comprehensively is necessary. For this purpose, several kinetic models have been developed (5,6). We developed a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum, which can explain the mechanism of skin permeation of various drugs and the effects of enhancers on it, based on physicochemical viewpoints (7).

In the present study, a method of percutaneous absorption enhancement of acyclovir is theoretically designed based on the action mechanism of a penetration enhancer 1-geranylazacycloheptan-2-one (GACH) which has already been revealed by the diffusion model analysis (7). Based on the relationship between the enhancement effect of GACH and physicochemical properties of the drug, skin permeation of the drug was hypothesized to be enhanced most effectively by synthesizing a prodrug with the optimal lipophilicity for the enhancer. In this experiment, acyclovir was used as a model compound which shows little enhancing effect by GACH. Various prodrugs of acyclovir were synthesized to evaluate their skin permeabilities in the presence of GACH and their permeabilities were compared with those predicted theoretically.

THEORETICAL BACKGROUND

Laplace-transformed equations representing drug permeation were previously derived from Fick's diffusion law based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum (7). The Laplace transform for the amount of drug appearing in the receptor across the skin under the finite-dosing condition is expressed as follows (7);

$$\tilde{Q} = Z_d X_0 (Z_{np} \sinh d_p + Z_p \sinh d_{np})/s/g(s) \qquad (1)$$

where s is the Laplace operator with respect to time and X_0 is the initially applied dose, and Z_d, Z_{np}, Z_p, g(s), d_p, and d_{np} substituted for the equations demonstrated in our previous report (7).

The penetration amount at any time was calculated from Eq. (1) using a fast inverse Laplace transform FILT algorithm (8) on the main frame computer M-382 of the Kyoto University Data Processing Center. In this analysis, the partition coefficient for the nonpolar route (K_{np}) was related to the octanol-water partition coefficient ($PC_{oct/w}$) based on a linear free-energy relationship (9).

$$\log K_{np} = \alpha \log K_{oct/w} + \beta \qquad (2)$$

where the values of α and β are 1.3 and -3.0 for control, 1.1 and -0.9 for treatment with 51.0 μmol of GACH, respectively. The partition coefficients for polar route (K_p) in the stratum corneum was assumed to be unity as we used water for the vehicle (10). Since there is little difference in the permeation of drugs with diverse lipophilicity through the tape stripped skin (7), we assume that the partition coefficient (K_d) between the vehicle and viable skin layer is one regardless of lipophilicity of the drug. The diffusion parameter in each route was independent of the species of drugs. Each parameter was referred from our previous report on the analysis of skin penetration of drugs with different lipophilicities (7).

Fig. 1 shows the relationship between the penetration amount over 24 hr in the finite dose system and the $PC_{oct/w}$ value of drug with or without GACH. Without the enhancer, the relationship between skin permeation and $PC_{oct/w}$ of drug is bell-shaped. The drug with a logarithm of $PC_{oct/w}$ of 1.7 shows the highest permeation under this condition. If we try

[1] Department of Drug Delivery Research, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.
[2] To whom correspondence should be addressed.

0724-8741/96/0300-0427$09.50/0

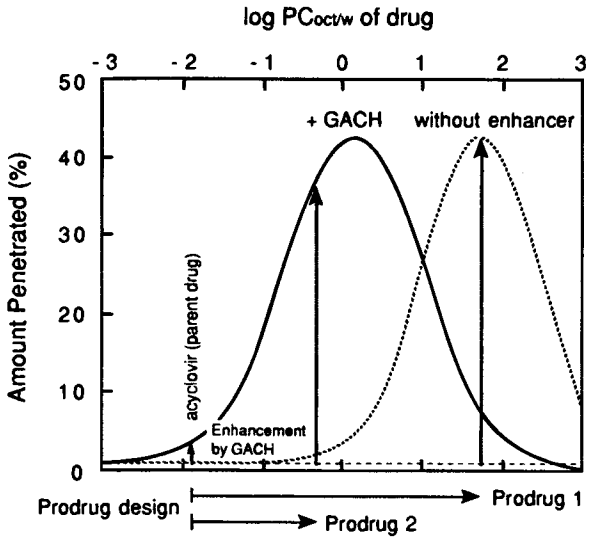


Fig. 1. Relationship between octanol/water partition coefficient of drugs and skin penetration with or without GACH calculated with penetration parameters reported previously (16). Dotted and solid lines represent skin penetration without and with GACH, respectively. Skin penetration is expressed as the amount of drug penetrating within 24 hr in the finite dose system. The following parameters were used in this simulation: $D_p/L_s^2 = 40(hr^{-1})$; $D_{np}/L_s^2 = 1.5(hr^{-1})$; $D_d/L_d^2 = 0.06(hr^{-1})$; $K_pV_p = 0.000015(cm^3)$; $K_dV_d = 0.7(cm^3)$; $\log K_{np}V_{np}(cm^3) = 1.3*\log PC_{oct/w} - 3.0$ (without enhancer); $\log K_{np}V_{np}(cm^3) = 1.1*\log PC_{oct/w} - 0.9$ (with GACH 25.5μmol).

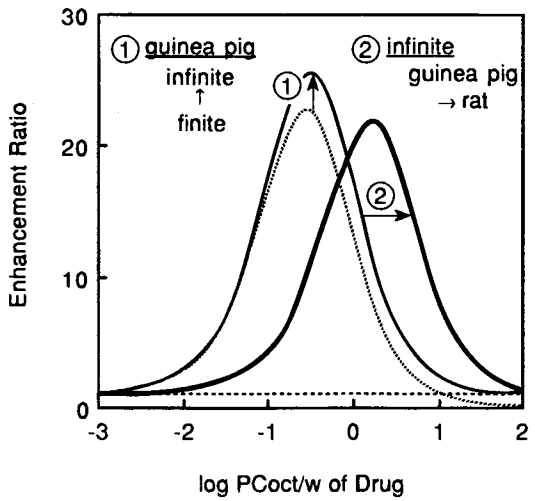


Fig. 2. Relationship between octanol/water partition coefficient of drug and enhancement effect of GACH under different conditions. In the finite dose system the enhancement effect is defined as the amount of drug penetrating within 24 hr with GACH divided by that without GACH, while in the infinite dose system it is defined as the ratio of permeability coefficient of drug with GACH to that without GACH treatment (control). The following parameters were used in this simulation: $D_p/L_s^2 = 40$(guinea pig) or $60(rat)(hr^{-1})$; $D_{np}/L_s^2 = 1.5(hr^{-1})$; $D_d/L_d^2 = 0.06$(guinea pig) or $0.08(rat)(hr^{-1})$; $K_pV_p = 0.000015$(guinea pig) or $0.00007(rat)(cm^3)$; $K_dV_d = 0.7$(guinea pig) or $3.5(rat)(cm^3)$; $\log K_{np}V_{np}(cm^3) = 1.3*\log PC_{oct/w} - 3.0$ (without enhancer); $\log K_{np}V_{np}(cm^3) = 1.1*\log PC_{oct/w} = 0.9$ (with GACH 25.5μmol).

to enhance the permeation of hydrophilic drugs by using a prodrug approach independently, it is necessary to drastically alter the physicochemical properties of the drug. However, in this case, the approach may be restricted by an increase in molecular size due to chemical modification or the more serious problem of a decrease in water solubility. Thus, percutaneous absorption of drugs could not be markedly improved by the prodrug approach alone, except in some cases (2).

GACH, which increases the partitioning of most drugs into the nonpolar route, enhances drug permeation by shifting the relationship between the penetration amount and $PC_{oct/w}$ to the left. The $PC_{oct/w}$ value of drug showing maximal permeation in the presence of GACH decreased in comparison to that without the enhancer. Here, skin permeation of highly hydrophilic drugs is still little enhanced, whereas that of drugs with an intermediate hydrophilicity/lipophilicity balance would be markedly enhanced. On the other hand, skin permeation of highly lipophilic drugs would be decreased by the enhancer. Thus, GACH would show the most extensive enhancement effect for drugs with a logarithm of $PC_{oct/w}$ of -0.3 under the present condition.

Based on these relationships, an effective approach to increasing drug absorption can be found. By synthesis of a prodrug with log $PC_{oct/w}$ of around -0.3, skin permeation of a drug can be markedly enhanced by GACH. This approach may be applicable to a wide range of drugs, because it is not necessary to alter the physicochemical properties of drugs greatly as in the simple prodrug approach.

We have further expanded our theoretical analysis to treat several problems relating to several experimental conditions such as finite/infinite dosing manner and animal species (7,11). Under an infinite dosing condition, the enhancement effect by the enhancer can be defined as an increase in permeability coefficient, while it is expressed by the penetration amount at a special time-point under the finite-dosing condition. Based on a two-layer model with parallel routes, the permeability coefficient through intact skin (P) is calculated from the following equation:

$$1/P = L_S/(K_pD_pAf + K_{np}D_{np}A(1 - f)) + L_d/K_dD_dA \quad (3)$$

Fig. 2 shows the relationship between the enhancement effect and $PC_{oct/w}$ of drugs in both finite and infinite dose systems in guinea pig skin, simulated by using the penetration parameters obtained previously (7). Here, the enhancement effect in the finite dose system was calculated at 24 hr. We can obtain the simulation curve for rat skin using the parameters obtained previously (11). In Fig. 2, the simulated relationship between enhancement effect of GACH and log $PC_{oct/w}$ values of drugs in the infinite dose system with rat skin is shown. In the present study, experiments were carried out under this condition (7,11).

In the theoretical design, we did not take metabolism modality in the skin into account, because if the rate-limiting step of drug permeation is through the stratum corneum, metabolism in the viable skin shows little effect on total penetration. Therefore, we can predict the penetration profile of hydrophilic prodrugs ($K_{oct/w} < 1$) regardless of regeneration manner.

MATERIALS AND METHODS

Materials

GACH was synthesized by Kuraray Co., Japan. Acyclovir was kindly supplied by Nippon Wellcome K.K., Japan. Acyl

chlorides and other materials were obtained commercially from Nacalai Tesque Inc., Japan.

Synthesis of Acyclovir Prodrugs

Acyclovir was esterified in dry N,N-dimethylformamide with 3 equivalents of corresponding acyl chloride and 4-dimetylaminopyridine at room temperature for 48 hr. The reaction mixture was evaporated under reduced pressure. The obtained residues were slurried in 15 ml of NaOH solution (pH of 9.0 ± 0.2). Upon standing at 4°C for 5 hr, the precipitate was filtered off, washed with cold NaOH solution, and recrystallized from ethanol to give a white crystal (12). The purity of the prodrugs was confirmed by HPLC to be more than 98%. Melting points were measured using a micro-melting point apparatus (MP-S3, Yanagimoto Co., Japan).

Determination of Solubility

The solubility of acyclovir and its prodrugs in water and octanol at 37°C were determined after suspending excess compounds in the solvents (3 ml) for 24 hr with agitation. The mixtures were centrifuged at 5000 rpm (RL-100, Tomy Seiko Co., Japan), filtered using a Cosmonice-filter with a pore diameter of 0.45μm (Nacalai Tesque Inc., Japan), and diluted with the corresponding solvents for determination. The ratio of solubility between octanol and water is considered to be a partition coefficient between them ($PC_{oct/w}$).

In Vitro Skin Penetration Experiment

The full-thickness abdominal skin of a Wistar strain male rat weighing about 200 g was excised after removal of hair with an electric clipper. In this procedure, we adhered to the "Principles of Laboratory Animal Care." After the underlying adipose tissue was removed, the skin was punched out into a 3-cm diameter disk and mounted on a flow-through type diffusion cell with the epidermal side facing the donor cell (exposed area 3.14 cm^2) as previously described (11). The apparatus was thermostated at 37°C in a water bath throughout the experiment.

The skin mounted on the diffusion cell was pretreated with 0.2 ml of an ethanolic solution containing 0, 6.4, and 25.5 μmol of GACH. Six hours later, ethanol remaining in the donor cell was evaporated with a hair dryer and a 2-ml aliquot of 5% (w/v) drug suspension in phosphate-buffered saline (PBS) was applied. The suspension was prepared by stirring PBS containing the compounds for 24 hr at 37°C. The dermal side of the skin was continuously washed with PBS containing streptomycin sulfate (50 mg/l, Sigma Chemical Co., MO) and penicillin G potassium salt (30 mg/l, Wako Pure Chemicals Industries, Ltd., Japan) which flowed at a rate of 5 ml/hr. The receptor fluid was collected every 60 min for 12 hr. Each sample was mixed with an equivalent amount of methanol for deproteination and analyzed by HPLC after filtration with a Cosmonice-filter with 0.45 μm of pore diameter (Nacalai Tesque Inc., Japan).

HPLC Analysis

The amounts of acyclovir and its prodrugs were determined using an HPLC system (Shimadzu Co., Japan), consisting of a Shimadzu Model LC-6A pump, a variable-wavelength Shimadzu SPD-6A UV detector operated at 250 nm, and a Shimadzu SIL-6B auto injector with a fixed injection volume of 20 μl. The stationary phase was Cosmosil $5C_{18}$ packed column (size, 4.6 × 150 mm), Nacalai Tesque, Inc., Japan. The mobile phases for acyclovir and its prodrugs were mixtures of methanol and water flowing at 0.8 ml/min.

RESULTS

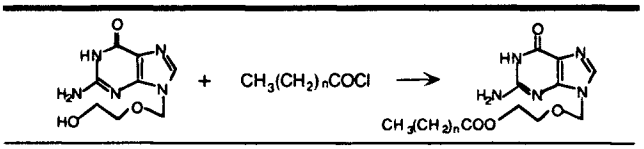
Table I summarizes the physicochemical properties of acyclovir and its prodrugs. The melting point of each ester is lower than that of acyclovir. The solubility of acyclovir acetate, propionate, and butyrate in water was about one half as high as that of acyclovir, while prodrugs with a longer acyl chain gave fairly decreased solubilities with an increasing number of carbons. The $PC_{oct/w}$ values of the prodrugs also markedly increased with an increase in the number of carbons.

Fig. 3 shows the penetration profiles of acyclovir and prodrugs through the rat skin pretreated with various amounts of GACH. In the case of prodrugs, both prodrugs and the regenerated acyclovir appeared in the receptor fluid. Table II summarizes the steady-state flux for penetration of acyclovir and acyclovir prodrugs through the skin. The ratio of intact prodrug flux appearing in the receptor fluid to total flux increased with the number of carbons of the prodrugs.

GACH only slightly enhanced skin penetration of acyclovir and its acetate (Table II), but it markedly improved the permeation of other prodrugs with an increasing dose of enhancer. Furthermore, GACH dose-dependently increased the ratio of intact prodrug appearance to total appearance for all the prodrugs.

In Fig. 4, the enhancement effects of prodrug approach alone (a) and prodrug/enhancer combination (b) on acyclovir penetration are presented against $PC_{oct/w}$ of prodrugs, together with the theoretical curves estimated based on the present consideration. Here, the enhancement effect is defined as the ratio of permeability coefficient of total acyclovir under each condition to the corresponding control value. The control values are permeability coefficient of acyclovir in Fig. 4a, and that of each prodrug without GACH in Fig. 4b. The theoretical curves were predicted using penetration parameters of each compound

Table I. Chemical Structures and Physicochemical Properties of Synthesized Acyclovir Prodrugs



	n	m.p. (°C)	Solubility (mM)[a]	PCoct/w[b]
Acyclovir (AC)		260~262	11.9	0.0123
AC acetate	0	242~245	4.89	0.0578
AC propionate	1	218~220	5.73	0.212
AC butyrate	2	220~222	4.64	0.402
AC valerate	3	206~208	2.50	0.702
AC hexanoate	4	213~215	0.741	3.352

[a]Drug solubility in water was determined at 37°C.

[b]n-Octanol/water partition coefficient ($PC_{oct/w}$) was calculated as a ratio of solubilities in water and n-octanol at 37°C.

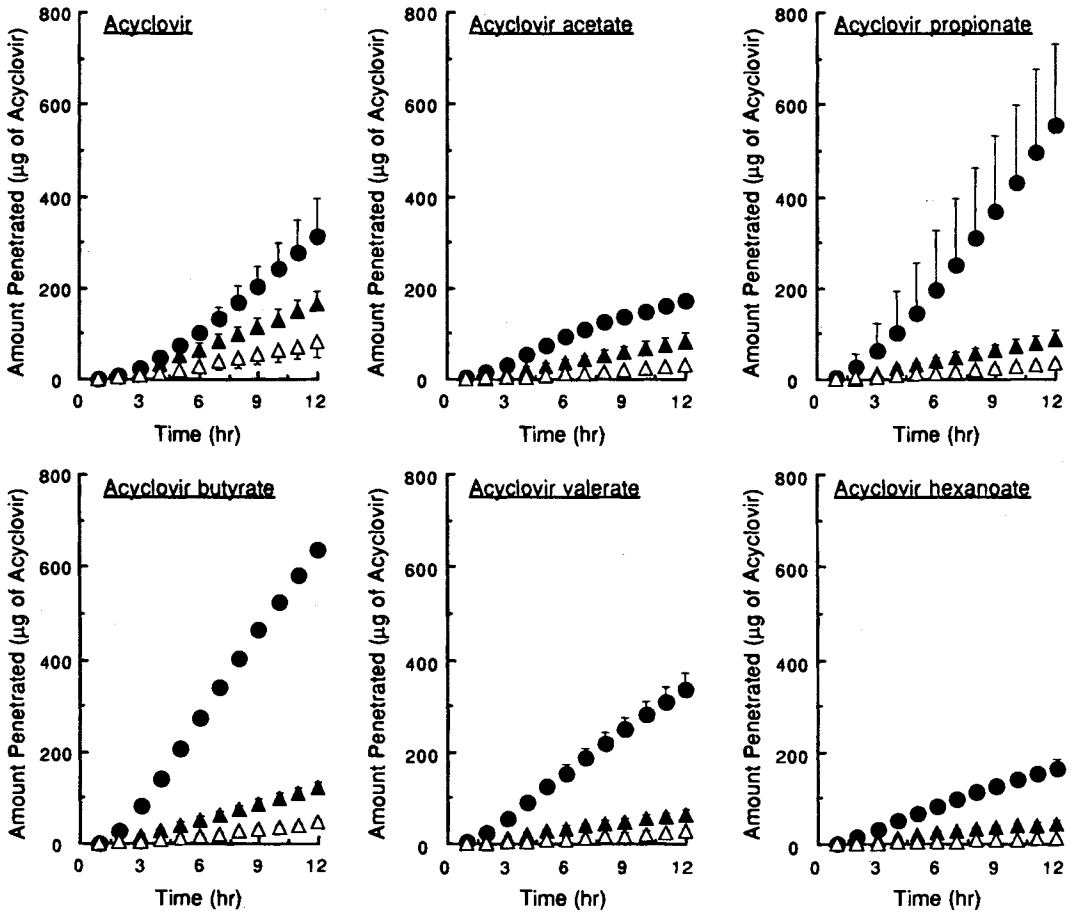


Fig. 3. Time courses of total acyclovir amount penetrating through the rat skin pretreated with ethanolic solution of 0 (△), 6.4 (▲), and 25.5 μmol (●) of GACH. Acyclovir and its prodrugs were applied in suspension. In the case of the prodrug application, the sum of acyclovir and prodrugs appearing in the receptor is shown. Each point represents the mean ± S.D. value of at least three experiments.

obtained previously with guinea pig and rat skin (7,11). The enhancement effects observed by the prodrug approach (Fig. 4a) and prodrug/enhancer combination approach (Fig. 4b) were in good agreement with the theoretical effects.

DISCUSSION

Our previous study on prodrug/enhancer combination revealed that the enhancement effect of GACH was larger for acyclovir butyrate ($PC_{oct/w}$ = 0.40) than for acyclovir ($PC_{oct/w}$ = 0.012) (13). This might not be enough to validify the theoretical basis of the designing prodrug/enhancer combination shown in Fig. 1. In the present study, therefore, additional prodrugs with different side chains were synthesized to evaluate their skin permeability. The physicochemical properties of the prodrugs summarized in Table I are similar to those reported by Shao *et al.* (14).

The enzymatic activity is sometimes decreased during in vitro experiments (15). Since the ratio of steady-state fluxes of both prodrug and acyclovir were constant for all prodrugs during the experimental period (18hr), we considered that the enzymatic activity was basically constant.

Without GACH, the acyclovir prodrugs showed similar skin permeability. The skin permeability of its hexanoate with a 300-fold larger $PC_{oct/w}$ value was only two times higher than acyclovir. This may correspond to the observation of Ackermann *et al.* (10) and our theoretical prediction that the skin permeability of highly hydrophilic drugs is constant regardless of their lipophilicities since they mainly permeate through an aqueous pathway in the stratum corneum. Actually, the skin permeability of these prodrugs could be precisely predicted by using our diffusion model considering a polar route (Fig. 4a). As suggested by the theoretical curve, extremely higher lipophilicity is needed for prodrugs for sufficient improvement of skin permeation. Even if a prodrug with sufficiently higher lipophilicity were synthesized, its low solubility in the vehicle may prevent its application. Although we synthesized a more lipophilic prodrug, decanoate ($PC_{oct/w}$ = 201), we could not measure its penetration amount due to its low solubility (data not shown). GACH improved skin permeation of propionate, butyrate, valerate, and hexanoate to be much greater than that of the hydrophilic drugs (acyclovir and acetate). This is because penetration across the nonpolar route, which is the main action site of GACH, becomes dominant as the lipophilicity of the prodrug increases. Although the prodrug/enhancer combination increased the original acyclovir absorption about six times, the effect of prodrug derivation was poor especially that evaluated

Table II. Steady-State Flux for Penetration of Acyclovir and Acyclovir Prodrugs

Drug	GACH Dose (μmol)	Flux (μg/hr)[a]			GACH treatment/ control ratio	Intact prodrug flux/total flux ratio
		acyclovir	prodrug	Total		
acyclovir	0	3.42 ± 1.18	—	3.42 ± 1.18	1	—
	6.4	5.58 ± 0.77	—	5.58 ± 0.77	1.63	—
	25.5	11.5 ± 3.6	—	11.5 ± 3.6	3.37	—
acyclovir acetate	0	0.99 ± 0.14	0.45 ± 0.18	1.43 ± 0.13	1	0.31
	6.4	1.24 ± 0.15	1.59 ± 0.29	2.82 ± 0.34	1.97	0.56
	25.5	2.73 ± 0.06	4.23 ± 1.04	6.96 ± 1.10	4.84	0.61
acyclovir propionate	0	0.88 ± 0.16	0.31 ± 0.08	1.19 ± 0.20	1	0.26
	6.4	1.55 ± 0.37	1.37 ± 0.34	2.92 ± 0.61	2.46	0.47
	25.5	6.98 ± 0.43	12.4 ± 2.5	19.4 ± 2.3	16.3	0.64
acyclovir butyrate	0	1.60 ± 0.19	0.11 ± 0.06	1.71 ± 0.17	1	0.06
	6.4	3.27 ± 0.31	0.53 ± 0.19	3.81 ± 0.28	2.21	0.14
	25.5	14.9 ± 0.5	6.48 ± 0.66	21.0 ± 0.6	12.3	0.31
acyclovir valerate	0	0.66 ± 0.07	0.06 ± 0.01	0.72 ± 0.07	1	0.08
	6.4	1.26 ± 0.17	0.93 ± 0.31	2.18 ± 0.42	3.04	0.42
	25.5	4.74 ± 1.29	3.48 ± 1.66	8.22 ± 1.41	11.5	0.43
acyclovir hexanoate	0	0.35 ± 0.10	0.03 ± 0.00	0.37 ± 0.10	1	0.07
	6.4	1.01 ± 0.19	0.35 ± 0.14	1.37 ± 0.27	3.66	0.26
	25.5	2.82 ± 0.37	1.62 ± 0.71	4.44 ± 0.66	11.86	0.36

[a]Steady-state flux was obtained from the slopes of the linear portions of penetration profiles.

according to the absolute acyclovir amount base. However, we expect that absorption enhancement becomes higher in the in vivo experiment with high enzymatic activity.

As shown in Fig. 4b, the observed enhancement effects of GACH on prodrugs are in good agreement with those predicted, showing that penetration enhancement by the prodrug/enhancer combination is realized as estimated based on diffusion model analysis.

For all the prodrugs, GACH increased the ratio of prodrug appearing in the receptor to total acyclovir. There are two possible reasons for this phenomenon, i.e., saturation of enzymatic reaction due to an increase in prodrug concentration and the direct effect of the enhancer on esterase. Previously (13), we did not consider the saturation of enzymatic reaction since oleic acid did not show such an effect although it also extremely increased the skin penetration of prodrugs. The direct effect on esterase might also be supported by the fact that even for acyclovir acetate for which the penetration was not greatly enhanced, GACH increased the appearance of prodrug form in the receptor (Table II).

Although we have systematically studied the action mechanism of GACH using guinea pig skin, we used rat skin in this

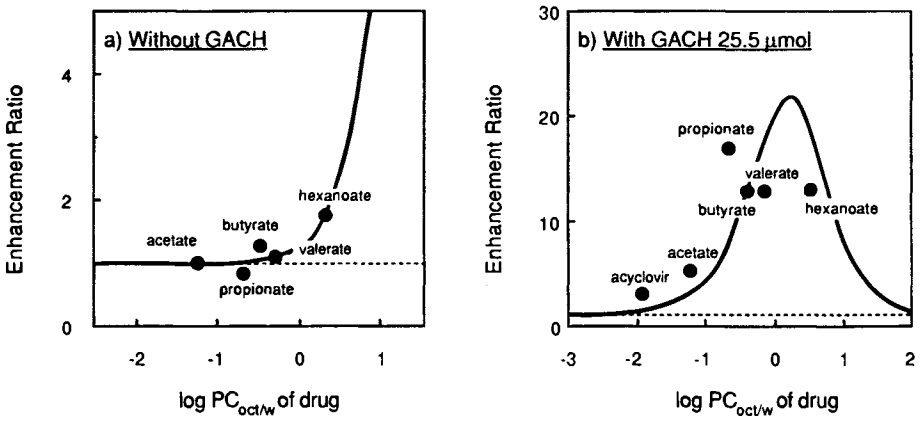


Fig. 4. Comparison of penetration enhancement ratio by prodrug derivation (a) and GACH (b) with the predicted one based on a diffusion model. Penetration enhancement ratio by prodrug derivation was defined as the permeability coefficient of prodrug divided by that of acyclovir. On the other hand, the enhancement ratio of GACH was defined as the permeability coefficient of each prodrug with GACH divided by that without the enhancer. To calculate the theoretical enhancement effects, the following parameters were used: $D_p/L_s^2 = 60(hr^{-1})$; $D_{np}/L_s^2 = 1.5(hr^{-1})$; $D_d/L_d^2 = 0.08(hr^{-1})$; $K_pV_p = 0.00007(cm^3)$; $K_dV_d = 3.5(cm^3)$; log $K_{np}V_{np}(cm^3) = 1.3*\log PC_{oct/w} - 3.0$ (without enhancer); $\log K_{np}V_{np}(cm^3) = 1.1*\log PC_{oct/w} - 0.9$ (with GACH 25.5 μmol).

experiment because rats are superior to guinea pigs in the in vitro/in vivo correspondence. In previous analysis of drug absorption between rats and guinea pigs based on a diffusion model, we found that there are no differences in the action mechanism of enhancers and the differences in apparent permeation of drugs can be corrected with the penetration parameters for the polar route and the viable layer. Taking these points into account, we could predict the skin penetration enhancement by a prodrug/enhancer combination (Fig. 4).

In conclusion, skin permeation of prodrugs applied with an enhancer can be predicted and optimized by model analysis. 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.

REFERENCES

1. A. C. Williams and B. W. Barry. The enhancement index concept applied to terpens penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. *Int. J. Pharm.* **74**:157–168 (1991).
2. K. B. Sloan. Prodrugs: Topical and Ocular Drug Delivery, Marcel Dekker Inc., New York, 1992.
3. P. G. Green, R. S. Hinz, A. Kim, F. C. Szoka, and R. H. Guy. Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm. Res.* **8**:1121–1127 (1991).
4. W. M. Shannon, L. Westbrook, W. I. Higuchi, K. Sugibayashi, D. C. Baker, S. D. Kumar, J. L. Fox, G. L. Flynn, N. F. H. Ho, and R. Vaidyanathan. Influence of 1-dodecylazacycloheptan-2-one (Azone) on the topical therapy of cutaneous herpes simplex virus type 1 infection in hairless mice with 2′,3′-di-o-acetyl-9-β-d-arabinofuranosyladenine and 5′-o-valeryl-9-β-d-arabinofuranosyladenine. *J. Pharm. Sci.* **74**:1157–1161 (1985).
5. H. Okamoto, F. Yamashita, K. Saito, and M. Hashida. Analysis of drug penetration through the skin by the two layer skin diffusion model. *Pharm. Res.* **6**:931–937 (1989).
6. A. H. Ghanem, H. Mahmoud, W. I. Higuchi, U. D. Rohr, S. Borsadia, P. Liu, J. L. Fox, and W. R. Good. The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. *J. Controlled Release.* **6**:75–83 (1987).
7. F. Yamashita, T. Yoshioka, Y. Koyama, H. Okamoto, H. Sezaki, and M. Hashida. Analysis of skin penetration enhancement based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum: Dose-dependent effect of 1-geranylazacycloheptan-2-one on drugs with different lipophilicities. *Biol. Pharm. Bull.* **16**:690–697 (1993).
8. Y. Yano, K. Yamaoka, and H. Tanaka. A nonlinear least squares program, MULTI(FILT), based on fast inverse Laplace transform for microcomputers. *Chem. Pharm. Bull.* **37**:1035–1038 (1989).
9. A. Leo, C. Hansch, and D. Elkins. Partition coefficients and their uses. *Chem. Rev.* **71**:525 (1971).
10. C. Ackermann and G. L. Flynn. Ether-water partitioning and permeability through nude mouse skin in vitro. I Urea, thiourea, glycerol and glucose. *Int. J. Pharm.* **36**:61–66 (1987).
11. F. Yamashita, H. Bando, Y. Koyama, S. Kitagawa, Y. Takakura, and M. Hashida. In vivo and in vitro analysis of skin penetration enhancement based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum. *Pharm. Res.* **11**:185–191 (1994).
12. H. Bundgaard, E. Jensen, and E. Falch. Water-soluble, solution-stable, and biolabile n-substituted (aminomethyl) benzoate ester prodrugs of acyclovir. *Pharm. Res.* **8**:1087–1093 (1990).
13. H. Bando, F. Yamashita, Y. Takakura, and M. Hashida. Skin penetration enhancement of acyclovir by prodrug-enhancer combination. *Biol. Pharm. Bull.* **17**:1141–1143 (1994).
14. Z. Shao, G. B. Park, R. Krishnamoorthy, and A. K. Mitra. The physicochemical properties, plasma enzymatic hydrolysis, and nasal absorption of acyclovir and its 2′-ester prodrugs. *Pharm. Res.* **11**:237–242 (1994).
15. P. Liu, W. I. Higuchi, A. H. Ghanem, and W. R. Good. Transport of b-estradiol in Freshly Excised Human Skin in Vitro: Diffusion and Metabolism in Each Skin Layer. *Pharm. Res.* **11**:1777–1784 (1994).