

Comparative Pharmacokinetics of Two Prodrugs of Zidovudine in Rabbits: Enhanced Levels of Zidovudine in Brain Tissue

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The pharmacokinetics of two prodrugs of zidovudine (AZT), 1,4-dihydro-1-methyl-3-[(pyridylcarbonyl)oxy] ester and isoleucinyl ester (DPAZT and IAZT, respectively), were investigated in a rabbit model to determine their potential utility as drugs against human immunodeficiency virus. Drugs were administered by intravenous infusion over 5 min at doses equal to 10 mg of AZT per kg of body weight. The levels of the prodrugs and of released AZT in plasma, cerebrospinal fluid (CSF), and brain were determined by high-performance liquid chromatography analysis. DPAZT disappeared rapidly from plasma, whereas IAZT maintained a sustained level in plasma for up to 4 h. The levels in plasma of AZT released from DPAZT were consistently lower than the levels of AZT released from IAZT or AZT itself. At 75 min after infusion of AZT, DPAZT, and IAZT, the CSF/plasma AZT ratios were 0.23, 0.30, and 0.25, while the brain/CSF AZT ratios were 0.32, 0.63, and 0.64, respectively. These results indicate that the administration of each of the prodrugs produced a higher concentration of AZT in the brain than did the direct administration of AZT. Both prodrugs therefore may be superior to AZT itself with respect to achieving anti-human immunodeficiency virus concentrations within the central nervous system.

Despite long-term treatment of AIDS patients with zidovudine (AZT), eradication of human immunodeficiency virus (HIV) from the central nervous system (CNS) remains a problem. AIDS dementia complex, a CNS complication, has been observed in up to 40% of AIDS patients and is associated with direct brain infection by HIV type 1 (HIV-1) (6, 14). The presence of HIV-1 in the cerebrospinal fluid (CSF) (8) and brain tissue (19) of AIDS patients has been confirmed. It is not obvious how the virus penetrates the blood-brain barrier (BBB), but it has been suggested that infected macrophages or monocytes may be one of the sources (10). It has been speculated that gp120 of HIV may interfere with an endogenous neurotropic substance, thereby resulting in neurological disorders (5). The brain may serve as a sanctuary for HIV-1-infected macrophages and may be a source of continuous reinfection of peripheral tissues, possibilities indicating that anti-HIV-1 drugs must penetrate the BBB to achieve effective therapy.

AZT has been shown to penetrate into the CNS, thereby producing levels in CSF that are approximately 35 to 70% of the levels in plasma at 2 to 4 h after intravenous administration (9). This observation suggested that higher-than-therapeutic doses of AZT should be given to result in adequate antiviral concentrations in the CSF, although higher doses may exacerbate AZT-induced bone marrow toxicity (15). It has been reported that, although AZT penetrates CSF through the blood-CSF barrier at the choroid plexus, its transport into the brain is restricted (17). This report supports the basic idea that CSF and brain interstitial fluid are not in equilibrium. In contrast, in a rat model, AZT has been shown to cross the BBB with a brain uptake index of 5.4, compared with the diffusible standard ¹⁴C-antipyrine (3). In that study, the brain uptake index of AZT was enhanced by pretreatment with 0.6 or 1.0 unit of insulin per rat, with

lower doses of insulin yielding insignificant effects (3). The potential benefit of diffusion modulators may be limited because of adverse effects associated with the modulators themselves.

A systematic effort has been made in our laboratory to synthesize a series of prodrugs of AZT in an attempt to overcome its limited penetration of the BBB (1). Two prodrugs of AZT, 1,4-dihydro-1-methyl-3-[(pyridylcarbonyl)oxy] ester (DPAZT) and isoleucinyl ester of the 5'-hydroxyl group of AZT (IAZT), were found to be the most effective anti-HIV-1 agents *in vitro* (1). Earlier results from our laboratory demonstrated that DPAZT has a higher therapeutic index than AZT *in vitro* as an anti-HIV-1 agent (7). Preliminary results from other laboratories showed that DPAZT was present in its oxidized form in rat brain tissue at 1 to 3 µg/g following the intravenous injection of DPAZT at a dose of 20 mg/kg of body weight (18), a result indicating the trapping of DPAZT in the brain. The 1,4-dihydropyridine derivative of 2',3'-dideoxy-2',3'-dideoxythymidine also has been reported to penetrate the BBB in mice and to achieve higher levels in its oxidized form in brain tissue (13). Other reports demonstrated that DPAZT resulted in a threefold-higher concentration of AZT in rat brain and a twofold-higher concentration in the CSF of dogs, compared with those seen following the administration of AZT itself (11). Another study reported that the relative concentration of AZT in the brain after the administration of DPAZT in a mouse model was approximately ninefold higher than that after the administration of AZT itself (4). These reports thus indicate that DPAZT can produce variable concentrations of AZT in brain tissue, based on the particular animal model used.

In the present investigation, we used a rabbit model to estimate the areas under the concentration-time curve (AUCs) of AZT and AZT derived from the prodrugs DPAZT and IAZT as well as to differentiate the tissue AZT concentrations in CSF and brain compartments.

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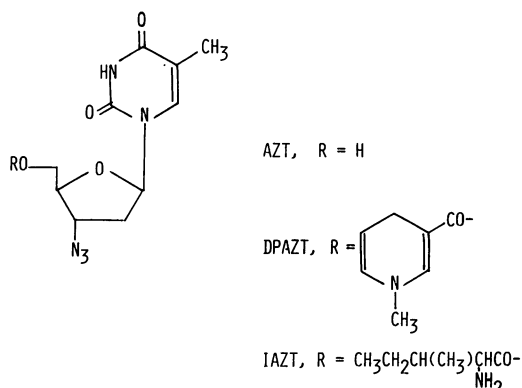


FIG. 1. Structures of AZT, DPAZT, and IAZT.

(This model and some of these data have been reported from our laboratory in a preliminary form [12].)

MATERIALS AND METHODS

Drugs and chemicals. AZT, DPAZT, and IAZT (structures shown in Fig. 1) were synthesized in our laboratory by previously described procedures (1). Acetonitrile (high-pressure liquid chromatography [HPLC] grade) was purchased from Aldrich Chemicals, Milwaukee, Wis. All other chemicals were reagent grade and were obtained from commercial sources. The drug solutions for intravenous infusion were made in dimethyl acetamide and were diluted with saline to make a final solvent concentration of 50%.

Animals. Male New Zealand White albino rabbits (Jackson Laboratory, Bar Harbor, Maine) weighing 2 to 3 kg were used. The rabbits were housed in cages under temperature-controlled conditions. Rabbit chow and water were available ad libitum. Food was removed from the cages the night before the experiment.

In vivo procedures. The animals were anesthetized with sodium pentobarbital. A catheter was placed in the right femoral vein and connected to a Sage infusion pump. The drugs were administered intravenously through this pump over a period of 5 min. Heparinized blood samples (1 ml) were taken from the left femoral vein at 5, 10, 15, 30, 60, and 120 min. CSF samples (50 to 100 μl , free of blood) were obtained by cisternal puncture at the same times. Whole-brain samples were obtained from separate groups of animals by a midline craniotomy at 30 min and at 75 min (at which time a pseudodistributional equilibrium was reached) for each treatment group.

Extraction and sample preparation. One milliliter of whole blood per plasma sample was centrifuged. For preparation of the specimens, 0.2 ml of plasma was mixed with 0.8 ml of methanol and the mixture was then centrifuged. For analysis of CSF, 50 to 100 μl of each sample was mixed with an equal volume of methanol and the mixture was then centrifuged. The resulting supernatant fractions for either plasma or CSF were then filtered through nylon-66 filters (pore size, 0.45 μm ; 13-mm diameter) prior to HPLC analysis. For analysis of whole brain, samples were perfused with saline to remove blood. The tissues were then blotted dry, weighed, minced, and homogenized in 30 ml of methanol by use of five strokes over 15 s with a motor-driven Teflon pestle. The homogenates were centrifuged, and the supernatants were filtered as indicated for plasma samples in preparation for HPLC analysis.

Analytical procedures. All samples were analyzed by HPLC with a reverse-phase Econosphere C18 column (150 by 4.6 mm; particle size, 5 μm) for DPAZT and an Absorbosphere C18 column (250 by 4.6 mm; 5 μm) for AZT and IAZT. The mobile phases and retention times were as follows: for AZT, 15% acetonitrile in 25 mM ammonium phosphate buffer (pH 7.4) and a peak retention time of 9.2 min at a flow rate of 1 ml/min; for DPAZT, 60% methanol and a peak retention time of 5.2 min at a flow rate of 1 ml/min; and for IAZT, 70% methanol and a peak retention time of 8.6 min at a flow rate of 1 ml/min. Absorbances for all compounds were measured at 267 nm with a Beckman UV detector (model 340). The lower limit of detection under these conditions for AZT and the prodrugs was approximately 100 ng/ml.

In vitro hydrolysis studies. Ten microliters of a solution of IAZT or DPAZT (10 mg/ml in dimethyl sulfoxide) was added to 990 μl of rabbit plasma or a solution of rabbit hepatic microsomes (prepared as described below; 15.5 mg of protein per ml). The mixture was incubated at 37°C in a water bath. At various times (0 to 4 h), 100- μl samples were withdrawn and added immediately to ice-cold methanol (400 μl). The samples were mixed and centrifuged. The supernatant fractions were filtered through nylon-66 filters (pore size, 0.45 μm) and analyzed by HPLC as described above.

Hepatic microsomes were isolated by a published procedure (16). The rabbits were fasted for 24 h (with water ad libitum) and then sacrificed by ethyl ether inhalation. The livers were excised and perfused with 0.9% saline to remove blood and until a pale perfusate appeared. The tissues were minced, homogenized in 0.25 M sucrose in 10 mM Tris-HCl buffer at pH 7.4, and centrifuged at 1,200 $\times g$ for 10 min. The pellets were discarded, and solid calcium chloride was added to the supernatants to reach a final concentration of 8 mM. The solutions were stirred and centrifuged at 25,000 $\times g$ for 15 min. The resulting pellets, consisting of microsomes, were resuspended in 150 mM KCl in 10 mM Tris-HCl buffer at pH 7.4 and centrifuged for an additional 15 min at 25,000 $\times g$, and the supernatants were discarded. All steps were carried out at 4°C. The washed microsomal fractions were resuspended in 10 mM Tris-HCl buffer and stored at -70°C. The protein concentration in the microsomes was determined by use of Bio-Rad reagent methodology. Bovine gamma globulin was used as the standard for the protein determination.

Pharmacokinetics and statistical analysis. The plasma drug concentration degradation curves for AZT, IAZT, and AZT released from IAZT were found to fit the exponential functions of a two-compartment mammillary pharmacokinetic model having constant intravenous input into and first-order output from the central compartment. The equation defining the plasma drug level-time plot for the drugs is $C_t = A(e^{-at} - e^{-at'}) + B(e^{-bt} - e^{-bt'})$, where C_t is concentration at time t , A is the distribution phase intercept, a is the distribution phase slope, B is the elimination phase intercept, b is the elimination phase slope, e is the natural logarithm base, t is time after infusion, and t' is the duration of infusion. A computer program (PCNONLIN, version 3.0; Statistical Consultants, Inc., Lexington, Ky.) was used to determine AUCs for the model. The adequacy of model fit was judged, in all cases, by noting (i) residual sums of squared deviations, (ii) degree of correlation between observed and predicted plasma drug levels resulting from the model, and (iii) the condition number generated by the computer program.

Statistical analysis of the data was conducted by use of STATA (The Computing Resource Center, Los Angeles,

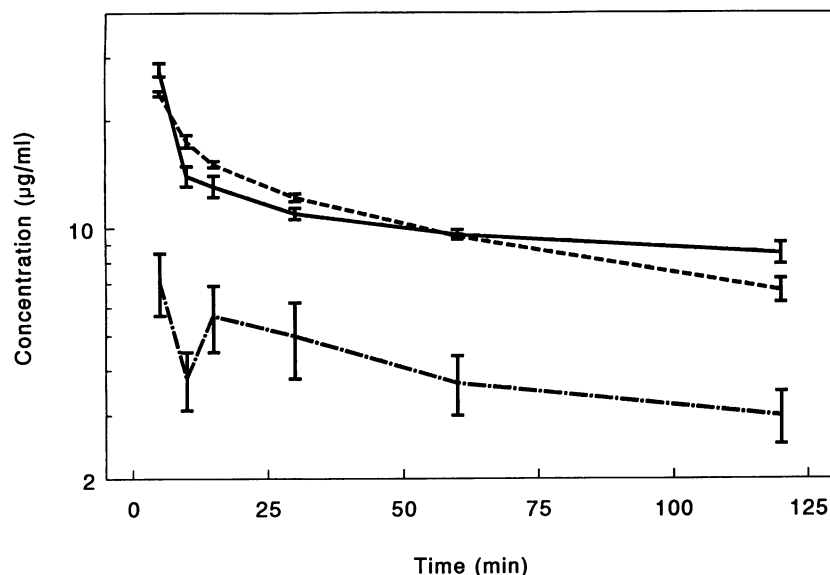


FIG. 2. Concentration-time curves for AZT in the plasma of rabbits after an intravenous infusion of AZT (10 mg/kg; —●—), DPAZT (14 mg/kg; - - -■-), or IAZT (18 mg/kg; —▲—). Each point is the mean \pm standard error for values obtained from at least six animals.

Calif.). The plasma, brain, and CSF drug concentrations and the CSF/plasma, brain/plasma, and brain/CSF drug ratios for AZT and its two prodrugs were compared by use of a one-way analysis of variance followed by a Bonferroni multiple-comparison test of mean values. Significant differences in means were defined at the level of $P = 0.05$.

RESULTS

Kinetics in plasma. The plasma drug concentration-time curves for AZT, AZT released from IAZT, and AZT released from DPAZT are shown in Fig. 2 ($n = 6$ for each group). A significant fraction of the prodrug IAZT was rapidly hydrolyzed, as demonstrated by the presence of 25 to 30 μg of AZT per ml in plasma at the end of the 5-min infusion. With our experimental estimate for the mean central volume of distribution in the rabbit (0.34 liters/kg), the mean level in plasma of AZT from IAZT at 5 min (27.8 $\mu\text{g}/\text{ml}$), the mean animal weight of 2.5 kg, and the mean amount of AZT not yet hydrolyzed from IAZT at 5 min (2.05 $\mu\text{g}/\text{ml}$), the *in vivo* hydrolysis of IAZT at 5 min was estimated to be approximately 90%. However, hydrolysis in plasma of IAZT *in vitro* was 22.2% at 5 min (Table 1). The plasma drug concentration-time curves for AZT from the prodrug IAZT followed a pattern similar to the one obtained

after the administration of AZT, but the levels of AZT from IAZT tended to be higher at 120 min. This result suggests a "sustained release" of AZT from the prodrug at 120 min. Even though IAZT was rapidly hydrolyzed, it was still detected in plasma at 120 min, although at lower concentrations (the amount of AZT not yet hydrolyzed from IAZT *in vivo* at 120 min is approximately 0.61 $\mu\text{g}/\text{ml}$). The pharmacokinetic behavior of AZT and AZT from IAZT allowed both to be fit to a two-compartment mammillary model having constant input into and first-order output from the central compartment. The AUC estimated for AZT (mean, 2,214 $\mu\text{g} \cdot \text{min}/\text{ml}$; standard deviation [SD], 254 $\mu\text{g} \cdot \text{min}/\text{ml}$) was comparatively smaller than the AUC estimated for AZT from IAZT (mean, 3,374 $\mu\text{g} \cdot \text{min}/\text{ml}$; SD, 221 $\mu\text{g} \cdot \text{min}/\text{ml}$). However, these estimates are based on extrapolation to infinity from data sampling through 120 min. It is most probable that estimates of AUC to infinity for AZT would be the same if data points covering five to six half-lives were supplied for PCNONLIN analysis, given the fact that equimolar doses of AZT and the prodrugs were administered.

The comparative profiles for AZT and AZT released from the prodrug DPAZT in plasma (Fig. 2) indicated that plasma AZT concentrations were higher at all time points after the administration of AZT than after the administration of DPAZT. The mean AUC for AZT for DPAZT was $974 \pm 226 \mu\text{g} \cdot \text{min}/\text{ml}$. However, the plasma drug concentration-time curve for the prodrug DPAZT (Fig. 3) was significantly different from that for the prodrug IAZT, since DPAZT was found to disappear rapidly from plasma and could not be detected beyond 15 min. IAZT, on the other hand, was present in plasma even at 2 h. The prodrug DPAZT could not be fitted to a single-exponential-function model because of the apparent nonlinearity of its plasma drug concentration-time profile.

A sharp decrease at 10 min in the level of AZT derived from DPAZT (Fig. 2) was noted in all experiments. This decrease was followed by a sharp increase in the level at 15 min. On the basis of the *in vitro* plasma drug hydrolysis experiments (with only 5% of DPAZT being hydrolyzed at 15

TABLE 1. *In vitro* hydrolysis of AZT prodrugs in rabbit plasma and hepatic microsomes

Time (min)	% Hydrolysis of the indicated prodrug in:			
	Plasma		Hepatic microsomes	
	IAZT	DPAZT	IAZT	DPAZT
5	22.2	0.0		40.8
15	33.3	5.0	38.7	56.0
30	44.4	12.0	85.7	77.0
60	66.6	35.0	100.0	100.0
120	88.8	61.0		
240	100.0	100.0		

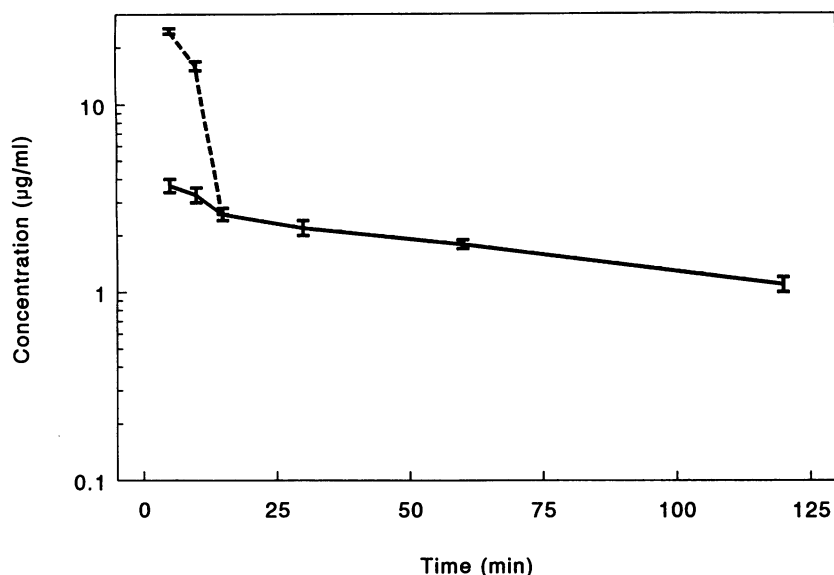


FIG. 3. Concentration-time curves for the prodrugs of AZT in the plasma of rabbits after an intravenous infusion of DPAZT (14 mg/kg; ---) or IAZT (18 mg/kg; —). Each point is the mean \pm standard error for values obtained from at least six animals.

min), this characteristic pattern of quick changes in the level of AZT in plasma may be due to the rapid distribution of the prodrug DPAZT from blood before the hydrolysis of AZT occurs. The redistribution of free AZT back into blood from the peripheral tissues receiving DPAZT may be responsible for the characteristic sharp increase in the level of AZT in plasma at 15 min.

Distribution in CSF and brain. The time courses of the distribution of AZT in CSF after the administration of AZT and the prodrugs IAZT and DPAZT ($n = 6$ for each drug) are shown in Fig. 4. AZT appearing in CSF following an intravenous injection of AZT itself reached a peak concentration of 4.8 (SD = 0.6) $\mu\text{g/ml}$ at 30 min. AZT released from the prodrug IAZT exhibited a mean peak concentration in

CSF of 4.1 (SD = 0.6) $\mu\text{g/ml}$ at 15 min. AZT released from the prodrug DPAZT exhibited a relatively constant mean concentration in CSF of 1.5 to 0.9 $\mu\text{g/ml}$ during the time period from 5 to 120 min following intravenous administration.

The prodrug IAZT could not be detected in CSF at the earliest times, 5 and 15 min, following intravenous infusion. The prodrug DPAZT diffused rapidly into CSF and, at 5 min following intravenous infusion, achieved a concentration of 32.6 (SD = 0.9) $\mu\text{g/ml}$. However, the concentration of DPAZT in CSF declined rapidly to 6.5 (SD = 0.3) $\mu\text{g/ml}$ at 15 min, similar to its rapid disappearance from plasma. The high level of DPAZT in CSF at 5 min supports the hypothesis derived from the level in plasma that DPAZT is distributed very rapidly from blood in an intact prodrug form.

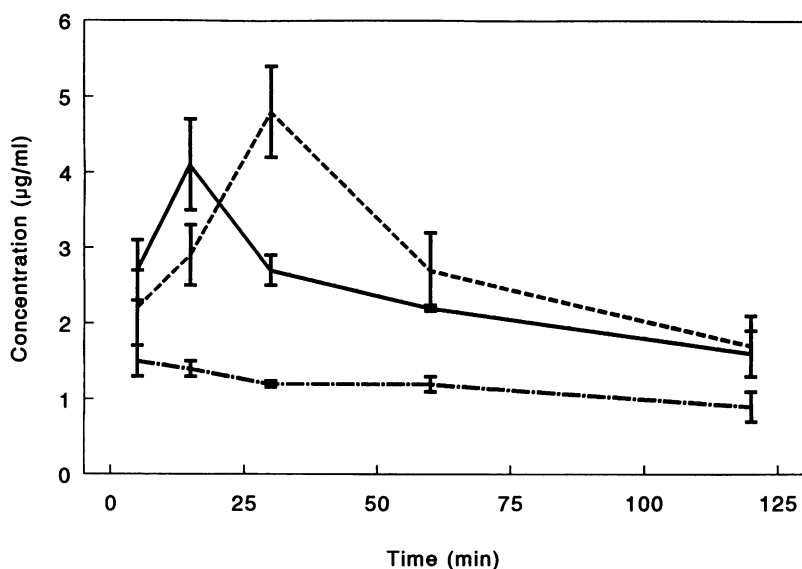


FIG. 4. Concentration-time curves for AZT in the CSF of rabbits after an intravenous infusion of AZT (10 mg/kg; ---), DPAZT (14 mg/kg; - · - · -), or IAZT (18 mg/kg; —). Each point is the mean \pm standard error for values obtained from at least six animals.

TABLE 2. Concentrations of AZT in plasma, CSF, and brain

Drug	Concn ^a at the indicated time (min) in:					
	Plasma		CSF		Brain	
	30	75	30	75	30	75
AZT	12.2 ± 0.2	8.4 ± 1.9	4.7 ± 0.6	1.9 ± 0.6	7.1 ± 1.0	0.6 ± 0.2
DPAZT	4.7 ± 0.2 ^b	4.6 ± 0.5 ^c	1.6 ± 0.4 ^b	1.4 ± 0.1	4.9 ± 1.3	0.9 ± 0.2
IAZT	11.8 ± 1.1	5.6 ± 1.4	2.2 ± 0.5 ^b	1.3 ± 0.2	6.1 ± 1.6	0.8 ± 0.2

^a Expressed in micrograms per milliliter for plasma and CSF and micrograms per gram for brain tissue ($n = 3$). Values are means ± SDs.

^b Statistically significant difference ($P < 0.01$) compared with AZT.

^c Statistically significant difference ($P < 0.05$) compared with AZT.

For determination of the levels of AZT achieved in brain tissue after the administration of either AZT itself or the prodrugs, a separate set of animals ($n = 3$) was used at each time because the animals were sacrificed to obtain AZT levels in brain tissue. These experiments were conducted at 30 and 75 min following the intravenous infusion of each drug. The 30-min time point was chosen since the concentration of AZT in CSF peaked at 30 min after the administration of the parent drug AZT. The second time point of 75 min represented the time at which the pseudodistributional equilibrium occurred. Along with the determination of concentrations of AZT in brain, the concentrations in plasma and CSF were determined simultaneously in these animals. The data are shown in Table 2. The plasma and CSF AZT concentrations at the 30-min time point in Table 2 compare favorably with the concentrations obtained for at least six animals per datum point in Fig. 2 and 4. The concentration of AZT in brain at the 30-min time point followed the pattern observed for plasma and CSF, achieving levels of 7.1 µg/g after AZT administration and 4.9 and 6.1 µg/g after administration of the prodrugs DPAZT and IAZT, respectively.

Similarly, the mean plasma AZT concentration at 75 min was higher after the administration of AZT itself (8.4 µg/ml) than after IAZT (5.6 µg/ml) or DPAZT (4.6 µg/ml) administration. A similar pattern was observed with free AZT in CSF. However, there was no significant difference in the concentrations of AZT in brain tissue among the three groups at 75 min, even though the CSF AZT levels after the administration of AZT itself exceeded significantly the levels in CSF of AZT derived from both of the prodrugs ($P < 0.05$).

The CSF/plasma, brain/plasma, and brain/CSF drug ratios of the AZT concentrations in each compartment obtained after the administration of either AZT or the prodrugs are shown in Table 3. The highest CSF/plasma drug ratio of 0.38 was observed with AZT itself at 30 min, whereas ratios of 0.35 and 0.19 were obtained after the administration of DPAZT and IAZT, respectively. However, as shown in Fig.

4, the peak AZT level in CSF after IAZT administration was observed at 15 min, and at this time, the CSF/plasma drug ratio was 0.30, in the same range as that observed after AZT or DPAZT administration. The data in Table 3 further demonstrate that the mean brain/CSF drug ratios and brain/plasma drug ratios for AZT released from the prodrugs at 75 min were consistently higher (approximately twofold) than the ratios for AZT itself. For example, the mean brain/CSF drug ratios for AZT from DPAZT of 0.63 and for AZT from IAZT of 0.64 were approximately twice the ratio of 0.32 for AZT itself.

In vitro hydrolysis. The time course of in vitro hydrolysis of the AZT prodrugs by plasma esterases and hepatic microsomes is shown in Table 1. Some of these data were discussed above in the context of explaining the rapid distribution of DPAZT from plasma to CSF. It was found that both prodrugs were completely hydrolyzed in plasma by 240 min. Hepatic microsomes required only 60 min to achieve complete hydrolysis. As previously described, DPAZT was relatively more stable against plasma esterases than IAZT.

DISCUSSION

These results provide a further comparative characterization of two prodrugs of AZT, namely, DPAZT and IAZT, developed in our laboratory (1, 7) and demonstrate the greater brain tissue penetration for DPAZT and IAZT, on the basis of observed levels of AZT derived from the prodrugs. AZT penetrates the CSF, and previous reports indicated a CSF/plasma drug ratio of 0.35 to 0.70 at the steady state, 2 to 4 h after the intravenous administration of AZT to patients (9). Our results with rabbits indicated a similar peak CSF/plasma drug ratio of 0.38 at 30 min, but this ratio declined to 0.23 at 75 min after the intravenous infusion of AZT. The corresponding brain/plasma drug ratios for AZT in our experiments were 0.58 and 0.07 at 30 and 75 min,

TABLE 3. Ratios of concentrations of AZT in plasma, CSF, and brain

Drug	Ratio ^a at the indicated time (min):					
	CSF/plasma		Brain/plasma		Brain/CSF	
	30	75	30	75	30	75
AZT	0.38 ± 0.05	0.23 ± 0.04	0.58 ± 0.08	0.07 ± 0.02	1.53 ± 0.06	0.32 ± 0.04
DPAZT	0.35 ± 0.13	0.30 ± 0.01	1.09 ± 0.37	0.19 ± 0.02 ^b	3.30 ± 0.43	0.63 ± 0.10 ^c
IAZT	0.19 ± 0.03 ^d	0.25 ± 0.07	0.52 ± 0.09	0.15 ± 0.03 ^c	2.78 ± 0.10 ^b	0.64 ± 0.18 ^c

^a Expressed as mean ± SD ratios of concentrations of AZT in each tissue after the administration of either AZT or the prodrugs.

^b Statistically significant difference ($P < 0.01$) compared with AZT.

^c Statistically significant difference ($P < 0.05$) compared with AZT.

^d Statistically significant difference ($P = 0.07$) compared with AZT.

respectively, results suggesting efficient entry to and removal from brain compared with CSF in rabbits. In contrast, an earlier published report (17) provided evidence that although AZT penetrates the CSF through the blood-CSF barrier at the choroid plexus in rats, its transport through the BBB is restricted.

The two-compartment model provides a description of the plasma pharmacokinetics for AZT and AZT released from IAZT. The longer terminal elimination half-life for AZT released from IAZT (186 min versus 106 min) might suggest a sustained release of AZT from the prodrug, at least during the 120-min time interval that was used in this study.

The rapid disappearance of the dihydropyridine prodrug, DPAZT, from plasma may be related to its rapid distribution from plasma (within approximately 15 min) to peripheral tissues, although the metabolism of DPAZT by hepatic microsomal enzymes (Table 1) may also play a role by increasing the systemic clearance of this prodrug. The characteristic dip in the plasma AZT level noted between 5 and 15 min following the administration of DPAZT (Fig. 2) and the rapid appearance of DPAZT itself in CSF suggest that this prodrug reaches the brain despite hepatic metabolism. The relatively high brain/CSF drug ratio for AZT from DPAZT, which is comparable to that for AZT from IAZT, confirms the movement of DPAZT into the brain.

These results also suggest that AZT released from prodrugs would achieve a higher concentration in the brain at the pseudodistributional equilibrium (75 min) than AZT itself. The brain/plasma and brain/CSF drug ratios for AZT released from the prodrugs were significantly higher (approximately twofold) than those for AZT itself, while the CSF/plasma ratios drug were essentially similar. These results support the theory of a greater penetration of the prodrugs into the brain directly from plasma rather than from plasma via CSF. However, no differences in the actual levels of AZT released from the prodrugs were found in brain tissue at 75 min (the time at which the pseudodistributional equilibrium occurred), even though the levels in plasma and CSF of AZT itself exceeded the levels of AZT derived from both prodrugs. The completion of microsomal hydrolysis of DPAZT and IAZT by 4 h in rabbit plasma and liver suggests that such mechanisms offer an efficient metabolic and elimination pathway for these prodrug esters of AZT.

In conclusion, although plasma AZT levels following the administration of DPAZT or IAZT were either lower than or comparable to the levels achieved with AZT itself, the AZT levels in the brain were increased, as shown by the higher brain/plasma drug ratios. The partition coefficient ratios of 1.2 for AZT, 7.2 for DPAZT, and 10.3 for IAZT in *n*-octanol-phosphate buffer (pH 7.4) (1) suggest that prodrugs with higher partition coefficient ratios may show increased penetration of the BBB. However, in contrast to the results for the DPAZT ester, the absence of the IAZT ester in CSF indicates that the latter prodrug was hydrolyzed immediately after crossing the blood-CSF barrier. Nevertheless, the data in this report suggest that it is possible to obtain effective brain AZT concentrations for the treatment of HIV infections of the CNS with these prodrugs. Simultaneously, the lower plasma AZT concentrations, although effective for anti-HIV activity, may result in reduced toxicity in tissues such as the bone marrow. We reported earlier that DPAZT was significantly less toxic for bone marrow cells than AZT in an assay based on the formation of CFU of erythroid progenitor cells (7). It was further demonstrated that the reduced toxicity of the prodrug in this *in vitro* assay was primarily due to incomplete hydrolysis of the prodrug in

progenitor cells, whereas the prodrug was hydrolyzed completely within 4 h in peripheral blood lymphocytes. Similarly, IAZT has been shown to possess a higher therapeutic index because of the reduced toxicity of this prodrug for bone marrow cells (2). Furthermore, sustained release of AZT from IAZT should provide an added advantage over AZT itself by reducing the frequency of drug administration and increasing the peripheral tissue drug distribution.

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