Dose-Dependent Brain Delivery of Zidovudine Through the Use of a Zidovudine Chemical Delivery System

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INTRODUCTION

Treatment of acquired immune deficiency syndrome (AIDS) encephalopathy and dementia requires that effective agents be delivered to the site of infection in an efficient and sustained fashion (1). Such a profile is often not possible for many potentially useful antiviral agents because of their poor membrane penetration and pharmacokinetic properties. A germane example is zidovudine (azidothymidine, AZT) which exerts significant activity against peripheral manifestation of the causative viral pathogen, human immunodeficiency virus (HIV). Because of its poor residence time in the CNS and resulting low central drug concentrations, AZT has only marginal antiretroviral activity against central complications (2). The need to access the centrally sequestered virus is, however, acute based on both clinical findings that suggest a majority of AIDS patients demonstrate symptomotology of HIV involvement in the brain and because more than 90% of AIDS patient express neurohistological alterations at autopsy (3). One method which may be applicable to improving CNS uptake and retention of AZT is the chemical delivery system (CDS), a methodology that provides for brain targeting of drugs through redox trapping (4). A derivative designed along these lines, AZT-CDS (Figure 1), was found to produce three to ten times as much AZT in brain than did AZT dosing in various species including the rat, rabbit, mouse and dog (5–7). Aggarwal et al. and Mizrachi et al. found that not only was the AZT-CDS more effective in inhibiting HIV replication than was AZT in vitro, but it was also less toxic to the host lymphocytes than AZT (7,8). The current study was designed to examine the effect of AZT-CDS dose on AZT delivery and linearity.

MATERIAL AND METHODS

Chemistry. The lead compound, 5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]-3′-azido-3′-deoxythymidine (AZT-CDS), was prepared according to previous published procedures (5). The prototype formulation used in animal work was prepared by adding 30 mg/mL of the AZT-CDS potassium salt (prepared by reacting the AZT-CDS with methanolic potassium hydroxide followed by solvent removal) to a solution of 5 mM Na_3PO_4 and 15% w/v 2-hydroxypropyl-β-cyclodextrin (9). The final pH for the solution was 10.5 and the system was isoosmotic (280 mOsm).

Analytical Methodology. High performance liquid chromatography (HPLC) was used to separate, detect and quantitate AZT, AZT-CDS and the AZT-CDS oxidation product (AZT-Q+). For all derivatives, the system configuration included a Perkin-Elmer Model ISS-100 autosampler, a SpectraPhysics Model SP8810 pump, a SpectraPhysics Model 100 variable wavelength (UV/Vis) detector and a SpectraPhysics Model SP 4270 integrator. For the AZT-CDS, a Spherisorb C8, 5 μm particle size, 25 cm × 4.6 mm i.d. analytical column was used with an upstream 5 μm in-line filter. The mobile phase contained 45:55 acetonitrile: 0.05 M ammonium acetate buffer (pH 7.2) and the flow rate was 1.0 mL/min. The AZT-CDS was detected at 266 nm at ambient temperature and eluted at 5.6 min. For the AZT and AZT-Q+, an Absorbosphere C18, 5 μm particle size 25 cm × 4.6 mm i.d. analytical column was used. The mobile phase contained 55:20:25 ammonium acetate (0.05 M): acetonitrile: water and was adjusted to a pH of 5.5 with glacial acetic acid. The flow rate was 1.0 mL/min; determinations were completed at ambient temperature and the derivatives were detected at 266 nm. Under these conditions, the retention times for AZT and the AZT-Q+ were 5.41 and 7.17 min, respectively. The relative standard deviation (RSD) was less than 4% for all measurements. The limits of detection (in μg/mL for blood or brain homogenate) ranged from 0.01 to 0.05 while the limits of quantitation ranged from 0.03 to 0.18.

Animal Studies. Groups (n = 5) of adult, male Sprague Dawley rats, weighing 175 to 225 g, were administered various doses (26, 53, 77, 103 and 130 μmol/kg) of either the AZT-CDS formulated as described above or AZT in a similar formulation via the tail vein. These doses corresponded to 10, 20, 30, 40 and 50 mg/kg of AZT-CDS and 6.9, 13.8, 22.6, 27.5 and 34.4 mg/kg of AZT. Animals were killed by rapid decapitation at 0.25, 1.0 and 4.0 hours after an injection of AZT and at 15, 30, 60, 120 and 240 min after administration of the AZT-CDS. Immediately upon sacrifice, trunk blood was collected into heparinized (0.15 mL per tube) tubes, capped and inverted thrice to thoroughly mix the blood and heparin to prevent clotting. The entire brain was removed, weighed and frozen on dry ice. The tissues or blood were then stored at −5°C prior to homogenization. One mL of deionized water was added to either 1.0 mL of whole blood or whole brain. Each tissue was homogenized for 3–5 min using a Polytron Model PT-1200C homogenizer. To each homogenate was then added 4.0 mL of acetonitrile and the system was vortexed. Concentrated saline (1.0 mL) was then added and the system was allowed to settle at −5°C for one hour. The organic phase which separated under these conditions was removed, filtered, diluted 1:4 with 0.05M ammonium acetate, transferred to autosampler vials and submitted for HPLC analysis as described above.

Pharmacokinetics. Area under the curve (AUC) values were generated using either the trapezoid rule or were obtained by model fitting using the RStrip software package (Micromath, Salt Lake City, Utah).

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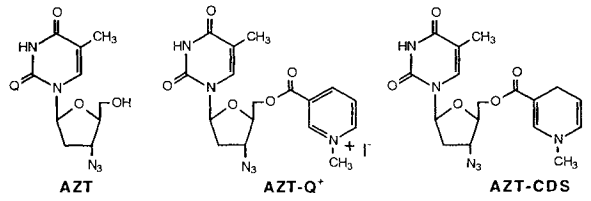

Fig. 1. Chemical structures for AZT, AZT-Q+, and AZT-CDS.

RESULTS AND DISCUSSION

Administration of the parent drug, AZT, to rats yielded a pharmacokinetic profile consistent with the polar nature of the compound. Blood concentrations of AZT after i.v. administration were initially high but fell rapidly as a function of time. Highly linear relationships between dose and blood area under the curve (AUC) have been observed by several investigators suggesting linear pharmacokinetics for AZT in rats over the 10 to 60 mg/kg dose range (10–12). The limited pharmacokinetic assessment provided for AZT herein is in good agreement with these published data. In addition, the current evaluations as well as previously reported studies have found that AZT levels in brain are low after AZT dosing. De Miranda et al. demonstrated that brain levels of AZT were less than 7% of plasma levels 15 min after a 10 mg/kg dose of ^{3}H-AZT (13). Likewise Galinsky et al. found brain to blood ratios (determined by comparisons of brain and blood AUC) of approximately 2–3% in rats after a 6.7 mg/kg dose, Gallo estimated a brain to blood ratio of AZT of 6.2% after multiple AZT administration to rats and Doshi et al. determined a brain to serum ratio of 6.4% after a 50 mg/kg dose to mice (12,14,15).

Administration of AZT-CDS provides for extensive distribution of the injected delivery system with rapid formation of CDS metabolites (the highest dose group is given in Figures 2 and 3). In blood, the AZT-CDS and AZT-Q+ fell rapidly in concentration between the 15 min and one hour time points and, in all cases, were totally cleared by 120 min. The AUC for AZT in blood derived from the AZT-CDS is 35 to 45% lower than that produced by the parent compound administration consistent with its designed lower availability. As with AZT dosing, the AZT produced from AZT-CDS is highly correlated with dose suggesting linear pharmacokinetics over the dosing range. In brain, AZT-CDS was detectable only at 15 min in agreement with its large volume of distribution and metabolic lability. The high AZT-Q+ levels in brain confirm that the AZT-CDS was readily incorporated by the CNS after which it was metabolically converted to the corresponding quaternary salt (Figure 3). The basis for this assignment is that administration of the polar AZT-Q+ salt resulted in undetectable brain levels of AZT-Q+ (M. Brewster, unpublished results). Importantly, while blood AUC values for AZT (derived from AZT-CDS dosing) were linearly related to dose, brain AUC values were not. As illustrated in Figure 4, higher AZT-CDS doses provided for disproportionately higher AZT brain AUC values.

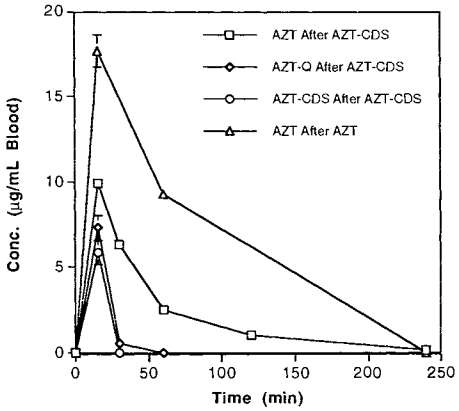

Fig. 2. Blood concentrations of AZT after i.v. administration of AZT (50 mg/kg, 130 mol/kg) of of AZT, AZT-Q+, and AZT-CDS after an equimolar dose of the AZT-CDS.

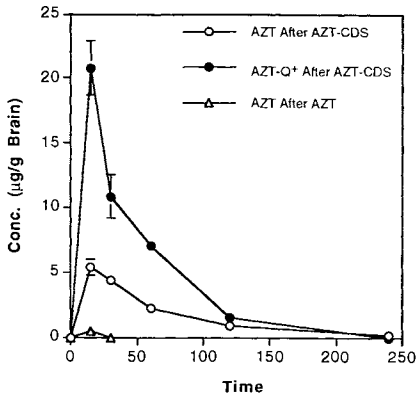

Fig. 3. Brain concentrations of AZT after i.v. administration of AZT (50 mg/kg, 130 mol/kg) of of AZT and AZT-Q+ after an equimolar dose of the AZT-CDs.

The observed nonlinearity may be associated with several events. The poor brain to blood ratio observed after administration of AZT has been attributed to a variety of causes. Terasaki and Pardridge, using a brain uptake index technique, found that uptake of AZT at the BBB was very low, presumably due to limited membrane permeability, suggesting that the majority of AZT accessed the CNS through the choroid plexus (16). Recent 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. Sawchuk et al. found that the elimination of AZT from brain and blood was reduced by probenicid, an inhibitor of weak acid transport at

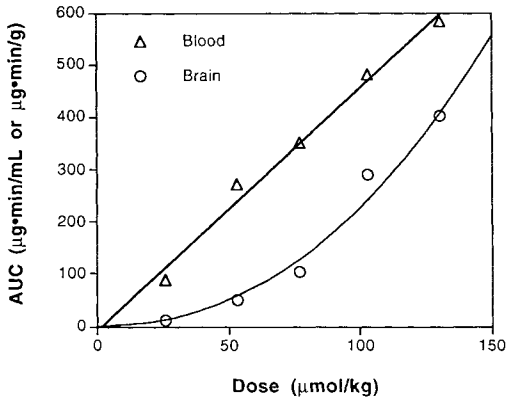

Fig. 4. A comparison of AUC values in blood and brain for AZT determined after AZT-CDS administration at various doses.

the level of the CSF and kidneys (17). Microdialysis studies have shown, for example, that probenicid increases the AUC for AZT in brain (thalamic extracellular fluid) by 5 to 6-fold. In rats, Dykstra et al. reported similar findings i.e., that the limited uptake of AZT was related to its efficient elimination from brain rather than to its poor brain uptake (18). These data would suggest that delivery of high AZT levels may saturate the active elimination mechanism providing for slower brain efflux and higher than expected AZT concentrations.

The combination of higher brain and lower blood levels provides for a large increase in the AZT brain to blood ratio for AZT-CDS as a function of dose with the ratio values ranging from 20% at the 10 mg/kg dose to almost 80% at the 50 mg/kg levels. These same results were hinted at by the work of Doshi et al. in mice. In these studies, increasing doses of AZT from 50 to 250 mg/kg resulted in a disproportionate increase in brain to blood ratios from 6.4% to 28% (33). The increased brain to blood ratio achieved by the AZT-CDS at relatively low doses is consistent with enhanced brain delivery and reduced peripheral levels of AZT.

In conclusion, these data indicate that the AZT-CDS induces a unique pharmacokinetic profile for AZT in the rat since higher doses of the AZT-CDS generate disproportionately higher brain levels of AZT while blood levels were dose-related. The result of this manipulation is a dramatic increase in the brain to blood ratio as a function of dose. The basis for this effect may be related to autoinhibition of AZT efflux due to the high AZT levels in the CNS. While such a mechanism is plausible, other effects such as interaction of AZT-CDS or AZT-Q+ with the active pump are also possible. These pharmacokinetic advantages offered by the AZT-CDS may be useful in the treatment of AIDS encephalopathy and dementia.

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