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Transdermal Absorption of Zidovudine from Ethanol–Isopropyl Myristate Mixed System and Influence of Probenecid on It in Rats

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ABSTRACT

Transdermal absorption of zidovudine (AZT) from an ethanol–isopropyl myristate (IPM) mixed system was examined in rats. For comparison of bioavailability (BA) after topical applications, 0.25 ml of the ethanol/IPM system containing 40% ethanol and 60 mM AZT was applied as a standard formulation. Values of the area under the plasma concentration–time curves of AZT for 8 hr (AUC_{0-8}), as indices of BA, following application of various formulations were compared with that of the standard formulation. Then the influence of content of the drug and ethanol, and application volume of the system was evaluated. BA was effectively improved only when the total amount of ethanol applied on the skin was increased. On the other hand, simultaneous transdermal application of AZT and probenecid increased the AUC_{0-8} of AZT without necessitating the increase in ethanol content in the formulation. In addition, coadministered probenecid improved cerebrospinal fluid/plasma concentration ratio of AZT.

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). In oral use, 100 to 200 mg of AZT should be administered 4 to 5 times a day in order to achieve effective therapy. Such a dosing regimen inevitably causes unwanted side effects as well as low patient acceptability or compliance. In search for more efficient delivery of AZT, coadministration of other drugs such as probenecid (3,4) has been shown to be a hopeful

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strategy. New administration routes such as rectal (5) and nasal cavity (6) have also been evaluated in our laboratory. The transdermal delivery system of AZT should be useful in providing less frequent dosing and high patient compliance (7–9). In our previous studies (10,11), in vitro permeation of AZT and probenecid through rat skin was shown to be significantly enhanced by using the ethanol–isopropyl myristate (IPM) mixed system, and the application volume of the formulation remarkably affected the permeability of the drugs.

In the present study, transdermal absorption of AZT from the ethanol–IPM system was examined in vivo using rats. As the standard formulation, 0.25 ml (0.70 ml/cm^2 × 3.6 cm^2) of the ethanol–IPM system containing 40% ethanol and 60 mM AZT was chosen. Bioavailabilities evaluated with the area under the plasma concentration–time curve of AZT following applications of formulations with various contents of the drug and ethanol were compared. The effect of application volume of the system was also observed. In addition, simultaneous application of AZT and probenecid was examined. Since probenecid should be useful in improving pharmacokinetics and distribution property of AZT (3,4), the effect of probenecid coadministered with AZT transdermally on the plasma concentration and cerebrospinal fluid (CSF)/plasma concentration ratio of AZT was evaluated.

EXPERIMENTAL

Materials

AZT and probenecid were purchased from Yamasa Shoyu Co. (Chiba, Japan) and Sigma Chemical (St. Louis, MO), respectively. All other chemicals were of reagent grade and were used as received.

Preparation of Ethanol–IPM Mixed Systems

For preparation of the ethanol–IPM mixed systems, ethanol was dissolved in IPM at a level of 20 or 40 w/w%. The resulting solutions were used for the preparation of the solution of AZT (30 mM or 60 mM in 40% ethanol–IPM) or the suspension (120 mM in 40% ethanol or 30 mM in 20% ethanol–IPM). For the simultaneous application, both drugs were dissolved (60 mM) in the ethanol–IPM system containing 40% ethanol. Solubilities of AZT and probenecid in several ethanol–IPM systems are shown in Table 1.

Table 1

Solubility of AZT and Probenecid in Ethanol–IPM Mixed Systems

	Solubility at 37°C, mM	
Media	AZT	Probenecid
IPM alone	1.5	6.2
20% ethanol–IPM	28.2	80.3
40% ethanol–IPM	116.0	130.0

Topical Application of Ethanol–IPM Mixed System on Rats

WBN/ILA-Ht male hairless rats (180–230 g, Ishikawa Laboratory Animals, Saitama, Japan) were anesthetized with urethane (1 g/kg, IP). A glass cell with an available diffusion area of 3.6 cm^2 was fixed on the abdominal skin of the rats. The ethanol–IPM mixed system containing the drug(s) was applied into the cell, and the top of the cell was covered for occlusion. Plasma (0.1 ml) was collected from the jugular vein at specified times after the application.

Examination of Distribution of AZT into CSF

The ethanol–IPM system containing AZT alone or AZT and probenecid was applied in the same way as stated in the previous paragraph. At 1 hr after the application, plasma (0.1 ml) was collected from the jugular vein and CSF (0.1 ml) was taken by a cisternal puncture (6).

Analysis of AZT and Probenecid in Plasma and CSF

A high-performance liquid chromatography (HPLC) system (10) was used for the determination of AZT and probenecid in plasma and CSF. The plasma or CSF sample (0.1 ml) was mixed with 10% acetic acid (0.1 ml), and the drugs were extracted with 0.4 ml of ethyl acetate containing internal standards, 7-(β-hydroxypropyl)theophylline for AZT and *n*-butyl *p*-hydroxybenzoic acid for probenecid. After centrifugation at 14,000 rpm, 0.3 ml of the organic layer was taken. To the residue was added 0.5 ml of ethyl acetate for reextraction of the drug. After centrifugation, 0.5 ml of the organic layer was taken. The two organic layers were combined and dried under N_2 gas flow, and the

residue was reconstituted with a mobile phase (0.1 ml) used in the HPLC system. For AZT analysis, the ultraviolet (UV) detector was operated at 256 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, 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. A reversed-phase column packed with LiChrospher® RP-18e (250 × 4 mm) was used.

RESULTS AND DISCUSSION

Transdermal Absorption from the Ethanol–IPM Mixed System

Table 2 shows the compositions of various formulations of the ethanol–IPM systems examined, and the plasma concentrations of AZT observed following application of the formulations on rat skin are shown in Figs. 1 and 2. A significant penetration enhancing effect of ethanol for AZT was readily seen when the profiles for formulations A and B (Fig. 1) were compared. Doubling the content of AZT, i.e., 60 mM B to 120 mM C, slightly increased the plasma level of AZT. When the solubility of AZT is considered (Table 1), formulation C initially contained AZT, nearly 50% of which as solid. Therefore AZT concentrations in solution are not so different in the two formulations. When the application volume was increased; 0.25 ml B to 0.5 ml D, the plasma level of AZT was significantly increased. In this case, increasing the application volume leads to increase in the total amount of "dissolved" AZT as well as ethanol in the system applied.

Comparing formulations B and E, the amount of "dissolved" AZT and concentration of ethanol are equal, but the application volume, and hence the amount

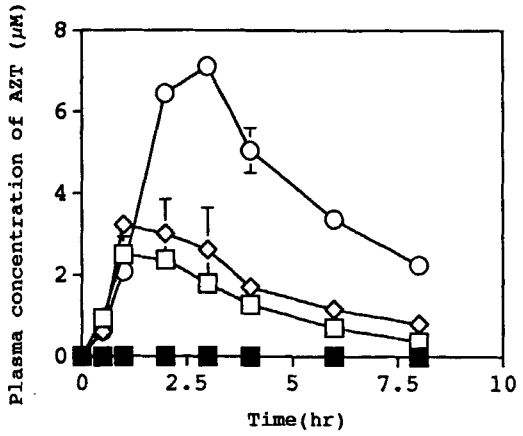

Figure 1. Plasma concentrations of AZT following application of various formulations on rat skin (3.6 cm^2). Formulations: ■, A, 60 mM AZT in 0.25 ml IPM; □, B, 60 mM AZT in 0.25 ml 40% ethanol–IPM; ◇, C, 120 mM AZT in 0.25 ml 40% ethanol–IPM; ○, D, 60 mM AZT in 0.50 ml 40% ethanol–IPM. Each point represents the mean ± *SE* of 3 determinations.

of ethanol, is different. Formulation E gave a much higher plasma level of AZT than that of Formulation B. Decreasing the concentration of ethanol, i.e. 40% E to 20% F, resulted in significant decrease in the plasma level of AZT.

These results suggests that the total amount of ethanol applied is a most important factor in the effective enhancement of transdermal absorption of AZT.

Table 3 shows the values of the area under the plasma concentration–time curves of AZT for 8 hr (AUC_{0-8}) and AUC_{0-8}/dose of AZT following application of various formulations on rat skin. The values of AUC_{0-8}/dose can be regarded as the fraction absorbed or the efficiency of absorption of the drug in 8 hr. The AUC_{0-8}/dose values for formulations C and F were

Table 2

Formulations Applied in the Transdermal Absorption Studies

Formulation Code	Applied Volume (ml)	Concn. of AZT (mM)	Amount of AZT (mg)	Concn. of Ethanol (%)	Amount of Ethanol (g)
A	0.25	60	3.75	0	0
B	0.25	60	3.75	40	0.1
C	0.25	120	7.5	40	0.1
D	0.50	60	7.5	40	0.2
E	0.50	30	3.75	40	0.2
F	0.50	30	3.75	20	0.1

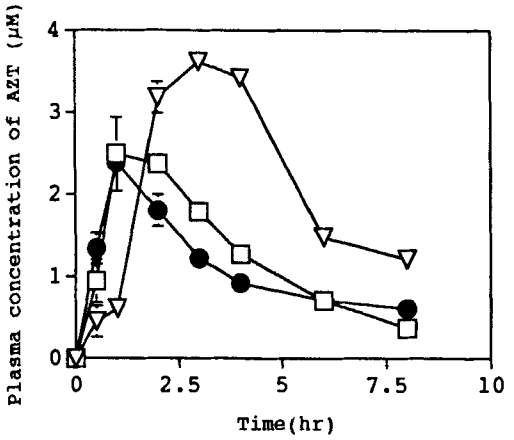

Figure 2. Plasma concentrations of AZT following application of various formulations on rat skin (3.6 cm^2). Formulations: □, B, 60 mM AZT in 0.25 ml 40% ethanol-IPM; ▽, E, 30 mM AZT in 0.50 ml 40% ethanol-IPM; ●, F, 30 mM AZT in 0.50 ml 20% ethanol-IPM. Each point represents the mean ± *SE* of 3 determinations.

smaller than that for B. The reason for the decrease in the values for C may be that the solid drug did not contribute to the penetration through the skin. As for formulation F, the content of ethanol might be too small to exert enough enhancing effect on skin permeation of AZT. On the other hand, the values for formulations D and E, both containing larger amount of ethanol, were remarkably higher than that for B, and hence the efficiency of absorption of AZT was high. Although efficient transdermal delivery can be achieved through the use of high dose ethanol as an enhancer, possible drawbacks such as irritation of the skin induced by ethanol would be a limiting factor.

Simultaneous Percutaneous Application of AZT and Probenecid

Plasma concentration profiles of AZT and probenecid following simultaneous transdermal application are shown in Fig. 3. The profile following the application of the formulation containing AZT alone is also included in the figure. Plasma concentration and AUC of AZT coadministered with probenecid were higher than those of the case without probenecid (Table 4). The increase in the plasma concentration of AZT should be due to the

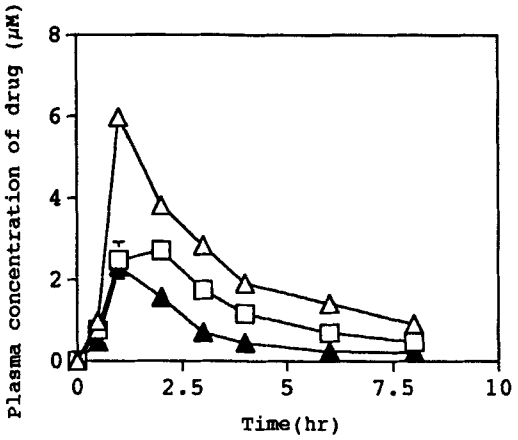

Figure 3. Plasma concentrations of AZT or probenecid following application of 0.25 ml of 40% ethanol-IPM containing the drug(s) on rat skin (3.6 cm^2): □, concn. of AZT formulation with 60 mM AZT; Δ, concn. of AZT formulation with 60 mM AZT + 60 mM probenecid; ▲, concn. of probenecid formulation with 60 mM AZT + 60 mM probenecid. Each point represents the mean ± *SE* of 3 determinations.

Table 3

Comparison of Bioavailabilities of AZT Following Application of Various Formulations in Rats

Formulation Code	AUC_{0-8} (μM · hr), Mean ± *SE* (*n* = 3)	AUC_{0-8}/Dose (μM · hr/mg)
B	10.1 ± 0.4	2.68
C	14.0 ± 2.3	1.87
D	32.1 ± 2.3	4.28
E	16.8 ± 2.1	4.48
F	8.9 ± 0.9	2.37

Table 4

Effect of Probenecid on AUC_{0-8} and Ratio of CSF/Plasma Concn. of AZT[a]

	AUC_{0-8} (μM · hr)	Concn. of AZT (μm)[b]		CSF/Plasma Ratio
		Plasma	CSF	
AZT alone	10.1 ± 0.4	3.92 ± 0.34	0.76 ± 0.04	0.196 ± 0.008
AZT with probenecid	17.0 ± 1.6[c]	6.50 ± 0.69	2.52 ± 0.18	0.393 ± 0.017[c]

[a]Each value represents the mean ± *SE* of 3 determinations.
[b]Determined at 1 hr after the application.
[c]Significantly different from those values for AZT alone ($p \leq 0.01$).

reduction of clearance of AZT in the presence of probenecid (3,4).

Efficient delivery of AZT to CSF has also been desired in treatment for patients with central nervous system dysfunctions. CSF concentration and CSF/plasma concentration ratio of AZT 1 hr after the applications are shown in Table 4. Probenecid coadministered with AZT increased the CSF/plasma concentration ratio of AZT with statistical significance. The effect of probenecid could be due to an inhibition of efflux of AZT from CSF to blood (3,4). Thus a promising coadministration effect of probenecid for the improvement of retention in the body as well as distribution to CSF of AZT may be obtained following transdermal administration.

In the present study, AZT was transdermally applied to rats in several formulations consisting of ethanol–IPM mixed systems. For the case of the AZT–ethanol–IPM systems, efficient delivery of the drug was achieved in the system containing a large amount of ethanol. For suitable transdermal delivery of drugs, both efficiency of drug absorption and safety for skin are important. Since degree of irritation of the skin should be related to the amount of ethanol applied, an application system must be designed carefully. On the other hand, transdermal coadministration of probenecid increases plasma concentration of AZT without necessitating the increase in the required amount of the penetration enhancer. Simultaneous application of AZT and probenecid should be useful in improving efficiency of transdermal delivery of AZT.

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