Analysis of Zidovudine Distribution to Specific Regions in Rabbit Brain Using Microdialysis

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The distribution of zidovudine (3′-azido-3′-deoxythymidine; AZT) into two regions of rabbit brain was investigated in crossover using microdialysis. Six rabbits had guide cannulas surgically implanted in the lateral ventricle and thalamus by stereotaxic placement. After recovery, microdialysis probes were positioned and i.v. bolus doses of 5, 10, 20, and 30 mg/kg were administered to each animal over a period of 2 weeks. Blood was drawn via a marginal ear vein catheter for 8 hr. Brain dialysate was collected at 3 μl/min from ventricle and thalamus dialysis probes every 10 min. Simulated cerebrospinal fluid (CSF), to which 3′-azido-2′,3′-dideoxyuridine (AZdU) was added, was used as perfusate. AZdU loss, which was measured during simultaneous retrodialysis, served as a marker for *in vivo* recovery of AZT. AZT concentrations in plasma, as well as in ventricle and thalamus dialysate, were determined using a sensitive HPLC assay, and AZdU was simultaneously analyzed in the dialysates. Calculation of *in vivo* recovery of AZT was based on loss of AZdU from the perfusate during retrodialysis and was used to estimate the concentration of drug at both sites in the brain. *In vitro* loss of AZdU and recovery of AZT showed good agreement, demonstrating a bivariate regression slope of 0.99. The half-lives and AUCs (normalized to dose) achieved in the plasma, ventricle, or thalamus were not significantly different for the four doses. The AUC ratios, which represent the ratio of clearances into and from the CNS, were not significantly different among the doses studied (AUC_v/AUC_p range, 0.16–0.19; AUC_t/AUC_p range, 0.05–0.09), providing further evidence that the kinetics of distribution into the thalamus and CSF are linear. The results also demonstrate that the time-averaged concentrations of AZT in thalamus ECF are about half of those in the CSF.

KEY WORDS: zidovudine (AZT); brain and ventricular cerebrospinal fluid distribution; microdialysis; retrodialysis.

INTRODUCTION

Zidovudine (AZT) is currently the only antiviral drug approved for the treatment of acquired immunodeficiency syndrome (AIDS). During early clinical studies, it was administered as oral doses of 250 mg every 4 hr (1). Because of the high incidence of bone marrow depression (2), and since a lower effective inhibitory concentration has now been postulated, reduced daily doses were recently recommended (3).

It has been reported that more than 50% of children with AIDS suffer progressive encephalopathy (4) and about 65% of adult patients develop dementia. These clinical observations correlate well with the degree of immunological abnormality (5). Even though the precise cause of this disorder is unknown, histological studies indicate that loosely organized cellular infiltrate including macrophages, microglia, astrocytes, and scattered multinucleated cells (6–8) are present in AIDS dementia complex (ADC). These cellular infiltrates are commonly found in basal ganglia, brain stem, and subcortical white matter, with the extent of infiltration correlating with the degree of dementia (9). Although the mode of HIV entry into the CNS remains unclear, HIV infection of the brain is suggested by the presence of HIV-1 antigen within the cerebrospinal fluid (CSF) and by intra-blood–brain barrier synthesis of HIV-1-specific antibodies (8).

These findings underscore the need for effective transport of AZT into the brain in the treatment of CNS disorders in AIDS patients. It is unclear if the recently adopted reductions in dosage alter the distribution of AZT into the brain, modulating the effectiveness of AZT treatment of ADC secondary to HIV infection.

Although different methods of quantifying drug distribution into the brain have been developed, most of these techniques are invasive and require anesthesia. In addition, the variance in the results frequently includes a major interanimal component, since continuous sampling within an animal is often not possible. Recently, noninvasive methods (10,11) such as positron emission tomography (PET) and single photon emission tomography (SPECT) have been developed to study drug transport. However, these methods are relatively expensive and difficult to apply in pharmacokinetic studies where a high degree of analytical precision and sensitivity are required.

Microdialysis, one of the several research techniques currently utilized *in vivo,* can be employed to examine drug concentrations in specific brain regions with the blood–brain barrier remaining intact following implantation. The principle of this technique is to create an "artificial blood vessel" where diffusion of chemical substances will flow in the direction of the lowest concentration (12), allowing extracellular concentrations of the compound of interest to be measured. Microdialysis causes minimal damage to brain tissue and can be performed in conscious animals. The advantages of this technique have been reviewed by several authors (12–14) and validated by comparison with *in vivo* electrochemical methods and tissue assay (15). The technique has recently been employed to study acetaminophen pharmacokinetics in the rat (16).

In addition to the relative noninvasive nature of microdialysis, it can be used in crossover studies performed in the same animal. This clearly cannot be achieved using other methods which require sacrifice of the test animal in order to sample the brain.

The aim of this research was to investigate the distribution characteristics of AZT across the blood–brain barrier (BBB) and the blood–CSF barrier in the conscious rabbit by microdialysis. In addition, the linearity of this distribution into the CNS and of the elimination kinetics of this agent was examined using a range of doses which produces plasma levels of zidovudine comparable to those observed clinically. In these studies, retrodialysis (17,18) was employed to

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determine the *in vivo* recovery of AZT during brain microdialysis. This approach utilizes the addition of a marker, with dialysis clearance similar to that of AZT, to the dialysate in order to determine the recovery of AZT during the microdialysis procedure.

MATERIALS AND METHODS

Chemicals

Zidovudine (3′-azido-3′-deoxythymidine; AZT) was donated by Burroughs Wellcome, Research Triangle Park, NC. 3′-Azido-2′,3′-dideoxyuridine (AZdU) was a gift from Triton Biosciences Inc., Alameda, CA. The following chemicals were purchased and used as received: β-hydroxypropyl theophylline (BHPT) from Sigma Chemical, St. Louis, MO; acetonitrile, chloroform, and isopropyl alcohol from Burdick and Jackson Labs, Muskegon, MI; ammonium phosphate monobasic and acetic acid from Mallinckrodt, St. Louis, MO; and methanol from EM Science, Gibbstown, NJ. Solvents were HPLC grade, and all other chemicals were AR grade.

Animal Surgery

Male New Zealand White rabbits (Birchwood Farm Rabbitry, Grantsburg, WI) weighing 3–3.5 kg were allowed to adapt to the animal housing environment for at least 1 week before surgery. On the day of surgery, the animal was weighed and a dose of potassium penicillin G (Marsam Pharmaceuticals Inc., Cherry Hill, NJ) equivalent to 0.30 million units (USP) was injected intramuscularly (i.m.) into the thigh of the rabbit. In the same thigh, 0.4 mg of atropine sulfate (Vedco, Inc., St. Joseph, MO) was also given i.m. Fifteen minutes later, 1 mg/kg of acepromazine maleate (Aveco Co., Inc., Fort Dodge, IA) and 45 mg/kg of ketamine (Fort Dodge Lab., Fort Dodge, IA) were given separately in the contralateral thigh, i.m. When the rabbit was anesthetized, the surgical area was shaved, and further hair removed with a depilatory (Nair; Carter Products, New York). The rabbit was then immediately placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) equipped with a rabbit adaptor and securely positioned. Iodophore (Davis and Geck, American Cynamid Company, Danbury, CT) was applied to the area. The animal was draped with sterile towels, with only the surgical area exposed. A midline incision was made parallel to the sagittal suture, starting 1 cm anterior and ending 2 cm posterior to the coronal suture. Sterilized gauze was used to control bleeding. Tissue covering the skull was gently averted, and the exposed skull was cleansed with 1% hydrogen peroxide. The position of the bregma was located and used as the reference point for fixing the ventricle and thalamus sites. Using a dental drill, two holes (2-mm diameter) were drilled in the frontal and parietal bone. Dialysis guide cannulas were lowered using an electrode manipulator (David Kopf Instruments, Tujunga, CA) into the right lateral ventricle (coordinates: anterior, 1.0 mm; lateral, 2.5 mm; and ventral, 7.0 mm) and thalamus (coordinates: posterior, 4.0 mm; lateral, 4.0 mm; and ventral, 10.0 mm) according to a rabbit stereotaxic atlas (19). Additional holes were drilled on the skull for placement of three stainless-steel screws (size, 1–72; length, ⅛ in.). The guide cannulas and the supporting screws were then anchored to the skull by dental cement (L. D. Caulk Company, Milford, DE). When implantation of the cannulas was completed, the incised tissue was sutured to cover the cement, leaving only the cannulas exposed. The entire surgical procedure was done aseptically. The animal was then allowed to recover for at least a week before the microdialysis experiment was performed.

Study Design

The animals ($n = 6$) were divided into two groups (A and B) and studied in crossover fashion, with four dose treatments for each animal. Group A animals ($n = 3$) received an intravenous bolus of AZT (5 mg/kg) on the first day, followed by a dose of 20 mg/kg on the second day. Group B animals ($n = 3$) received an intravenous bolus of AZT 10 mg/kg on the first day and a dose of 30 mg/kg on the second day. One week later, Group A and Group B animals received the alternate doses in a similar order.

Dialysate Preparation and Drug Administration Procedure

At least 1 week after surgery, the rabbit was positioned in a recovery cage (Harvard Apparatus Inc., South Natick, MA). One marginal ear vein was cannulated and the catheter (I-Cath Delmed, New Brunswick, NJ) was positioned without anesthesia in the anterior vena cava.

On the day of the experiment, simulated cerebrospinal fluid (1.1 m*M* Mg^{2+}, 1.35 m*M* Ca^{2+}, 3.0 m*M* K^+, 144.48 m*M* Na^+, 20 m*M* HCO_3^-, 0.242 m*M* HPO_4^{2-}, 131.9 m*M* Cl^-, pH 7.6) was prepared (20) and filtered (PC membrane, 0.4 μm; Nuclepore, Pleasanton, CA) immediately. Dummy cannulas from both the ventricle and the thalamus guide cannulas were replaced by 3-mm CMA/10 probes (Bioanalytical Systems, West Lafayette, IN). Simulated CSF was then perfused (Gilson Minipuls 3; Gilson Medical Electronics, Inc., Middleton, WI) at a flow rate of 3 μl/min for at least 1 hr before drug administration.

The AZT dosing solution was prepared in normal saline at room temperature just prior to intravenous bolus dosing. After administration of drug, the catheter was flushed with 2–3 ml of heparinized normal saline and subsequently used for blood sampling. Throughout the experimental period, the animal was conscious but restrained.

Microdialysis Procedure and Probe Calibration

An AZdU solution was prepared in 10 ml of simulated cerebrospinal fluid (CSF) solution to produce a concentration of 0.1 μg/ml at room temperature. This solution was utilized for perfusion of the dialysis probes using the Minipuls 3 peristaltic pump at a flow rate of 3 μl/min. The pump was coupled via PE10 tubing to the probes inserted into the ventricle and thalamus positions through the guide cannulas.

In vivo probe recovery of AZT was calculated as the loss of AZdU into ventricular and thalamus probes by retrodialysis during the microdialysis procedure, as described below in Eq. (2). Probe recovery *in vitro* was determined at the end each experiment. After the dialysis probes were carefully removed from the brain region through the guide cannulas, they were immersed in a beaker containing AZT in

simulated CSF (1.0 μg/ml). *In vitro* dialysate samples were collected as described below.

Microdialysate and Blood Sampling

The effluent from the dialysis probes, *in vivo* and *in vitro*, was collected via low-dead volume tubing into glass HPLC injection vials spiked with a standard (2.5 ng BHPT per vial) every 10 min through a Gilson fraction collector (Model FC 203; Gilson Medical Electronics, Inc.).

Blood samples (0.5 ml) were drawn into heparinized tubes at predetermined times and immediately centrifuged to obtain plasma. Samples were then stored at −20°C until analysis. Total blood loss over a 2-day experiment represented less than 8% of the blood volume.

Analysis of Plasma and Dialysate

AZT concentrations in plasma were quantitated according to an HPLC method reported previously (21). Briefly, the method involves a liquid–liquid extraction and requires only 100 μl of plasma. The reconstituted samples were injected into the HPLC (Hewlett-Packard 1081B, Palo Alto, CA), where separation was achieved using a flow rate of 1.5 ml/min on a Supelcosil (Supelco, Bellefonte, PA) ODS column (15 × 0.46 cm, 5 μm) fitted with a 2-cm Supelguard LC-18 precolumn. Column effluent was monitored at 266 nm using a variable-wavelength UV detector (Model SPD-6A; Shimadzu, Kyoto, Japan). Assay precision between days was characterized by a coefficient of variation ranging from 1.4 to 14% for the plasma standard curves (0.05 to 50.0 μg/ml) using 100 μl of sample.

Analysis of AZT concentrations in dialysates was performed on the same day of the dialysis experiment. Standard curves were prepared by spiking appropriate amounts of AZT in methanol stock solution into glass injection vials containing BHPT. Solvent was evaporated at 55°C and the residue reconstituted with 30 μl of simulated CSF to obtain concentrations ranging from 5 to 1000 ng/ml. Standards and samples were vortex-mixed and analyzed by HPLC (Model 1090; Hewlett-Packard). The method described above was modified to employ a microbore hypersil ODS column (15 × 0.46 cm, 5 μm; Hewlett-Packard) to increase sensitivity. The mobile phase, 10.5% acetonitrile and 89.5% 10 m*M* monobasic ammonium phosphate, by volume, was mixed on line and delivered at 0.2 ml/min. This modification allowed quantitation as low as 5 ng/ml with a 5-μl injection. The day-to-day coefficient of variation was 4.8 to 20% for dialysate concentrations ranging from 5 to 1000 ng/ml when 5 μl of sample was injected.

Data Analysis

In vitro recovery of the probes, calculated by Eq. (1), was expressed as the ratio of the AZT concentration in the effluent dialysate to that in the reservoir, in terms of the peak-height ratio (PHR) of AZT to BHPT. The *in vivo* recovery of AZT was estimated (17) by the loss of AZdU relative to the reservoir concentration as expressed in Eq. (2).

$$\text{Recovery}_{in\ vitro} = \frac{C_{\text{dial}}}{C_{\text{res}}} = \frac{\text{PHR(AZT/BHPT)dialysate}}{\text{PHR(AZT/BHPT)reservoir}} \quad (1)$$

$$\text{Recovery}_{in\ vivo} = 1 - \frac{\text{PHR(AZdU/BHPT)dialysate}}{\text{PHR(AZdU/BHPT)reservoir}} \quad (2)$$

The mean *in vitro* and *in vivo* recoveries were calculated and compared. Concentrations of AZT in the dialysates from the lateral ventricle and thalamus were then corrected by the *in vivo* recovery to obtain the corresponding concentrations in ventricular CSF and thalamus ECF over the dialysate collection interval.

Terminal half-lives of AZT in plasma, ventricle, and thalamus were estimated by linear regression of the logarithm of the values using the last five measurable data points. Statistical tests were then performed to compare differences between sites and doses. AZT areas under the curve in plasma (AUC_p) were calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of C_n/β, where C_n is the concentration of the last measured sample and β is the terminal elimination rate constant. Because the concentration of AZT measured in dialysate is a time-averaged concentration, the AZT area under the curve of ventricle (AUC_v) and thalamus (AUC_t) dialysates were obtained as the sum of the products of the measured concentrations and the collection time interval, with the addition of the residual area, as

$$\text{AUC} = \sum_{i=1}^{n} C_i \Delta t + \frac{C_n}{\beta} \quad (3)$$

The residual areas for AUC_v and AUC_t were obtained as C_n/β, defined as above. For all the AUCs calculated in different sites, the residual area was always less than 10% of the total AUC. Statistical analysis for differences in half-lives between doses and sites utilized a two-way ANOVA. A *P* value less than 0.05 was considered statistically significant.

Noncompartmental analysis (22) was used to obtain the total-body clearance of AZT (Cl_{tot} = dose/AUC_p) in the plasma and volume of distribution ($V_{d_{ss}}$ = dose * AUMC/AUC^2). Mean residence time (MRT = AUMC/AUC) for AZT was obtained and compared among doses.

The clearance ratio, Cl_{in}/Cl_{out}, describes transfer between the brain (both the ventricle and the thalamus) and the plasma. It reflects the distribution of the drug between plasma and the specific brain region and is measured as the ratio of time-averaged concentrations calculated as

$$\frac{Cl_{in}}{Cl_{out}} = \frac{AUC_{brain}^{0\text{–inf}}}{AUC_{plasma}^{0\text{–inf}}} \quad (4)$$

where AUC_{brain} is the area under the CSF or thalamus concentration–time curve (from $t = 0$ to ∞), AUC_{plasma} is the area under the plasma concentration–time curve (from $t = 0$ to ∞), Cl_{in} is the intercompartmental clearance from plasma to CSF (or thalamus), and Cl_{out} is the intercompartmental clearance from CSF (or thalamus) to plasma.

RESULTS

The relationship between AZdU loss and AZT recovery *in vitro* was characterized by an orthogonal regression slope

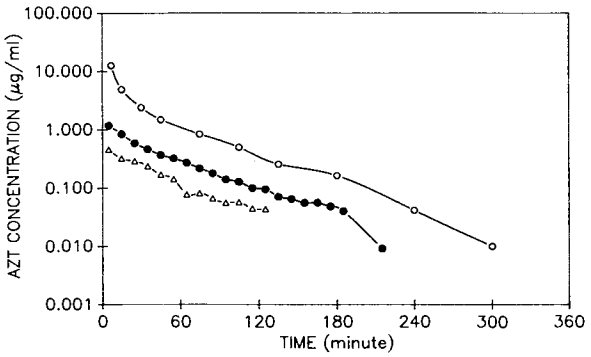

Fig. 1. AZT concentration–time profiles in plasma (○), ventricle (●), and thalamus (△) for a representative animal (5-mg/kg dose).

of 0.99 and a correlation coefficient of 0.82, indicating that loss of AZdU closely estimates the microdialytic recovery of AZT. The *in vitro* recoveries of AZT from the ventricle and thalamus probes determined immediately after the studies *in vivo* were 24 ± 5 and 20 ± 4%, respectively, across all doses and all animals. The *in vivo* losses of AZdU into the ventricle and thalamus probes were 18 ± 5 and 16 ± 4%, respectively. There was no statistical difference between calibrating the probes *in vitro* before and after use in the brain distribution study ($P > 0.05$).

Figure 1 shows semilogarithmic plots of concentrations of AZT in plasma (C_p), ventricular CSF (C_v), and thalamus ECF (C_t) versus time for the 5-mg/kg dose in a representative rabbit. The data for the other doses showed a similar pattern in all animals. AZT concentrations in the brain (ventricle and thalamus) have been corrected for *in vivo* recovery as described above. The plasma concentrations of AZT declined biexponentially in all rabbits in this study in accord with previous reports (23,24). The CSF concentrations of AZT declined with a profile similar to that in plasma suggesting rapid equilibration between plasma and CSF. The thalamus ECF concentrations of AZT declined in an apparent log-linear fashion, parallel to that in plasma and CSF in the terminal phase at all doses, indicating that there is no saturable transport from thalamus in this dose range.

Figure 2 depicts semilogarithmic plots of mean plasma concentrations of AZT. The concentrations were generally measurable up to 5 and 7 hr for the 5- and 30-mg/kg doses,

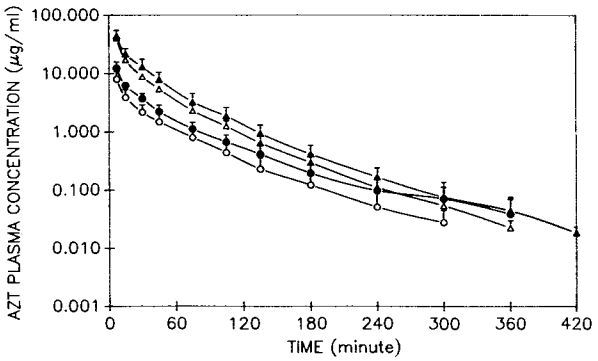

Fig. 2. AZT plasma concentration–time profiles: (○) 5 mg/kg; (●) 10 mg/kg; (△) 20 mg/kg; (▲) 30 mg/kg. Each point is the mean ± SD for three to six observations, from 240 min on.

Table I. Terminal AZT Half-Lives (Mean ± SD) Related to Dose and Sampling Sites

Dose (mg/kg)	Plasma (min)	Ventricle (min)	Thalamus (min)
5	38.9 ± 6.8	32.9 ± 7.5	29.5 ± 4.3
10	42.4 ± 11	37.3 ± 11	39.4 ± 11
20	41.4 ± 2.9	38.7 ± 7.4	43.1 ± 13
30	43.2 ± 6.7	40.2 ± 9.1	45.6 ± 7.4

respectively. That the curves are parallel to each other indicates linear pharmacokinetics in this dose range. The mean half-lives ($n = 6$) for the four doses ranged from 38.9 to 43.2 min (Table I), with no statistical difference (one-way ANOVA, $P > 0.05$).

Areas under the curve for AZT plasma concentrations were linearly related to dose, further suggesting that linear pharmacokinetics are applicable in this dosing range. Table II summarizes the noncompartmental pharmacokinetic parameters for AZT in this study. The total-body clearances, which ranged from 19.8 to 25.5 ml/min.kg, are similar to those previously observed in rabbits in both i.v. bolus and i.v. infusion studies (23,25). Thus neither the surgical procedure nor the probe placement had any discernible effect on the disposition kinetics of zidovudine.

Figure 3 depicts the semilogarithmic plot of mean ventricular CSF concentrations of AZT versus time. Although each data point reported here is a time-averaged concentration over a 10-min interval, a biexponential decline in AZT concentrations in this physiological space is apparent. CSF concentrations of AZT were measurable up to 240 min following the 30-mg/kg dose. All the curves are superimposible between doses. The AUC_v values normalized for dose ranged from 7.3 to 9.8 kg · min/liter (Fig. 5). The half-lives for the four doses measured in ventricle dialysates are 33–40 min (Table I). These results suggest that there is no dose dependency in the distribution kinetics of AZT into CSF over the dose range studied.

Figure 4 depicts a plot of the mean AZT thalamus concentrations versus time. Although there is greater variability in these concentrations, the profile generally follows those of plasma and ventricular CSF. Transport of AZT into and from the thalamus was not saturable in this dose range as evidenced by the similar half-lives (Table I) and the dose-normalized AUCs (Fig. 5).

Table I summarizes the terminal half-lives for the four doses studied at each sampling site. There was no statistical difference related to either dose or site (two-way ANOVA, $P > 0.05$). Figure 5 depicts the dose-normalized AUCs for AZT in plasma, ventricle, and thalamus. No dose-related

Table II. AZT Noncompartmental Parameters (Mean ± SD)

Dose (mg/kg)	Cl_{tot} (ml/min · kg)	$V_{d_{ss}}$ (L/kg)	MRT (min)
5	21.4 ± 7.0	0.82 ± 0.2	39.2 ± 6.1
10	25.5 ± 5.2	1.0 ± 0.07	41.3 ± 7.5
20	19.8 ± 4.7	0.62 ± 0.2	31.2 ± 2.1
30	23.1 ± 4.7	0.78 ± 0.1	34.4 ± 4.9

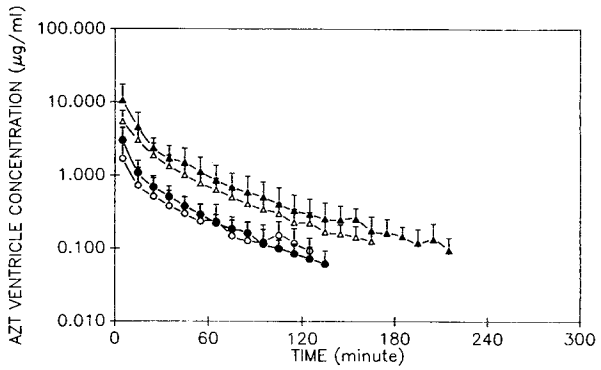

Fig. 3. AZT ventricular CSF concentration–time profiles: (○) 5 mg/kg; (●) 10 mg/kg; (△) 20 mg/kg; (▲) 30 mg/kg. Each point is the mean ± SD for three to six observations.

difference was observed at any site (one-way ANOVA, $P > 0.05$).

Variation in the total-body clearance of AZT in the study animals would lead to a significant variation in plasma AUC and therefore affect AUC values in the specific brain region. Calculation of the AUC achieved in the ventricle or the thalamus relative to the AUC in plasma eliminates the effects of interanimal variability in elimination clearance [Eq. (4)]. Table III shows mean values of AUC_v/AUC_p and AUC_t/AUC_p in individual animals for each dose. These ratios ranged from 0.15 to 0.19 and from 0.05 to 0.09 for ventricle and thalamus, respectively. Although the data suggest that AZT diffuses into thalamus via the BBB less well than it does into CSF, this interpretation is not well supported since the possibility of active transport from brain parenchyma to plasma cannot be ruled out.

The ratios C_v/C_p and C_t/C_p were examined as a function of time. Although there was a gradual increase in the mean value of C_t/C_p at the highest dose, no statistical difference was observed over time.

DISCUSSION

Distribution of drugs into the CNS depends on the extent of protein binding, lipophilicity, and the existence of transport systems. AZT exhibits a partition coefficient (octanol/buffer, pH 7.4) close to 1.1 (26), and this is five times

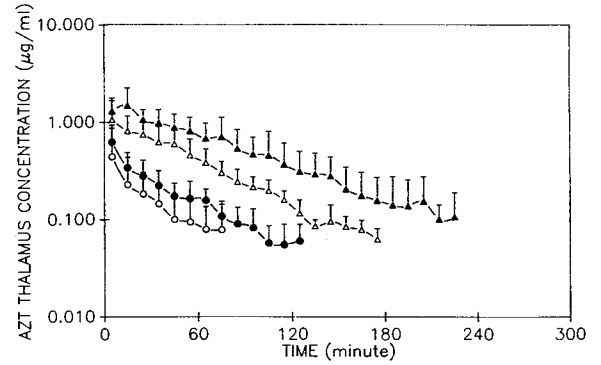

Fig. 4. AZT thalamus ECF concentration–time profiles: (○) 5 mg/kg; (●) 10 mg/kg; (△) 20 mg/kg; (▲) 30 mg/kg. Each point is the mean ± SD for three to six observations.

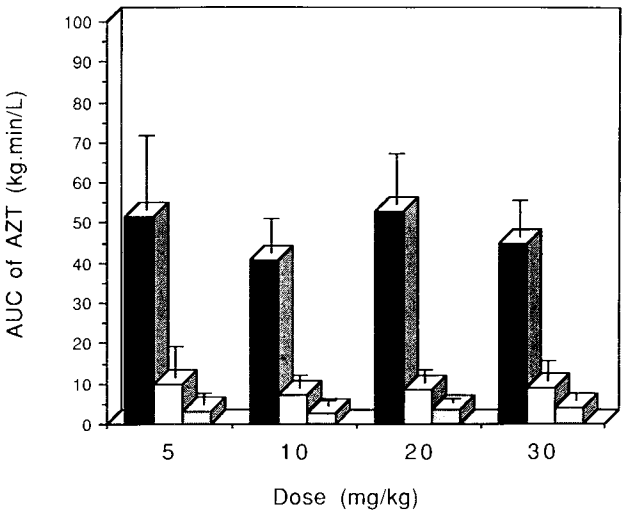

Fig. 5. Dose-normalized AUC for AZT (mean, SD) in plasma (■), ventricle (□), and thalamus (▨), as a function of dose.

that for thymidine. With this increased lipophilicity, which is due to the presence of the 3′-azido group, AZT is capable of permeating erythrocyte and lymphocyte membranes by nonfacilitated diffusion (27). Since AZT is 93 ± 3% unbound in human plasma over a concentration range of 0.1–2.5 μg/ml (28), almost all the drug in plasma is available for diffusion across biological membranes.

Concentrations of AZT in CSF have been measured in adult (29) and pediatric patients (30). Reported CSF/plasma concentration ratios range from 0.15 to 1.35. However, studies using carotid artery injection technique in rats showed that less than 1.0% of the injected AZT (31) or thymidine (32) penetrated into brain. It was concluded that these compounds did not cross the blood–brain barrier via the nucleoside transport system (31) and it has been speculated that AZT diffuses readily across the blood–CSF barrier, distributes into the CSF, reaches the ependymal lining of the ventricle, and then diffuses into brain parenchyma. However, the use of anesthesia in these studies may have altered transport of the drug, leading to reduced extraction of this agent into the brain (33).

The primary goal of the present study was to investigate the usefulness of microdialysis as a technique for studying distribution of AZT into the brain of conscious rabbits. Microdialysis allows continuous monitoring of extracellular AZT concentrations in the brain. Moreover, simultaneous measurement of microdialysis recovery *in vivo* using retrodialysis of a closely related compound shown to exhibit similar dialysis clearance permits the calculation of extracellular

Table III. AZT AUC in Ventricle and Thalamus Normalized to AUC in Plasma (Mean ± SD)

Dose (mg/kg)	AUC_v/AUC_p	AUC_t/AUC_p
5	0.173 ± 0.070	0.052 ± 0.027
10	0.177 ± 0.053	0.067 ± 0.030
20	0.157 ± 0.048	0.064 ± 0.013
30	0.193 ± 0.096	0.092 ± 0.037

fluid concentrations from the measured concentration of drug in dialysate. We had previously investigated retrodialysis *in vitro* and demonstrated (17) the equivalence of recovery of AZT and loss of simultaneously perfused AZdU over a range of concentrations. In addition, this relationship was validated *in vivo* (17,18) by coperfusing AZT and AZdU into probes implanted in rabbit brain and comparing the microdialysis clearance of both compounds simultaneously over the entire experimental period. Four dose levels (5–30 mg/kg) were used in this study to examine AZT brain distribution over a range of concentrations that may be encountered clinically (26,34). Based on a comparison of terminal half-lives and AUC ratios, no dose-related differences in distribution or elimination kinetics were noted in this study. The relatively high initial concentrations and reasonably rapid equilibration of AZT between thalamus ECF and plasma suggest that this drug reaches brain tissue at least in part by transport through the blood–brain barrier. That the AUC_v/AUC_p ratio (0.18) in this study is consistent with those observed at steady state during constant-rate infusion in the rabbit (0.152), where CSF was sampled from the cisterna magna (23), suggests that the recovery of AZT estimated by retrodialysis is reliable. These results are also comparable to the steady-state CSF:plasma ratio (0.24) obtained during continuous intravenous infusion of AZT in children (29). The mean thalamus ECF to plasma AUC ratio (AUC_t/AUC_p) observed in the present study was 0.090, similar to the brain/plasma ratio (0.062) measured in a study evaluating osmotic pump delivery of AZT (35).

In summary, microdialysis was successfully utilized to examine the distribution of AZT into the brain at doses ranging from 5 to 30 mg/kg. These studies employed simultaneous retrodialysis of AZdU as a calibrator of microdialysis recovery of AZT *in vivo*. Using this technique, crossover studies of brain drug distribution using multiple treatments in the same animal are possible. Continuous monitoring of the extracellular fluid allows a comparison of concentration–time profiles in specific regions of the brain with those in plasma, providing a means of studying drug transport into the central nervous system.

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