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Evaluation of a Brain-Targeting Zidovudine Chemical Delivery System in Dogs

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AIDS encephalopathy is an insidious complication of human immunodeficiency virus infection which is difficult to treat because of the poor uptake of many potentially useful antiretroviral drugs through the blood-brain barrier. A chemical delivery system (CDS) for zidovudine (AZT) based on redox trapping within the brain has been prepared and tested in several animal models to circumvent this limitation. The behavior of the AZT-CDS in the dog was considered. Parenteral administration of AZT resulted in rapid systemic elimination and poor uptake by the central nervous system. Ratios of the area under the concentration-time curve of AZT for cerebrospinal fluid to that for blood were 0.32, and ratios of the area under the concentration-time curve of AZT for brain to that for blood were approximately 0.25. Administration of an aqueous formulation of the AZT-CDS resulted in rapid tissue uptake and conversion of the CDS to the corresponding quaternary salt with the subsequent production of AZT. Delivered in this way, the levels of AZT in brain were 1.75- to 3.3-fold higher than those associated with conventional AZT administration. In addition, the levels of AZT in blood were 46% lower than those associated with AZT administration. The higher concentrations in brain and lower concentrations in blood combined to significantly increase the ratio of the concentration of AZT in the brain to that in blood after AZT-CDS administration compared to that after AZT dosing.

AIDS is a pernicious virally mediated disorder, the prognosis of which is usually death (24, 29, 37). The affliction is caused by human immunodeficiency virus type 1, a lentivirus that targets and destroys CD4 lymphocytes and in the process robs its victims of both cellular and humoral immunities (18, 43). One of the most destructive components of this disease is an encephalopathy and associated dementia spawned by direct viral infection of the brain (6, 17, 27, 28, 36, 39). Antiviral agents must therefore gain access to the central nervous system (CNS) in therapeutically relevant concentrations if AIDS dementia is to be adequately treated. Such pharmacokinetic considerations are often not met because of the ostensibly protective blood-brain barrier (BBB) which effectively prevents or reduces the entry of many hydrophilic drugs, including antiviral ribosides (35, 38).

The first drug approved for the treatment of AIDS was zidovudine (azidothymidine; AZT), an agent which exerts antiviral action through inhibition of reverse transcriptase, the enzyme responsible for transcription of viral RNA to proviral DNA (20, 46, 47). A variety of data suggest that improved delivery of AZT to the brain, which is not achieved concurrently with increased peripheral levels of the drug, may be helpful in combating the CNS component of AIDS. One method which may be useful in this regard is the chemical delivery system (CDS), a methodology that provides for targeting of drugs to the brain (3–5, 11, 12). The CDS method involves covalent attachment of a molecular targetor to the drug of interest and has been applied to many drugs including AZT (4, 16, 31). The synthesized conjugate is more lipophilic than the parent drug, resulting in improved BBB penetration and extensive distribution into tissue. Importantly, the targetor (a 1-methyl-1,4-dihydronicotinate) is readily converted from a lipophilic, membrane-permeable species to a hydrophilic, membrane-impermeable derivative through the formation of a nicotinate salt (the quaternary salt of AZT [AZT-Q^+]) (Fig. 1).

While the BBB acts as a barrier to the rapid loss of that portion of the oxidized targetor conjugate that is formed in the brain, the polar salt is readily lost from other compartments in the body resulting in selective CNS retention. The "lock-in" salt can then degrade to give rise to the parent drug, after which the spent carrier (1-methylnicotinic acid) is actively eliminated by efflux systems located at the choroid plexus. Various animals studies have confirmed the ability of the AZT-CDS to increase the levels of AZT in the brain relative to those achieved by administration of the parent drug. In addition, acceptable aqueous formulations of the AZT CDS have been developed (14). The current study was designed to examine the pharmacokinetic and tissue distribution behaviors of AZT-CDS in the dog relative to those of AZT.

MATERIALS AND METHODS

Chemistry and materials. AZT CDS (5′-[(1-methyl-1,4-dihydropyridin-3-yl)-3′-azido-3′-deoxythymidine) was synthesized by previously published procedures (13, 31). Briefly, AZT (ACIC, Inc., Brantford, Ontario, Canada) was acylated with nicotinic anhydride, generating the 5′-O-nicotinate. Alkylation of the ester with methyl iodide generated the *N*-methylnicotinate salt (AZT-Q^+) which was reduced with sodium dithionite to give the dihydronicotinate or AZT-CDS. The AZT-CDS potassium salt was produced by dissolving 20 g of the AZT-CDS (0.052 mol) in a methanolic solution containing a molar equivalent of potassium hydroxide. After 20 min, the solvent was removed and the residue was dried at 30°C for 2 h. The intravenous (i.v.) formulation was prepared by adding 30 mg of the AZT-CDS potassium salt per ml to a solution of 5 mM Na_3PO_4 and 15% (wt/vol) 2-hydroxypropyl-β-cyclodextrin (Roquette, Lestrem, France) (degree of substitution, 4.2). The final pH for the solution was 10.5, and the dosage form

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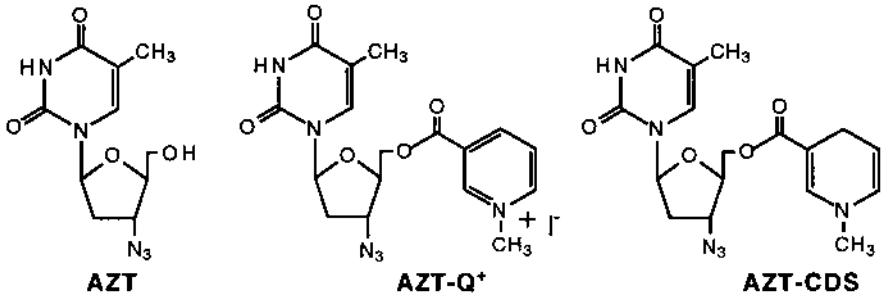

FIG. 1. Chemical structures of AZT, AZT-Q^+, and AZT-CDS.

was isoosmotic (282 ± 3 mosmol/kg) (14). A similar formulation of AZT was also prepared.

Analytical methodology. High-performance liquid chromatography was used to quantitate AZT, AZT-CDS, and the AZT-CDS oxidation product, AZT-Q^+, in blood, cerebrospinal fluid (CSF), urine, and brain. For all derivatives, the system configuration included a Waters model 510 pump, a Kratos Spectraflow 757 variable-wavelength detector, a Spectra-Physics model SP 8880 Autosampler, and a SpectraPhysics model SP 4270 integrator. Two analytical methods were used in this study: one for AZT-CDS and a second one for both AZT and the AZT-Q^+. For analysis of AZT-CDS, a C_8 Spherisorb analytical column (5-μm particle size, 25 cm by 4.6 mm [inner diameter]) was used; the column was fitted with a guard column containing pellicular C_{18} packing material. The mobile phase contained 55% ammonium acetate (0.05 M) and 45% acetonitrile. For AZT and the AZT-Q^+, analyses were completed with a C_{18} Adsorbosphere column (5-μm particle size, 25 cm by 4.6 mm [inner diameter]) fitted with a guard column by using a mobile phase containing 55% ammonium acetate (0.05 M), 25% deionized water, and 20% acetonitrile. In addition, the composite mobile phase contained 5 mM tetraethylammonium perchlorate. The pH of the system was adjusted to 5.5 with acetic acid. In all determinations, the flow rate was 1.0 ml/min, the injection volume was 20 μl, the compounds were detected at 266 nm, and analyses were conducted at ambient temperature.

Under the assay conditions used, AZT-CDS demonstrated a retention time of 5.8 min (relative standard deviation [RSD], 2%), and repeated injections gave reproducible peak heights (RSD, 4%). AZT and AZT-Q^+ gave retention times of 5.4 min (RSD, 1%) and 6.9 min (RSD, 2%), respectively, with peak area RSDs of 3%. The estimated limits of detection (LODs) and limits of quantitation (LOQs) were determined on the basis of signal-to-noise ratios of 3 and 10, respectively, by using the low concentration calibrant. LODs and LOQs for AZT were 40 and 100 ng/ml, respectively, those for AZT-Q^+ were 140 and 460 ng/ml, respectively, and those for AZT-CDS were 20 and 40 ng/ml, respectively. Concentrations in blood, CSF, and urine were determined by using an externally standardized curve (by using calibrants ranging from 0.01 to 10 μg/ml) corrected for extraction recoveries. Percent recoveries were determined by analyzing nondosed biological samples spiked with known quantities (1 to 10 μg/ml) of each analyte. For AZT, recoveries from blood, CSF, and urine were 89% ± 3%, 90% ± 3%, and 88% ± 7%, respectively, while those for AZT-Q^+ were 45% ± 5%, 43% ± 8%, and 48% ± 4%, respectively. AZT-CDS was extracted from all matrices in an essentially quantitative manner. Samples were stable in the described matrices over the time course of the analysis.

For preparation of animal samples for analysis, 0.5 to 1.0 ml of the appropriate biological matrix (blood, CSF, or urine) was treated with 1 volume of water and 4 volumes of ice-cold acetonitrile and homogenized (Polytron homogenizer). One milliliter of saturated saline was then added, and the samples were vortexed and stored at −20°C for 1 h. The organic phase was separated and injected as such in the case of AZT-CDS determinations. For AZT and AZT-Q^+, 0.25 ml of the acetonitrile extract was diluted with 0.75 ml of an aqueous solution of ammonium acetate (0.75 ml, 0.05 M [pH 2.5]), followed by high-performance liquid chromatographic analysis. The ammonium acetate served to improve chromatographic properties as well as to stabilize the AZT-Q^+ in solution. For brain tissue from animals in terminal studies, the material was homogenized in 1 volume of water, deproteinized with 4 volumes of acetonitrile, and then treated with 1 ml of saturated saline. After storage for 1 h at −20°C, the organic layer was removed and divided into two portions, one of which was assayed without further manipulations for AZT-CDS as described above. The second was diluted 1:4 with ammonium acetate (0.05 M) and was assayed for AZT and AZT-Q^+.

Animal studies. Healthy mongrel dogs weighing 20 to 35 kg were acclimatized and conditioned in the core facility at the University of Florida's Department of Animal Resources. Animals were fasted the night before the initiation of dosing and were anesthetized for the procedure with isoflurane. Dogs were catheterized bilaterally with over-the-needle catheters in both cephalic veins: one for drug administration and one for blood sampling. In addition, the urinary bladder was fitted with a Foley catheter. For CSF sampling, a sterile 22-gauge epidural cannula was inserted into the cisterna magnum to allow for serial withdrawal. In addition, dogs were hydrated i.v. with lactated Ringer's solution, which was administered continuously through the experimental session. Vital signs (blood pressure and heart rates) were measured noninvasively during treatment. Drugs (either AZT or AZT-CDS) were administered according to a two-way crossover paradigm with a 1-week washout period. Dogs were administered the AZT or AZT-CDS (potassium salt) formulations as short (5-min) infusions at doses of 17.2 and 27.5 mg/kg of body weight, respectively (equimolar to 25 mg of AZT CDS per kg). The injection volume was approximately 15 ml. Prior to the infusion, at the end of the infusion, and at 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, and 1,440 min after drug administration, 5 ml of blood was removed, collected in heparinized tubes, and immediately frozen. Similarly, at the end of the infusion period and at 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min, 1.0-ml samples of CSF were collected, as were urine samples at various times. In addition to these evaluations, a set of eight dogs was examined in a series of terminal studies. Dogs were prepared as indicated above. Four dogs received AZT (17.2 mg/kg; equimolar to 25 mg of AZT-CDS per kg), and four dogs received AZT-CDS potassium salt (27.5 mg/kg; equimolar to 25 mg of AZT-CDS per kg) as a short i.v. infusion. Blood and CSF samples were withdrawn from two animals from each group at the end of the infusion and at 5, 15, and 30 min. These four animals were then sacrificed 30 min after the infusion ended by pentobarbital overdose, and samples of cortex and cerebellum were removed, weighed, and frozen. The remaining four dogs were treated similarly, except that they were sacrificed 90 min after the infusion ended, with blood and CSF collected at the end of the infusion and at 15, 30, 60, and 90 min.

Pharmacokinetic analysis. Blood drug concentration and CSF drug concentration profiles were analyzed by using a nonlinear regression model (RSTRIP; 1989 version; MicroMath, Inc., Salt Lake City, Utah). A two-compartment model was used for compartmental kinetic analysis. Model selection was based on screening of average blood concentration-time data with five different exponential models, with subsequent analysis of the generated statistical parameters (including a model selection criterion). The area under the blood or CSF concentration-time curves extrapolated to infinite time (AUC_∞) were obtained from the regression program by using the linear trapezoidal rule. Parameters including the distribution or appearance half-life ($t_{1/2\alpha}$) and terminal elimination half-life ($t_{1/2\beta}$) were obtained directly from the regression software. The significance of differences in the means of calculated pharmacokinetic data was assessed by analysis of variance with post-hoc Tukey's comparison, with $P \leq 0.05$ considered statistically significant.

RESULTS

AZT dosing (crossover study). The administration of AZT to dogs resulted in high initial AZT levels (maximum concentration of drug in blood [C_{max}], 71 μg/ml), which fell in a biexponential manner (Fig. 2). The distribution phase was characterized with a $t_{1/2}$ of less than 5 min, while the elimination phase manifested a $t_{1/2}$ of approximately 130 min, which was linear over at least seven $t_{1/2}$s. Various compartmental and noncompartmental pharmacokinetic parameters are presented in Table 1. In the CSF, AZT appeared rapidly, with an estimated $t_{1/2}$ of 13 min. The elimination of AZT from the CSF was not statistically different from the $t_{1/2\beta}$ in blood. On the basis of the AUC ratios, levels in CSF were 32% of the concentrations in blood, while C_{max} values were 10% of the corresponding concentrations in blood. AZT was also detected in the urine at high levels starting at the first sampling time (60 min) and continuing at least until the 8-h collection point. As indicated in Fig. 3, almost 80% of the administered dose could be accounted for in the urine as unchanged AZT.

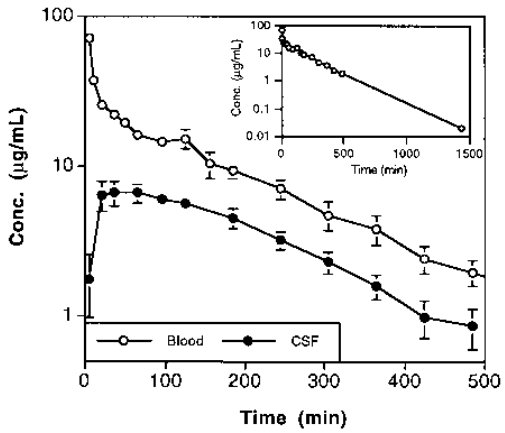

FIG. 2. Average concentrations (mean ± standard error of the mean) of AZT in the blood and CSF of five dogs after the administration of a 17.2-mg/kg i.v. dose of AZT. The insert includes the 24-h time point.

AZT-CDS dosing (crossover study). AZT-CDS (given as its potassium salt) was administered to a group of five dogs at a dose of 27.5 mg/kg (equimolar to 25 mg of AZT-CDS per kg), with the levels of AZT-CDS and its major metabolites, AZT-Q^+ and AZT, determined in blood, CSF, and urine. No AZT-CDS was detected in dogs 1 and 3. In other animals, material could be detected for up to 30 min, with average levels in CSF of 2.77, 1.34, and 0.4 μg/ml at the end of the infusion and at 15 and 30 min after the infusion, respectively, and average concentrations in blood of 2.38, 0.36 and 0.12 μg/ml at the end of the infusion and at 5 and 30 min after the infusion, respectively. The rapid disappearance of the AZT-CDS was correlated with an expeditious appearance of the AZT-Q^+ in both the blood and CSF compartment (Fig. 4 and Tables 2 and 3). Maximum concentrations of the quaternary salt were 41 μg/ml in blood and 3.9 μg/ml in CSF. In both cases, an apparent biphasic disposition was observed. Analysis of the urine indicated that AZT-Q^+ was directly excreted and that almost 15% of the AZT-CDS dose was eliminated as the quaternary salt. The ratio of the AZT-Q^+ concentration in CSF to that in blood was 0.36. The disappearance of AZT-Q^+ from blood and CSF was associated with an increase in the AZT concentration in both compartments. The AZT AUC as well as C_{max} values in CSF were 41% of the comparable values in blood, and as in the AZT dosing case, AZT was eliminated with equal efficiency from brain and CSF after AZT-CDS administration. The accumulation of AZT in urine after AZT CDS dosing was similar to that observed after AZT dosing, and when AZT-Q^+ and AZT amounts were summed, cumulative excretion curves for AZT and AZT-CDS administration were superimposable.

Brain drug concentrations. In a terminal study, eight dogs naive to either AZT or AZT-CDS were treated with either AZT (four dogs) or AZT-CDS (four dogs) and were sacrificed at either 30 or 90 min after treatment. Blood, brain (cortex and cerebellum), and CSF were sampled and analyzed, and the data are presented in Table 4. Since in most cases assay results were similar for both brain regions (cortex and cerebellum), comparisons were made by using the data for the cortex. AZT penetrated the brain poorly, giving concentrations that were approximately one-quarter of those detected in blood. AZT-CDS administration generated high AZT-Q^+ levels in the brain, amounting to 35 μg/g at 30 min (or close to 5% of the administered dose) and 22.2 μg/g at 90 min. These concentrations were more than 10- and 35-fold the corresponding levels

TABLE 1. Pharmacokinetic parameters for AZT in blood and CSF after i.v. administration of a 17.2-mg/kg dose

Dog no.	Blood				CSF			
	AUC (μg · min/ml)	C_{max} (μg/ml)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	AUC (μg · min/ml)	C_{max} (μg/ml)	T_{max} (min)[a]	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)
1	7,071	76.10	2.46	157.3	1,957	6.64	53.6	14.4	166.3
2	7,062	78.96	4.90	168.6	2,737	7.78	34.8	4.37	219.9
3	5,346	58.51	2.73	112.2	1,123	5.30	63.8	28.4	85.1
4	4,761	74.25	2.10	84.3	2,440	12.47	20.3	4.82	124.9
5	5,297	67.88	1.84	127.4	1,287	4.91	52.1	15.5	143.6
Mean ± SEM (SD)	5,907 ± 432 (964)	71.16 ± 3.24 (7.25)	2.81 ± 0.48 (1.09)	130.2 ± 13.7 (30.7)	1,908 ± 281 (628)	7.42 ± 1.22 (2.72)	44.9 ± 6.9 (15.4)	13.5 ± 3.9 (8.8)	148.0 ± 20.0 (44.8)

[a] T_{max}, time to C_{max}.

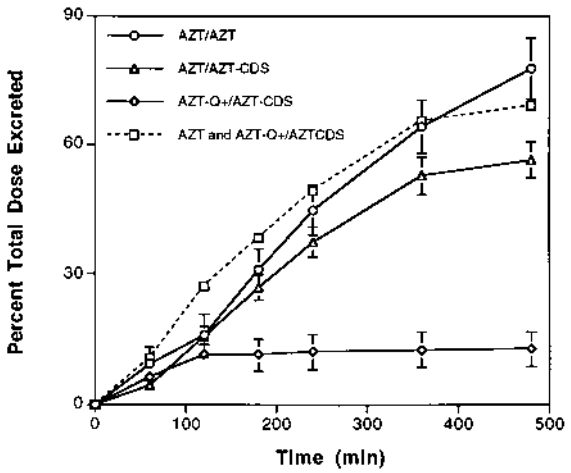

FIG. 3. Average cumulative urinary excretion (mean ± standard error of the mean) of AZT (after the administration of a 17.2-mg/kg dose of AZT) or AZT and AZT-Q^+ (after the administration of an equimolar 27.5-mg/kg dose of AZT-CDS, potassium salt) for a group of five dogs. The dotted line gives the sum of the amounts of AZT and AZT-Q^+ eliminated.

in blood at 30 and 90 min, respectively. Declining AZT-Q^+ levels were associated with increasing AZT levels, which were found to be 7.8 μg/g at 30 min and 10.9 μg/g at 90 min. These brain AZT levels were 23 and 100% higher than the corresponding levels in blood at 30 and 90 min, respectively, and 6.7- and 3.0-fold higher than the levels in CSF at 30 and 90 min, respectively. A comparison of AZT and AZT-CDS dosing indicates that AZT-CDS delivered 75% more AZT at 30 min and 3.3 times more AZT CDS at 90 min than did AZT administration.

DISCUSSION

Adequate treatment of AIDS encephalopathy requires delivery of antiviral agents to the brain at therapeutically relevant concentrations. In an attempt to provide for improved distribution of AZT in tissue while reducing systemic levels of the drug in blood, a brain-targeting redox CDS has been applied to AZT (1, 2, 9, 13, 14, 19, 23, 31, 32, 34). Experimental studies with this system suggest that AZT CDS has a pharmacokinetic advantage over AZT. Little et al. (31) found that systemic administration of AZT-CDS to rats produced three to four

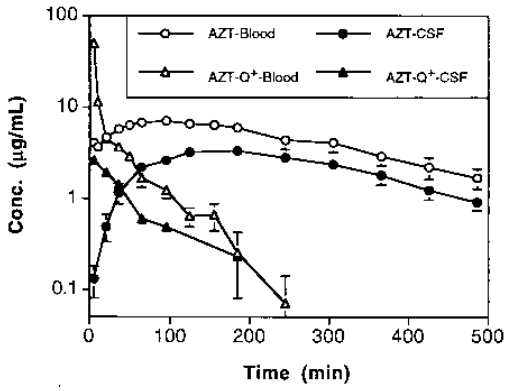

FIG. 4. Average concentrations (mean ± standard error of the mean) of AZT and AZT-Q^+ in the blood and CSF of five dogs after the administration of a 27.5-mg/kg i.v. dose of AZT-CDS, potassium salt.

TABLE 2. Pharmacokinetic parameters for AZT and AZT-Q^+ in blood after i.v. administration of AZT-CDS[a]

Dog no.	AZT					AZT-Q^+		
	AUC (μg · min/ml)	C_{max} (μg/ml)	T_{max} (min)[b]	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	AUC (μg · min/ml)	C_{max} (μg/ml)	T_{max} (min)[b]
1	2,932	7.53	64.4	25.8	196.9	378	9.67	3.65
2	3,352	8.67	52.9	88.4	123.1	604	80.6	0.16
3	2,202	6.30	14.5	59.8	91.3	226	10.5	1.65
4	4,035	8.82	27.6	46.4	199.2	853	87.8	3.08
5	3,366	7.95	36.1	49.7	125.2	674	16.4	0.01
Mean ± SEM (SD)	3,177 ± 269 (602)	7.86 ± 0.40 (0.91)	39.1 ± 7.9 (17.8)	54.0 ± 9.1 (20.4)	147.0 ± 19.3 (43.3)	547 ± 99 (221)	41.0 ± 15.8 (35.4)	1.71 ± 0.66 (1.48)

[a] AZT-CDS was administered as the potassium salt at 27.5 mg/kg.
[b] T_{max}, time to C_{max}.

TABLE 3. Pharmacokinetic parameters for AZT and AZT-Q^+ in CSF after i.v. administration of AZT-CDS[a]

Dog no.	AZT					AZT-Q^+	
	AUC (μg · min/ml)	C_{max} (μg/ml)	T_{max} (min)[b]	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	AUC (μg · min/ml)	C_{max} (μg/ml)	T_{max} (min)[b]
1	1,099	3.10	139	73.8	108.7	192	1.70	7.33
2	1,700	3.51	215	116	169.2	107	1.20	1.34
3	828	2.11	162	92.7	129.1	ND[c]	ND	ND
4	1,736	4.29	168	94.6	119.5	305	9.10	3.35
5	1,123	3.05	149	81.6	113.6	182	3.75	5.97
Mean ± SEM (SD)	1,297 ± 160 (359)	3.21 ± 0.32 (0.70)	166 ± 12 (26)	91.9 ± 6.5 (4.5)	128.0 ± 9.7 (21.7)	197 ± 35 (71)	3.93 ± 1.56 (3.13)	4.49 ± 1.15 (2.30)

[a] AZT-CDS was administered as the potassium salt at 27.5 mg/kg.
[b] T_{max}, time to C_{max}.
[c] ND, not determined.

TABLE 4. Individual and mean AZT levels after AZT administration or AZT-Q^+ and AZT concentrations after AZT-CDS administration in blood, CSF, cortex, and cerebellum 30 and 90 min after administration of doses of 17.2 mg of AZT per kg or 27.5 mg AZT-CDS (potassium salt) per kg, respectively

Formulation and dog no.	Time (min)	AZT or AZT-Q^+ concn (μg/ml or μg/g)			
		Blood	CSF	Cortex	Cerebellum
AZT					
A	30	18.49	3.92	4.93	5.10
B	30	14.52	3.18	3.63	3.89
Avg		16.51	3.55	4.28	4.50
C	90	9.52	3.59	3.17	3.61
D	90	11.77	8.30	2.93	4.50
Avg		10.65	5.95	3.05	4.06
AZT-CDS[a]					
E	30	5.83	0.71	6.88	7.04
F	30	6.33	1.52	8.10	8.29
Avg		6.08	1.12	7.49	7.67
G	90	5.11	3.72	12.64	13.09
H	90	5.19	3.08	7.82	8.62
Avg		5.15	3.40	10.23	10.86
AZT-CDS[b]					
E	30	3.23	0.40	36.74	35.63
F	30	3.20	1.28	34.13	34.35
Avg		3.22	0.84	35.44	35.00
G	90	0.66	1.21	25.05	26.06
H	90	0.58	0.49	19.40	18.30
Avg		0.62	0.85	22.23	22.18

[a] Concentrations are those of AZT.
[b] Concentrations are those of AZT-Q^+.

times more AZT in brain than did AZT dosing. Other studies were similarly encouraging in demonstrating an increase in the ratios of the concentration in brain to the concentration in blood after AZT-CDS treatment compared with those after AZT administration. Chu et al. (19), for example, found a 10-fold increase in the AZT AUC for the brain after AZT-CDS dosing compared to that after AZT treatment in mice, while Brewster et al. (9) demonstrated three- to fourfold increased delivery to the brain in rabbits. In rats, the improvement in AZT delivery after AZT-CDS administration was found to be dose dependent, with the ratios of the concentration in the brain to the concentration in blood increasing from 20% at the 10-mg/kg dose to almost 80% at the 50-mg/kg dose (15). Mizrachi et al. (34) and Aggarwal and colleagues (1, 25) found not only that AZT-CDS is more effective in inhibiting human immunodeficiency virus replication than AZT in vitro but also that it is less toxic to the host lymphocytes than AZT. A major advancement in the development of AZT-CDS was the configuration of an aqueous dose form based on a cyclodextrin excipient (2, 7, 8, 10). This formulation is produced by using the potassium salt of AZT-CDS and is nonirritating and isoosmotic and does not contain organic cosolvents.

Administration of AZT to dogs yields a pharmacokinetic profile in keeping with the hydrophilic character of the drug. It is readily eliminated from the blood in a biphasic manner, consistent with other published reports (22, 30). Penetration into the CSF is rapid, but the drug levels in CSF are only one-third of the concentrations in blood. Similarly, the con-

centrations of AZT in brain are lower than the levels in blood, with ratios of the concentration in the brain (cortex) to that in blood being of 0.26 and 0.29 at 30 and 90 min, respectively. The poor uptake of AZT by the dog brain has been similarly reported by Gallo et al. (22). These lower levels of drug in the CNS may reflect two simultaneous effects: poor uptake and active elimination. Terasaki and Pardridge (41) found that the uptake of AZT at the BBB was very low and suggested that the nucleoside gained access to the CSF by way of the choroid plexus. Other studies, however, suggest that the uptake of AZT may in fact be BBB mediated and that the low concentrations observed are not due to poor penetration but rather to active AZT loss from the brain (33, 42). Sawchuk and colleagues (40, 44, 45) found that the elimination of AZT from brain and blood was reduced by probenicid, an inhibitor of weak acid transport at the level of the CSF and kidneys. Microdialysis studies have shown, for example, that probenicid increases the AUC for AZT in brain (thalamic extracellular fluid) by five- to sixfold. Dykstra et al. (21) reported similar findings in rats, i.e., that the limited uptake of AZT is related to its efficient elimination from brain rather than to its poor uptake by the brain. While AZT undergoes extensive glucuronidation in humans and monkeys to the 5′-O metabolite (G-AZT), it is principally excreted unchanged in lower species including dogs, rabbits, rats, and mice. In the current study, the cumulative urinary excretion of AZT amounted to approximately 75% by 8 h, consistent with several published reports (26).

The administration of AZT-CDS provided for extensive distribution of the injected delivery system as well as its metabolites. AZT-CDS rapidly disappeared from blood and CSF, with detectable levels present only at early times, consistent with its designed metabolic lability and large octanol:water partition coefficient (log $P = \sim 1.5$). This disposition is also consistent with the rapid appearance, at high concentrations, of AZT-Q^+ in the blood, CSF, and brain compartments. The disappearance of AZT-Q^+ from each of the three compartments is related to both metabolism, i.e., hydrolysis to AZT, and elimination. In blood, AZT-Q^+ is eliminated as such into the urine, accounting for approximately 15% of the administered AZT-CDS dose. Urinary data suggest that the majority of the AZT-Q^+ being disposed of by the kidney is eliminated during the first 2 h after dosing, which correlated with data on the levels in blood. In CSF, AZT-CDS was also efficiently converted to AZT-Q^+, as evidenced by high initial levels. In the brain, AZT-Q^+ is highly concentrated, reaching levels in the cortex of 35 μg/g at 30 min and 22.2 μg/g at 90 min. These concentrations are significantly greater than those generated in blood and CSF, indicating that their source is the delivered AZT-CDS (administration of the quaternary salts themselves do not result in significant uptake by the brain). The very large ratio of the concentration in the brain to the concentration in blood for AZT-Q^+ is consistent with AZT-Q^+ trapping behind the BBB, which is predicted for CDS. Decreases in AZT-Q^+ as a function of time are associated with increasing AZT levels in the brain, even though levels in blood are falling. It is clear, therefore, that a significant portion of AZT in the brain is derived from AZT-Q^+ and not from AZT in blood. This is punctuated by results from studies of AZT administration in which the calculated ratios of the concentration in blood to the concentration in the brain are on the order of 0.2 to 0.3. AZT-CDS generates AZT ratios of the concentration in the brain to the concentration in blood for AZT of almost 2 at 90 min.

A comparison of the AZT levels after delivery by either AZT-CDS or AZT dosing suggests an advantage for the CDS. Thus, while the levels of AZT in the brain were almost 2- and 3.3-fold higher at 30 and 90 min after AZT-CDS dosing, respectively, the levels in blood were on average 46% lower after delivery by AZT-CDS than by AZT dosing. This combination of lower concentrations in blood and higher concentrations in the brain contributed to a dramatic increase in the ratios of the concentration in the brain to the concentration in blood, as illustrated in Fig. 5. Data for the concentrations in CSF were interesting in this context. After AZT administration, the levels of the parent compound in CSF are a relatively good estimator of the concentrations in brain tissue, while they are a poor reflection of the levels in blood. By contrast, AZT-CDS administration generates AZT levels in CSF that poorly mirror brain AZT concentrations, while they are a relatively good indicator of the levels in blood. This disconnection of traditional pharmacokinetic dependencies is a consequence of the trapped AZT-Q^+ in CNS, which acts as the major AZT source for the brain after AZT-CDS administration. In the case of AZT dosing, blood-borne and CSF-laden AZT represents the sole source of the antiviral agent for the brain. Another interesting finding with AZT-CDS is that it appears to become more efficient in higher species. In comparing similar doses (25 mg/kg) and sampling times (30 min), the ratio of the concentration in the brain to that in blood increases from 0.2 in rats, to 1.0 in rabbits, to 1.2 in dogs (11, 18). The ratios are even higher at later times, but the relative levels of enhancement are similar. The improved functioning of CDS may be due to different enzymatic (esterase or oxidoreductase) activities, which are known to be highly species specific.

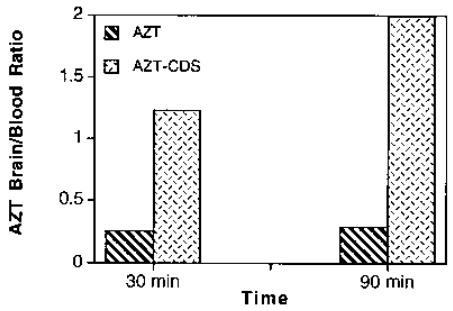

FIG. 5. Ratios of the concentrations of AZT in the brain (cortex) to that in blood AZT at 30 and 90 min subsequent to the administration of either a 17.2-mg/kg dose of AZT or an equimolar dose (27.5 mg/kg) of AZT-CDS.

In conclusion, a CDS for targeting AZT to the brain was tested in dogs. Relative to AZT dosing, AZT-CDS delivered higher concentrations to the brain and lower concentrations to blood, resulting in significant increases in the ratios of the concentrations in blood to the concentrations in the brain. In addition, CNS trapping of the AZT-CDS metabolite AZT-Q^+ and slower elimination of the salts from the CSF were demonstrated. The improved delivery to the brain and decreased potential for dose-limiting side effects suggest that AZT-CDS may be a useful adjunct for the treatment of AIDS encephalopathy and related dementias.

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