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RESEARCH PAPERS

Comparison of Skin Permeation of Dideoxynucleoside-Type Anti-HIV Drugs: Alone Versus Combination

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ABSTRACT

The effects of vehicles and skin permeation enhancer on the skin permeation of dideoxynucleoside-type anti-HIV drugs, Zalcitabine (DDC), Didanosine (DDI), and Zidovudine (AZT), alone and in combination, were compared using hairless rat and human cadaver skins. Each drug alone or a combination of three drugs was added to various compositions of ethanol/water or ethanol/tricaprylin cosolvent system to saturation, and in vitro skin permeation studies were conducted using Valia–Chien skin permeation cells. In both ethanol/water and ethanol/tricaprylin systems, the hairless rat skin permeation rates achieved by each drug alone and three drugs in combination were not significantly different. Addition of oleic acid [1.0% (v/v) for each drug alone and 5.0% (v/v) for drug combination] in ethanol/tricaprylin (50:50) could not significantly enhance the skin permeation of these drugs. In hairless rat skin permeation of each drug alone, the permeation rates of all three drugs were dramatically enhanced with the addition of oleic acid in ethanol/water (60:40) cosolvent system and reached plateau level with oleic acid as low as 0.3% (v/v). However, in the case of drug combination, the enhancement of skin permeation rates of these drugs with the addition of oleic acid in ethanol/water (80:20) cosolvent system was not as high as that observed for each drug alone, and plateau level was not observed even at 5.0% (v/v) of oleic acid. Human cadaver skin permeation rates of each drug alone saturated in ethanol/water (60:40) cosolvent system containing 1.0% (v/v) of oleic acid were 3–4 times lower than those of hairless rat skin. However, in skin permeation of three drugs in combination, saturated in ethanol/water (80:20) cosolvent system containing 5.0% (v/v) of oleic acid, human cadaver skin permeation rates of DDC and DDI

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were slightly lower than those of hairless rat skin, and there was no significant difference between the two skins for AZT. These results show that mutual skin permeation-enhancing effects of oleic acid and an ethanol/water cosolvent system made the transdermal delivery of anti-HIV drugs, alone and in combination, feasible.

INTRODUCTION

FDA-approved 2′,3′-dideoxynucleoside-type anti-HIV drugs, such as 2′,3′-dideoxycytidine (DDC), 2′,3′-dideoxyinosine (DDI), and 3′-azido-3′-deoxythymidine (AZT), showed beneficial results in treating human immunodeficiency virus (HIV) infection (1). However, the treatment of acquired immunodeficiency syndrome (AIDS) by a single-drug therapy has limitations due to the dose-dependent toxicity and the development of resistant strains. Moreover, because of the short biological half-life and considerable hepatic "first-pass" degradation of these drugs (2), conventional oral or IV routes require frequent high-dose administration to maintain a suitable blood concentration. Therefore, it was proposed that a noninvasive zero-order delivery, such as the transdermal route, is desirable to overcome the problems of conventional delivery and to avoid the strong side effects which may be attributed to an excessive plasma level of these drugs immediately after oral or IV administration (3). Transdermal delivery can enhance the antiviral activity and reduce the frequency and severity of side effects by optimizing blood concentration profiles within the therapeutic range for longer duration. Transdermal delivery can also improve the bioavailability by circumventing the hepatic first-pass elimination (4).

Moreover, combination of two or more anti-HIV drugs has been shown to achieve synergistic inhibition of HIV replication in vitro (5–9) and in clinical studies (10–13). These synergistic combination could reduce the risk of toxicity by reducing dose, while maintaining a good antiviral effect and reducing the risk of resistance development. This study compared the effect of vehicles and skin permeation enhancer on the transdermal delivery of DDC, DDI, and AZT for each drug alone and three drugs in combination. Various compositions of ethanol/water and ethanol/tricaprylin cosolvent systems were chosen as a hydrophilic and lipophilic vehicles, respectively. Effect of oleic acid on the permeation of each drug alone and three drugs in combination across hairless rat skin and human cadaver skin was also studied.

MATERIALS AND METHODS

Materials

DDC, DDI, and AZT were kindly supplied by Hoffmann-La Roche (Nutely, NJ), Bristol-Myers Squibb (Wallingford, CT), and Burrough Wellcome Co. (Research Triangle Park, NC), respectively. Ethanol USP (200 proof) was obtained from Florida Distiller Co. (Lake Alfred, FL). Tricaprylin (TCP) and oleic acid (OA) were purchased from Sigma Chemical Co. (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals were reagent grade and were used as received.

Preparation of Skins

Hairless rats (Fuzzy strain, 7-8 weeks), purchased from Harlen Sprague Dawley Inc. (Indianapolis, IN), were sacrificed in a CO_2 chamber on the day of experiments. A full-thickness skin was surgically removed from the dorsal site of each rat and carefully cleaned with normal saline.

Human cadaver skin was purchased from Ohio Valley Tissue and Skin Center (Cincinnati, OH) and stored in a liquid nitrogen tank until use. On the day of experiment, skin was thawed in warm normal saline for 20 min before the permeation study.

Preparation of the Saturated Solutions

An excess amount of DDC, DDI, and AZT (alone or in combination) was added to the various volume fractions of ethanol/water or ethanol/TCP cosolvent system (15 ml each) with or without OA. The solution was immersed in a shaking water bath at 37°C and allowed to equilibrate for 48 hr.

In Vitro Skin Permeation Studies

Hydrodynamically well-calibrated Valia–Chien permeation systems were used to conduct in vitro skin per-

meation studies of DDC, DDI, and AZT for 30 hr at 37°C. The donor half-cells, which faced the stratum corneum surface, contained saturated solution of DDC, DDI, and AZT (alone or in combination) in various compositions of cosolvent systems with or without OA (3.5 ml). The receptor half-cells, which faced the dermis side, were filled with isotonic phosphate buffer (pH 7.4, 3.5 ml) containing 0.01% (w/v) of gentamicin and *p*-chloromercuribenzoic acid to minimize the degradation during HPLC analysis and permeation study (14). At predetermined time intervals, a sample (0.1–2 ml) was taken from the receptor solution, which was refilled with the same volume of fresh receptor solution. Samples were kept in the freezer (–20°C) until analyzed by HPLC.

Analytical Methods

Concentrations of DDC, DDI, and AZT were determined using a gradient reverse-phase HPLC system (HP 1050 Liquid Chromatograph) equipped with a HP 1050 UV detector and a HP 3396A integrator (Hewlett Packard, Mountainview, CA). A HP Hypersil ODS column (5 μm, 200 × 4.6 mm, Hewlett Packard) maintained at 37°C was used as the analytical column. The injector volume was 15 μl, and the wavelength was 265 nm. Acetonitrile–phosphate buffer (pH 7.0, 20 mM dibasic sodium phosphate) combination was used as the mobile phase at a flow rate of 1.0 ml/min. Retention times of DDC, DDI, and AZT were 3.89, 3.85, and 4.18 min when 5%, 10%, and 20% acetonitrile was used, respectively. DDC, DDI, and AZT were analyzed simultaneously by programming the volume fraction of acetonitrile to increase from 5% to 30% over 10 min and maintaining 30% acetonitrile for 5 min at a constant flow rate of 1.0 ml/min. Retention times of DDC, DDI, and AZT were 4.62, 5.21, and 7.16 min, respectively.

Data Analysis

The cumulative amount of each drug permeated per unit area was plotted as a function of time, and the steady-state permeation rate was calculated from the slope of the linear portion.

Each condition was run in triplicate and expressed as the mean (± standard deviation). Statistical comparisons were made using Student's *t* test, and differences were considered to be significant at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Vehicle on the Hairless Rat Skin Permeation

The hairless rat skin permeation rates of DDC, DDI, and AZT, saturated in ethanol/water cosolvent system alone or in combination, were compared as a function of ethanol volume fraction (Fig. 1). In both cases, the skin permeation rates increased with increasing volume fraction of ethanol, reached maximum values at approx. 60–70% of ethanol in water, and then decreased with further increase in ethanol volume fraction. The permeation rates achieved by each drug alone and three drug combination were not significantly different at the same volume fraction of ethanol.

Figure 2 shows the hairless rat skin permeation rates of DDC, DDI, and AZT saturated in the ethanol/TCP cosolvent system, alone and in combination, as a function of volume fraction of ethanol. In both cases, hairless rat skin permeation rates of these drugs increased as the volume fraction of ethanol increased, reached the maximum values at 50% (v/v) of ethanol in TCP, and then decreased with further increase in volume fraction of ethanol. The skin permeation rates of these drugs achieved by each drug alone and three drugs in combination were not significantly different.

Effect of Oleic Acid on the Skin Permeation

Ethanol/TCP (50:50) cosolvent system was chosen (from Fig. 2) to study the effect of OA as a skin permeation enhancer. As shown in Fig. 3, addition of OA in ethanol/TCP (50:50) cosolvent system [1.0% (v/v) for each drug alone and 5.0% (v/v) for drug combination] could not significantly enhance the skin permeation of these drugs. It is known that the extent of the effect of OA on lipid perturbation and flux is quantitatively related to the amount of OA incorporated into the stratum corneum bilayer (15). Thus in can be speculated that the incorporation of viscous T into ethanol seems to reduce the thermodynamic activity of OA to distribute from the vehicle to the skin, which resulted in no significant enhancing effect of OA in ethanol/TCP (50:50) cosolvent system.

From Fig. 1, two best vehicle combinations—ethanol/water (60:40) for each drug alone and ethanol/water (80:20) for drug combination—were chosen to investigate the effect of OA. Addition of OA in ethanol/wa-

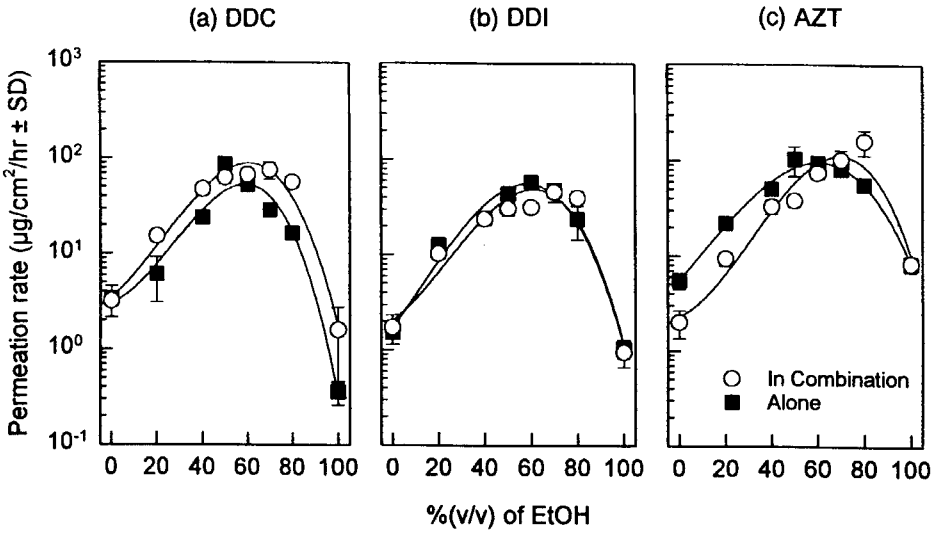

Figure 1. Comparison of the permeation rate of (a) DDC, (b) DDI, and (c) AZT, alone and in combination, across hairless rat skin as a function of volume fraction of ethanol in ethanol/water cosolvent system.

ter (60:40) dramatically enhanced the permeation rate of all three drugs [Fig. 4(a)] with reduced lag time (Table 1). Moreover, hairless rat skin permeation rates of these drugs had already reached plateau level with the addition of 0.3% (v/v) of OA [Fig. 4(a)]. However, in case of drug combination [Fig. 4(b)], higher than 1.0% (v/v) of OA concentration was required to observe significant skin permeation enhancement, and the hairless rat skin permeation rates of these drugs kept increasing even to 5.0% (v/v) of OA. In addition, although addition of OA in ethanol/water (80:20) cosolvent system significantly enhanced the skin permeation rates of these

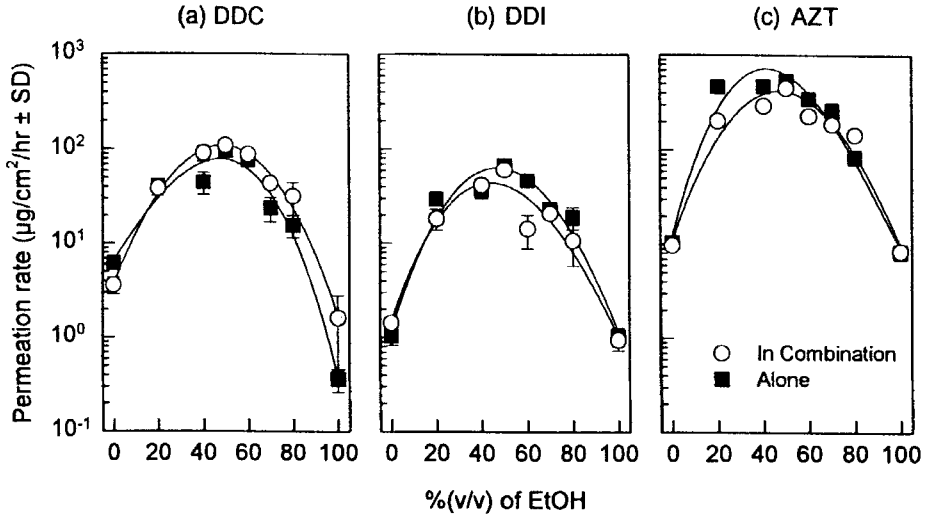

Figure 2. Comparison of the permeation rate of (a) DDC, (b) DDI, and (c) AZT, alone and in combination, across hairless rat skin as a function of volume fraction of ethanol in ethanol/tricaprylin cosolvent system.

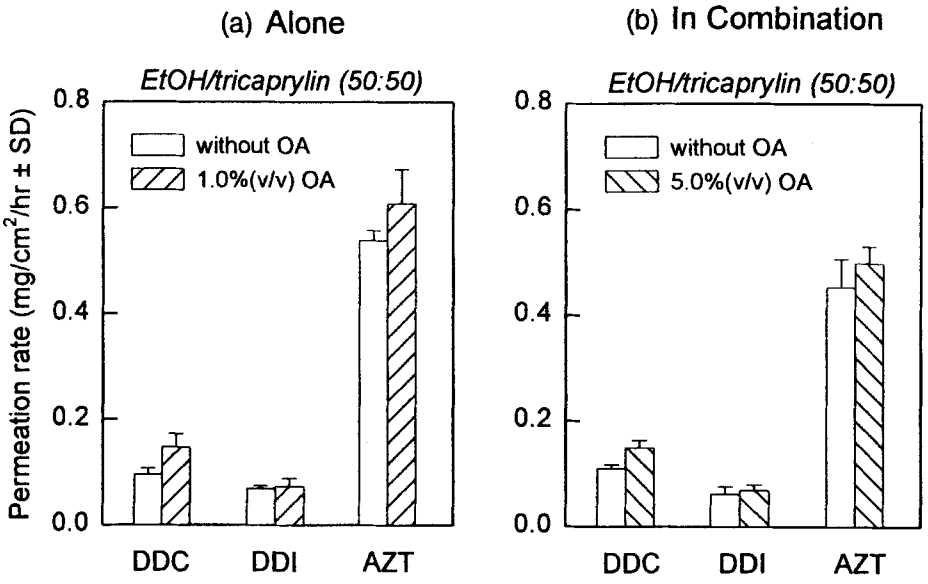

Figure 3. Effect of oleic acid (OA) on the skin permeation rate of DDC, DDI, and AZT, saturated in ethanol/tricaprylin (50:50) cosolvent system: (a) alone or (b) in combination.

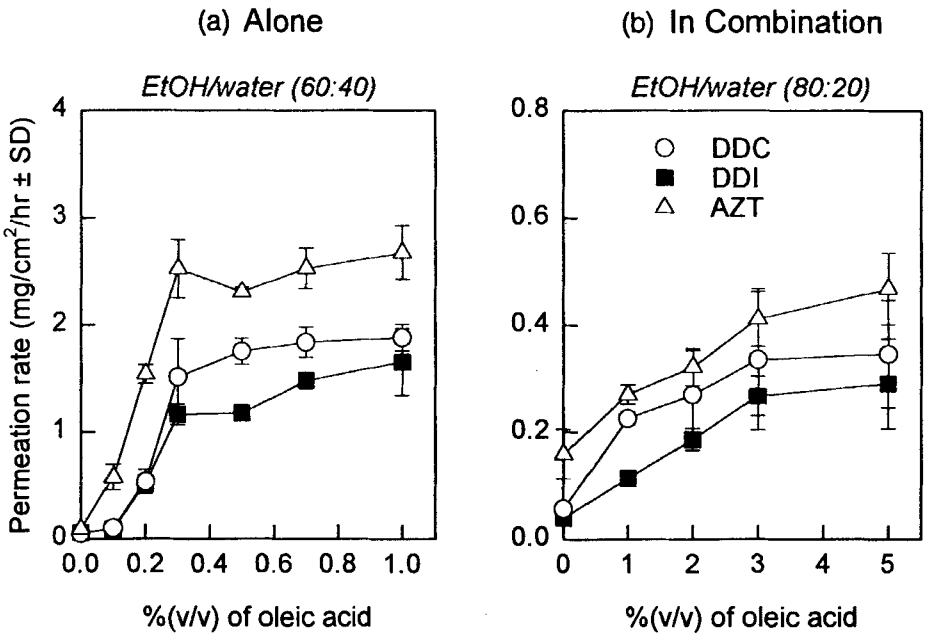

Figure 4. Effect of oleic acid concentration on the permeation rate of DDC, DDI, and AZT, saturated in ethanol/water cosolvent system, across the hairless rat skin: (a) alone or (b) in combination.

Table 1

Effect of Oleic Acid Concentration on the Lag Time of Hairless Rat Skin Permeation

Formulation (Ethanol/Water)	Oleic Acid [% (v/v)]	Time (hr ± *SD*) DDC	DDI	AZT
Each drug alone (60:40)	0.0	10.31 (0.52)	11.57 (0.98)	10.40 (0.57)
	0.1	8.60 (0.35)	8.24 (1.02)	11.06 (1.54)
	0.2	6.91 (0.49)	6.15 (0.48)	9.81 (1.32)
	0.3	4.26 (1.57)	2.42 (0.32)	5.53 (0.14)
	0.5	1.82 (0.67)	0.72 (0.10)	1.90 (0.15)
	0.7	0.55 (0.14)	0.69 (0.24)	1.80 (0.11)
	1.0	0.65 (0.07)	0.64 (0.08)	1.69 (0.42)
Three drugs in combination (80:20)	0.0	9.57 (0.69)	10.64 (1.07)	11.09 (0.82)
	1.0	9.50 (0.31)	10.48 (0.58)	10.03 (1.00)
	2.0	2.20 (0.19)	2.45 (0.20)	1.91 (0.44)
	3.0	2.39 (0.32)	2.88 (0.52)	2.06 (0.29)
	5.0	1.38 (0.03)	2.37 (0.16)	1.68 (0.54)

drugs with reduced lag time (Table 1), the enhancement of the skin permeation rates was not as high as that observed with each drug alone. It can be speculated that each drug seems to compete with others for the skin permeation through the same pathway enhanced by OA when all three drugs are saturated in combination.

Skin Permeation Through Human Cadaver Skin

Human cadaver skin permeation of DDC, DDI, and AZT—each drug alone and three drugs in combination—was compared with hairless rat skin permeation. Figure 5 shows the permeation profiles of each drug, saturated alone in ethanol/water (60:40) cosolvent system containing 1.0% (v/v) of OA, across hairless rat skin and human cadaver skin. Since the cumulative amount permeated was decreased after 12 hr, the permeation rates were calculated from the initial linear portion; these are summarized in Table 2. In general, the human skin permeation rates of these drugs were 3–4 times lower than hairless rat skin. Lag time was not significantly different between two skins (Table 2).

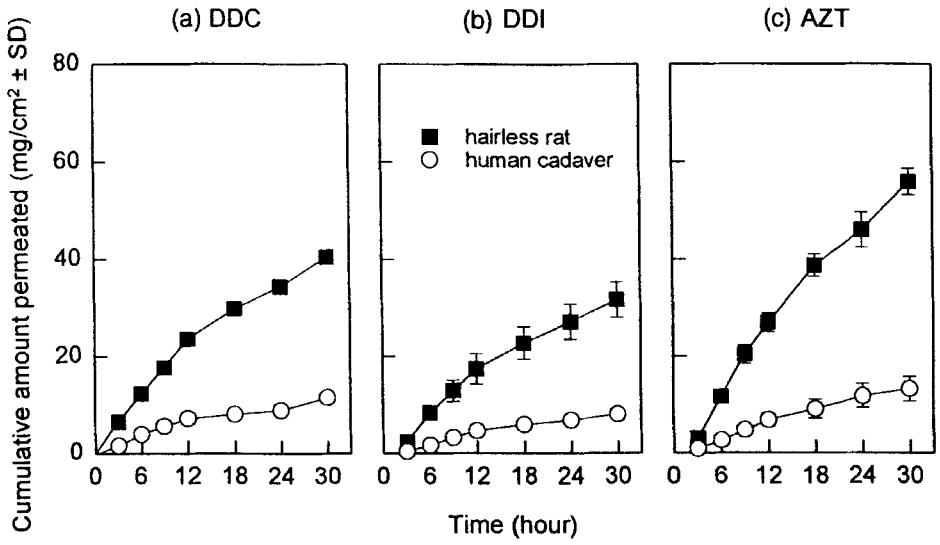

Figure 5. Comparison of the hairless rat skin and human cadaver skin permeation profiles of (a) DDC, (b) DDI, and (c) AZT, each drug alone saturated in ethanol/water (60:40) cosolvent system containing 1.0% (v/v) oleic acid.

Table 2

Comparison of Skin Permeation Rate and Lag Time Between Hairless Rat Skin and Human Cadaver Skin: Each Drug was Saturated Alone in Ethanol/Water (60:40) Cosolvent System Containing 1.0% (v/v) of Oleic Acid

	DDC		DDI		AZT	
	Rat	Human	Rat	Human	Rat	Human
Permeation rate	1.88	0.61	1.66	0.47	2.68	0.67
($mg/cm^2/hr \pm SD$)	(0.12)	(0.02)	(0.31)	(0.06)	(0.25)	(0.11)
Lag time	0.65	0.46	0.64	1.08	1.69	1.89
($hr \pm SD$)	(0.07)	(0.23)	(0.08)	(0.63)	(0.42)	(0.57)

When three drugs were simultaneously saturated in ethanol/water (80:20) cosolvent system containing 5.0% (v/v) of OA (Fig. 6), human cadaver skin permeation ratres of DDC and DDI were slightly lower than the rates for hairless rat skin (Table 3). However, there was no significant difference between the two skins for AZT. Lag time was not significantly different between the two skins (Table 3). Since the human cadaver skin permeation rate was not much lower than the hairless rat skin in simultaneous skin permeation of three drugs,

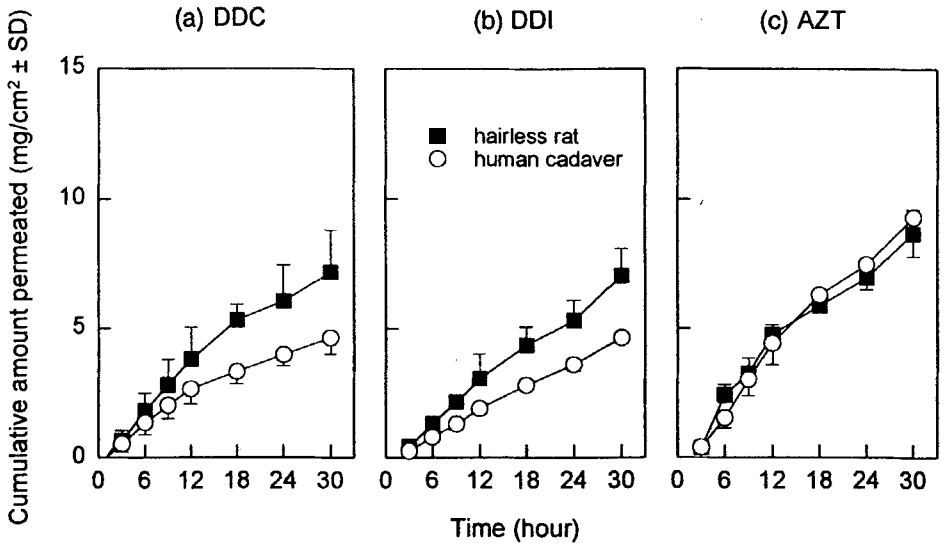

Figure 6. Comparison of the hairless rat skin and human cadaver skin permeation profiles of (a) DDC, (b) DDI, and (c) AZT, three drugs in combination saturated in ethanol/water (80:20) cosolvent system containing 5.0% (v/v) oleic acid.

Table 3

Comparison of Skin Permeation Rate and Lag Time Between Hairless Rat Skin and Human Cadaver Skin. All Three Drugs Are Saturated Simultaneously in Ethanol/Water (80:20) Cosolvent System Containing 5.0% (v/v) of Oleic Acid

	DDC		DDI		AZT	
	Rat	Human	Rat	Human	Rat	Human
Permeation rate	0.35	0.23	0.29	0.18	0.47	0.45
($mg/cm^2/hr \pm SD$)	(0.10)	(0.03)	(0.08)	(0.01)	(0.07)	(0.09)
Lag time	1.38	1.44	2.37	2.27	1.68	2.24
($hr \pm SD$)	(0.03)	(0.34)	(0.16)	(0.15)	(0.54)	(0.43)

further investigations seem to be necessary to understand the exact mechanism(s) of the mutual permeation-enhancing effort of ethanol and OA across human cadaver skin.

CONCLUSIONS

The hairless rat skin permeation rates of DDC, DDI, and AZT, saturated simultaneously in various compositions of ethanol/water or ethanol/TCP cosolvent system, were not significantly different from those achieved by each drug alone. Addition of OA in ethanol/water cosolvent system enhanced the skin permeation rate of DDC, DDI, and AZT with shorter lag time. However, the effect of OA on the simultaneous skin permeation of these drugs was not prominent. The human skin permeation rates of each drug alone was 3–4 times lower than the rate for hairless rat skin but was not significantly decreaed when three drugs were delivered simultaneously. Since combination therapy for HIV infection with two or more drugs may allow a reduction of dose, the mutual skin permeation-enhancing effect of OA and ethanol/water cosolvent system showed the feasibility of transdermal delivery of anti-HIV drugs.

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