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Report

Percutaneous Penetration of Acyclovir Through Excised Hairless Mouse and Rat Skin: Effect of Vehicle and Percutaneous Penetration Enhancer

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The effects of vehicle and percutaneous penetration enhancer on the penetration of acyclovir through excised hairless mouse and rat skin were investigated. Four solvents, propylene glycol (PG), ethanol (ET), isopropanol (IPA), and isopropyl myristate (IPM), were employed as vehicles, in combination with four enhancers, 1-farnesylazacycloheptan-2-one (7FU), 1-geranylazacycloheptan-2-one (7GU), 1-geranylazacyclopentan-2-one (5GU), and 1-dodecylazacycloheptan-2-one (Azone). Acyclovir was suspended in vehicles to avoid the effect of the thermodynamic activity of acyclovir in the vehicle. The penetration of acyclovir through hairless mouse skin from IPA was enhanced by 7GU, whereas that from IPM was not affected. All combinations of vehicle and penetration enhancer were examined using rat skin. No effect of the enhancers was observed in the IPM vehicle. The estimated solubility parameters of vehicles and enhancers indicated that the polarities of IPM and the enhancers are similar, which prevents effective penetration of the enhancers from IPM. However, the penetration of acyclovir from the other vehicles was increased by the enhancers. The combination of hydrophilic vehicle and hydrophobic enhancer resulted in a large enhancing effect. The disappearance of the enhancers from the vehicle correlated with their enhancing activity, but other factors also seemed to affect the penetration enhancement of acyclovir.

KEY WORDS: percutaneous absorption; percutaneous drug delivery; acyclovir; percutaneous penetration enhancer; vehicle.

INTRODUCTION

Acyclovir has marked antiviral activity on herpes virus infections with a low host toxicity (1), and its intravenous, oral, and topical formulations have been introduced into clinical field. However, limitations of topical application of acyclovir for recurrent cutaneous herpes simplex virus infections have been documented in some cases (2). For example, percutaneous penetration of acyclovir is insufficient for treatment with a polyethylene glycol vehicle, while it can be improved with dimethyl sulfoxide (2) and propylene glycol including oleic acid or oleyl alcohol (3) as the vehicles.

Percutaneous penetration enhancers may further serve to improve percutaneous penetration of acyclovir. We have developed 1-alkyl- and 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers (4–6). The pretreatment of excised guinea pig skin with these compounds enhanced the penetration of acyclovir, possibly by raising the affinity of acyclovir to the skin, which leads to increased drug penetration and accumulation in the skin (unpublished data).

In this study, we formulated these enhancers and 1-dodecylazacycloheptan-2-one (Azone) in various vehicles and examined their effect on acyclovir penetration. The disappearance of the enhancer from vehicle was also determined and related to the extent of the enhancing activity.

MATERIALS AND METHODS

Materials

Acyclovir was a gift from Wellcome Foundation, UK. Four solvents, propylene glycol (PG), ethanol (ET), isopropanol (IPA), and isopropyl myristate (IPM) were purchased from Nacalai Tesque, Inc., Japan, and employed as vehicles. Azone was kindly supplied from Nelson Research Center, USA. 1-Geranylazacycloheptan-2-one (7GU), 1-farnesylazacycloheptan-2-one (7FU), and 1-geranylazacyclopentan-2-one (5GU) were synthesized by Kuraray Co., Japan.

Determination of Lipophilic Index of Enhancer

The lipophilic index was determined by high-performance liquid chromatography (HPLC) (7). An HPLC system (TRI ROTAR, Japan Spectroscopic Co., Ltd.) equipped with a UV detector (UVIDEC-100-III, Japan Spectroscopic Co., Ltd.) operating at 210 nm was used in a reverse-phase mode. The stationary phase was silica gel

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bonded chemically with octadecyl chains prepacked into a 10-cm stainless-steel column (Cosmosil 5C18 packed column, Nacalai Tesque, Inc., Japan). A mixture of methanol (95–75%) and distilled water (5–25%) was employed as mobile phase. The elution time of a solvent (t_0) and the retention time of an enhancer (t_R) were determined at each of the mobile phase composition. The log k' value defined by Eq. (1) was plotted against the methanol concentration in the mobile phase and the extrapolated log k' value to 0% methanol was obtained as an index of lipophilicity of the enhancer (log k'_0).

$$\log k' = \log [(t_R - t_0)/t_0] \quad (1)$$

Calculation of Solubility Parameter

The theoretical solubility parameters of vehicles and enhancers were calculated by the method of Fedors (8) from their chemical structures.

In Vitro Percutaneous Penetration Experiment

Transdermal delivery rates of acyclovir were determined by *in vitro* diffusion experiments. The full-thickness dorsal skin of female hairless mice (9–10 weeks) was excised in one piece, and adherent fat and other visceral debris were removed from the undersurface. Also, the full-thickness abdominal skin of male Wistar rats (ca. 300 g) was obtained after the removal of the hair and adipose tissue. The freshly excised skin was mounted on a diffusion cell with an available diffusion area of 8.04 cm^2 as described by Loftsson and Bodor (9). The receptor compartment of each cell was filled with 48 ml of saline containing streptomycin sulfate (50 mg/liter, Sigma Chemical Co.) and penicillin G potassium salt (30 mg/liter, Toyo Jozo, Japan) and stirred with a magnetic stirrer. Test formulations were prepared by suspending acyclovir in PG, ET, IPA, and IPM to a total concentration of 20 m*M*. The enhancers were added to these suspensions at a concentration of 0.1 *M*. The acyclovir suspension (2 ml) was applied to the donor compartment, which was sealed with a silicone stopper. The diffusion cell was thermostated at 32°C in a water bath. At appropriate intervals, 1 ml of the receptor medium was withdrawn and this volume was replaced with fresh medium. At the end of experiment, the drug and enhancer remaining in the donor phase were recovered with methanol. Twenty-five milliliters of methanol was sufficient to dissolve acyclovir, enhancer, and vehicle.

Determination of Acyclovir and Enhancers

Acyclovir was determined using the HPLC system described above with a UV detector operating at 252 nm. The mobile phase was a mixture of methanol and distilled water (10:90) and flowed at 0.8 ml/min. The sample from receptor compartment was analyzed after filtration with Milliporefilter (pore size, 0.45 μm). The sample obtained from donor compartment (methanol solution) was analyzed after evaporation of methanol and resolubilization in water. The enhancers recovered from donor phase were determined by the same manner as in determination of lipophilic index after adequate dilution with methanol. The mobile phase was a mixture of methanol and water (90:10) and flowed at 1.0 ml/min.

RESULTS

Lipophilicity of Enhancers and Vehicles

The plots of log k' values of enhancers and IPM against the methanol concentration of the mobile phase show a linear relationship (Fig. 1). The lipophilic indices determined by the extrapolation of these plots to 0% methanol are listed in Table I. A large lipophilic index means high lipophilicity of the compound. Table I also lists the solubility parameters estimated by the method of Fedors (8). The solubility parameters express the cohesion between like molecules (10), used as an index of compound polarity, which is supported by its correlation with dielectric constants (11). These values of the enhancers (9.07–9.30) are closer to that of IPM (8.54) than to the other vehicles (11.6–14.8). The solubility parameters inversely follow the order of the determined lipophilic indices in enhancers. The solubility parameters of the vehicles show an order of polarity predicted from their chemical structure.

Vehicle Effect on Enhancer Activity on Acyclovir Penetration

The penetration profiles of acyclovir through hairless mouse skin from IPA and IPM are illustrated in Figs. 2A and B, respectively. The penetration of acyclovir from IPA was remarkably increased by 7GU, whereas that from IPM was not affected, suggesting the importance of vehicle property for the activity of the enhancer.

The effects of the enhancers formulated in the four vehicles were examined using excised rat skin. Figures 3A–D show the penetration profiles of acyclovir through rat skin from PG, ET, IPA, and IPM vehicles with or without the enhancers. Table II summarizes the recovery percentages of acyclovir and enhancers at the end of the 8-hr diffusion experiment.

Theoretically, all vehicles containing finely ground sus-

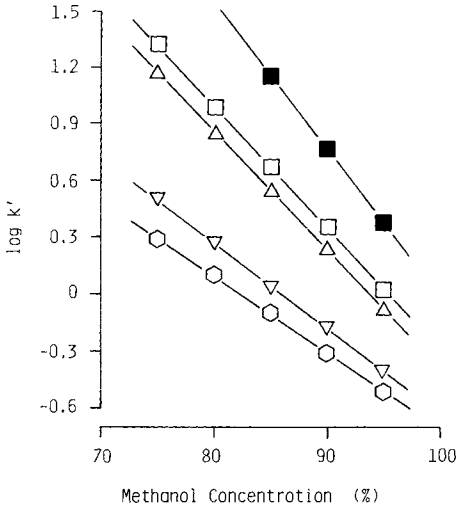

Fig. 1. Relationship between log k' values of IPM (■), Azone (□), 7FU (△), 7GU (▽), and 5GU (○) and methanol concentration (v/v, %) in the mobile phase.

Table I. Enhancers and Vehicles Employed in this Study

Compound	Solubility parameter $(cal/cm^3)^{1/2}$ [a]	Lipophilic index
Enhancer		
Azone	9.07	6.14
7FU	9.10	5.86
7GU	9.21	3.86
5GU	9.30	3.30
Vehicle		
Propylene glycol	14.8	—[b]
Ethanol	12.6	—[b]
Isopropanol	11.6	—[b]
Isopropyl myristate	8.54	7.75

[a] Estimated value by the method of Fedors (8).
[b] Not determined.

pensions of the drug will give the same rate of penetration because the thermodynamic activity is that of the solid drug (12). When results of control experiments are compared, however, the acyclovir penetration from the vehicle with a higher lipophilicity was faster (IPM > IPA > ET > PG). Also, the amount of acyclovir remaining in the hydrophobic vehicle was smaller than that in the hydrophilic vehicle. Thus, the vehicles themselves affected the permeability of the skin to some extent. However, the variation of acyclovir penetration observed in different vehicles was much smaller than that with different enhancers.

The variation of control values suggests a direct effect of the vehicles on the skin permeability. To normalize the contribution of the direct effect of vehicles, "increasing ratio in acyclovir penetration" was calculated by dividing the amount of acyclovir penetrating from vehicle containing enhancer by that from pure vehicle, and results are plotted against the solubility parameter of the vehicle and lipophilic index of the enhancer in Fig. 4. This figure reveals that the enhancer potential is higher in the vehicle with high hydrophilicity (with larger solubility parameter). This phenomenon was more remarkable for the enhancer with higher lipophilicity: the effect of 5GU was large in ET. 7GU showed a large effect in PG and ET. The effects of 7FU and Azone were increased with increasing hydrophilicity of the vehicle,

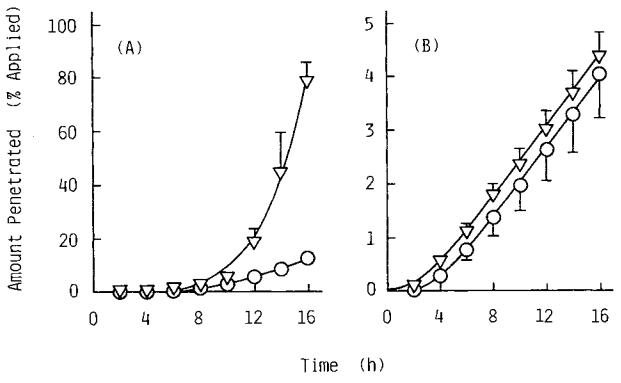

Fig. 2. Percutaneous penetration of acyclovir through excised hairless mouse skin. Acyclovir was applied as suspensions in IPA (A) and IPM (B) with (▽) and without (○) of 7GU.

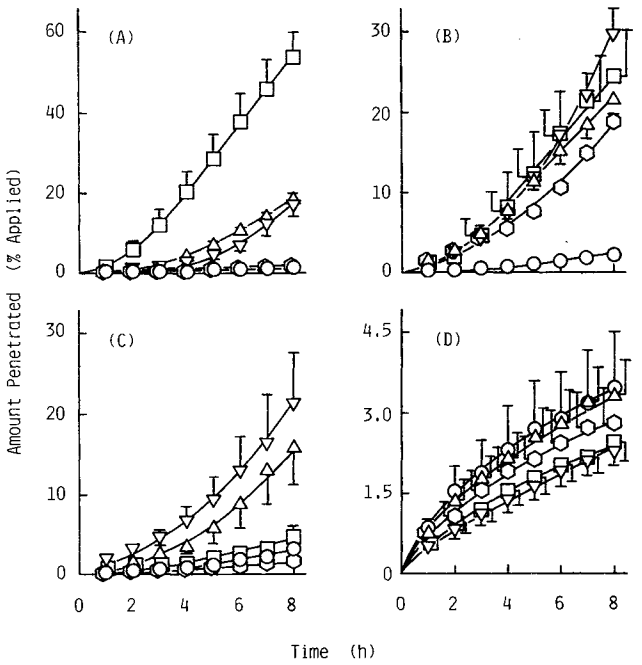

Fig. 3. Percutaneous penetration of acyclovir from PG (A), ET (B), IPA (C), and IPM (D) through excised rat skin. Acyclovir was suspended in the vehicles without enhancer (○) or with Azone (□), 7FU (△), 7GU (▽), and 5GU (○).

and maximum enhancement of acyclovir penetration was observed with the combination of PG and Azone, the most hydrophilic vehicle and the most lipophilic enhancer. IPM was an unfavorable vehicle for all enhancers examined. In IPA, 7GU (the enhancer with the lipophilic index of 4) showed the greatest activity, while all enhancers had similar enhancing activity in ET. PG raised the activity of enhancer with higher lipophilicity.

During the 8-hr diffusion experiments, 2–4 $\mu mol/cm^2$ of enhancer disappeared from the vehicle. The disappearance of the enhancer correlated with the enhancing activity (Table II). The activity of 5GU was demonstrated when formulated in ET, from which 5GU disappeared more rapidly than from the other vehicles. The use of IPM resulted in less activity of all enhancers and also showed a larger enhancer retention. However, a large enhancement of acyclovir penetration did not always coincide with a large enhancer disappearance (for instance, with the combination of Azone and PG), suggesting that other factors may also affect enhancer activity.

DISCUSSION

The physicochemical properties of vehicle, drug, and skin are the main determinants of percutaneous penetration of drug (13), and changes in one of them is expected to alter their dynamic interactions and affect percutaneous drug penetration. Examining the penetration of acyclovir through excised guinea pig skin preloaded with 7GU, we have found a remarkable enhancement when the drug was applied with rather large amounts of 7GU (unpublished data). In the present study, the enhancer effect was also remarkably affected by the type of vehicle, and it was suggested that the vehicle altered the skin penetration of the enhancer itself (Table II). Enhancer concentrations did not reach saturation

Table II. Recovery Percentages of Enhancers and Acyclovir at the End of Diffusion Experiment[a]

Vehicle	Enhancer	N	Recovery from donor (%)		Recovery of acyclovir from receptor (%)
			Enhancer	Acyclovir	
PG	Control	3	—	103.86 ± 4.98	1.30 ± 0.43
	Azone	3	89.03 ± 4.32	44.64 ± 9.79	53.78 ± 5.79
	7FU	3	86.65 ± 1.35	79.33 ± 2.21	18.38 ± 1.46
	7GU	3	82.75 ± 4.46	79.01 ± 16.66	16.66 ± 2.62
	5GU	3	88.43 ± 1.60	105.82 ± 7.20	2.07 ± 0.66
ET	Control	3	—	104.76 ± 7.11	2.17 ± 0.37
	Azone	3	91.69 ± 3.14	58.16 ± 7.08	24.39 ± 5.91
	7FU	3	84.71 ± 6.10	71.93 ± 6.46	21.81 ± 2.01
	7GU	3	87.39 ± 0.86	60.77 ± 6.39	29.53 ± 3.32
	5GU	3	84.06 ± 2.14	68.73 ± 2.33	18.77 ± 0.34
IPA	Control	3	—	91.13 ± 2.43	3.13 ± 0.96
	Azone	4	87.72 ± 1.65	101.60 ± 4.40	4.83 ± 1.24
	7FU	3	86.47 ± 3.28	72.04 ± 6.86	15.90 ± 4.83
	7GU	3	65.91 ± 25.06	73.97 ± 21.93	21.18 ± 6.41
	5GU	3	89.05 ± 4.26	94.63 ± 7.46	1.63 ± 0.32
IPM	Control	3	—	87.85 ± 14.66	3.44 ± 1.08
	Azone	4	91.61 ± 2.17	93.17 ± 3.93	2.43 ± 0.33
	7FU	4	89.90 ± 1.46	96.29 ± 4.60	3.34 ± 0.64
	7GU	3	92.66 ± 1.51	96.29 ± 4.60	2.26 ± 0.22
	5GU	3	90.02 ± 2.19	91.86 ± 6.29	2.80 ± 0.66

[a] Mean ± SE.

in these vehicles, and their thermodynamic activities differed among the vehicles. Sloan et al. investigated the penetration of theophylline (14), salicylic acid (15), and 6-mercaptopurine (16) in various vehicles and demonstrated that the flux and permeability coefficient were minimized when the solubility parameter of the vehicle was close to that of the penetrant. Since the penetration enhancers tested in this study have solubility parameters close to that of IPM, they should display a relatively small penetration and activity in IPM.

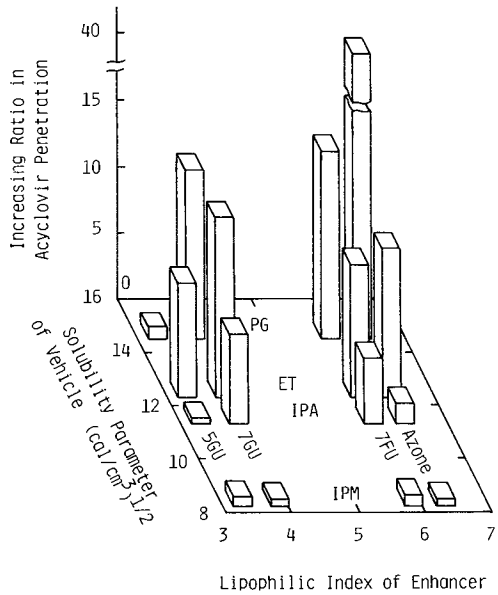

Fig. 4. Effect of Azone, 7FU, 7GU, and 5GU on the percutaneous penetration of acyclovir from various vehicles with different physicochemical properties.

IPM caused unusual diffusion profiles of acyclovir (Fig. 3D). One explanation may be that the rate-limiting step is not the passage through the skin but the movement in the vehicle. It is possible that the dissolution rate of suspended acyclovir determines its penetration rate. However, these phenomena were observed under the suppressed penetration conditions, where a decrease in acyclovir concentration was negligible. In the experiment with hairless mouse skin, normal penetration profiles were obtained for 7GU in IPM (Fig. 2).

The great enhancing effect observed with the combinations of a relatively hydrophilic vehicle and a hydrophobic enhancer is not fully explained by the extent of skin penetration by the enhancer. It has been reported that PG augmented the percutaneous penetration enhancing activity of oleic acid (17), lauric acid, and lauryl alcohol (18). Barry proposed a synergistic action of PG and Azone (19), which may also be the case for acyclovir penetration in the present investigation. A large disappearance of PG from the donor phase was also observed at the end of the present diffusion experiment when Azone was added to it. In spite of the notable improvement of acyclovir penetration, the transport of enhancers from PG to skin was smaller than expected from the difference between the solubility parameters of the enhancers and PG. The movement of hydrophilic vehicle, i.e., PG, into the stratum corneum should increase the polarity of skin and result in a decrease in enhancer affinity to the skin.

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