Microdialysis Studies of the Distribution of Stavudine into the Central Nervous System in the Freely-Moving Rat

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Purpose. To study the extent and time course of distribution of stavudine (d4T) into the central nervous system (CNS) and to investigate the transport mechanisms of antiviral nucleosides in the CNS.

Methods. Microdialysis with on-line HPLC analysis was used to measure drug concentrations in the brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) in the freely-moving rat. The *in vivo* recovery of d4T and zidovudine (AZT) was estimated by retrodialysis, which was validated by the zero-net flux method. The CNS distribution of d4T was investigated during iv and intracerebroventricular (icv) infusion. In the subsequent studies, the effect of AZT on CNS distribution of d4T was examined.

Results. During iv infusion, d4T distributed rapidly into the CNS. Its brain ECF/plasma and CSF/plasma steady-state concentration ratios were 0.33 ± 0.06 and 0.49 ± 0.12, respectively (n = 15). During icv infusion, the steady-state d4T concentrations in the brain ECF were 23-fold higher than those during iv infusion, whereas its steady-state plasma levels were about the same for these two routes. Coadministration of AZT with d4T did not alter their respective brain distribution and systemic clearance at the concentrations examined. More importantly, the steady-state brain ECF/plasma and CSF/plasma concentration ratios of d4T were about 2-fold higher than those of AZT (0.15 ± 0.04 and 0.25 ± 0.08) determined in the same animals.

Conclusions. d4T readily crosses the blood-brain barrier (BBB) and blood-CSF barrier. An active efflux transport system in the BBB and blood-CSF barrier may be involved in transporting d4T out of the CNS. Direct icv administration of d4T can be used to enhance its brain delivery. Moreover, d4T exhibits a more favorable penetration into the CNS than AZT and therefore may be useful in the treatment of AIDS dementia complex.

KEY WORDS: stavudine; zidovudine; microdialysis; retrodialysis; central nervous system; brain uptake; blood-brain barrier; intracerebroventricular administration; AIDS.

INTRODUCTION

Stavudine (d4T) is a thymidine analog used for AIDS treatment. Its pharmacokinetics have been extensively studied (1–5). However, little is known concerning the extent and time course of the distribution of d4T into the central nervous system (CNS).

The rationale for delivery of anti-HIV drugs into the CNS is that HIV enters the CNS early, probably concomitant with initial systemic infection (6). The dementia caused by HIV infection in the CNS has been estimated to affect up to 60% of all individuals in the late stages of HIV disease (7). On the other hand, the brain may act as a "pharmacologic sanctuary" for the virus. The observed decrease in peripheral viral loads by combination therapy underscores the importance of enhancing the access of anti-HIV drugs to the brain. Knowledge on the CNS distribution of d4T would have practical implication in treating AIDS dementia complex, and also is essential for developing new approaches to enhance its brain delivery.

Microdialysis represents a unique tool to evaluate drug transport processes in the CNS (8), offering significant advantages over conventional tissue sampling techniques. In the present work, we first evaluated the feasibility of using microdialysis to study the CNS distribution of d4T in a freely-moving rat model. The use of retrodialysis to estimate the relative recovery of d4T was validated by the zero-net flux method both *in vitro* and *in vivo*. Subsequently, we investigated the CNS distribution of d4T during iv and intracerebroventricular (icv) infusion. Lastly, the effect of zidovudine (AZT) on CNS distribution of d4T was examined.

MATERIALS AND METHODS

Drugs and Reagents

d4T was provided by Bristol-Myers Squibb Co. (Syracuse, NY). AZdU was a gift from Dr. C. K. Chu, University of Georgia. AZT was generously donated by Glaxo Wellcome (Research Triangle Park, NC). Solvents were of HPLC grade, and all other chemicals were AR grade.

Probes, Artificial CSF, and Drug Solutions

For the *in vitro* calibration experiments, 2- and 3-mm CMA-12 probes (CMA/Microdialysis, Acton, MD) were used; for the *in vivo* studies, 1- and 2-mm probes were implanted in the lateral ventricle and frontal cortex of the rat brain, respectively. All probes were calibrated using retrodialysis during their use. Artificial CSF (9) was freshly prepared and filtered (0.2 μm) before use.

Immediately before each experiment, the following solutions were prepared: 250 ng/ml of AZdU in artificial CSF; d4T and AZT solutions for iv administration in normal saline; and d4T solution for icv infusion in artificial CSF. The AZdU solution was used for perfusion of probes to calibrate the relative recovery of d4T and AZT *in vivo*.

Surgery

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing approximately 300 g (n = 15) were used in the studies. The detailed surgical procedures for implantation of the probe guide cannulas and cannulation of femoral artery and vein have been described elsewhere (10). The stereotaxic coordinates for the frontal cortex were 3.2 mm anterior and 3.2 mm lateral (left) to the bregma; the tip of guide cannula was 0.5 mm ventral from the brain surface. For the lateral ventricle, they

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were 0.9 mm posterior and 1.5 mm lateral (right) to the bregma, and 3.0 mm ventral from the brain surface (11). The femoral artery and vein were cannulated for drug sampling and dosing, respectively. In the studies where AZT was coadministered with d4T, a second femoral vein was cannulated for AZT dosing to allow simultaneous iv infusion of d4T in the crossover studies.

The microdialysis probes were slowly implanted into the brain to replace the guide cannulas 18–24 hr before beginning the experiment. During this period, the probes were perfused with blank artificial CSF at a flow rate of 0.2 μL/min.

Set-Up of a Microdialysis System with On-Line HPLC Analysis

A microdialysis system with on-line HPLC analysis was established as described by Malhotra *et al.* (10)

In Vitro Probe Calibration Studies

These studies were performed at ambient temperature.

Effect of Perfusion Flow Rates on Recovery/Loss

Various perfusion flow rates (0.1, 0.2, 0.4, 0.8, 1.0, 2.0, and 5.0 μL/min) were used to examine the effect of flow rate on the recovery of d4T and the loss of AZdU.

Effect of Medium Concentrations on Recovery/Loss

The recovery of d4T and the loss of AZdU were determined at different concentrations of d4T in the dialyzed medium (25, 100, 200, and 500 ng/mL) at a flow rate of 0.2 μL/min.

Effect of Diffusion Directions Across the Probe Membrane on Recovery/Loss

Both the recovery and the loss of d4T and AZdU were determined and compared at a flow rate of 0.2 μL/min.

Simultaneous Zero-Net Flux Method and Retrodialysis In Vitro

To determine the probe recovery by the zero-net flux method, microdialysis probes were placed into a reservoir containing about 250 ng/mL of d4T. The perfusates containing d4T at concentrations of 0, 20, 100, 300, and 400 ng/mL were perfused through the probes; the concentrations of d4T in the dialysate were then quantified. Retrodialysis was simultaneously performed with the zero-net flux method. The reservoir concentration of d4T was also directly measured by on-line HPLC.

Simultaneous Zero-Net Flux Method and Retrodialysis *In Vivo*

d4T was given to rats (n = 2, 1 and 2) as a constant-rate iv infusion (1.75 mg/hr/kg) to produce a steady-state plasma level of about 1.0 μg/mL. Once the concentrations of d4T in the brain ECF and CSF reached steady state, the perfusates with various d4T concentrations (250, 500, and 1000 ng/mL) were perfused through the probes. The gain or loss of d4T in the perfusates was then determined by on-line HPLC. Retrodialysis was simultaneously performed. Blood samples (0.3 mL) were collected at 360, 540, 720, 930, 1110, and 1390 min during, and at 10, 30, 60, and 120 min post iv infusion.

Determination of d4T Free Fraction in Rat Plasma

Ultrafiltration studies were conducted in an environmental room at 37°C as described elsewhere (10).

CNS Distribution of d4T During Intravenous and Intracerebroventricular Infusion

A crossover study was used. In Phase I, d4T was administered to rats (n = 7, 3–9) as a constant-rate iv infusion (1.75 mg/hr/kg) for 600 min. On the day following iv infusion, icv administration of d4T into the lateral ventricle of these rats was performed (Phase II) at the same infusion rate. Microdialysate samples were collected alternatively from the brain ECF and CSF every 10 min and analyzed by on-line HPLC. Blood samples (0.3 mL) were collected at 480, 540, and 600 min during, and at 5, 15, 30, 45, 60, 120 and 180 min post iv infusion, and; at 480, 540, and 600 min during icv infusion.

Effect of Coadministered AZT on the Distribution of d4T into the CNS

A crossover study was again used. In Phase I, rats (n = 6, 10–15) received a constant-rate iv infusion of d4T at 1.75 mg/hr/kg for 480 min. In Phase II, the iv infusion of d4T was continued; AZT was administered as an iv bolus (10 mg/kg), followed by an iv infusion of 2.55 mg/hr/kg for another 480 min. The steady-state plasma levels of both d4T and AZT were targeted to be about 1.0 μg/mL. d4T and AZT in the CNS were continuously monitored by microdialysis with on-line HPLC analysis. Blood samples (0.3 mL) were collected at 360, 420, 480, 840, 900, and 960 min during, and at 10, 30, 60, 120, 180 min post iv infusion.

Sample Analysis

Plasma samples (0.1 mL) were analyzed for d4T and AZT using an off-line HPLC method reported by Wong *et al.* (3) with modification. The mobile phase consisted of methanol-acetonitrile-10 mM monobasic ammonium phosphate (5:4:91, v/v/v) at a flow rate of 1 mL/min. AZdU was used as an internal standard in the assay. The limit of quantification for d4T and AZT was 30 and 75 ng/mL, respectively. For d4T and AZT, within-run and run-to-run accuracy ranged from 94 to 109% and from 96 to 109%, respectively; within-run and run-to-run precision (%CV) was less than 10% and 12%, respectively.

The d4T and AZT concentrations in microdialysate samples were determined by a sensitive on-line HPLC assay coupled with microdialysis sampling. A Hewlett-Packard hypersil ODS column (20 cm × 2.1 mm, 5 μm, Wilmington, DE) was used at 35°C. The mobile phase consisted of acetonitrile and 10 mM monobasic ammonium phosphate (6.5:93.5, v/v) at a flow rate of 0.3 mL/min (Shimadzu LC-10AD, Columbia, MD). The compounds were detected at a wavelength of 266 nm (Shimadzu SPD-6A). With a injection volume of 2 μL, the limit of quantification for d4T and AZT was 20 and 30 ng/mL, respectively. For d4T and AZT, within-run and run-to-run accuracy ranged from 97 to 102% and 96 to 104%, respectively; the %CV

was less than 11% and 10% for within-run and run-to-run precision, respectively.

Data Analysis

All the data were expressed as mean ± SD ($n \geq 3$). Differences were considered significant when $P < 0.05$ using a pair or nonpair Student's t-test. For the zero-net flux method, simple linear regression was performed to determine the recovery of d4T as described by Wang *et al.* (12). The intercept on the X-axis equals to the analyte concentration outside of the probe. The absolute value of the slope corresponds to the recovery of d4T. The loss (L_c) of the retrodialysis calibrator from the probe to the dialyzed medium is calculated as (12):

$$L_c = 1 - \frac{C_{ec}}{C_{ic}}$$

where C_{ec} and C_{ic} are concentrations of the retrodialysis calibrator in the dialysate and the perfusate, respectively. The analyte concentration outside of the probe is then calculated as its concentration in the dialysate divided by L_c.

The total body clearances of d4T and AZT were calculated as the infusion rate divided by their respective steady-state plasma concentrations. The elimination half-lives in plasma, brain ECF and CSF were determined by linear regression of the terminal part of the semilogarithmic concentration-time curve. The distribution advantage was determined as the ratio (icv/iv) of the steady-state brain ECF concentration of d4T normalized to the corresponding infusion rate. The ratio of brain influx to efflux clearance of d4T was calculated as the brain ECF (or CSF) concentration of d4T divided by its plasma concentration at steady state, assuming negligible exchange between the brain ECF and CSF during iv infusion, and negligible protein binding in the blood.

RESULTS

In Vitro Probe Calibration Studies

Effect of Perfusion Flow Rates on Recovery/Loss

The recovery of d4T and the loss of AZdU were similar at each flow rate examined (Fig. 1a). As the flow rate increased, the recovery of d4T and the loss of AZdU decreased from 0.83 to 0.06, and from 0.97 to 0.09 in 2- and 3-mm probes, respectively. A perfusion flow rate of 0.2 μL/min was selected based on the results. The recovery or loss of a compound is a function of its effective permeability-area product (PeA) and the perfusion flow rate. The inset in Fig. 1a shows dependence of the PeA of the analytes on perfusion flow rates. The PeA of the analytes was also proportional to the size of the probe.

Effect of Concentration on Recovery/Loss

The results show that the recovery of d4T and the loss of AZdU are comparable, and concentration-independent (Fig. 1b).

Effect of Diffusion Direction Across the Probe Membrane on Recovery/Loss

The recovery of d4T and AZdU was 0.69 ± 0.03 and 0.66 ± 0.02 (n = 12), respectively. The loss of d4T and AZdU was

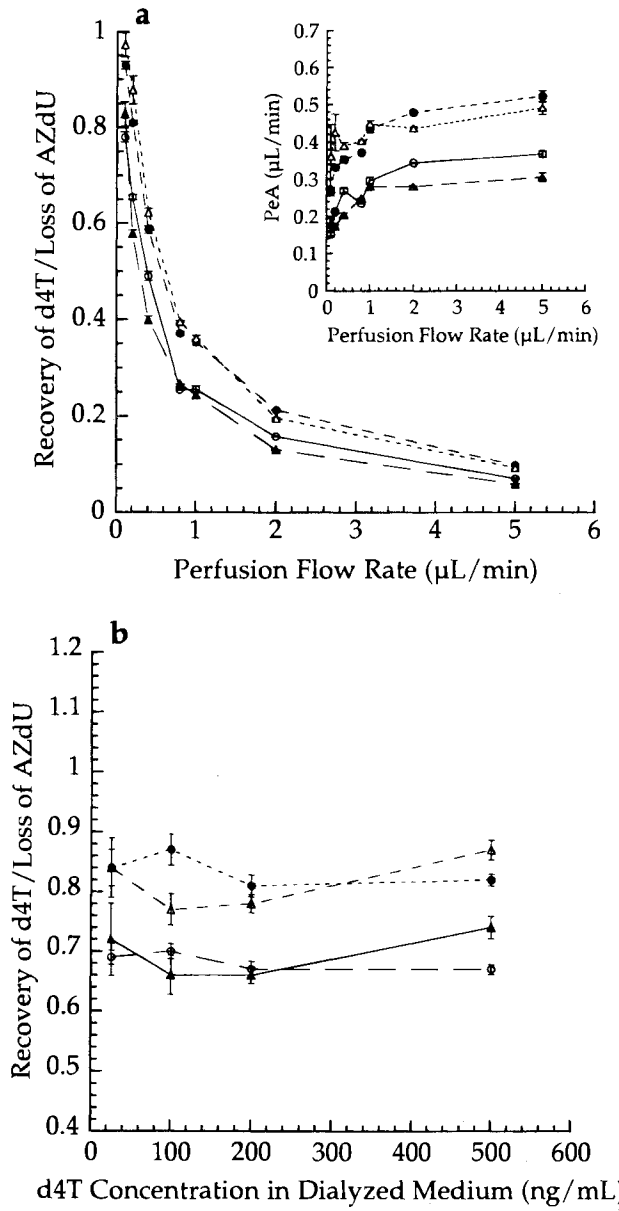

Fig. 1. a. Effect of perfusion flow rates on the recovery of d4T and the loss of AZdU *in vitro* (mean ± SD, n = 4). Inset: Dependence of the PeA of d4T and AZdU on perfusion flow rates. b. Effect of medium concentrations of d4T on the recovery of d4T and the loss of AZdU *in vitro* (mean ± SD, n = 4 or 5). 2-mm probe: d4T (▲), AZdU (○); 3-mm probe: d4T (△), AZdU (●).

0.69 ± 0.01 and 0.68 ± 0.03 (n = 12), respectively. There is no directional dependence of diffusion of d4T or AZdU across the probe membrane.

Simultaneous Zero-Net Flux Method and Retrodialysis In Vitro

The *in vitro* recovery of d4T estimated by the zero-net flux method were 0.74 and 0.88 for 2- and 3-mm probes, respectively. The values are comparable to the loss of AZdU determined by retrodialysis (0.72 ± 0.01 and 0.84 ± 0.02). The reservoir concentrations of d4T determined by retrodialysis

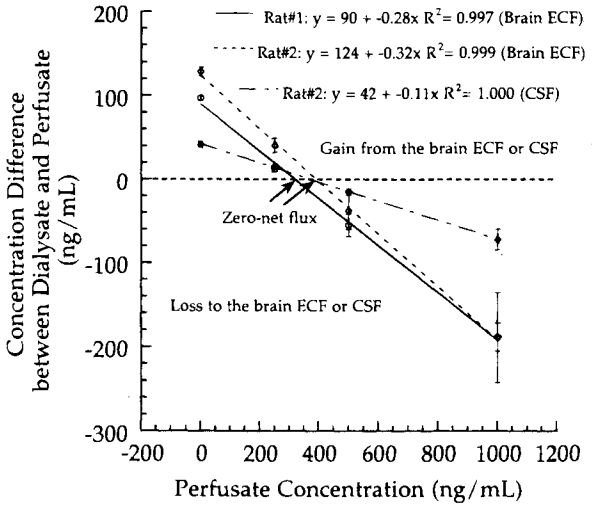

Fig. 2. d4T zero-net flux plot *in vivo* (mean ± SD, n ≥ 3). Rat #1: brain ECF (○); Rat #2: brain ECF (◇), CSF (◆).

(230 ± 10 and 232 ± 10 ng/mL) and the zero-net flux method (229 and 231 ng/mL) for 2- and 3-mm probes, were similar to that measured directly from the reservoir (230 ng/mL). The results demonstrate that retrodialysis is a feasible method for estimating drug concentrations in the dialyzed medium *in vitro*.

Simultaneous Zero-Net Flux Method and Retrodialysis *In Vivo*

The loss of AZdU to the brain determined by retrodialysis in rats #1 and #2 was 0.28 ± 0.04 and 0.29 ± 0.02, respectively, similar to the recovery of d4T obtained by the zero-net flux method (0.28 and 0.32) (Fig. 2). The loss of AZdU in CSF determined by retrodialysis in rat #2 was 0.11 ± 0.02, and the recovery of d4T by the zero-net flux method was 0.11 (Fig. 2). The loss of AZdU was constant over the entire study period (26 hr). These results agree with those observed *in vitro*, indicating that AZdU is a reliable retrodialysis calibrator for d4T. It is noted that the *in vivo* recovery and loss of both compounds are less than one-half of their *in vitro* values.

The d4T concentrations in the brain ECF and CSF determined by retrodialysis were similar to those obtained by the zero-net flux method (Table I). The brain ECF/plasma concentration ratio of d4T at steady state was 0.30 and 0.40 for rats #1 and #2, respectively; the CSF/plasma ratio was 0.35 for rat #2 (Table I). These results demonstrate that d4T penetrates into the brain to an appreciable extent.

Determination of d4T Free Fraction in Rat Plasma

The free (unbound) fraction of d4T determined in the studies was 0.95 ± 0.10. It was constant over the d4T concentration range of 0.1–4.0 μg/mL.

CNS Distribution of d4T During Intravenous and Intracerebroventricular Infusion

During iv infusion, d4T distributed rapidly into the CNS (Fig. 3). It can be readily detected in the dialysates of the brain ECF and CSF immediately after onset of iv infusion. The d4T ECF/plasma and CSF/plasma steady-state concentration ratios were 0.34 ± 0.04 and 0.50 ± 0.09, respectively (Table II).

During icv infusion, there was a significant delay in the distribution of d4T from CSF into the probed brain tissue (Fig. 3). This reflects the time required for the drug to diffuse into the brain parenchyma during this route of administration. The steady-state concentrations of d4T in the brain ECF were 23-fold higher than those during iv infusion, whereas its steady-state plasma levels were not significantly different ($P > 0.05$) between these two routes (Table II). This clearly demonstrates an administration route-based distribution advantage. Similar steady-state plasma levels of d4T between iv and icv infusion indicate that the brain is not an organ which metabolizes d4T to any measurable extent.

Effect of Coadministered AZT on the Distribution of d4T into the CNS

Fig. 4 displays a typical d4T concentration-time profile in the brain ECF and CSF before and during the coadministration of AZT. In the absence of AZT, the d4T ECF/plasma and CSF/plasma steady-state concentration ratios were 0.30 ± 0.07 and 0.51 ± 0.15, respectively (Table III). These ratios in the presence of AZT were 0.30 ± 0.07 and 0.54 ± 0.24, respectively. The results indicate that AZT has no effect on the CNS distribution of d4T in the rat, in plasma concentration ranges considered to be therapeutic in man. Vice versa, d4T has no effect on the CNS distribution of AZT. The CSF/plasma steady-state concentration ratios of AZT (0.25 ± 0.08) determined in this work are in an excellent agreement with the results by Mastereeuw *et al.* (13) where the CSF samples were taken from cisterna magna cannulas. More importantly, the steady-state concentration ratios of d4T were 2-fold higher than those of AZT determined simultaneously in both probed sites (Table III). Therefore, d4T exhibits greater CNS distribution than AZT.

The total body clearance of d4T in plasma was not significantly different ($P > 0.05$) between Phases I and II (Table III). Both d4T and AZT total body clearances (1.99 and 2.62 L/hr/

Table I. Results of Microdialysis Using Zero-Net Flux Method (ZNF) and Simultaneous Retrodialysis (RD) *In Vivo*

	Cecf,ss (ng/mL)		Ccsf,ss (ng/mL)		Cp,ss[b]	Cecf,ss/Cp,ss		Ccsf,ss/Cp,ss	
Rat No.	By RD[b]	By ZNF	By RD[b]	By ZNF	(ng/mL)	By RD	By ZNF	By RD	By ZNF
1	274 ± 33	319	—[a]	—[a]	988 ± 46	0.28	0.32	—[a]	—[a]
2	395 ± 62	392	323 ± 89	370	994 ± 80	0.40	0.39	0.32	0.37

[a] Not measured due to the probe failure.
[b] Data are expressed as mean ± SD (n = 3 or 4 for brain ECF and CSF, n = 6 for plasma).

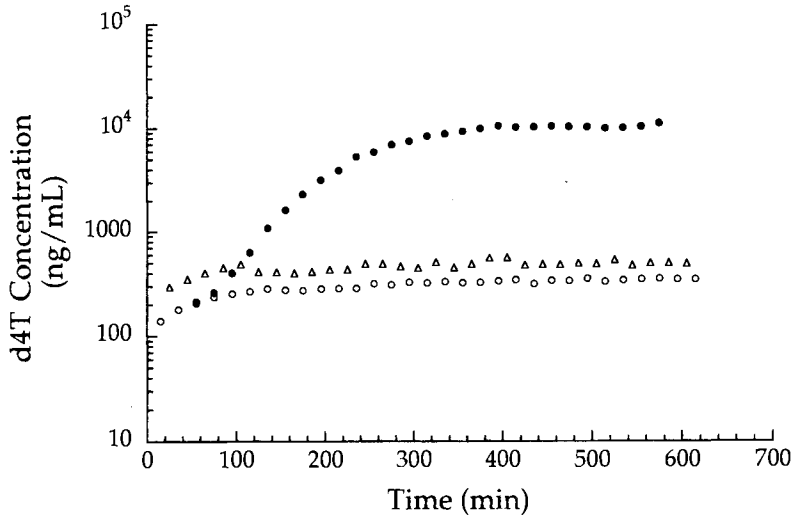

Fig. 3. d4T concentration-time profile in brain ECF and CSF during iv and icv infusion in rat #7. iv infusion: Cecf (○); Ccsf (△). icv infusion; Cecf (●).

kg, respectively) determined in the present studies are consistent with the reported values (4,13). The results suggest that, under the current experimental conditions, no interaction exists between d4T and AZT with respect to their systemic clearance as measured in plasma. Similar results have recently been reported by Odinecs *et al.* (14) in the monkey.

Summary of Pharmacokinetic Parameters of d4T

In all studies (n = 15), the steady-state brain ECF/plasma and CSF/plasma concentration ratios of d4T during iv infusion were 0.33 ± 0.06 and 0.49 ± 0.12, which were equal to the ratios of influx to efflux clearances of d4T for the brain ECF and CSF, respectively. The total body clearance of d4T measured in plasma was 1.99 ± 0.23 L/hr/kg. The half-life of d4T in the brain ECF (93 ± 37 min) was significantly longer than that in the CSF (52 ± 20 min) and the plasma (40 ± 8 min) ($P < 0.01$ in both cases).

DISCUSSION

A freely-moving rat model was employed in these studies to investigate the CNS distribution of d4T. The distribution of anti-HIV nucleosides into the CNS appears to be species-independent. For example, the ECF/plasma and CSF/plasma steady-state concentration ratios of AZT determined in this work are in good agreement with previous results in rabbits and humans (15,16). Therefore, this model may be useful in predicting the CNS distribution of d4T in humans.

The pathway of entry of anti-HIV nucleosides into the brain is controversial. Early work on AZT suggested that the drug was transported into the CSF by a thymidine transport system in the blood-CSF barrier (17), from which it may diffuse into the brain parenchyma. Such a transporter is not available in the BBB (17). Studies in our laboratory, however, suggest direct entry of AZT into the brain across the BBB (15,18). In the present work, d4T rapidly distributed into the CNS, suggesting that it not only permeates the blood-CSF barrier, but also readily crosses the BBB. As a thymidine analog, d4T is about 10-fold more polar than AZT as measured by octanol-water partition coefficients (19). d4T may enter the brain via the CSF pathway, but this route alone cannot account for the simultaneous increase of d4T concentrations in brain ECF and CSF as plasma concentration increases during iv infusion. Indeed, in subsequent icv studies, a significant delay was noted in the distribution of d4T from CSF into brain ECF. Studies in human placenta and cell lines have shown that d4T crosses

Table II. d4T Steady-State Concentrations and Ratios in Brain ECF, CSF and Plasma During IV and ICV Infusion[a]

	Cecf,ss (ng/mL)	Ccsf,ss[b] (ng/mL)	Cp,ss (ng/mL)	Cecf,ss/ Cp,ss	Ccsf,ss/ Cp,ss[b]	{Cecf,icv/ Cecf,iv}ss[c]	{Cp,icv/ Cp,iv}ss
Phase I (iv infusion)							
n	7	7	7	7	7		
d4T	290 ± 52	431 ± 101	850 ± 90	0.34 ± 0.04	0.50 ± 0.09		
Phase II (icv infusion)							
n[d]	6	—	5	4	—	6	5
d4T	6421 ± 2277	—	821 ± 118	9.3 ± 3.1	—	22.8 ± 7.0	0.96 ± 0.07

[a] Data are expressed as mean ± SD.
[b] CSF data were not available during icv infusion.
[c] {Cecf,icv/Cecf,iv}ss is defined as the administration route-based distribution advantage after normalized to the corresponding infusion rate.
[d] Data were not available in some animals because of failure of probes or blood sampling catheters during icv infusion.

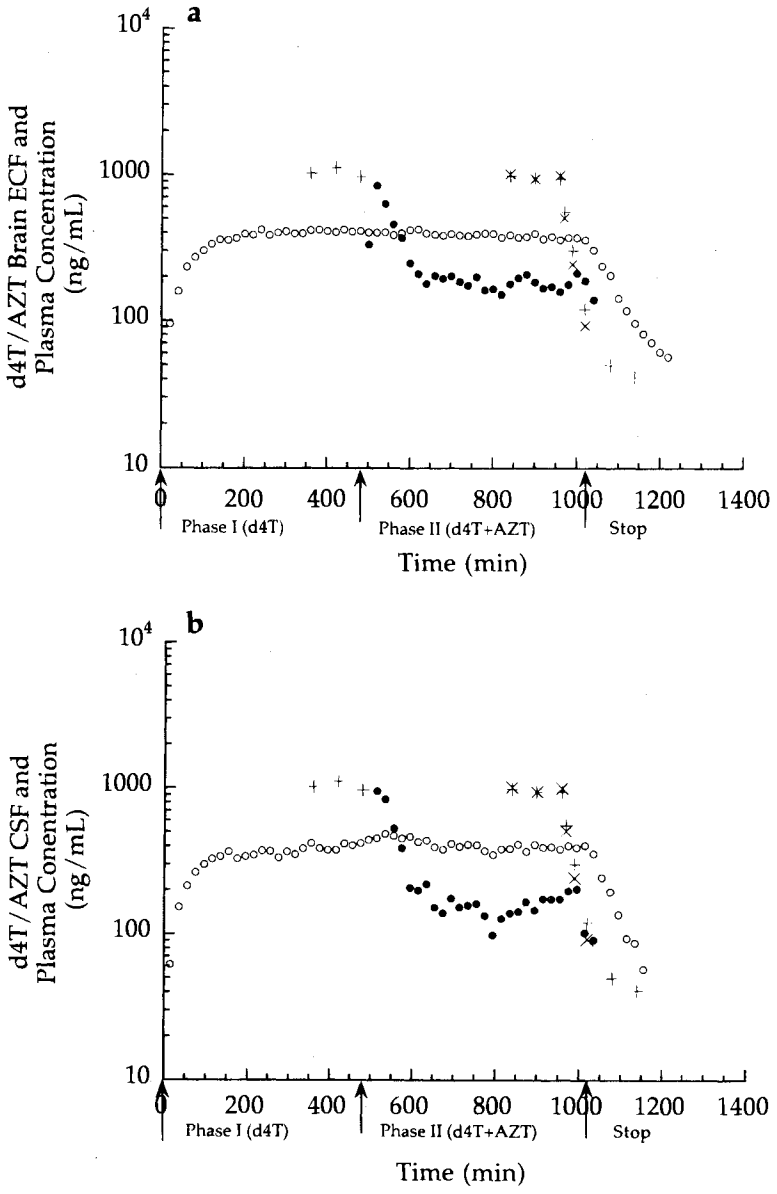

Fig. 4. a. d4T/AZT brain ECF and plasma concentration-time profile in rat #12. b. d4T/AZT CSF and plasma concentration-time profile in rat #12. The arrows indicate the onset or termination of the infusion. d4T: brain ECF or CSF (○); plasma (+). AZT: brain ECF or CSF (●); plasma (×).

membranes by passive diffusion (19,20). In view of similar permeability characteristics of the cell membrane and the BBB, it is possible that d4T enters the CNS by the same transport mechanism.

The higher efflux clearance of d4T relative to its influx counterpart from the brain ECF and CSF was observed during iv infusion. A high efflux clearance from the brain can be attributed to a number of mechanisms. For a polar drug such as d4T, convective flow in the turnover of brain ECF and CSF may contribute to its efflux clearance. However, the observed steady-state concentrations of d4T in the CSF were even higher than those in the brain ECF, suggesting that this mechanism may contribute only marginally to the high efflux clearance observed. Extensive metabolism in the brain may cause one to overestimate the efflux clearance; this is unlikely in the case of d4T, since the results of the icv infusion study suggest that the brain does not show measurable metabolism of d4T. The most likely explanation for these results is that there exists an active efflux transport system in the BBB, which transports d4T out of the brain. Such an efflux transporter for d4T may also exist in the blood-CSF barrier.

There is indirect evidence supporting the hypothesis that d4T is actively transported out of the brain. The steady-state concentrations of d4T in brain ECF were lower than those in CSF. This concentration gradient between CSF and brain ECF cannot be explained by an active efflux transporter in the blood-

Table III. Summary of d4T/AZT Interaction Study[a]

	Cp,ss (ng/mL)	CLtot (L/hr/kg)	Cecf,ss/ Cp,ss	Ccsf,ss/ Cp,ss
Phase I (d4T)				
d4T	895 ± 132	1.99 ± 0.29	0.30 ± 0.07	0.51 ± 0.15
Phase II (d4T + AZT)				
d4T[c]	911 ± 93	1.94 ± 0.20	0.30 ± 0.07	0.54 ± 0.24
AZT[c]	994 ± 153	2.62 ± 0.41	0.15 ± 0.04[b]	0.25 ± 0.08[b]
Ratio of Steady-state Concentration Ratio of d4T relative to that of AZT in Phase II[d]				
	ECF			CSF
	2.1 ± 0.1			1.9 ± 0.5

[a] Data are expressed as mean ± SD (n = 6).
[b] Data were not available in first 2 animals because of analytical problems measuring AZT in the brain ECF and CSF.
[c] The ECF/plasma and CSF/plasma concentration ratios of d4T at steady state were significantly different from those of AZT ($p < 0.05$).
[d] The ratios were determined from each animal and then averaged.

CSF barrier, unless such a transporter is also present in the BBB. Such a hypothesis is further supported by the fact that AZT, which is structurally similar, is well known to be actively transported out of the brain (9,12,15,18). The nature of this efflux transporter in the BBB is not well understood. A recent study by Miller *et al.* (21) has shown possible involvement of the multidrug resistance-associated protein in mediating AZT efflux transport in the brain endothelial cells.

Drug concentrations in the brain ECF may result from the direct BBB transport and/or diffusion from the CSF. The important role of the CSF as an entry point for polar agents into the brain parenchyma has been previously demonstrated (10,15). In the present icv infusion studies, the diffusion of d4T from the CSF to the brain ECF was found to be substantial. It suggests that direct CSF administration may be useful in enhancing the brain delivery of d4T while minimizing its systemic toxicity. It should be noted that, unlike what is observed during systemic infusion, drug concentrations in the brain ECF during icv dosing are likely very heterogeneous; they are not only a function of time, but also markedly dependent on the distance from the CSF-brain interface. This may explain the greater variability seen in brain ECF concentrations during icv as compared with iv infusion.

While a combination of d4T and AZT in antiviral therapy may not be beneficial because of their competing activities with cytosolic thymidine kinase, the interaction studies between these two drugs provide important insight into transport processes of nucleosides in the CNS. The rationale is that, because of their similar structures, d4T and AZT are likely to be substrates of the same efflux transporter in the BBB and/or the blood-CSF barrier. If this is so, competitive inhibition of the efflux of one nucleoside by the other may be observed; thus the CNS distribution of d4T may be altered when coadministered with AZT. However, no interaction was observed between d4T and AZT with respect to their brain distribution, even when the AZT concentrations in the brain ECF were as high as 1 μg/mL (a loading dose of AZT). It may be that these nucleosides are not transported by the same efflux mechanism. Another possibility is that d4T and AZT still share the same efflux transporter in the BBB and blood-CSF barrier, but their concentrations in the CNS achieved in the studies were well below their respective dissociation constants with the transporter. In this linear range, no interaction would be observed with respect to their brain distribution.

It is of interest that the steady-state CSF/ECF ratios (1.5) of d4T observed in these studies are almost identical to those (1.4–1.5) of AZT (12,15). It indicates that the relative contribution of efflux via the blood-CSF barrier and the BBB is similar for d4T and AZT. If we assume the influx of the drugs into the brain ECF and CSF is a passive diffusion process, this efficiency may be directly related to the density or concentration of transporter available at the BBB and blood-CSF barrier. In spite of the fact that no inhibition of d4T efflux was exhibited by AZT, they may nevertheless share the same efflux transport system in the BBB and blood-CSF barrier. Further studies are needed to directly test this hypothesis.

One important finding from the interaction studies is that d4T exhibits greater CNS distribution than AZT. This is unexpected if passive diffusion is to explain the transport into and out of the CNS, because d4T is much more polar than AZT. One possibility is that, because of the difference in their lipophilicity, d4T may have a lower affinity for the efflux transporter in the BBB and blood-CSF barrier than AZT. This may result in a lower efflux clearance of d4T from the CNS compared to that of AZT, assuming they share the same transporter and have similar transport capacity. These results emphasize the importance of studying transport mechanisms of anti-HIV nucleosides in the CNS.

In conclusion, microdialysis is a feasible method for studying the distribution of d4T and AZT into the CNS in a freely-moving rat model. d4T readily crosses the BBB and blood-CSF barrier. An active efflux transport system in the BBB and blood-CSF barrier may be involved in transporting d4T out of the CNS. Direct icv administration of d4T enhances its brain delivery. Coadministration of AZT with d4T does not alter their respective brain distribution and systemic clearance in the rat. Moreover, d4T exhibits a more favorable penetration into the CNS than AZT and therefore may be useful in the treatment of AIDS dementia complex.

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REFERENCES

1. C. M. Horton, M. N. Dudley, S. Kaul, K. H. Mayer, K. Squires, L. Dunkle, and R. Anderson. *Antimicrob. Agents Chemother.* **39**:2309–2315 (1995).
2. M. N. Dudley, K. K. Graham, S. Kaul, S. Geletko, L. Dunkle, M. Browne, and M. Kenneth. *J. Infect. Dis.* **166**:480–485 (1992).
3. S. L. Wong and R. J. Sawchuk. *Pharm. Res.* **8**:619–623 (1991).
4. F. D. Boudinot, S. G. Smith, E. D. Funderburg, and R. F. Schinazi. *Antimicrob. Agents Chemother.* **35**:747–749 (1991).
5. R. F. Schinazi, F. D. Boudinot, K. J. Doshi, and H. M. McClure. *Antimicrob. Agents Chemother.* **34**:1214–1219 (1990).
6. L. Resnick, J. R. Berger, P. Shapshak, and W. W. Tourtellotte. *Neurology* **38**:9–14 (1988).

7. J. C. McArthur, O. A. Selnes, J. D. Glass, D. R. Hoover and H. Bacellar. In R. W. Price and S. W. Perry (eds.), *HIV, AIDS and the Brain*, Raven Press, New York, 1993, pp. 251–272.
8. W. F. Elmquist and R. J. Sawchuk. *Pharm. Res.* **145**:267–288 (1997).
9. K. H. Dykstra, A. Arya, D. M. Arrioa, P. M. Bungay, P. F. Morrison, and R. L. Dedrick. *J. Pharmacol. Exp. Ther.* **267**:1227–1236 (1993).
10. B. K. Malhotra, M. Lemaire, and R. J. Sawchuk. *Pharm. Res.* **11**:1223–1232 (1994).
11. G. Paxinos and C. Watson. *The Rat Brain in Stereotaxic Coordinates*, Academice Press, California, 1986.
12. Y. Wang, S. L. Wong, and R. J. Sawchuk. *Pharm. Res.* **10**:1411–1419 (1993).
13. R. Masereeuw, U. Jaehde, M. W. E. Langemeijer, A. G. de Boer, and D. D. Breimer. *Pharm. Res.* **11**:324–330 (1994).
14. A. Odinecs, C. Nosbisch, and J. D. Unadkat. *Antimicrob. Agents Chemother.* **40**:1569–1571 (1996).
15. Y. Wang and R. J. Sawchuk. *J. Pharm. Sci.* **84**:871–876 (1995).
16. F. M. Balis, P. A. Pizzo, R. F. Murphy, J. Eddy, P. F. Jaronsinski, J. Falloon, S. Border, and D. G. Poplack. *Ann. Intern. Med.* **110**:279–285 (1989).
17. R. Spector. *Pharmacology* **40**:1–7 (1990).
18. S. L. Wong, K. Van Belle, and R. J. Sawchuk. *J. Pharmacol. Exp. Ther.* **264**:899–909 (1993).
19. E. M. August, E. M. Birks, and W. H. Prusoff. *Mol. Pharmcol.* **39**:246–249 (1991).
20. R. E. Bawdon, S. Kaul, and S. Sobhi. *Gynecol. Obstet. Invest.* **38**:1–4 (1994).
21. D. W. Miller and J. L. Maier. *Pharm. Res.* **12**:S381 (1995).