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Formulation development for a zidovudine chemical delivery system
1. Parenteral dosage forms

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Abstract

A chemical delivery system for zidovudine (AZT-CDS) has been shown to increase brain levels of the parent antiretroviral agent while at the same time reducing blood concentrations. Such selectivity may improve the therapeutic index for AZT. Unfortunately, the AZT-CDS is lipophilic and labile to oxidative and hydrolytic degradation thereby complicating the development of a convenient formulation. The configuration of several potentially acceptable parenteral dosage forms using cyclodextrin-based systems are described herein. A prototype formulation was developed using the AZT-CDS potassium salt in an aqueous matrix of 2-hydroxypropyl-β-cyclodextrin (HPβCD) (15% w/v) and Na_3PO_4 (0.005 M). While alkaline, the formulation was associated with a low buffering capacity and was not irritating in a rat tail model of extravasation. Systemic administration of this dosage form provided for, in addition to improved brain levels of AZT and an increased brain to blood ratio, improved bioavailability compared to a dimethyl sulfoxide (DMSO) vehicle.

Keywords: Zidovudine; Chemical delivery system; Parenteral dosage form

1. Introduction

Preclinical evaluation of a brain-targeting zidovudine (azidothymidine, AZT) chemical delivery system (CDS) suggests that this agent may be a useful adjunct in the treatment of acquired immune deficiency syndrome (AIDS) and associated collateral complications such as AIDS encephalopathy and dementia (Little et al., 1990; Chu et al., 1990; Gallo et al., 1990; Brewster et al., 1991, 1993; Lupia et al., 1993). The object compound, 5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3′-azido-3′,5′-dideoxythymidine or AZT-CDS, is designed to readily penetrate the blood-brain barrier (BBB) after which the targe-

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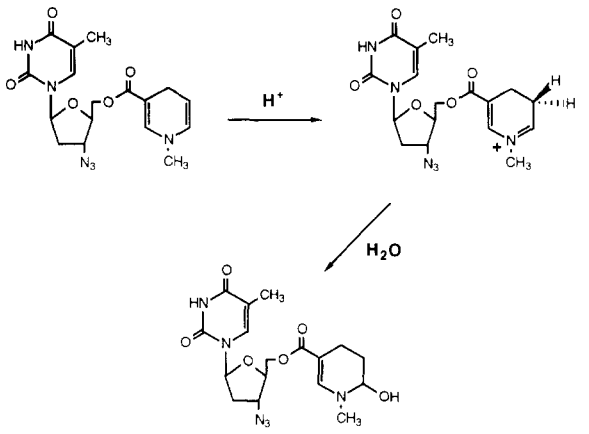

Structure 1–3.

tor function is converted, through oxidation, to the corresponding nicotinate salt (AZT-Q+). The dramatic reduction in lipophilicity associated with this conversion provides for trapping of the AZT-Q+ in the central nervous system (CNS) relative to peripheral sites. The centrally delivered salt can then hydrolyze releasing the parent antiviral agent. Studies have demonstrated that the AZT-CDS selectively increases brain versus blood levels of AZT in a variety of test animals including mice, rats, rabbits and dogs (Chu et al., 1990; Gallo et al., 1990; Little et al., 1990; Brewster et al., 1991, 1993; Lupia et al., 1993). Furthermore, the AZT-CDS is more active in vitro in inhibiting human immunodeficiency virus (HIV) replication than is the parent drug due to better intracellular penetration and AZT delivery (Gogu et al., 1989; Aggarwal et al., 1990; Mizrachi et al., 1994).

The AZT-CDS is designed to be lipophilic to allow for rapid BBB transit and enzymatically labile to provide for AZT-Q+ formation and hydrolysis. These features, while essential for CDS operation, complicate the development of useful formulations in that the increased lipophilicity of the AZT-CDS reduces water solubility and the designed lability can act to attenuate shelf-life. The herein reported efforts were undertaken to identify potentially acceptable formulations for the AZT-CDS with the current paper describing parenteral dosage forms and the subsequent communication describing approaches that may be applicable to oral and other non-parenteral formulations. While the majority of AZT produced is intended for oral use, the development of an acceptable parenteral product was deemed important for several reasons. First, AIDS encephalopathy affects a majority of AIDS patients and there is presently no generally effective approach to treat this complication (Grant and Heaton, 1990; Graham et al., 1991; Reinvang et al., 1991; Pajeau and Roman, 1992). Any product which provides for amelioration of this infection including i.v. systems would find acceptance. Second, an i.v. formulation is necessary as a pharmaceutical tool to assess bioavailability of the AZT-CDS after drug administration through other administration routes. Third, while specialized, there are clear circumstances where an i.v. product is indicated. In a recently completed human evaluation sponsored by the AIDS Clinical Trial Group (ACTG 076), a combination of i.v. and oral dosing of peripartum women and their offspring resulted in a significant reduction in the rate of HIV vertical transmission from approx. 24 to 8%. An i.v. preparation of the AZT-CDS may therefore find ready acceptance in such clinical situations.

Several potential vehicles were considered for the AZT-CDS including organic co-solvents, electroneutral detergents, the use of pH adjustments and chemically modified β-cyclodextrins. Criteria for an acceptable formulation included adequate solubilization of the AZT-CDS (10–50 mg/ml), low potential for venous irritation and extravasation injury, similar or improved distributional characteristics compared to AZT-CDS solubilized in organic co-solvents (dimethyl sulfoxide, DMSO) and a low systemic toxicological profile.

2. Materials and methods

2.1. Chemistry and supplies

The AZT-CDS (5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]-3′-azido-3′-deoxythymidine) was prepared according to previous published proce-

dures (Little et al., 1990; Brewster et al., 1993). Briefly, AZT (ACIC, Inc., Canada) is acylated with nicotinic anhydride generating the 5′-*O*-nicotinate. Alkylation of the nicotinate with methyl iodide generates the nicotinate salt (AZT-Q+) which upon sodium dithionite reduction gives rise to 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 (52 ml of a 1.0 N solution). After stirring the solution for 10 min, the solvent was removed and the residue dried at 30°C for 2 h. The potassium salt (22 g, 99% yield) was obtained as a yellow powder which was > 97% pure by HPLC. Organic co-solvents including dimethyl sulfoxide (DMSO), dimethyl acetamide (DMA), benzyl alcohol, propylene carbonate, polyethylene glycol, tetraglycol, ethanol, ethylene glycol, propylene glycol, detergents including Tween 80 and Cremophor and pharmaceutically acceptable oils including safflower and corn oil were obtained from Sigma Chemical Co. and were used without further purification. 2-Hydroxypropyl-β-cyclodextrin (HPβCD, degree of substitution = 7) was obtained from Pharmos, Corp., heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCD) was obtained from Cyclolabs, Inc., Budapest, Hungary and maltosyl-β-cyclodextrin (G2βCD) was obtained from Ensuiko Sugar Co., Yokohama, Japan.

2.2. 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 acetonitrile 0.05 M ammonium acetate buffer (pH 7.2) (45:55) and the flow rate was 1.0 ml/min. The AZT-CDS was detected at 266 nm at ambient temperature. Under the above indicated conditions the AZT-CDS eluted at 5.6 min. For the AZT and AZT-Q+, a different chromatographic system was necessary. In this case, 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.

2.3. Solubilization studies

The extent of solubilization of AZT-CDS in various organic co-solvents was estimated by several methods. For solvent systems in which AZT-CDS demonstrated relatively poor solubility, 100 mg of the AZT-CDS were equilibrated in 10 ml of the solvent. The system was then centrifuged and the supernatant assayed by HPLC with or without dilution with acetonitrile or mobile phase. For more soluble systems, 1.0 ml aliquots of an appropriate solvent were added to samples of 50, 100, 250, 500 and 750 mg of AZT-CDS. Once a solubility estimate was determined, the degree of solubilization of the AZT-CDS was calculated by addition of 1.0 ml of solvent to AZT-CDS at 25 mg increments near the initial solubility estimate. For example, if 100 mg of AZT-CDS were soluble (in 1.0 ml of solvent) but 250 mg were not, samples containing 125, 150, 175 mg, etc., would be prepared, treated with 1.0 ml of solvent and assessed for solubilization.

2.4. Cyclodextrin studies

Several solubilization techniques were applied. First, an excess of AZT-CDS was added to a 43.5% w/v solution of HPβCD or 40% solutions of DMβCD or G2βCD after which the samples were stirred or sonicated for one to 4 h. The suspensions were then filtered and the filtrate freeze-dried (Labconco Freeze Dryer Model 4.5)

to produce the powdered complex. The rate of incorporation was then determined by analysis of a weighed amount of the powder (Higuchi and Connors, 1965). A second approach for solubilization of the AZT-CDS in cyclodextrin utilized preparations of AZT-CDS in appropriate cosolvents such as DMSO (500 mg/ml), followed by addition of small volumes of these solutions to aqueous HPβCD (43.5% w/v). The systems were then stirred, filtered, lyophilized and assayed. The third type of complex formation involved dissolution of AZT-CDS in ethanol (50 mg/1.0 L) or other organic solvents followed by an addition of 1.0 g of HPβCD. After stirring, the solvent was removed under reduced pressure and the residue reconstituted with water (2.0 ml), filtered and lyophilized (Pitha and Hoshino, 1992; Pitha et al., 1992). The degree of incorporation was determined by HPLC analysis. Solutions of the AZT-CDS potassium salt were prepared in aqueous HPβCD containing Na_3PO_4. The amount of HPβCD varied from 5 to 43.5% while Na_3PO_4 concentrations varied from 5 to 500 mM. Solution osmolality was determined by freezing point depression using an Advanced Instrument Digimatic Osmometer Model 3D2.

2.5. Venous irritation

Adult, Sprague-Dawley, male rats (body weight 250 g, $n = 5$) were administered several test solutions in the tail vein including polyethylene glycol/ethanol (2:1), propylene glycol/ethanol/water (3:1:1), propylene glycol/ethanol/water (4:1:5), HPβCD (15% w/v) in 50 mM Na_3PO_4, 25 mg/ml AZT-CDS potassium salt in HPβCD (15% w/v) in 50 mM Na_3PO_4 and normal saline. A 27 gauge (1/2 inch) needle was used for all administrations. Treatments (0.5 ml/kg) were given twice daily (09:00 and 15:00 h) for five consecutive days. At various times during and after the injection series, the tail was examined for signs of distress by an observer blinded to the treatments. Venous irritation was estimated using the following scoring system: 1, normal; 2, slight erythema at the injection site; 3, inflammation surrounding the injection site and evidence of edema; 4, contusions surrounding the injection site; 5, necrosis at the distal end of the tail and general deterioration of the entire tail.

2.6. Tissue distribution

Groups ($n = 5$) of adult, male Sprague-Dawley rats, weighing 175–225 g were administered various doses (26, 53, 77, 103 and 130 μmol/kg) of either the AZT-CDS formulated in DMSO, the AZT-CDS potassium salt prepared in 15% w/v HPβCD containing 0.005 M Na_3PO_4 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, 11, 22, 33, 44 and 55 mg/kg of the K + AZT-CDS and 6.9, 13.8, 22.6, 27.5 and 34.3 mg/kg of AZT. Animals were killed by rapid decapitation at 0.25, 1.0 and 4.0 h after injection of the DMSO or cyclodextrin-based systems. Immediately upon death, trunk blood was collected into heparinized (0.15 ml per tube) tubes, capped and inverted thrice to thoroughly mix the blood and heparin and prevent clotting. The entire brain or, in some cases, approximately 1.0 g of liver was removed, weighed and frozen on dry ice. The tissues or blood were then stored at −5°C prior to homogenization.

1 ml of deionized water was added to either 1.0 ml of whole blood, whole brain or 1.0 g of liver. Each tissue was thoroughly homogenized using a Polytron Model PT-1200C homogenizer. To each homogenate was then added 4.0 ml of acetonitrile and the system was vortexed. Concentrated brine (1.0 ml) was then added and the system was allowed to settle at −5°C for 1 h. The organic phase which separated under these conditions was removed, filtered, diluted 1:4 with 0.05 M ammonium acetate, transferred to autosampler vials and submitted for HPLC analysis. Sample stability was assessed during the storage conditions (acetonitrile: buffer at freezer, refrigerator and room temperatures). No degradation was observed for periods in excess of 1 week of low temperature storage or for 24 h at room temperature.

2.7. Pharmacokinetics and statistics

Area under the curve (AUC) values were generated using the trapezoid rule. In all cases, the

significance of differences between data means were analyzed using one-way ANOVA with post-hoc Tukey's comparison. For all tests, the level of probability was $p < 0.05$.

3. Results and discussion

3.1. Formulation development

The solubility of AZT-CDS in a variety of solvents, including several deemed to be pharmaceutically acceptable, is presented in Table 1. The greatest degree of solubilization was afforded by DMSO, the solvent used in preliminary animal trials, followed by dimethylacetamide which has been used in experimental systems for solubilization of anticancer agents (Trissel, 1992). The solubility of the AZT-CDS in ethanol was poor (< 0.5 mg/ml), and in water almost negligible. Electroneutral detergents such as Cremophor EL (polyoxyethylenated caster oil) have been used in parenteral formulations for cyclosporin and taxol (Trissel, 1992). Unfortunately, both Cremophor EL and Tween 80 provided an unacceptable degree of solubilization for the AZT-CDS. Another approach which has found acceptance in the pharmaceutical area for solubilization of poorly water-soluble drugs is the use of lipid-based formulations including emulsions as exemplified by Diprivan® (propofol) (Glen and Hunter, 1984). As indicated in Table 1, the solubility of AZT-CDS is low in various pharmaceutically acceptable oils precluding de novo emulsification as a formulation option.

Cyclodextrins are cyclic oligomaltose derivatives that have been shown to improve drug solubility by the formation of dynamic inclusion complexes (Szejtli, 1982). While β-cyclodextrin is poorly water-soluble limiting its pharmaceutical usefulness in parenteral formulations, chemically modified β-cyclodextrins are highly water-soluble and therefore more applicable (Pitha et al., 1988; Brewster et al., 1990; Brewster, 1991). A variety of cyclodextrins were therefore examined as possible solubilizers. Unfortunately, as indicated in Table 2, traditional solubilization techniques provided for only low degrees of complexation. 2-Hydroxypropyl-β-cyclodextrin (HPβCD), for example, resulted in a complex formation in the 2–4 mg/g range.

Table 1
Solubility of AZT-CDS in various organic co-solvents, electroneutral detergents or pharmacetically acceptable oils

Solvent	Solubility (mg/ml)
Water	≪ 0.01
Dimethyl sulfoxide	625
Dimethyl acetamide	150
Benzyl alcohol	140–150
Propylene carbonate	15–25
Polyethylene glycol	10–20
Tetraglycol	< 1
Ethanol	< 0.1
Ethylene glycol	< 5
Propylene glycol	< 5
Cremophor EL	< 5
Tween 80	< 5
Diluent 12 (50:50 Cremophor/ethanol)	< 5
Safflower oil	< 2
Corn oil	< 2

Since the rate-determining step in complex formation for poorly water-soluble compounds is often the phase-to-phase transition, an acceleration of this process was considered by using water-miscible organic co-solvents (Pitha and Hoshino, 1992). Small volumes of a concentrated AZT-CDS solution in DMSO, for example, were added to aqueous solutions of HPβCD followed by sonication and stirring. Lyophilization of these systems provided for degrees of incorporation in the 2–4 mg/g range (Table 3). An alternative approach was suggested by Pitha et al. (1992) who

Table 2
Solubilization of AZT-CDS in various cyclodextrins and their derivatives without the use of co-solvents or other system additives

Cyclodextrin	Degree of incorporation (mg/g complex)
α-Cyclodextrin	1.2
γ-Cyclodextrin	> 1
Hydroxyethyl-β-cyclodextrin	2.2
2-Hydroxypropyl-β-cyclodextrin	4.0
Maltosyl-β-cyclodextrin	1.2
Dimethyl-β-cyclodextrin	4.0

Table 3
Solubilization of AZT-CDS in aqueous cyclodextrins using co-solvents and/or other system additives or solubilization of AZT-CDS in non-aqueous systems followed by solvent removal, aqueous reconstitution, filtration and freeze-drying

Cyclodextrin	Solvent or co-solvents	Additive	AZT-CDS (mg)	Mixing time (°C)	Incorporation (mg/g)
HPβCD	water (100 ml)	DMSO	50	2.5 h (25°C)	3.7
HPβCD	water (100 ml)	DMSO	50	2.5 h (25°C)	3.9
HPβCD	water (100 ml)	DMSO	50	2.5 h (25°C)	3.0
HPβCD	water (100 ml)	DMSO	50	2.5 h (25°C)	2.4
HPβCD	water (100 ml)	urea	50	2.5 h (25°C)	< 1.0
HPβCD (1 g)	*t*-butanol (100 ml)	–	50	0.5 h (25°C)	6.2
HPβCD (1 g)	methanol (100 ml)	–	50	0.5 h (25°C)	25.6
HPβCD (1 g)	acetonitrile (100 ml)	–	50	0.5 h (25°C)	14.5
HPβCD (1 g)	isopropanol (100 ml)	–	50	0.5 h (25°C)	14.7
HPβCD (1 g)	ethanol (15 ml)	–	50	2 h (25°C)	3.4
HPβCD (1 g)	ethanol (50 ml)	–	50	2 h (25°C)	3.2
HPβCD (1 g)	ethanol (50 ml)	–	50	57 h (25°C)	3.8
HPβCD (1 g)	ethanol (100 ml)	–	50	2.5 h (25°C)	4.1
HPβCD (1 g)	ethanol (100 ml)	–	50	57 h (25°C)	3.7
HPβCD (1 g)	ethanol (200 ml)	–	50	2.5 h (25°C)	5.2
HPβCD (1 g)	ethanol (200 ml)	–	50	57 h (25°C)	5.7
HPβCD (1 g)	ethanol (500 ml)	–	50	2.5 h (25°C)	12.5
HPβCD (1 g)	ethanol (1.0 l)	–	50	2.5 h (25°C)	21.3

Table 4
Solubilization of AZT-CDS using buffers, pH alterations, cyclodextrins, co-solvents and/or other system additives

Cyclodextrin	Additive	Buffer (M, pH)	Mixing time (°C)	Incorporation (mg/g)
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	2 h (4°C)	32.6
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	3 h (4°C)	33.6
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	4 h (4°C)	37.0
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	2 h (4°C)	33.4
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	1 h (60°C)	36.5
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	2.5 h (60°C)	50.8
HPβCD	–	Na_3PO_4 (0.05 M, pH 11)	2.5 h (70°C)	55.0
HPβCD	–	K_2CO_3 (0.05 M, pH 13)	1 h (4°C)	31.1
HPβCD	–	K_2CO_3 (0.05 M, pH 13)	1.5 h (25°C)	45.0
HPβCD	–	Tris (0.05 M, pH 10)	1.5 h (25°C)	55.1
HPβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	4.5 h (35°C)	40.1
HPβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	3.5 h (50°C)	41.2
HPβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	4.5 h (65°C)	39.5
HPβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	4.5 h (75°C)	50.5
DMβCD	–	Na_3PO_4 (0.05 M, pH 11)	4.0 h (35°C)	52.2
DMβCD	–	Na_3PO_4 (0.05 M, pH 11)	4.0 h (45°C)	70.8
DMβCD	–	Na_3PO_4 (0.05 M, pH 11)	4.0 h (75°C)	66.0
DMβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	4.0 h (47°C)	39.1
DMβCD	PVP	Na_3PO_4 (0.05 M, pH 11)	4.0 h (47°C)	40.0
G2βCD	–	Na_3PO_4 (0.05 M, pH 11)	3.0 h (55°C)	40.0
G2βCD	–	Na_3PO_4 (0.05 M, pH 11)	3.0 h (75°C)	39.9

indicated that dissolution of HPβCD and the agent to be complexed in a solvent such as aqueous ammonia or ethanol followed by solvent removal, reconstitution with water and lyophilization, provided for improved solubilization over classical methods. In applying this technique, large volumes of ethanol had to be used due to the low solubility of AZT-CDS. Optimal results were obtained when 50 mg of the AZT-CDS and 1.0 g of HPβCD were dissolved in 1.0 l of ethanol, producing a solid containing 15 to 20 mg of AZT-CDS incorporated into 1 g of complex. As illustrated in Table 3, the variables that affected the degree of complexation included stirring time, temperature and the quantity of solvent used to dissolve the AZT-CDS. Other solvents including *t*-butanol, methanol, acetonitrile and isopropanol were examined. Of this group, methanol gave the best degree of solubilization (25.6 mg/g).

The AZT-CDS, like the parent nucleoside thymidine, has an ionizable proton at the imine N3 nitrogen suggesting improved water solubility through the use of alkaline vehicles or AZT-CDS salt formation. That such manipulations were possible were indicated by the high solubility of the AZT-CDS in dilute solutions of NaOH or KOH. Use of alkaline systems associated with low buffering capacity, such as 0.05 M Na_3PO_4 (pH 12), provided for increased solubility of the AZT-CDS but such systems were not physically stable. Several investigators, including Loftsson and Bodor (1989), Liu et al. (1992) and Tinwalla et al. (1993), have found that the combination of pH adjustment and cyclodextrin use can provide for two advantageous events: synergistic solubilization and stabilization to chemical and physical degradation. The benzodiazepine, medazepam, for example, demonstrated a significantly higher apparent stability constant in its ionized form ($K_{1:1} = 1380\ M^{-1}$) as compared to the unionized species ($K_{1:1} = 150\ M^{-1}$) (Loftsson and Bodor, 1989). The solubilization of AZT-CDS was therefore examined in solutions of HPβCD (43.5% w/v) containing various alkaline buffers such as Na_3PO_4, K_2CO_3 and Tris. The use of such conditions greatly increased the degree of incorporation of the AZT-CDS into cyclodextrin. As illustrated in Tables 2 and 4, a combination of pH adjustment and cyclodextrin use provided for increases in solubilization on the order of 10-fold compared to solubilization in cyclodextrin without pH modification. Manipulation of stirring time and temperature generally increased the extent of solubilization. The data also suggest that dimethyl-β-cyclodextrin (DMβCD) is a slightly better solubilizing agent than HPβCD. Unfortunately, the surface activity of DMβCD reduces its attractiveness as an i.v. excipient. The maltosyl-β-cyclodextrin (G2βCD) is similar to its solubilizing potency as is the benchmark HPβCD. Finally, Loftsson et al. (1994a,, 1994b) have suggested that addition of water-soluble polymers such as polyvinylpyrolidone (PVP) or hydroxypropylcellulose may improve the extent of cyclodextrin complexation. In this vein, 0.25% PVP was incorporated into HPβCD solutions. In the case of AZT-CDS, no improvements in AZT-CDS solubilization were observed.

In extending this line of research, salts of the AZT-CDS were considered. While several possibilities were examined including the sodium, potassium and ammonium salts, the potassium salt was found to be superior based on its ease of preparation and stability. The potassium salt was generated by dissolving equimolar amounts of the CDS and potassium hydroxide in methanol followed by solvent removal. The AZT-CDS was stable under the reaction conditions and quantitative yields of the pure (> 97%, HPLC) salt were obtained. The potassium salt of the AZT-CDS (K^+ AZT-CDS) was stable at refrigerator temperatures when protected from moisture and light. The K^+ AZT-CDS was soluble in water but solutions were alkaline (pH 11–12) and precipitated within 10 min of preparation. Addition of the AZT-CDS potassium salt to solutions of Na_3PO_4 (pH 12, 0.05 M) provided for increased solubility but the physical stability of the solutions was still limited. As indicated above, both pH adjustment and the presence of cyclodextrins appeared to be necessary to generate potentially useful systems in that simply adding the AZT-CDS potassium salt to a solution of HPβCD in the absence of a buffer did not provide for a stable system. Thus, the tendency of AZT-CDS to precipitate from the prototypes formulations

Table 5
Preparation and characteristics of prototype formulations containing the AZT-CDS potassium salt, 2-hydroxypropyl-β-cyclodextrin and Na_3PO_4

[K^+ AZT-CDS] (mg/ml)	[Cyclodextrin] (% w/v)	[Buffer] (M)	pH	mOsm/kg	Comments
25	0.0	0.0	12	–	precipitation within 5 min
25	0.0	0.05	12	–	precipitation within 5 min
20	15	0.0	–	255	precipitation within 5 min
35	15	0.05	11.9	–	some precipitation after 24 h
35	15	0.025	11.7	–	some precipitation after 24 h
35	15	0.01	11.3	323	some precipitation after 24 h
35	15	0.005	11.1	300	some precipitation after 24 h
35	15	0.0025	11.0	299	some precipitation after 24 h
35	15	0.001	10.9	294	some precipitation after 24 h
30	15	0.005	10.7	282 ± 3	stable for 72 h
40	15	0.005	10.8	–	some precipitation after 4.0 h
50	15	0.005	11.0	–	some precipitation after 3.0 h

could be modified by dissolving the potassium salt in solutions of HPβCD containing Na_3PO_4 (Table 5). System optimization was achieved with the production of a 30 mg/ml solutions of the AZT-CDS (potassium salt) which contained 15% w/v HPβCD and 0.005 M Na_3PO_4. This system was physically stable for a period exceeding 72 h. In addition, HPLC examination of these solutions at room temperature showed no significant degradation for periods exceeding 48 h. The formulation has a low buffering capacity which may reduce irritation associated with the alkaline nature of the prototype dosage form and was isoosmotic (280 mOsm/kg). This scheme was superior to solubilization of the parent compound (AZT-CDS) in the buffered cyclodextrin because of the long stirring times required for dissolution in the latter case.

3.2. Biological testing

The potential of several of the above-described formulations to induce venous irritation was examined in a rat tail vein model of extravasation. Saline, various potential drug vehicles, as well as the AZT-CDS potassium salt formulation were examined as shown in Table 6. The degree of irritation produced by the AZT-CDS potassium salt/HPβCD prototype formulation was similar to that seen with the vehicle, suggesting little or no effect of the drug. In addition, this prototype formulation was superior to systems containing

Table 6
Venous irritation in a rat tail extravasation model (1–5 irritation score) for various drug vehicles, formulated drugs and saline

Treatment	Day					
	1	2	3	4	5	7
PEG/ethanol (2:1)	2.0 ± 0.0	2.5 ± 0.3	4.5 ± 0.3	4.5 ± 0.3	4.8 ± 0.3	4.5 ± 0.5
PG/ethanol:H_2O (3:1:1)	1.0 ± 0.0	1.0 ± 0.0	4.0 ± 0.6	4.5 ± 0.3	4.8 ± 0.3	5.0 ± 0.0
PG/ethanol:H_2O (4:1:5)	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.4	1.8 ± 0.5
HPβCD (15% w/v) in Na_3PO_4 (0.05 M)	1.8 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	2.0 ± 0.0	1.5 ± 0.3
K^+ AZT-CDS (25 mg/ml) in HPβCD and Na_3PO_4	1.0 ± 0.0	1.0 ± 0.0	1.8 ± 0.3	1.8 ± 0.3	1.5 ± 0.3	1.5 ± 0.5
Saline	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

Table 7
Tissue and blood concentrations of AZT ± SE after various i.v. doses of AZT administered in HPβCD

Dose (μmol/kg)	Time (min)	Blood (μg/ml)	Brain (μg/g)	Liver (μg/g)
26	15	3.7 ± 1.1	0.11 ± 0.01	1.7 ± 0.1
	60	0.0	0.0	0.0
	240	0.0	0.0	0.0
53	15	5.7 ± 0.4	0.14 ± 0.06	4.0 ± 0.2
	60	0.66 ± 0.43	0.0	0.12 ± 0.04
	240	0.0	0.0	0.0
77	15	7.1 ± 0.5	0.20 ± 0.20	3.3 ± 0.5
	60	1.5 ± 0.1	0.0	0.10 ± 0.01
	240	0.0	0.0	0.0
103	15	11 ± 1.2	0.23 ± 0.23	5.8 ± 0.8
	60	2.5 ± 0.3	0.0	0.37 ± 0.26
	240	0.0	0.0	0.0
130	15	18 ± 1.0	0.45 ± 0.07	11 ± 0.8
	60	2.1 ± 0.2	0.0	0.40 ± 0.24
	240	0.0	0.0	0.0

organic co-solvents and was close in its irritation profile to that of normal saline.

3.3. AZT administration

In order to provide a baseline against which the various formulation could be compared in vivo for the rat, the tissue distribution of AZT was examined. AZT, given i.v. in a vehicle containing 15% HPβCD and 5 mM Na_3PO_4, was administered at five doses ranging from 26 to 130 μmol/kg (6.9–34.4 mg/kg). At 15, 60 and 240 min, blood, brain and liver were obtained and AZT concentration determined by HPLC. Results are given in Table 7. The blood and tissue levels are consistent with the disposition of a polar compound. Blood levels at 1 h were 10–20% of the 15 min values. Brain concentrations of

Table 8
Brain and blood concentrations of AZT-CDS, AZT-Q + and AZT ± SE and after various doses of AZT-CDS administered in a DMSO vehicle

Dose (μmol/kg)	Time (min)	Blood (μg/ml)			Brain (μg/g)		
		AZT-CDS	AZT-Q +	AZT	AZT-CDS	AZT-Q +	AZT
26	15	0.0	0.0	1.2 ± 0.2	0.0	1.1 ± 0.3	0.42 ± 0.02
	60	0.0	0.0	0.80 ± 0.08	0.0	0.49 ± 0.12	0.32 ± 0.02
	240	0.0	0.0	0.0	0.0	0.0	0.0
53	15	0.47 ± 0.33	2.0 ± 0.3	2.6 ± 0.1	0.0	4.2 ± 0.6	0.77 ± 0.06
	60	0.0	0.0	0.80 ± 0.08	0.0	0.61 ± 0.21	0.30 ± 0.08
	240	0.0	0.0	0.0	0.0	0.0	0.0
77	15	0.98 ± 0.24	2.6 ± 0.1	2.6 ± 0.4	0.0	2.6 ± 0.5	0.79 ± 0.10
	60	0.0	0.07 ± 0.07	1.4 ± 0.5	0.0	1.1 ± 0.2	0.43 ± 0.14
	240	0.0	0.0	1.2 ± 0.2	0.0	0.0	0.0
103	15	1.2 ± 0.1	4.4 ± 1.0	3.8 ± 0.7	0.0	4.0 ± 1.6	0.88 ± 0.29
	60	0.0	0.20 ± 0.12	2.4 ± 0.4	0.0	1.4 ± 0.4	1.0 ± 0.2
	240	0.0	0.0	1.4 ± 0.5	0.0	0.0	0.0
130	15	0.55 ± 0.10	6.8 ± 1.5	5.9 ± 0.6	0.0	4.0 ± 1.0	1.1 ± 0.2
	60	0.0	0.18 ± 0.01	2.9 ± 0.4	0.0	2.3 ± 0.9	1.2 ± 0.3
	240	0.0	0.0	1.7 ± 0.6	0.0	0.0	0.0

AZT were low and detectable only at the 15 min time point. Initial liver concentrations of AZT were similar to early blood values but tended to be more rapidly eliminated (Little et al., 1990; Brewster et al., 1993).

3.4. AZT-CDS / DMSO administration

Preliminary animal studies of the AZT-CDS were conducted using DMSO as a vehicle. The solvent provided for excellent solubilization and was relatively nontoxic (Brewster et al., 1993). To critically evaluate this system, as well as to provide a framework for comparing this co-solvent to others developed during the course of this work, AZT-CDS in DMSO was administered at five doses (10–50 mg/kg; 26–130 μmol/kg) to rats. At 15, 60 and 240 min, blood and brain were assayed for AZT-CDS, the AZT-Q + and AZT. Results are given in Table 8. In blood, the AZT-CDS was detected in most treatment groups at 15 min but levels were low and were cleared from the blood by 60 min. The AZT-Q + was present through the 1 h time point in the three highest dose groups. The parent compound, AZT, demonstrated its C_{max} at 15 min after which levels slowly declined. At the highest three doses, AZT was present throughout the 4 h time course of the experiment at concentrations greater than 1.0 μg/ml. Compared to AZT dosing, AZT levels subsequent to AZT-CDS/DMSO administration were generally lower in blood with the greatest differences observed at early time points. Thus, the 130 μmol/kg dose of AZT produced 15 min blood levels of almost 18 μg/ml while AZT-CDS administration generated levels of almost 6 μg/ml. These changes in C_{max} provided for a decreased blood AUC for AZT after AZT-CDS administration (Fig. 1).

In brain, high levels of both the AZT-Q + and AZT were detected after all five doses of the AZT-CDS in DMSO. Quaternary salt concentrations were variable and not strictly dose-related. AZT levels were dose-related and tended to increase from the 15 min time point to 60 min. No AZT was detected at 240 min subsequent to AZT-CDS administration. Brain levels of AZT were significantly higher after AZT-CDS dosing

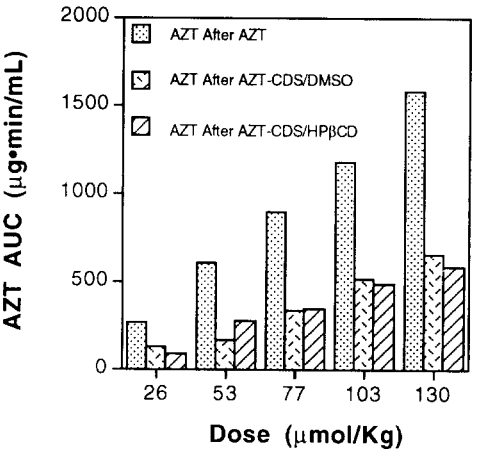

Fig. 1. Area under the curve (AUC) in blood for AZT after administration of AZT, AZT after administration of the AZT-CDS in a DMSO vehicle or AZT after administration of AZT-CDS (as its potassium salt) in an HPβCD vehicle at five doses.

as compared to AZT administration with AUC values for AZT 10 to 100-fold higher subsequent to CDS administration (Fig. 2). The result of the higher brain and lower blood levels was an improvement in the blood to brain ratio which increased from approx. 2–5% for AZT to 20–40% for AZT after AZT-CDS administration. These

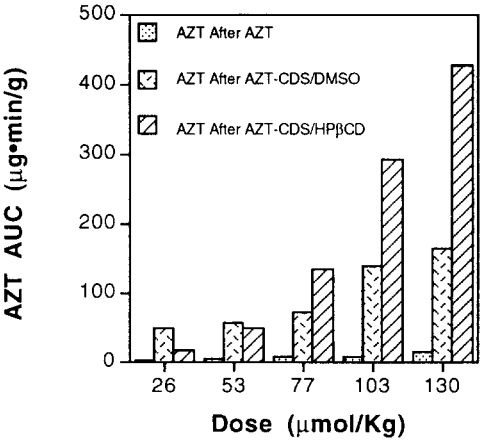

Fig. 2. Area under the curve (AUC) in brain for AZT after administration of AZT, AZT after administration of the AZT-CDS in a DMSO vehicle or AZT after administration of AZT-CDS (as its potassium salt) in an HPβCD vehicle at five doses.

data are consistent with earlier examinations of the AZT-CDS using a DMSO vehicle.

3.5. AZT-CDS potassium salt / HPβCD administration

The aqueous cyclodextrin formulation for the K^+ AZT-CDS (15% HPβCD w/v and 5 mM Na_3PO_4) was examined at various doses (11–55 mg/kg; 26–130 μmol/kg) in the rat. At 15, 60 and 240 min subsequent to dosing, brain and blood were examined and analyzed for AZT-CDS, AZT-Q + and AZT. Results are given in Table 9. Blood levels of AZT-CDS were detectable through 60 min unlike in the case of AZT-CDS/DMSO administration where detectable levels were present only at 15 min. Furthermore, AZT-Q + concentrations in blood tended to be higher and more persistent after use of the cyclodextrin vehicle compared to the DMSO system. The AZT-CDS/HPβCD system produced similar blood AUC values of AZT as did the AZT-CDS/DMSO system although the dynamics of AZT distribution were different. As illustrated in Fig. 3, the AZT-CDS/HPβCD tended to generate higher C_{max} values but also shorter blood half-lives. Thus, the 15 min values for AZT were higher and 240 min values lower after administration of the AZT-CDS in HPβCD as compared to use of the DMSO vehicle.

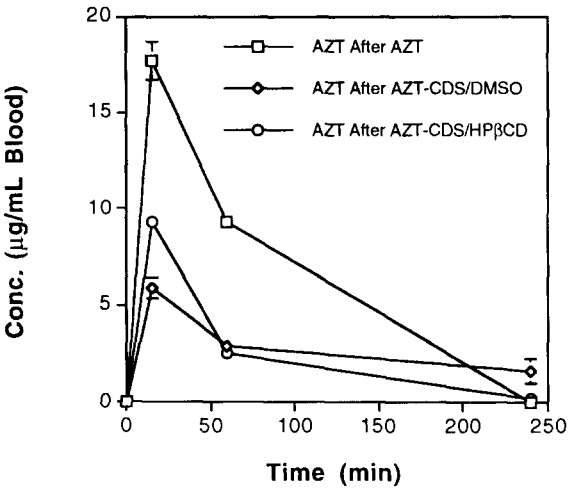

Fig. 3. AZT concentrations in blood for AZT after administration of AZT, AZT after administration of the AZT-CDS in a DMSO vehicle or AZT after administration of AZT-CDS (as its potassium salt) in an HPβCD vehicle at a 130 μmol/kg dose.

In brain, AZT-CDS was detectable at the 15 min time point but levels were low. As with blood, significantly higher AZT-Q + levels were generated in brain compared to AZT-CDS dosing

Table 9
Brain and blood concentrations of AZT-CDS, AZT-Q + and AZT ± SE and after various doses of AZT-CDS administered in an HPβCD/Na_3PO_4 vehicle

Dose (μmol/kg)	Time (min)	Blood (μg/ml)			Brain (μg/g)		
		AZT-CDS	AZT-Q +	AZT	AZT-CDS	AZT-Q +	AZT
26	15	1.1 ± 0.2	3.3 ± 0.02	1.4 ± 0.2	0.0	0.0	0.15 ± 0.04
	60	0.05 ± 0.5	0.21 ± 0.21	0.43 ± 0.02	0.0	0.0	0.11 ± 0.01
	240	0.0	0.0	0.0	0.0	0.0	0.0
53	15	1.0 ± 0.1	5.7 ± 0.3	3.9 ± 0.5	0.03 ± 0.03	3.5 ± 1.2	0.52 ± 0.20
	60	0.17 ± 0.04	0.60 ± 0.37	1.4 ± 0.1	0.0	1.3 ± 0.4	0.29 ± 0.09
	240	0.0	0.0	0.0	0.0	0.0	0.0
77	15	2.0 ± 1.2	5.8 ± 1.7	3.8 ± 1.2	0.01 ± 0.01	7.7 ± 1.2	1.15 ± 0.15
	60	0.10 ± 0.04	3.29 ± 0.62	1.8 ± 0.25	0.0	3.0 ± 0.5	0.86 ± 0.14
	240	0.0	0.0	0.0	0.0	0.0	0.04 ± 0.04
103	15	6.7 ± 0.6	11 ± 1.8	7.6 ± 0.5	0.15 ± 0.02	12 ± 2.5	3.5 ± 0.2
	60	0.11 ± 0.11	0.74 ± 0.20	2.1 ± 0.2	0.0	4.4 ± 0.7	1.6 ± 0.2
	240	0.0	0.0	0.15 ± 0.01	0.0	0.0	0.12 ± 0.01
130	15	5.9 ± 0.7	7.4 ± 0.8	9.9 ± 0.3	0.12 ± 0.03	21 ± 2	5.4 ± 0.6
	60	0.30 ± 0.02	0.55 ± 0.24	2.5 ± 0.04	0.0	7.0 ± 0.5	2.2 ± 0.2
	240	0.0	0.0	0.21 ± 0.20	0.0	0.0	0.19 ± 0.03

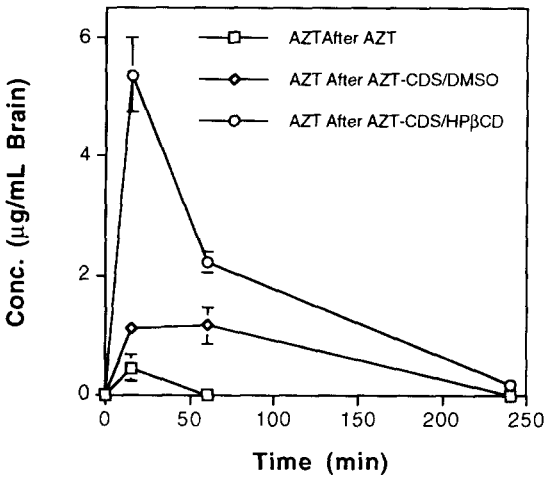

Fig. 4. AZT concentrations in brain for AZT after administration of AZT, AZT after administration of the AZT-CDS in a DMSO vehicle or AZT after administration of AZT-CDS (as its potassium salt) in an HPβCD vehicle at a 130 μmol/kg dose.

with the DMSO vehicle (Fig. 4). At the 130 μmol/kg dose, for example, brain AZT-Q + levels were 20.8 μg/g after administration of AZT-CDS in the HPβCD vehicle while maximum levels were only 4.0 μg/g after dosing with the DMSO vehicle. Areas under the curve showed similar trends. The transit time for AZT in brain after AZT-CDS/HPβCD was significantly increased over that associated with AZT-CDS/DMSO. As illustrated in Fig. 3, the HPβCD vehicle provided for a higher C_{max} (15 min), higher concentrations at 60 min and measurable AZT levels at 240 min in the case of the three highest dose groups. These effects provide an advantage in brain AUC after AZT-CDS/HPβCD administration. These effects are somewhat dose-related with higher AZT-CDS dosing showing the greatest differences between treatments. Thus, at the 50 mg/kg dose, AZT is almost 3-times more bioavailable in brain after use of the HPβCD formulation compared to the DMSO vehicle. When brain to blood (AUC) ratios are examined, use of the HPβCD vehicle provides for an increased ratio as a function of dose which approached 0.8 at the highest AZT-CDS dose.

There are several factors which may explain the vehicle effect observed in the case of AZT-CDS. One possibility is that although DMSO is a good solubilizing agent for the lipophilic CDS, i.v. administration leads to compound precipitation in vivo. This is a consequence of the log-linear solubilization achieved using organic co-solvents, a property which can be derived from the extended Hildebrand solubility approach (Martin et al., 1982). Given this solubility relationship, dilution of a solution of the AZT-CDS in DMSO will rapidly reduce the solubilizing power of the system resulting in rapid precipitation. This process may act to acutely decrease the drug availability by removing it from the circulation, thus reducing blood levels of AZT-CDS, AZT-Q + and AZT at early times and attenuating brain levels of both the AZT-Q + and AZT. In addition, slow redissolution of the precipitated AZT-CDS could explain the longer half-life in blood and higher terminal (240 min) blood concentrations. Such effects have been previously documented with other CDS-modified drugs (Brewster et al., 1988; Bodor, 1991). In the case of the AZT-CDS solubilized in HPβCD, such precipitation is not expected due to the nature of cyclodextrin-based solubilization which produces a linear-linear diagram rather than a log-linear relationship. Thus, in the linear portion of the AZT-CDS/HPβCD phase-solubility curve, i.e., for the 1:1 complex, dilution of the solubilizer provides for an equivalent dilution of the drug which results in maintenance of solubility.

The data suggest that potentially acceptable parenteral formulations for a zidovudine chemical delivery system can be prepared. While organic co-solvents do not appear to be optimal, cyclodextrin-based systems provide for reasonable solubilization and are not acutely toxic when administrated i.v. The use of an AZT-CDS potassium salt, together with solutions of HPβCD and a dilute Na_3PO_4 buffer, resulted in the preparation of a prototype formulation containing 30 mg of AZT-CDS per ml. Administration of the prototype formulation to animals not only demonstrated the brain-enhancement offered by the CDS but also showed that there was a significant formulation effect with organic co-solvents such

as DMSO reducing bioavailability compared with aqueous systems. The basis for this decreased bioavailability may be in situ AZT-CDS precipitation from the vehicle. These data, in addition to in vitro antiviral activity studies, suggest that the AZT-CDS may be a useful tool in fighting CNS complications of AIDS.

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