Pharmaceutical Research, Vol. 7, No. 9, 1990

Report

Enhanced Delivery of Zidovudine Through Rat and Human Skin via Ester Prodrugs

Toshinobu Seki,[1] Takeo Kawaguchi,[1,2] and Kazuhiko Juni[1]

Received January 5, 1990; accepted April 8, 1990

In an attempt to improve the skin delivery characteristics of Zidovudine (AZT, azidothymidine), five aliphatic esters (acetate, butyrate, hexanoate, octanoate, and decanoate) of AZT were synthesized and assessed as prodrugs of AZT. While the water solubility of the esters is lower than that of AZT, the solubilities in isopropylmyristate (IPM) and the partition coefficients (*n*-octanol:buffer) are higher. Susceptibility to enzymatic hydrolysis in the rat skin homogenate increases as the acyl chain of the ester is lengthened. Among the esters, acetate (C2-AZT) and hexanoate (C6-AZT) showed 2.4- and 4.8-fold enhanced permeation in human skin from an apolar vehicle (IPM) relative to application of AZT itself, respectively.

KEY WORDS: Zidovudine, azidothymidine (AZT); skin permeation; prodrug; esterase; hydrolysis; isopropylmyristate (IPM).

INTRODUCTION

Zidovudine (azidothymidine; AZT) is an inhibitor of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS) (1). Although AZT has been used orally to treat patients with AIDS or AIDS-related complex, its toxicity has been reported to be significant, necessitating dose reductions or discontinuation of the treatment (2,3). Since the thymidine analogue acts as a metabolic antagonist and the antiviral effects can be time dependent, an adequate inhibitory concentration should be maintained in the body for the antiviral effect while avoiding side effects such as bone marrow toxicity, attributable to an excessive plasma concentration following oral administration.

Transdermal drug delivery is suitable for maintaining a constant plasma level. Since the delivery of drugs through the skin is usually low, optimization of the formulations, including the prodrug approach, may be required (4). In a previous study, several aliphatic ester prodrugs of Zidovudine were synthesized and evaluated as long-acting prodrugs for parenteral use (5). Since their physicochemical properties and susceptibility to enzymatic hydrolysis differed notably, the skin delivery characteristics of the esters were of interest. The present paper evaluates the ability of the Zidovudine esters to increase transdermal delivery of the parent drug.

[1] Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 35002, Japan.

[2] To whom correspondence should be addressed.

EXPERIMENTAL

Materials

Zidovudine was purchased from Yamasa Shoyu Co. (Chiba, Japan). The 5′ esters of Zidovudine were synthesized as reported previously (5); their chemical structures are shown in Fig. 1. All the esters were more than 98% pure as shown by a single major peak in HPLC.

Male Wistar rats (190–220 g) were obtained from Saitama Laboratory Animals (Saitama, Japan), and were sacrificed to obtain the entire abdominal skin. The skin was homogenized with pH 7.0 isotonic phosphate buffer (0.1 *M*) containing 0.19 *M* sucrose at 0°C to give a concentration of 1.0% (w/v). The homogenate was centrifuged at 1000*g* for 20 min, and the resulting supernatant was stored at −40°C until use.

Determination of Solubilities and Partition Coefficients

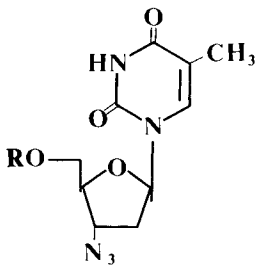
The solubilities of Zidovudine and the esters were determined in triplicate in water and isopropylmyristate (IPM) at 37°C by placing excessive amounts of the compounds in 1–1.5 ml of the solvent. The mixtures were stirred for 24 hr and centrifuged at 14,000 rpm. The concentration of the compounds in their saturated solutions was determined by the HPLC methods described below.

The partition coefficients of the compounds were determined at 24°C in an *n*-octanol–buffer (pH 7.0) system. Their concentration in the aqueous phase was determined by HPLC analysis.

Susceptibility to Enzymatic Hydrolysis

The enzymatic hydrolysis rates of the esters in the pres-

0724-8741/90/0900-0948$06.00/0



I	R = H
II	R = $COCH_3$
III	R = $CO(CH_2)_2CH_3$
IV	R = $CO(CH_2)_4CH_3$
V	R = $CO(CH_2)_6CH_3$
VI	R = $CO(CH_2)_8CH_3$

Fig. 1. Chemical structure of Zidovudine esters.

ence of the rat skin enzyme system were measured at 37°C. Hydrolysis was initiated by adding the ester solution (4×10^{-3} *M* in ethanol) to the preheated enzyme preparation in screw-capped test tubes to an initial concentration of 4 or 8 $\times 10^{-5}$ *M*. Changes in the concentration of the ester and Zidovudine were followed by HPLC and the pseudo-first-order rate constants were determined from the slopes of linear plots of the logarithm of residual ester against time. The enzymatic reaction was not saturated at a higher substrate concentration (8×10^{-5} *M*) and the results were standardized to the homogenate concentration of 1.0% (w/v).

Preparation of Donor Solutions

For the rat skin experiments, water and IPM were used as a polar and an apolar vehicle, respectively. Zidovudine esters (II–VI) were applied as solutions in IPM (5 m*M*) because of their very high solubility (>200 m*M*). Other systems (i.e., Zidovudine and its esters in water and Zidovudine in IPM) were applied as suspensions to maintain a constant driving force for diffusion.

For the human skin experiments, suspensions of Zidovudine in IPM and water, II in IPM, and a solution of IV in IPM (20%, w/w) were used, for the same reason as described above. All solutions and suspensions were shaken for 16 hr prior to application to the skin surface.

Procedure of Skin Permeation Experiments

Rat skin was obtained as described above. Whole human breast skin was obtained from two cancer patients (50 and 78 years) with total breast extirpation. Normal parts of the skin were stored at −40°C and allowed to thaw gradually at 24°C before use. All subcutaneous fat was removed and the skin cut into pieces. The excised skin was mounted in two-chamber diffusion cells of the same type as those used by Sugibayashi *et al.* (6); these cells have an available diffusion area of 0.95 cm^2. The dermal and epidermal sides of the skin were exposed to the receptor medium (saline) and the donor phase (suspension or solution of the compound), respectively. Both the receptor and the donor phase were stirred mechanically at 150 rpm and kept at a constant temperature of 37°C with a circulating water bath. At a specified time, samples of the whole receptor phase (2.3 ml) were removed and replaced with fresh saline. The samples were diluted with saline to 10 ml and stored at 4°C until their analysis for Zidovudine and ester content by HPLC.

Determination of Permeability Coefficient (K_p) of Zidovudine Esters in Rat Skin

To evaluate the changes in skin permeability during the experiments, permeability coefficients were determined at two stated 1-hr periods, 9.5–10.5 and 23.5–24.5 hr. In the suspension systems, permeability coefficients (K_p) were obtained by dividing the flux (F) by the corresponding solubility (C_s) of the compound (i.e., $K_p = F/C_s$). In the solution systems, permeability coefficients ($K_{p,sol}$) were obtained by dividing the fluxes (F) by the measured concentration in the donor phase (C_d). That is, permeability coefficients at 10 hr ($K_{p,sol,10}$) were calculated as follows.

$$K_{p,sol,10} = \frac{F_{(9.5-10.5)} \times 2}{C_{d\,9.5} + C_{d\,10.5}}$$

Where C_{dn} is the measured concentration in the donor phase, obtained from 50 µl of donor solution withdrawn at n hours. Degradation in the donor phase was not observed in any of the esters. In order to compare the flux data to those in the suspension systems, the maximum fluxes for the solution systems were estimated by multiplication of permeability coefficient ($K_{p,sol}$) and solubility (C_s).

HPLC Analysis

Zidovudine and its esters were determined by HPLC using a system consisting of a Shimadzu Model LC-6A pump, a variable-wavelength Shimadzu SPD-6A UV detector operated at 265 nm, and a Rheodyne 7125 injection valve with a 20-µl loop. A reversed-phase HPLC column (LiChrospher RP-18, 250 × 4 mm) was eluted at 40°C with a mixture of acetonitrile–water (25:75 for I, 30:70 for II, 50:50 for III, 60:40 for IV, 70:30 for V, 80:20 for VI) containing 0.1% acetic acid, the flow rate being 1.0 ml/min.

RESULTS AND DISCUSSION

Physicochemical Properties

The solubilities of Zidovudine and its esters II–VI in water and IPM are shown in Table I; all esters show improved lipid solubility over that of the parent compound. The solubilities of the esters in water are lower than that of Zidovudine, which inherently has a higher water solubility (30 mg/ml) and a low melting point (124°C). As expected from the above data, all the esters are also more lipophilic than the parent compound in terms of partition coefficients between *n*-octanol and pH 7.0 buffer. The solubilities of the esters in IPM are higher than that of Zidovudine and those of

Table I. Physicochemical Properties and Susceptibility to Enzymatic Hydrolysis of Zidovudine and Its Esters

Compound	m.p. (°C)	Solubility[a] Water	Solubility[a] IPM	log P^b	Enzymatic hydrolysis rate constant (min^{-1})[c]
I	124	113	1.50	0.02	—[d]
II	Amorphous solid	69.1	15.6	0.36	0.00066
III	Amorphous solid	8.77	15.6	1.50	0.011
IV	Oil	1.44	>200	2.45	0.13
V	Oil	0.087	>200	3.63	0.49
VI	Oil	0.0072	>200	4.45	1.56

[a] At 37°C, in m*M*.
[b] At 24°C, *n*-octanol/buffer (pH 7.0).
[c] At 37°C, standardized to 1% (w/v) enzyme preparation.
[d] No degradation was observed up to 4 hr.

the longer esters (IV–VI) are too high (>200 m*M*) to determine.

Susceptibility to Enzymatic Hydrolysis

The enzymatic hydrolysis rates of the esters were measured in the presence of rat skin enzyme preparation (supernatant of 0.025–1.0%, w/v, skin homogenate). The rate constants, standardized to 1.0% (w/v) enzyme preparation, increased as the alkyl chain lengthened from methyl (II) to nonyl (VI) (Table I). This result is consistent with those obtained using mouse enzyme systems (5).

Permeation of Zidovudine

The transdermal delivery characteristics of Zidovudine were examined in excised rat skin. Cumulative amounts (μmol) of Zidovudine measured in the receptor phase divided by the surface area of the diffusion cell (0.95 cm^2) were plotted against the time of sampling (Fig. 2). Although the suspensions were employed as the donor phase, the penetration rate of Zidovudine from IPM was higher than that from water. Since the suspensions (in IPM and in water) should provide the same driving force or activity, such a difference may be attributable to the effect of IPM on skin permeability (7,8).

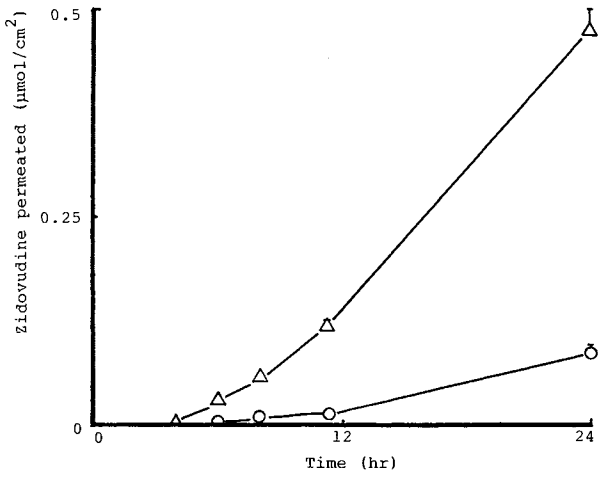


Fig. 2. Permeability of Zidovudine through rat skin from suspension in water (○) and IPM (△). Data are the means ± SE (n = 3).

Permeation of Zidovudine Esters

In order to characterize the skin permeability of the compounds, the permeability coefficients (K_p) were calculated. The relationships between the partition coefficients and the permeability coefficients of the compound are shown in Fig. 3. The permeability of the compounds from water increases as the lipophilicity of the compounds increases. This result is not unexpected, since other studies have shown that skin acts as a lipophilic membrane for the diffusion of lipophilic compounds (9). The higher permeability of the hydrophilic esters (II and III) and the lower permeability of the lipophilic esters (V and VI) at the later period (23.5–24.5 hr) than those at the earlier period (9.5–10.5 hr) may be attributable to the possible hydration of the skin, which may affect both its lipophilic and its hydrophilic pathways (10,11). The permeability of the compounds from IPM decreases as the lipophilicity of the esters increases. Since IPM can be more lipophilic than the skin, the partition of the compounds to the skin from IPM, which seems to be a driving force in the delivery, is assumed to decrease as the lipophilicity of the esters increases. If the compounds show similar diffusion constants in the skin, the supposed changes in the partition coefficients could mainly affect the permeability changes.

In the case of butyrate (III) to decanoate (VI), only Zidovudine was found in the receptor phase, indicating the efficient enzymatic hydrolysis of these esters during the transport through the skin. Acetate (II) was present both as

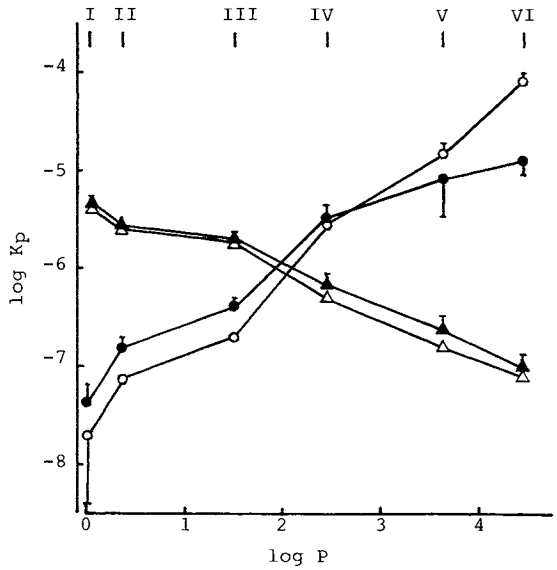


Fig. 3. Relationship between partition coefficient of the compound and permeability coefficient (K_p) in rat skin. From water at 9.5–10.5 hr (○) and 23.5–24.5 hr (●); from IPM at 9.5–10.5 hr (△) and 23.5–24.5 hr (▲). Data are the means ± SE (n = 3). The results of II showed the total values of intact ester and Zidovudine.

intact ester and as Zidovudine in the receptor phase at the various sampling times. These different abilities of the esters to undergo cutaneous hydrolysis parallel their relative susceptibility to the rat skin enzyme system, and the difference in susceptibility may partly affect the skin permeability of the esters. The ratio of the acetate ester (II)-to-Zidovudine fluxes into the receiver chamber was 0.34 at 9.5–10.5 hr and 0.33 at 23.5–24.5 hr in water and 0.44 at 9.5–10.5 hr and 0.43 at 23.5–24.5 hr in IPM.

The penetration rate from saturated solutions was compared to estimate the maximum flux (Table II). In II and III in IPM, the estimated maximum fluxes were calculated by the multiplication of permeability coefficients and corresponding solubilities (12,13). The penetration rates of the compound permeated from IPM were higher than those from water. Calculated fluxes of the ester were much higher than the maximum flux of Zidovudine from IPM; especially, acetate (II) showed about 6.2-fold higher flux relative to Zidovudine itself at the period of 23.5–24.5 hr. Although the maximum flux could not be calculated for hexanoate (IV) because of its high solubility, the higher penetration rate at a saturated condition (>200 m*M*) was estimated for IV rather than I–III, because of its high solubility in IPM and relatively high permeability coefficient from IPM. Although the permeation of the esters from water was not so promising, II showed about twofold higher flux than Zidovudine.

Human Skin Permeation

The skin permeation of Zidovudine and its acetate (II) and hexanoate (IV), which are esters showing promising delivery characteristics in rat skin, were studied in excised human skin (Fig. 4). Since IPM was a better vehicle in the rat skin study, acetate (II) and hexanoate (IV) were applied as a suspension and a solution (20%) in IPM, respectively. From the results in rat skin, the solution of IV (20%) in IPM was expected to show a higher penetration rate than a II suspension in IPM. The esters in IPM showed 4.8-fold (II) and 2.4-fold (IV) higher permeation than Zidovudine in terms of

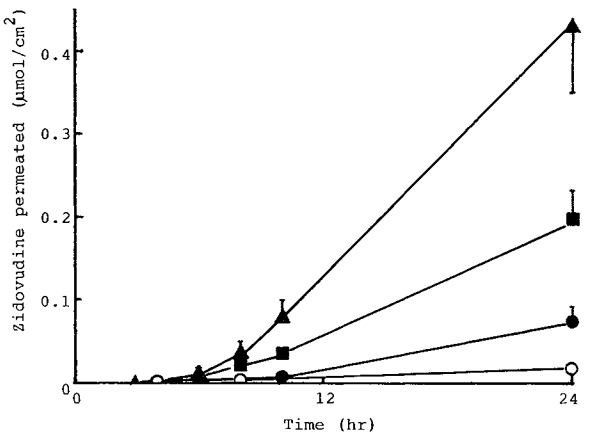


Fig. 4. Permeability of Zidovudine (I; water, ○; IPM, ●), its acetate (II; IPM, ▲), and hexanoate (IV; IPM, ■) through human skin. Data are the means ± SE (n = 3).

the fluxes, which were obtained from the amount of the compound permeating during the period of 10–24 hr. This discrepancy suggests that the driving force or activity of IV in IPM can be lower than estimated at such a high concentration (>200 m*M*). In the case of Zidovudine itself, IPM was also a better vehicle than water. Although the hexanoate (IV) was recovered only as Zidovudine, the acetate (II) was present both as intact ester and as Zidovudine in the receptor phase. Further, the ester (II)/Zidovudine ratio was different between the skin samples: 0.3 in the 50-year skin and 3.8 in the 78-year skin. This difference in ratio suggests that the enzyme activities in human skin differ among individuals. Both the susceptibility of the prodrugs and the enzyme activity in human skin may play a role in controlling drug permeation.

In conclusion, the acetate or hexanoate of Zidovudine has been shown to function as a prodrug capable of increasing the delivery of Zidovudine from the apolar vehicle, IPM. Further studies of the Zidovudine esters as a transdermal therapeutic system for AIDS patients are warranted.

Table II. Fluxes of Delivery of Zidovudine and Its Esters Through Rat Skin from Isopropylmyristate (IPM) and Water

	Maximum flux (nmol/cm²-hr)			
	Water		IPM	
Compound	9.5–10.5 hr	23.5–24.5 hr	9.5–10.5 hr	23.5–24.5 hr
I	8.1 ± 6.6	18.0 ± 8.2	23.6 ± 2.3	27.0 ± 2.9
II	18.1 ± 1.9	38.3 ± 10.2	141 ± 12[a]	168 ± 8[a]
			(51.3 ± 4.6)[b]	(58.5 ± 3.0)[b]
III	6.6 ± 0.9	13.6 ± 2.3	109 ± 4[a]	122 ± 11[a]
			(37.4 ± 1.2)[b]	(40.0 ± 3.6)[b]
IV	14.4 ± 1.0	17.4 ± 6.2	—	—
			(9.4 ± 1.8)[b]	(12.5 ± 2.9)[b]
V	4.7 ± 0.4	2.0 ± 0.9	—	—
			(3.6 ± 0.5)[b]	(5.9 ± 1.9)[b]
VI	2.2 ± 0.3	0.4 ± 0.1	—	—
			(1.7 ± 0.4)[b]	(2.0 ± 0.4)[b]

[a] Maximum flux calculated by multiplication of permeability coefficient and solubility.
[b] Observed values at actual condition. Data are the means ± SD (n = 3).

ACKNOWLEDGMENTS

We are grateful to Drs. Yasunori Morimoto and Kenji Sugibayashi for helpful discussions.

REFERENCES

1. H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, O. Bolognesi, D. W. Barry, and S. Broder. *Proc. Natl. Acad. Sci. USA* 82:7096–7100 (1985).
2. N. Mir and C. Costello. *Lancet* 2:1195–1196 (1988).
3. E. Dournon, W. Rozenbaum, C. Michon, C. Pettonne, P. De Truchis, E. Bouvet, M. Levacher, S. Matheron, S. Gharakhanian, P. M. Girard, D. Salmon, C. Leport, M. C. Dazza, and B. Regnier. *Lancet* 2:1297–1302 (1988).
4. R. W. Klecker, J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers. *Clin. Pharmacol. Ther.* 41:407–412 (1987).
5. T. Kawaguchi, T. Seki, and K. Juni. *J. Pharm. Sci.* 79 (in press).
6. K. Sugibayashi, K. Hosoya, and Y. Morimoto. *J. Pharm. Pharmacol.* 37:578–580 (1984).
7. K. Sato, K. Sugibayashi, and Y. Morimoto. *Int. J. Pharm.* 43:31–40 (1988).
8. T. Seki, K. Sugibayashi, K. Juni, and Y. Morimoto. *Drug Design Deliv.* 4:69–75 (1989).
9. R. P. Waranis and K. B. Sloan. *J. Pharm. Sci.* 76:587–595 (1987).
10. A. H. Ghanem, H. Mahmoud, W. I. Higuchi, U. D. Rohr, S. Borsadia, P. Liu, J. L. Fox, and W. R. Good. *J. Control. Release* 6:75–83 (1987).
11. K. Sato, T. Oda, K. Sugibayashi, and Y. Morimoto. *Chem. Pharm. Bull.* 36:2232–2238 (1988).
12. N. V. Sheth, D. J. Freeman, W. I. Higuchi, and S. L. Spruance. *Int. J. Pharm.* 28:201–209 (1986).
13. T. Seki, K. Sugibayashi, and Y. Morimoto. *Chem. Pharm. Bull.* 35:3054–3057 (1987).