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COMMUNICATION

Effect of Application Volume of Ethanol–Isopropyl Myristate Mixed Solvent System on Permeation of Zidovudine and Probenecid Through Rat Skin

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ABSTRACT

Permeation of zidovudine (AZT) and probenecid from an ethanol–isopropyl myristate (IPM) mixed system through rat skin was studied in a finite system. Several volume sizes of the ethanol-IPM mixed systems containing AZT and probenecid, both as suspensions, were applied on the skin of the hairless rat using a vertical glass cell, and the fractions of the drugs permeated in 8 hr $Q_{\%,8hr}$ *were determined. For the systems containing 40% ethanol, the* $Q_{\%,8hr}$ *value decreased with the reduction of volume of the system applied, and the decreasing profile was similar to that calculated on the assumption that the permeability of the drug does not change with the volume of the sample applied. On the other hand, in the systems containing 10% or 20% ethanol, the* $Q_{\%,8hr}$ *value showed a maximum when a specific volume of the sample was applied. Therefore, the effect of sample volume on the* $Q_{\%,8hr}$ *value was different between the 40% ethanol-IPM system and the 10% or 20% ethanol-IPM system. Following pretreatment of the skin with 0.105 ml/cm*^{2} *of drug-free 40% ethanol-IPM for 2 hr, several volume sizes of 10% ethanol-IPM systems containing the drugs were applied on the skin to explain why the different profiles were observed in the system containing 10% or 20% ethanol. The results for pretreated skin suggest*

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that the amount of ethanol in the systems with low ethanol concentration and small application volume is too small to exert an effect that enhances permeation of the drugs. In those systems, the integrated effect of ethanol on the skin would be important for the enhancing effect. Total volume, as well as concentration, of an enhancer should be set precisely in designing an efficient transdermal delivery system.
Key Words: *Ethanol–isopropyl myristate mixed system; Probenecid; Rat skin; Skin permeation; Zidovudine.*

INTRODUCTION

Zidovudine (3′-azido-3′-deoxythymidine; AZT), an inhibitor of the reverse transcriptase of the human immunodeficiency virus, has primarily been administered orally (1,2). New administration routes (3,4) and coadministration of other drugs (5,6) were widely studied for efficient delivery of AZT. The transdermal delivery system of AZT should be useful to provide less-frequent dosing and high patient compliance since frequent dosing (6 times/day) is required in oral administration (7–9).

In the present study, permeation of AZT and probenecid from an ethanol–isopropyl myristate (IPM) mixed system (10) through rat skin is examined in a finite system. Probenecid is known to improve the pharmacokinetics and distribution property of AZT (5). Probenecid, coadministered with AZT transdermally, may increase the plasma concentration and cerebrospinal fluid/plasma concentration ratio of AZT. In this study, ethanol as a penetration enhancer was added to IPM at 10–40% levels, and several volume sizes of the ethanol-IPM mixed systems containing AZT and probenecid, both as suspensions, were applied on excised skin of the hairless rat. The fractions of the drug permeated in 8 hr ($Q_{\%,8hr}$) were determined and compared to the difference in the volume applied. For establishing an efficient transdermal drug delivery system, both the composition and application volume of drug in the system should be set carefully.

EXPERIMENTAL

Materials

The AZT was purchased from Yamasa Shoyu Company (Chiba, Japan). Probenecid was purchased from Sigma Chemical (St. Louis, MO). All other chemicals were reagent grade and were used as received.

Preparation of Ethanol–Isopropyl Myristate Mixed Systems

For the preparation of the ethanol-IPM mixed systems, ethanol was dissolved in IPM at concentrations of 10%, 20%, and 40% (w/w). The resulting solutions were used for the preparation of the suspension containing both AZT and probenecid. The initial concentrations of AZT and probenecid were 60 mM and 140 mM, respectively, in the 10% and 20% ethanol systems and 150 mM and 180 mM, respectively, in the 40% ethanol system. Those values are 1.5–3 times higher than their solubilities in the corresponding systems (Table 1).

In Vitro Drug Permeation Studies

The abdominal skin of WBN/ILA-Ht male hairless rats (180–230 g, Ishikawa Laboratory Animals, Saitama, Japan) was excised under anesthesia and immediately mounted in a vertical-type glass cell (11) with 17 ml of receiver cell volume and 2.83 cm^2 of effective diffusion area. The receiver cell was filled with isotonic phosphate buffered solution (pH 7.4) and was kept at 37°C. The ethanol-IPM mixed systems containing both AZT and probenecid were applied on the skin at volumes of 0.035, 0.070, 0.105, 0.175, or 0.350 ml/cm^2. A sample (1 ml) was withdrawn from the receiver cell hourly for up to 8 hr after the application, and the same volume of the fresh buffer solution was added to the receiver cell to keep the volume constant.

Pretreatment of the Skin

In some permeation studies, a drug-free ethanol-IPM mixed system containing 40% ethanol was applied on the

Table 1

Solubilities of AZT and Probenecid

	Solubility at 37°C (mM)	
Media	AZT	Probenecid
Water	113	0.175
Ethanol	228	189
10% Ethanol/IPM	19.8	47.0
20% Ethanol/IPM	28.2	80.3
40% Ethanol/IPM	116	130
IPM	1.5	6.2

skin as a pretreatment. The solution was removed 2 hr after the application, and then the different volume of the 10% ethanol-IPM system containing the drugs was applied on the pretreated skin. The following procedures were the same as those described in the in vitro drug permeation studies.

Determination of the Drugs

A high-performance liquid chromatography (HPLC) system (10) was used for the determination of AZT and probenecid in the receiver solution. Briefly, the samples were mixed with the same volume of acetonitrile containing two internal standards, 7(β-hydroxypropyl)theophylline for AZT and *n*-butyl *p*-hydroxybenzoic acid for probenecid. After centrifugation at 14,000 rpm, the supernatant (20 μl) was applied to the HPLC system twice. For AZT analysis, the ultraviolet (UV) detector was operated at 265 nm, and a mobile phase of water : acetonitrile : acetic acid (84.9 : 15.0 : 0.1 by volume) was flowed at 1 ml/min. For probenecid analysis, the UV detector was operated at 244 nm, and a mobile phase of water : acetonitrile : acetic acid (49.9 : 50.0 : 0.1 by volume) was flowed at 1 ml/min. The reversed-phase column packed with LiChrospher™ RP-18 (250 × 4 mm) was used.

Calculation of $Q_{\%8hr}$ Values

The $Q_{\%8hr}$ values (fractions of the drug permeated in 8 hr) were calculated using following equation:

$$Q_{\%8hr} = A \times Q_{8hr}/(C_0 \times V) \quad (1)$$

in which Q_{8hr} is the cumulative amount of drug permeated per unit area in 8 hr, and A, C_0, and V are the effective diffusion area of the cell, the initial concentration of the drug, and the applied volume of sample, respectively. If the permeability coefficient does not change with the difference in V, the values of Q_{8hr} for drug suspension would not be influenced by V, and the values of $Q_{\%8hr}$ should be inversely proportional to V.

RESULTS AND DISCUSSION

Permeation profiles of AZT and probenecid from the ethanol-IPM mixed system containing 10%, 20%, and 40% ethanol are shown in Figs. 1–3. Although the contents and solubilities of AZT were smaller than those of probenecid in these media, the permeation rates (cumulative amount permeated with time) of AZT were greater than those of probenecid in all cases. This result agrees with that described in our previous report (10) in which a larger application volume (2.4 ml/cm^2) of the ethanol-IPM mixture was employed. The Q_{8hr} values of both AZT and probenecid increased with an increase in the applied volume of sample irrespective of the difference in the ethanol contents.

The changes in $Q_{\%8hr}$ values with the applied volume are shown in Fig. 4. In the case of the system containing 40% ethanol, the $Q_{\%8hr}$ values of AZT and probenecid decreased as the applied volume V increased. Such a rela-

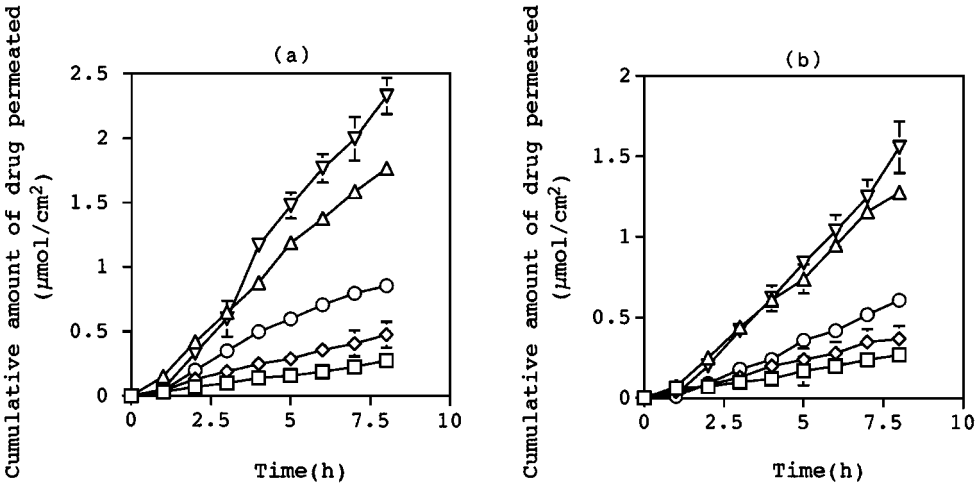

Figure 1. Effect of applied volume on permeation of (a) AZT and (b) probenecid from IPM containing 10% ethanol, 60 mM AZT (as suspension), and 140 mM probenecid (as suspension) through hairless rat skin. Applied volume (ml/cm^2): □, 0.035; ◇, 0.070; □, 0.105; △, 0.175; ▽, 0.350. Each point represents the mean ± SE of three experiments.

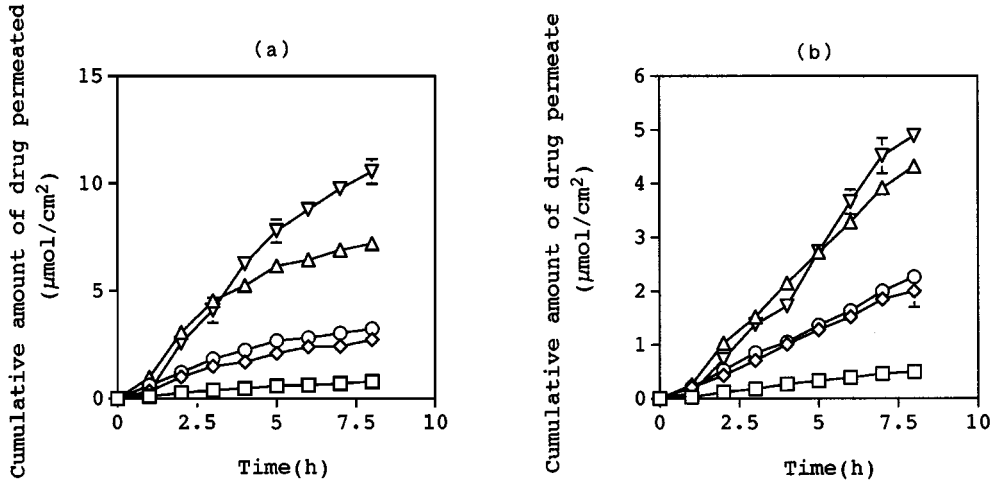

Figure 2. Effect of applied volume on permeation of (a) AZT and (b) probenecid from IPM containing 20% ethanol, 60 mM AZT (as suspension), and 140 mM probenecid (as suspension) through hairless rat skin. Applied volume (ml/cm^2): □, 0.035; ◇, 0.070; □, 0.105; △, 0.175; ▽, 0.350. Each point represents the mean ± SE of three experiments.

tionship between $Q_{\%8hr}$ and V qualitatively agrees with that predicted by Eq. 1, although the actual Q_{8hr} values were different when V was changed. On the other hand, the profiles of both AZT and probenecid in the systems containing 10% and 20% ethanol showed the maximum $Q_{\%8hr}$ values at $V = 0.175$ ml/cm^2 and 0.070 ml/cm^2, respectively. These results suggest that both concentration of enhancers and the application volume of the system are important for efficient transdermal drug delivery.

The enhancing effect of ethanol on the skin permeation of drugs has been shown to consist mainly of the action on skin lipids (12) and solvent drag effect (13–15) of ethanol. Although these mechanisms may function in parallel or concurrently, the former can be characterized as a rather ''static effect'' governed mainly by the integral amount of ethanol acting on the skin lipids. This effect mainly modifies (lowers) the barrier function of the stratum corneum. On the other hand, the latter may

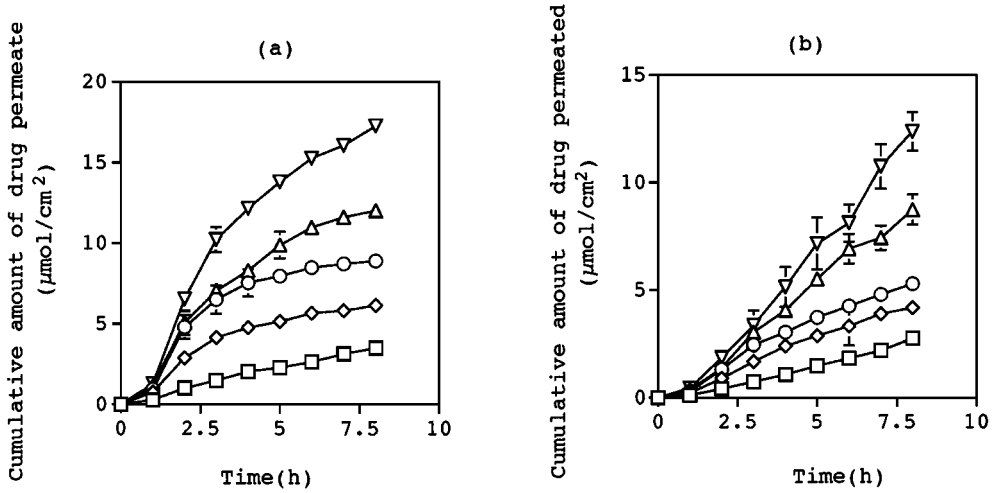

Figure 3. Effect of applied volume on permeation of (a) AZT and (b) probenecid from IPM containing 40% ethanol, 60 mM AZT (as suspension), and 140 mM probenecid (as suspension) through hairless rat skin. Applied volume (ml/cm^2): □, 0.035; ◇, 0.070; ○, 0.105; △, 0.175; ▽, 0.350. Each point represents the mean ± SE of three experiments.

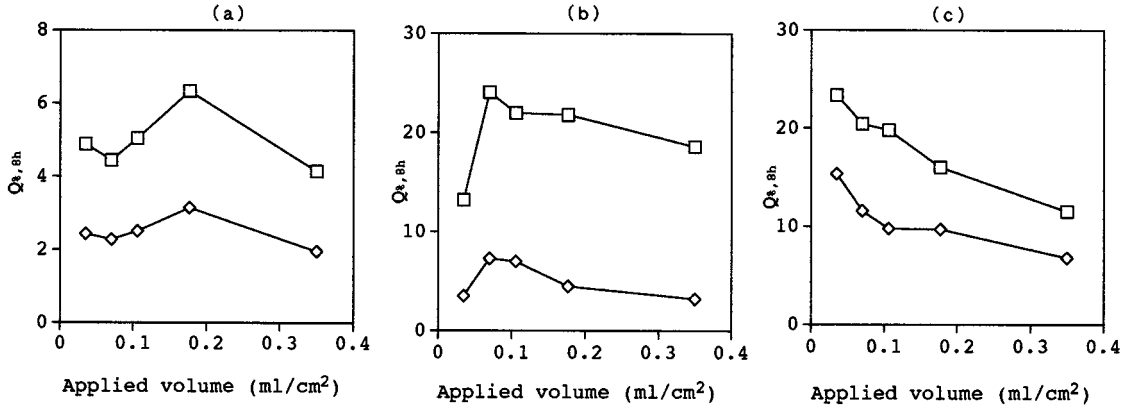

Figure 4. Relationship between percentage of drug permeated in 8 hr $Q_{\%8hr}$ and applied volume of ethanol-IPM systems containing AZT and probenecid on hairless rat skin (□, AZT; ◇, probenecid): (a) 10% ethanol, 60 mM AZT, 140 mM probenecid; (b) 20% ethanol, 60 mM AZT, 140 mM probenecid; (c) 40% ethanol, 150 mM AZT, 180 mM probenecid.

be characterized as a ''dynamic'' or ''kinetic effect'' exerted by the flow of ethanol through the skin. The reasons why Q_{8hr} values are low in the systems with lower ethanol contents (10% and 20%) and smaller applied volume can be explained as follows: First, an effect of ethanol on skin lipids was not enough to change permeability of the drugs in the skin since the total amount of ethanol permeated into the skin is low; second, permeation rate and solvent drag effect of ethanol were decreased since ethanol content in the applied system decreased rapidly to exhaustion due to the permeation of ethanol itself through the skin.

To clarify which effect is dominant in each system, the effect of pretreatment was observed. A drug-free ethanol-IPM mixed system (0.105 ml/cm^2) containing 40% ethanol was applied on hairless rat skin for 2 hr to get enough static effect of ethanol in the skin. After removing the pretreatment solution, the ethanol-IPM mixed systems of different volume sizes containing the drugs and 10% ethanol were applied on the skin. Figure 5 shows the perme-

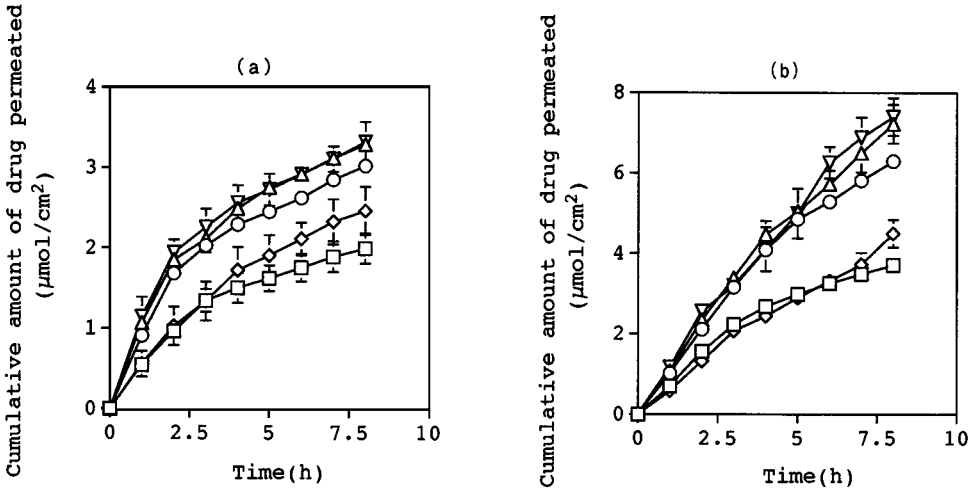

Figure 5. Effect of applied volume on permeation of (a) AZT and (b) probenecid from IPM containing 10% ethanol, 60 mM AZT (as suspension), and 140 mM probenecid (as suspension) through hairless rat skin pretreated for 2 hr with IPM containing 40% ethanol. Applied volume (ml/cm^2): □, 0.035; ◇, 0.070; ○, 0.105; △, 0.175; ▽, 0.350. Each point represents the mean ± SE of three experiments.

ation profiles of the drugs through the pretreated skin. Compared with the profiles shown in Fig. 1 (10% ethanol-IPM system without pretreatment), release rates of both drugs were increased, and the differences in Q_{8hr} values among systems with various applied volumes became smaller.

Figure 6 shows the relationship between the applied volume and the $Q_{\%8hr}$ values. Compared with the profiles shown in Fig. 4a (10% ethanol-IPM system without pretreatment), the $Q_{\%8hr}$ value decreased with an increase in the applied volume without showing any peak at medium applied volume. These results suggest that the static effect of ethanol is important and should be considered in the effective enhancement of skin permeation of the drugs. Once nearly maximum static effect is achieved with enough enhancer, the conditions corresponding to the 40% ethanol-IPM system, the barrier function of the stratum corneum becomes minimal, and the influence of application volume and the concentration of ethanol (enhancer) in the formulation should be critical factors in the system design.

Tata et al. (15) investigated the penetration of minoxidil through hairless mice skin from an ethanol–propylene glycol mixed system as functions of application volume and occlusion. In both the occluded and nonoccluded applications, the permeation of the drug from the 2% solution increased as the application volume increased. In addition, the permeation of the drug in the occluded applications was higher than that in the corresponding nonoccluded applications. These results suggest the importance of the ethanol remaining in the system on the skin. Our present results on the permeation of AZT and probenecid from the ethanol-IPM systems point out similar importance as stated by Tata et al. Tata et al. also reported that the in vitro results were parallel closely the in vivo data.

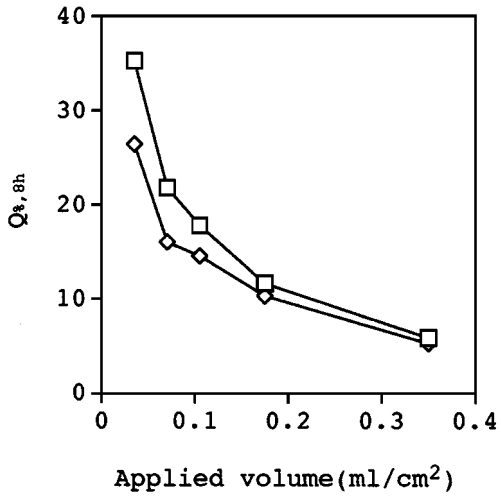

Figure 6. Relationship between percentage of drug permeated in 8 hr $Q_{\%8hr}$ and applied volume of 10% ethanol-IPM systems containing 60 mM AZT (as suspension) and 140 mM probenecid (as suspension) on hairless rat skin pretreated for 2 hr with IPM containing 40% ethanol (□, AZT; ◇, probenecid).

In conclusion, the application volume is an important factor to determine the fraction of AZT and probenecid permeated following application of the ethanol-IPM mixed systems on the skin. An effective enhancement of skin permeation of AZT and probenecid should be profitable for the design of an efficient transdermal delivery system for the treatment of AIDS.

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