Skin Penetration Enhancement of Acyclovir by Prodrug–Enhancer Combination

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The effectiveness of prodrug–enhancer combination in skin penetration enhancement was studied using acyclovir and its lipophilic prodrug, acyclovir butyrate, with octanol/water partition coefficient of 0.0123 and 0.402, respectively. In the *in vitro* diffusion experiment with rat skin, the total amount of acyclovir appearing in the receptor phase after administration of the aqueous suspension of acyclovir butyrate was smaller than that obtained after administration of acyclovir, but their permeability coefficients were almost equal. An enhancer, 1-geranylazacycloheptan-2-one (GACH) did not show a large penetration enhancement of acyclovir (3.37-fold) but demonstrated extensive enhancement effect on the prodrug (12.3-fold). Most of the prodrug appeared in the form of acyclovir in the receptor phase without GACH, but the appearance ratio of acyclovir to total flux decreased with an increase in pretreatment doses of GACH.

Keywords prodrug–enhancer combination; percutaneous absorption; acyclovir; acyclovir butyrate; 1-geranylazacycloheptan-2-one; penetration enhancement

Although various approaches have been developed to improve percutaneous drug absorption, they are sometimes applicable only for a limited range of drugs and particular means such as their combination are needed to achieve sufficient enhancement. For example, it has been reported that significant improvement in the performance of prodrugs could be obtained in combination with enhancers.^{1,2)} This approach, however, should be carried out through rational design based on a comprehensive understanding of each enhancing mechanism.

In a series of investigations, we have systematically studied the effects of skin penetration enhancers^{3–6)} and reported that the enhancement effect of azacycloalkanone derivatives gave a bell-shaped relationship against lipophilicities of the subjected drug.^{4,5)} Analysis of the action mechanism of 1-geranylazacycloheptan-2-one (GACH) based on a skin diffusion model, suggested that this enhancer shows the largest enhancing effect for a drug with octanol/water partition coefficient ($PC_{\text{oct/w}}$) of 0.5.⁵⁾

Using this as a theoretical basis, we developed an antiviral agent acyclovir⁷⁾ to a more lipophilic prodrug, acyclovir butyrate, which has a $PC_{\text{oct/w}}$ value suitable for combination with GACH, and evaluated its skin penetration.

MATERIALS AND METHODS

Materials GACH was synthesized by Kuraray Co., Okayama, Japan. Acyclovir was generously supplied by Nippon Wellcome K.K., Osaka, Japan. Butyryl chloride and other materials were obtained commercially from Nacalai Tesque Inc., Kyoto, Japan.

Synthesis of Acyclovir Prodrug Esterification of acyclovir was carried out in dry *N,N*-dimethylformamide with 3 eq of butyryl chloride and 4-dimethylaminopyridine at room temperature for 48 h. The reaction mixture was evaporated under reduced pressure. The obtained residue was slurried in 15 ml of NaOH solution (pH of 9.0 ± 0.2). Upon standing at 4 °C for 5 h, the precipitate was filtered

off, washed with cold NaOH solution, and recrystallized from ethanol to give white crystals.⁸⁾ The purity was confirmed by HPLC to be more than 98%.

Determination of Solubilities Solubilities of acyclovir and the prodrug in water and octanol at 37 °C were determined 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 Co., Tokyo, Japan), filtered using a Cosmonice-filter with a 0.45 μm pore diameter (Nacalai tesque Inc., Kyoto, Japan), and diluted with the corresponding solvents for determination. The ratio of solubility of octanol to water is considered to be equal to the partition coefficient between them ($PC_{\text{oct/w}}$).

Hydrolysis of the Prodrug in Skin Homogenate Hydrolysis of acyclovir butyrate in the presence and absence of rat skin homogenate was measured. One gram of full-thickness abdominal skin was homogenized in 5 ml of pH 7.4 phosphate buffered saline (PBS) at 4 °C and the supernatant was obtained after centrifugation at 10000 *g* (SCR20B, Hitachi Koki Co., Tokyo, Japan). To the prodrug solution in 25 ml of PBS (pH 7.4), 5 ml of the supernatant was added to give an initial concentration of 0.005, 0.05, and 0.5 mM. One milliliter of sample was periodically withdrawn from the reaction mixture for determination. The chemical hydrolysis was estimated in pH 7.4 PBS.

In Vitro Skin Penetration Experiment Full-thickness abdominal skin was excised from a Wistar strain rat weighing about 200 g after removal of hair with a hair clipper. After removal of adipose tissue, 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 cm²) as previously described.⁹⁾ 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 PBS was applied. The suspension was prepared by suspending the compounds in PBS for 24 h at 37°C with agitation. 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., Osaka, Japan) which flowed at a rate of 5 ml/h. The receptor fluid was collected every 60 min for 12 h. Each sample was mixed with equivalent methanol for deproteination and analyzed by HPLC after filtration with a Cosmonice-filter with a pore diameter of 0.45 μm (Nacalai Tesque Inc., Kyoto, Japan).

HPLC Analysis Amounts of acyclovir and its butyrate were determined using a HPLC system (Shimadzu Co., Kyoto, 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 fixed injection volume with 20 μl . The stationary phase was Cosmosil 5C₁₈ packed column (size, 4.6 $\times$ 150 mm), Nacalai Tesque, Inc., Kyoto, Japan. The mobile phases for acyclovir and the prodrug were mixtures of methanol and water (3:97 and 30:70, respectively) flowing at 0.8 ml/min.

RESULTS

Solubility and Hydrolysis Rate of Prodrug Water solubilities of acyclovir and acyclovir butyrate were 11.9 and 4.64 mM and their $PC_{\text{oct/w}}$ values were 0.0123 and 0.402, respectively. Hydrolysis of the prodrug in PBS with or without skin homogenate was examined (Fig. 1). Hydrolysis rate constant of the prodrug at 0.05 mM in pH 7.4 PBS was $1.73 \pm 0.08 \times 10^{-3} \text{ h}^{-1}$. While, those at 0.005, 0.05, and 0.5 mM in PBS with skin homogenate were $2.46 \pm 0.44 \times 10^{-1}$, $2.64 \pm 0.03 \times 10^{-1}$, and $1.87 \pm 0.31 \times 10^{-1} \text{ h}^{-1}$, respectively. No significant difference in the rate constant was observed within the tested concentration range. The regeneration of acyclovir was confirmed based

on the corresponding amount of the acyclovir butyrate that disappeared from the incubation medium.

Effect of Enhancer on Skin Penetration of Drugs in Diffusion Experiment In diffusion experiments, skin was pretreated with GACH to avoid drug-enhancer interaction in the vehicle. Figure 2 shows the penetration profiles of acyclovir and acyclovir butyrate through the skin pretreated with various amounts of GACH. Penetration of administered acyclovir butyrate is expressed as total acyclovir amount in Fig. 2.

Table I summarizes steady-state flux and lag time calculated from penetration profiles. Without GACH, total penetration of the prodrug was about half of that of acyclovir. The permeability coefficients of acyclovir and acyclovir butyrate calculated from their solubilities were 1.28×10^{-3} and $1.64 \times 10^{-3} \text{ cm/h}$, respectively. On the other hand, GACH remarkably enhanced total penetra-

Fig. 1. Hydrolysis of Acyclovir Butyrate in PBS (pH 7.4) at Initial Concentration of 0.5 (□), 0.05 (△), 0.005 (○), mM with or of 0.05 mM (▲) without Rat Skin Homogenate at 37°C

Each point represents the mean $\pm$ S.D. value of three experiments.

Fig. 2. Time Courses of Total Acyclovir Amount Penetrated through Rat Skin Pretreated with Ethanolic Solution of 0 (△), 6.4 (▲), 12.7 (■), 25.5 (●), μmol of GACH

Acyclovir (a) and acyclovir butyrate (b) were applied in forms of suspension. The data represent the sum of acyclovir and acyclovir butyrate in the case of the prodrug application. Each point represents the mean $\pm$ S.D. value of at least three experiments.

TABLE I. Steady-State Flux and Lag Time for Penetration of Acyclovir and Acyclovir Butyrate

Drug	GACH dose (μmol)	Acyclovir butyrate		Acyclovir		GACH treatment /control ratio	Acyclovir butyrate /total flux ratio
		Flux ^{a)} ($\mu\text{g/h}$)	Lag time ^{b)} (h)	Flux ^{a)} ($\mu\text{g/h}$)	Lag time ^{b)} (h)		
Acyclovir	0	—	—	3.42 $\pm$ 1.18	3.28 $\pm$ 0.90	1	—
	6.4	—	—	5.58 $\pm$ 0.77	2.49 $\pm$ 0.94	1.63	—
	12.7	—	—	6.43 $\pm$ 1.42	2.49 $\pm$ 0.77	1.88	—
	25.5	—	—	11.5 $\pm$ 3.6	3.09 $\pm$ 0.85	3.37	—
Acyclovir butyrate	0	0.11 $\pm$ 0.06	1.78 $\pm$ 0.31	1.60 $\pm$ 0.19	2.98 $\pm$ 0.23	1	0.06
	6.4	0.53 $\pm$ 0.19	1.32 $\pm$ 0.14	3.27 $\pm$ 0.31	2.31 $\pm$ 0.39	2.21	0.14
	12.7	3.12 $\pm$ 1.30	1.16 $\pm$ 0.21	6.78 $\pm$ 0.64	1.60 $\pm$ 0.05	5.85	0.32
	25.5	6.48 $\pm$ 0.66	0.98 $\pm$ 0.05	14.9 $\pm$ 0.5	2.36 $\pm$ 0.01	12.3	0.31

a) Steady-state flux was obtained from the slopes of the linear portions of penetration profiles. b) Lag time was obtained by extrapolating the linear portions of penetration profiles to X-axis. Values are the mean $\pm$ S.E. of at least three experiments.

tion of acyclovir from the prodrug suspension depending on the pretreatment dose of GACH up to 12.3-fold, whereas the enhancer improved it at most 3.37-fold from acyclovir suspension.

When the prodrug was applied, lag time for the appearance of acyclovir was significantly longer than that for the prodrug form ($p < 0.05$). In both acyclovir and the prodrug, the effect of GACH on their lag time was less than that on their penetration flux.

Without GACH, most of the prodrug appeared in an acyclovir form. The ratio of the penetrated amount of acyclovir in a prodrug form to total penetrated amount was greater with an increased GACH dose.

DISCUSSION

Although the prodrug has about 30-times greater $PC_{\text{oct/w}}$ than acyclovir, the skin permeability was not increased by chemical modification. In our model analysis, this is theoretically confirmed⁵⁾: Because lipophilicity of acyclovir butyrate is not very high, the contribution of the polar route, where the penetration is independent of lipophilicity of drugs,¹⁰⁾ on the total penetration process is still large.

GACH showed much larger enhancing effect for acyclovir butyrate (Fig. 2), corroborating the theory previously presented.⁵⁾ GACH might accelerate drug partitioning into the nonpolar route in the stratum corneum and consequently show more extensive enhancing effect for drugs with $PC_{\text{oct/w}}$ values closer to 0.5.⁵⁾ On the other hand, the lag time of acyclovir and the prodrug was little altered by GACH (Table I), corresponding to our previous finding that GACH increases not drug diffusivity but its partitioning.⁵⁾

In general, a larger penetrant should diffuse in skin more slowly and result in a longer lag time. Nevertheless, the lag time of the intact prodrug form was significantly shorter than that of the parent drug, acyclovir, when administered in a form of acyclovir butyrate suspension. Acyclovir butyrate is converted to the parent drug by esterases existing in skin, as revealed in an *in vitro* experiment with skin homogenate (Fig. 1). It has been theoretically shown that the lag time becomes shorter when a drug is metabolized in skin.¹¹⁾ Longer lag time of an

acyclovir form, however, would correspond to the metabolic process and subsequent diffusion of regenerated acyclovir in skin.

The ratio of the intact prodrug flux to total flux was increased by GACH as shown in Table I. Higo *et al.* found a similar phenomenon for nitroglycerin with 2% Azone in propylene glycol.¹²⁾ There are two possibilities which may be responsible for this type of phenomenon: (1) decrease in esterase activity by GACH and/or ethanol, and (2) saturation of metabolic activity by increased flux of the prodrug. Since pretreatment with oleic acid in ethanol gave the same total flux from the prodrug suspension as that with GACH without changing the ratio of the prodrug to total flux (data not shown), saturation of metabolic activity seems to be less important. Therefore, GACH may decrease drug metabolic activity in the skin.

Thus the combination of a prodrug and an enhancer based on theoretical considerations resulted in great enhancement of the skin penetration of acyclovir. However, it is also necessary to take into account the metabolic process from prodrug to drug in the skin to achieve optimal delivery of the drug with this approach.

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