Enhanced Transdermal Delivery of Zidovudine in Rats and Human Skin

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Zidovudine (azidothymidine, AZT), a potent antiviral agent acting on acquired immunodeficiency syndrome virus, was examined with regard to permeation through rat and human skin. A steady state plasma concentration of AZT after transdermal application in rats estimated from both pharmacokinetics data after i.v. administration and penetration rate through excised rat skin from 10% oleic acid (OA) aqueous solution shows penetration about 85 times higher compared to that from 10% OA would be needed for therapeutic efficacy. A mixed-solvent system consisted of 5% Sefsol-318® (S-318), 10% OA, 10% *N*-methyl-2-pyrrolidone (MP), 20% propylene glycol (PG) and water showed promising characteristics as a vehicle in terms of permeability of AZT through excised rat skin. The maximum flux of 0.41 μmol/cm²/h was observed in excised human skin after application of a gel formulation including S-318, OA, MP and PG. The result suggests a possible use of the gel formulation to gain an effective plasma concentration in humans.

Keywords Zidovudine; transdermal application; acquired immunodeficiency syndrome; human skin; gel formulation

Zidovudine (azidothymidine, AZT) is a strong inhibitor of the reverse transcriptase isolated from the acquired immunodeficiency syndrome (AIDS) virus,[1] and its therapeutic potency for AIDS or the AIDS-related complex has been recognized clinically. Although orally administered AZT is rapidly absorbed from intestinal mucosa, it loses considerable potency during its first pass (40%) and then is rapidly eliminated from the body with a half-life of 1 h.[2] In addition, orally administered AZT often shows strong side effects on bone marrow,[3] which may be attributable to an excessive plasma level of AZT immediately after administration. Non-oral zero-order delivery systems, if possible, would be useful to decrease the high daily dose of AZT (5—10 mg/kg, every 4 h), to maintain an expected anti-viral effect, which can be time-dependent, to reduce the strong side effects, and to improve patient compliance. The skin has been recently recognized as a potent application site for non-oral zero-order delivery systems. We previously reported the percutaneous absorption of AZT and the penetration enhancing effect of oleic acid (OA) in rats.[4] In the present study, we examined the skin permeation of AZT both *in vitro* and *in vivo* in rats to select an appropriate solvent system or a practical formulation. The skin permeation of AZT from a gel formulation was also measured by using excised human skin.

Materials and Methods

Materials Sefsol-318® (S-318), medium chain monoglyceride, was kindly supplied by Nikko Chemical Co. (Tokyo, Japan). AZT was purchased from Yamasa Shoyu Co. (Chiba, Japan). *N*-Methyl-2-pyrrolidone (MP) was purchased from Tokyo Kasei Kogyo Co., Ltd. OA, propylene glycol (PG) and D-limonene (LM) were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were used as received.

***In Vitro* Permeation Experiments** Abdominal skin of Wistar rats (190—220 g, Saitama Laboratory Animals, Saitama, Japan) or human breast skin were used for the permeation experiments. The human skins were obtained from cancer patients (female, age 43—66) with total breast extirpation. Normal parts of the skin were stored at −20 °C and allowed to thaw gradually at room temperature before use. After removal of all subcutaneous fat, the skin was mounted in a two-chamber diffusion cell; it has an available diffusion area of 0.95 cm² and a half-cell volume of 2.3 ml.[5] The dermal and epidermal sides of the skin were exposed to the receptor medium (saline) and donor solution, respectively. Both the receptor and donor phases were stirred mechanically at 150 rpm and were kept at 37 °C with a water bath. Samples of the whole receptor medium were removed at specified times and replaced with fresh saline.

To examine the penetration of AZT from a gel, the excised human skin was mounted in a vertical-type diffusion cell[6]; it has an available diffusion area of 4.9 cm² and a receptor phase volume of 21 ml. The cell-set was kept at 37 °C in a water bath, and then 0.44 g of gel was applied on the skin, and the saline solution was added in the receptor phase.

The samples were analyzed for AZT content by a high performance liquid chromatography (HPLC, LC-6A, Shimadzu Seisakusho, Kyoto, Japan). The conditions for the HPLC analysis were as follows: column, LiChlospher RP-18 (4.0 × 250 mm); detector, UV 265 nm; flow rate, 1 ml/min; mobile phase, 25% acetonitrile in 0.1% acetic acid.

***In Vivo* Experiments** For determination of the enhancing effect of OA, a Wistar rat was anesthetized with ether and its abdominal hair was removed by clippers. A drug-free 10% OA solution was applied on the abdominal skin, and the rat was kept in a Bollman cage for 24 h before drug application. Abdominal hair was removed without any pretreatment and used for the study with mixed-enhancer systems. The donor compartment of the vertical-type cell was fixed on the abdominal skin of rats anesthetized with urethane, and 1 ml of test solution was applied in the cell.

In order to estimate total body clearance (Cl_t) of AZT in the rat, AZT (3 mg/kg) was intravenously administered to urethane anesthetized rats.

A 100 μl sample of plasma was collected from the jugular vein at specified times after application, and was analyzed for drug level by HPLC. The conditions for HPLC analysis were similar to those for the samples of the *in vitro* experiments except for the mobile phase, 15% acetonitrile in 0.01 M phosphate buffer (pH 7.0).

Relationship between *in Vitro* and *in Vivo* Data At a steady state, plasma concentration (C_{ss}) after transdermal application is expressed as follows:

$$C_{ss} = \frac{A \times F}{Cl_t} \quad (1)$$

where A and F are the application area and the steady state penetration rate through the skin, respectively.

The correlation between *in vitro* and *in vivo* results were evaluated by comparing the observed C_{ss} and calculated C_{ss} obtained from the *in vitro* steady state penetration rate and the total body clearance after i.v. injection in rats.

The required application areas were calculated from the *in vitro* steady state penetration rate in human skin, and the total body clearance (1.3 l/h/kg) and the minimum effective concentration (1 μM) for AIDS virus reported by Klecker *et al.*[2]

Results and Discussion

Skin Permeation of AZT from 10% OA Emulsion Figure 1 shows *in vitro* penetration of AZT from water or 10% OA emulsion (AZT content of 5 mg/ml) in rat or human skin. Since solubility of AZT at 37 °C is 30 mg/ml and the partition coefficient (OA/water) at 24 °C is 0.11, AZT should be mainly distributed to the aqueous phase in 10% OA emulsion. The permeation of AZT was enhanced in both

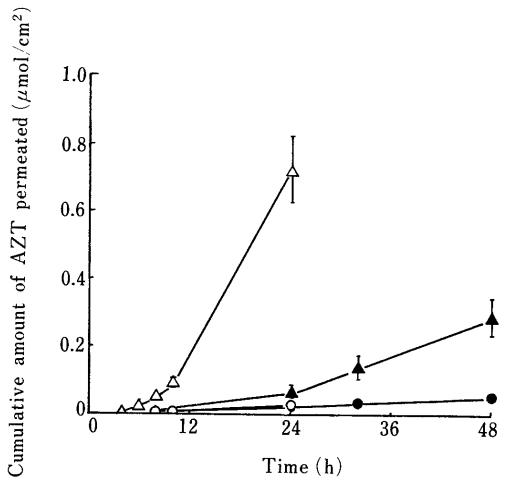

Fig. 1. Effect of OA on the Permeation of AZT through Excised Rat or Human Skin

Rat skin: ○, water; △, 10% OA. Human skin: ●, water; ▲, 10% OA. Data are the mean ± S.E. ($n=3$). Initial AZT concentration in the donor side is 5 mg/ml.

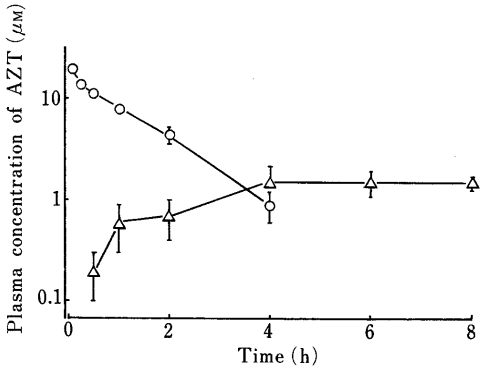

Fig. 2. Plasma Concentration of AZT after Intravenous or Percutaneous Administration to Rats

○, intravenous administration (3 mg/kg); △, percutaneous application (25 mg/kg). Data are the Mean ± S.E. ($n=3$).

rat and human by the addition of OA. The steady state flux in water as a vehicle was observed as 1.5×10^{-3} and 1.3×10^{-3} μmol/cm²/h for rat and human skin, respectively. The flux at 10—24 h from 10% OA in rat skin was 4.5×10^{-2} μmol/cm²/h and the value was 30 times higher than the steady state flux from water. In human skin, the penetration of AZT was enhanced 7.1 times by OA; the steady state flux from 10% OA was 9.2×10^{-3} μmol/cm²/h. Such enhancing effects of OA could be caused by its effect on stratum corneum lipid. OA may act as a solvent of the hard sebum in the stratum corneum to lower its viscosity.[7]

Figure 2 shows plasma concentrations of AZT after i.v. (3 mg/kg) or percutaneous (25 mg/kg) administration to rats. We previously reported that percutaneous absorption of AZT in the rat was enhanced by OA and the steady state plasma concentration of AZT was not obtained until 8 h after application unless skin was pretreated by 10% OA.[4] In the application to pretreated skin with 10% OA, plasma concentration of AZT from 10% OA solution (5 mg/ml) was detectable even at 0.5 h and the detectable level persisted for at least 8 h. The average plasma concentration of AZT at 2—8 h in the pretreated rat was similar to that at 23—24 h in the non-pretreated rat (1.3 μM) in the previous study. The

Table I. Effect of Vehicle on the Permeation of AZT through Excised Rat Skin

Composition of vehicle (%)							Skin permeation[a] ($n=3$)		
Rp.	OA	S-318	LM	MP	PG	Water	Q	F	E
1	10	0	0	0	0	ad. 100	9.7×10^{-2}	0.045	1
2	0	0	0	0	0	100	7.6×10^{-3}	0.002	0.04
3	0	0	0	10	10	ad. 100	4.0×10^{-3}	0.001	0.02
4	10	0	0	10	10	ad. 100	2.0×10^{-1}	0.10	2.2
5	0	5	0	10	10	ad. 100	1.3	0.25	5.5
6	10	5	0	0	10	ad. 100	2.4	0.46	10.2
7	10	5	0	10	0	ad. 100	2.7	0.54	12.0
8	10	5	0	10	10	ad. 100	3.8	0.63	14.0
9	10	0	5	10	10	ad. 100	1.7	0.43	9.5

Q, cumulative amount of AZT permeated at 10 h (μmol/cm²); F, maximum flux of AZT observed within 24 h (μmol/cm²/h); E, enhancing ratio to 10% OA. *a*) From AZT solution (5 mg/ml).

earlier steady state of AZT permeation was made possible by skin pretreatment. The value of Cl_t in the anesthetized rat obtained from i.v. data is 0.50 l/h/kg. By using Eq. 1, the steady state plasma concentration of AZT can be calculated from Cl_t, "A" (4.9 cm²), and the penetration rate of AZT through the excised skin (4.5×10^{-2} μmol/cm²/h). In a rat weighing 200 g, the calculated value of plasma concentration of AZT is 2.2 μM, and the value is similar to the values observed at 4—8 h. The result suggests a good correlation between *in vitro* and *in vivo* permeation of AZT from 10% OA. Since experiments *in vitro* are usually easier than those *in vivo*, the *in vitro* permeation experiments could be useful for an initial screening.

When the human skin permeability for AZT *in vivo* was comparable to that *in vitro*, the required application area was calculated from the penetration rate of AZT from 10% OA through the excised human skin and Cl_t in humans reported by Klecker *et al.*,[2] and the value was about 8500 cm² for a human weighing 60 kg. Since the desirable application area would be below 100 cm², at least 85 times more penetration enhancement would be needed for an effective delivery of AZT in clinical application.

Effect of Solvent Compositions on Permeation of AZT through Rat Skin OA, S-318 and LM were used as penetration enhancers acting on a lipophilic pathway in stratum corneum, and PG and MP were used as cosolvents. The effect of various ingredients on the permeation of AZT are shown in Table I. Since the solubilities of S-318, LM and OA in water are small, these preparations without Rp.2 were applied as emulsions. The simultaneous use of several components shows the high penetration rates; especially, the flux from a mixed solvent consisting of 5% S-318, 10% OA, 10% MP, 20% PG and water (Rp. 8) was 14 times higher than that from 10% OA. S-318 and LM may modify the viscosity of sebum in the stratum corneum or the structure of skin, and thus alter the diffusivity of AZT in the stratum corneum.[8] S-318 and LM can enhance not only AZT permeation but also MP and PG permeation. Since MP and PG are soluble in both water and oil and AZT is a hydrophilic compound, the dissolution of MP and PG into the stratum corneum may bring a high partition of AZT into the skin, which is naturally lipophilic.

Table II shows the effect of AZT concentration in the donor side on its permeation from the mixed system

TABLE II. Effect of AZT Concentration in the Donor Side on the Permeation through Excised Rat Skin ($n=3$)

Concentration in donor (mg/ml)	Q	F	E
1	1.3	0.20	4.4
5	3.8	0.63	14.0
15	15.0	2.41	53.6
30	31.3	4.85	107.8

The vehicle consists of 5% S-318, 10% OA, 10% MP, 20% PG and water (Rp. 8). Q, cumulative amount of AZT permeated at 10 h (μmol/cm^2); F, maximum flux of AZT observed within 24 h (μmol/cm^2/h); E, enhancing ratio to 10% OA.

TABLE III. Plasma Concentration of AZT after Percutaneous Application of AZT Solution Containing 5% S-318, 10% OA, 10% MP and 20% PG

Dose (mg/kg)	Plasma concentration (μM)				
	Time (h)				
	2	4	6	8	10
5	0.1±0.1	1.0±0.4	2.0±0.4	2.5±0.9	2.9±1.2

Data are the mean±S.E. ($n=3$). AZT concentration in the solution applied was 1 mg/ml.

TABLE IV. Components of Gel Formulation

	(%)		(%)
AZT	3	Gelatin	3
S-318	5	Polyvinylalcohol	2
OA	10	Sodium polyacrylate	6
MP	10	Water	41
PG	20		

consisting of 5% S-318, 10% OA, 10% MP, 20% PG and water. Since AZT is soluble in this mixed system within the range of tested concentrations, the penetration rates were proportional to the donor concentrations of AZT. The observed flux from this system at the donor concentration of 30 mg/ml was about 100 times higher than that from 10% OA at the donor concentration of 5 mg/ml.

The absorption-enhancing effect of this mixed system was investigated in rats. Though the highest penetration of AZT was observed at 30 mg/ml of the donor concentration in Table II, the concentration of AZT in the vehicle for the application to rats was 1 mg/ml, since the plasma concentration of AZT calculated from the penetration rate of AZT from 30 mg/ml AZT solution by using Eq. 1 was too high. Plasma concentrations of AZT after application of this system (5 mg/kg) are shown in Table III. Although the applied dose (concentration in the vehicle) was 5 times lower than that tested in skin pretreated with 10% OA, higher plasma concentrations of AZT were observed. Inflammation of the application site was not observed visually in the treatment of either the mixed system or the 10% OA. These results suggest that this system would be promising as a vehicle for the transdermal delivery of AZT for AIDS treatment.

Preparation of AZT Gel According to the results of the rat *in vitro* experiments, AZT gel formulation was prepared as shown in Table IV. Gelatin and polyvinylalcohol were dissolved in hot water (about 80 °C), and then S-318, OA, MP, PG and sodium polyacrylate were added. The resulting high viscous emulsion was finally mixed with AZT after cooling. Figure 3 shows the permeation of AZT through excised human skin from the gel. The flux of AZT at 5—10 h was 0.41 μmol/cm^2/h. The required application area (190 cm^2) for clinical use was calculated from the value. The value (190 cm^2) may be acceptable as an application area for AIDS patients. A larger application area than expected (100 cm^2) may be attributable to a difference in the enhancing effect between rats and humans and also to an under-estimation of the *in vitro* steady state permeation rate of AZT from the gel through human skin. Skin permeability would increase to a certain level after the addition of the enhancers. On the other hand, the driving force for permeation of AZT decreases as the donor concentration of AZT decreases. Therefore the observed flux was obtained at an apparent steady state, and the true steady state flux may be higher than the observed value.

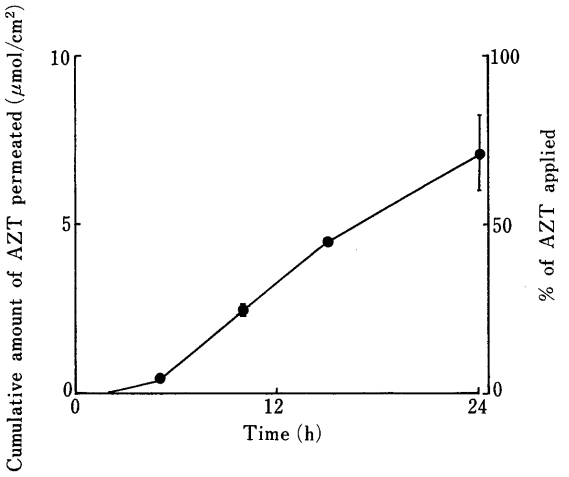

Fig. 3. Human Skin Permeation of AZT from the Gel Formation
Data are the mean±S.E. ($n=3$).

Conclusions

The results obtained in this study show that the transdermal absorption of AZT in rats can be estimated from both pharmacokinetics data after i.v. administration and the *in vitro* penetration rate through excised rat skin, and it can be synergistically enhanced by several enhancers. In addition, the human skin penetration rate of AZT from the AZT gel formulation consisting of the mixed system may be high enough to gain an effective plasma level in humans.

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