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Formulation development for a zidovudine chemical delivery system
2. Towards oral and non-parenteral dosage forms

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Abstract

Steps toward the development of an oral dosage form for a dihydronicotinate chemical delivery system for zidovudine (AZT-CDS) were examined. Administration of the AZT-CDS by gavage to rats indicated poor bioavailability consistent with the acid lability of the CDS. Furthermore, administration of the AZT-CDS in dimethyl sulfoxide (DMSO) intraintestinally did not result in therapeutically relevant brain or blood levels of the AZT-CDS or its metabolites. Use of a liposome formulation, however, did provide for significant uptake with administration to the jejunum more effective than AZT-CDS administration to the ileum or colo-caecum. Invasive administration of AZT-CDS complexed with various chemically modified cyclodextrins to the intestine also resulted in good bioavailability. Perfusion of a section of jejunum with a solution of AZT-CDS in 2-hydroxypropyl-β-cyclodextrin (HPβCD) resulted in demonstrable AZT-CDS uptake and pre-liver/post-liver blood concentration ratio of approx. 0.5. These results suggest that an enterically coated AZT-CDS tablet may provide for pharmacologically useful oral bioavailability. A second route of administration considered was rectal dosing. AZT was significantly bioavailable from prototype suppositories in the rat and although AZT-CDS could be detected after AZT-CDS treatment, the absolute bioavailability for AZT after such treatment was low.

Keywords: Zidovudine; Chemical delivery system; Parenteral dosage form

1. Introduction

Acquired immune deficiency syndrome (AIDS) encephalopathy and dementia are serious complications of AIDS that contribute detrimentally to the morbidity and mortality of the infection (Purdy and Plaisance, 1989; Reinvang et al., 1991; Pajeau and Roman, 1992). While antiretroviral drugs such as zidovudine (azidothymidine, AZT) can assault the peripheral components of this

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malady, the poor permeability and/or retention of many antiviral nucleosides in the brain limits their effectiveness in treating central nervous system (CNS) complications (Schmitt et al., 1988; Graham et al., 1991). One approach which may target antiviral drugs to the brain is the redox-based chemical delivery system (CDS) which modifies the parent drug through covalent attachment of a molecular targetor (Bodor et al., 1981; Brewster and Bodor, 1992; Brewster et al., 1993b). The result of this manipulation is facile uptake and drug accumulation in the CNS as a function of time. In the case of AZT, a dihydronicotinate system was designed, prepared and tested (Little et al., 1990; Brewster et al., 1991, 1993a). Parenteral administration of the AZT-CDS to test animals provided results consistent with the CDS concept in that such treatment leads to improved brain and reduced blood levels of the parent drug compared to conventional AZT administration (Chu et al., 1990; Little et al., 1990; Brewster et al., 1991).

While i.v. formulations have an important place in AIDS therapy, the majority of therapeutic options are available per os making the development of an oral dosage form of AZT-CDS highly desirable. Such development is not straightforward. The AZT-CDS is poorly water-soluble and labile to acid suggesting that enteric coating will be required to give a useful formulation. Basic questions must be addressed, however, prior to the development of an enterically coated system. These include whether the AZT-CDS is sufficiently water-soluble for significant intestinal absorption, what excipients may improve absorption and whether the molecule is sufficiently stable to be efficiently absorbed through the gastrointestinal tract and survive its transit through the presystemic circulation, liver and systemic circulation.

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 procedures (Little et al., 1990; Brewster et al., 1993a). 2-Hydroxypropyl-β-cyclodextrin (HPβCD, degree of substitution = 7) was purchased from Pharmos, Corp. and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCD) was obtained from Cyclolabs, Inc., Budapest, Hungary. Commercially available buffers (Fisher Scientific, NJ) were used to assay the sensitivity of the AZT-CDS to acidic conditions. All buffers were prepared at 0.05 M of the buffering species and included: pH 3, potassium biphthalate and hydrochloric acid; pH 4, potassium biphthalate; pH 5, potassium biphthalate and sodium hydroxide; pH 6, monobasic potassium phosphate and sodium hydroxide; pH 7, monobasic potassium phosphate and sodium hydroxide. Simulated gastric fluid was prepared according to compendial sources (2.0 g NaCl, 3.2 g pepsin and 7.0 ml of concentrated hydrochloric acid diluted to 1.0 l with water, pH 1.2) (US Pharmacopeia, 1990).

2.2. Liposome preparation

Liposomes encapsulating AZT-CDS were prepared as a lyophilized powder which were reconstituted with water for injection prior to administration. The lipid phase consisted of egg lecithin (Lipoid E-80, Merck, Germany), cholesterol (Sigma Chemical Co., St. Louis, USA) and α-tocopherol (Merck, Germany) at a phospholipid to cholesterol molar ratio of 10:2. All lipids were dissolved in chloroform in a round-bottom flask. AZT-CDS or AZT was added to the lipid mixture at a drug to phospholipid molar ratio of 1:10. Glass beads (100 g) were added to the flask and the chloroform solution was evaporated to complete dryness under reduced pressure using a rotary evaporator (Heidolph WB 2000, Germany). To the dry lipid film formed around the glass beads on the flask wall was added an aqueous solution containing lactose (0.25 M as a cryoprotectant) and the system was hydrated by mechanical shaking for 1.0 h using a Multi-Wrist shaker (Lab-Line Instruments, USA). The formed multilamellar liposomes were sized to submicron range by homogenization using a Gaulin Micron Lab 70 high pressure homogenizer (APV Gaulin Interna-

tional SA, Holland) at 800 bar (Brandl et al., 1993). The resulting liposomal formulation, which contained 100 mg of lipid per ml of buffer, was then lyophilized overnight using a Christ Beta 1-8K freeze-drier (Germany). After reconstitution of the lyophilized powder, the liposomes were sized using a Coulter N4MD Particle Size Analyser (Coulter Electronics, UK) which indicated that > 95% of the liposomes were below 100 nm in diameter. The concentration of AZT-CDS in this reconstituted system was 5 mg/ml.

2.3. Cyclodextrin preparations

Complexes of AZT-CDS and either HPβCD or DMβCD were prepared by equilibrating an excess of the CDS with 100 ml of a 40% w/v solution of the appropriate cyclodextrin in 0.05 M Na_3PO_4. The system was stirred for 2.5 h at 70°C under argon at which time the suspension was filtered and lyophilized (Labconco Freeze Dryer Model 4.5). The degree of incorporation was 54 mg/g HPβCD complex and 87.4 mg/g DMβCD complex. Solutions of AZT used for intestinal perfusion were prepared in aqueous 20% w/v HPβCD containing 0.05 M borate buffer. AZT-CDS solutions were generated by dissolving the AZT-CDS/HPβCD complex in HPβCD (to give a final HPβCD concentration of 20% w/v) prepared in borate buffer (0.05 M).

2.4. Stability of AZT-CDS in buffers and simulated gastric fluids

Various buffer solutions or simulated gastric fluid (2.75 ml) were placed in a cuvette fitted with a Teflon septum (Spectrocell, Inc., PA). The cuvette was then placed in the thermostated cell holder of a Hewlett-Packard 8451A diode array spectrophotometer and equilibrated at 37°C for 10 min. At time zero, 27.5 μl of a 5×10^{-3} M solution the AZT-CDS in DMSO were introduced into the cuvette via a Hamilton syringe. The disappearance of the band III absorbance (370 nm) was monitored. The pseudo first-order rate constants were calculated by plotting the change in the $\log(\text{absorbance}_{370})$ with time using software written for the HP 85-dedicated microprocessor.

2.5. 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^+ as described previously (Brewster et al., 1995).

2.6. Tissue analysis

1 ml of deionized water was added to tissue collected from the in vivo studies including whole blood (1.0 ml), whole brain or in some cases kidneys. 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.

2.7. Animal studies

2.7.1. Oral administration

Fasted (18 h), male Sprague-Dawley rats weighing 220–250 g were used in this study. Animals were anesthetized with a mixture of ketamine and xylazine (50:5 mg/kg) i.p. at a volume of 0.75 ml/kg. Suspensions of AZT and AZT-CDS were prepared in either polyethylene glycol 400 (PEG400) or a 4% aqueous solution of carboxymethyl cellulose (25–45 cP) prepared using a pH 9, 0.01 M borate buffer. The suspensions contained 25 mg/ml of AZT-CDS or 17.2 mg/ml of AZT and were designed to provide either 100 mg/kg AZT-CDS or equimolar (68.8 mg/kg) AZT at a 4 ml/kg dose. Both systems generated a stable suspension through 1 h. All preparations were administered by gavage using an intubation tube (Davol® Infant Feeding Tube, Plastic, 8 French, 15 inch) which was calibrated for suspen-

sion delivery to the stomach of a 250 g rat (distance of tube end to incisors: 12 or 8.2 cm from the factory marking). The feeding tube was fitted to a 6 ml syringe and the systems were resuspended prior to each syringe filling. At 1, 4 or 24 h subsequent to gavage treatment, brain and blood were removed and assayed.

2.7.2. Intestinal administration

Fasted (18 h) male Sprague-Dawley rats weighing 150–175 g were anesthetized using a mixture of ketamine and xylazine (50:5 mg/kg) i.p. A single midline incision was made and the stomach and intestine minimally exteriorized. For drug administration to various regions, a single ligature (3-0 silk) was placed either 3 cm distal to the pyloric sphincter (duodenum), 2 cm distal to the ligament of Treitz (jejunum), 6 cm proximal to the caecum (ileum) or 2 cm distal to the colocaecal junction (colo-caecum) (Park and Mitra, 1992). Vehicle or drug (AZT or AZT-CDS) was then given via injection using a 27 gauge needle. After injection, the abdominal muscle layer was sutured with two to three ligatures and the skin folds were closed with one to two stainless-steel (11 mm) wound clips. Rats were then killed at various times (1, 4 or in some cases 24 h) after drug administration and blood and organs removed, weighed and frozen on dry ice.

2.7.3. Jejunal perfusion (in situ single-perfusion method)

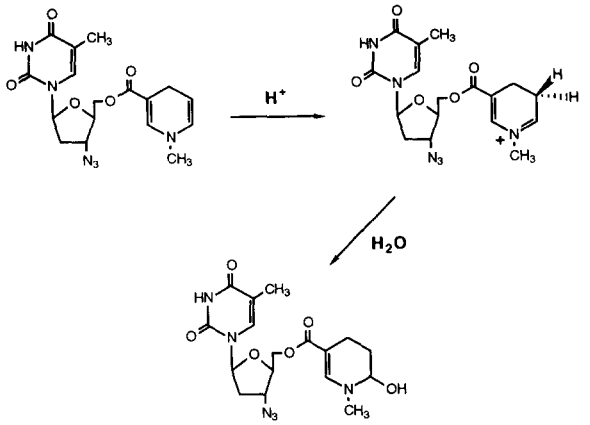
Male Sprague-Dawley rats weighing 175–210 g were fasted overnight and anesthetized on the morning of the experiment with pentobarbital (37.5 mg/kg, i.p.) and laporotomized. The jejunum was exposed and a single ligature was placed 2 cm distal to the ligament of Treitz. A Silastic catheter (19 gauge i.d.) was inserted into the jejunum and secured with a silk (3-0) ligature. A second catheter of the same type was placed 10 cm distal to the first catheter and secured with a ligature. The intestinal segment was irrigated with drug vehicle (20% HPβCD in 0.05 M borate buffer (pH 9.1)) at a perfusion rate of 1.2 ml/min (for 5 min). After the initial irrigation, animals were treated with AZT-CDS or AZT in the drug vehicle at a dose of 130 mmol/kg per h. Animals were perfused for 1.0 h and the flow rate was 20.7 ml/h. The concentration of AZT-CDS was determined at 15 min intervals in both the pre and post-intestine perfusion fluid. At 1 h after initiation of the perfusion, 1.0 ml of blood was removed from the anterior mesenteric vein approx. 3.5 cm anterior to the perfused section of jejunum as was 1.0 ml of blood from the vena cava. Both samples were collected into heparinized tubes and stored at −90°C prior to sample work-up and analysis. After collection of blood, the intestine was perfused with 20 ml of 20% w/v HPβCD in 0.05 M borate buffer over a period of 2–4 min. At that time, animals were decapitated and brains were removed, weighed and stored at −90°C until prepared for analysis.

2.7.4. Suppository administration

Blank or drug-laden rectal devices were prepared using Polybase® (Paddock Laboratories, Minneapolis, MN), a suppository base containing polyethylene glycol 400, polyethylene glycol 800 and polysorbate 80. The base was melt-loaded into 1.5 ml conical microcentrifuge tubes for the purpose of molding suitably shaped devices with a 250 mg suppository being optimal for rectal placement. Suppositories were then configured to contain 10 mg of the AZT-CDS or equimolar AZT in the 250 mg device and were inserted 2 cm into the rectum after which the recti were sealed with surgical cement to prevent leakage. The insertion distance was calibrated using the plunger from a 1 cm^3 Tuberculin syringe. In studies, six rats (body weight = 200 g) were anesthetized with ketamine, xylazine and administered blank suppositories or suppositories containing AZT-CDS (10 mg/250 mg device) or AZT (7.2 mg/250 mg device). At various times post insertion (0.5, 1.0, 1.5, 2.0, and 4.0 h), animals were killed and trunk blood and brain collected and frozen prior to sample work-up and analysis.

3. Results and discussion

Initial approaches to the oral delivery of AZT-CDS were direct and accomplished by suspending finely powdered AZT-CDS or AZT in either polyethylene glycol (PEG) 400 or carboxymethyl



Scheme 1.

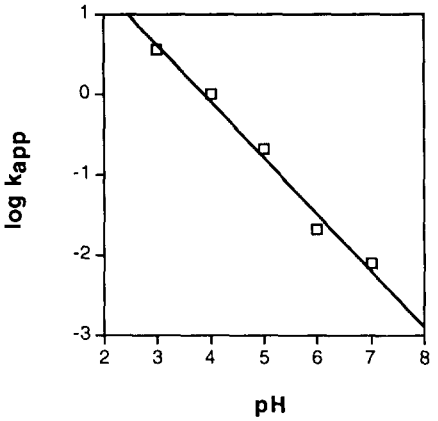


Fig. 1. pH-rate (log of the apparent disappearance rate constant (k min^{-1})) profile for AZT-CDS in various buffers. All buffers contained 0.05 M of the buffering species.

cellulose (CMC) followed by administration of a 100 mg/kg of AZT-CDS or equimolar AZT by gavage. While such treatment produced AZT at high concentrations in blood at 1 and 4 h after administration of AZT, little evidence of AZT-CDS uptake was apparent (Table 1). Several possible conditions may contribute to the observed poor oral bioavailability of AZT-CDS including: (1) that AZT-CDS was destroyed in the stomach; (2) the AZT-CDS underwent extensive metabolism at the level of the intestinal wall; (3) the AZT-CDS was so poorly water-soluble that intestinal uptake was dissolution-limited; or (4) a significant first-pass effect occurred. These possibilities were considered.

The AZT-CDS utilizes a dihydronicotinate targetor to achieve brain-enhanced delivery of AZT. This dihydropyridine CDS is susceptible to acid-catalyzed degradation through protonation β-to the dienamine nitrogen followed by water attack on the iminium double bond (Johnson et al., 1963; Johnson and Tuazon, 1977). This gives rise to a 6-hydroxytetrahydropyridine which is no longer capable of acting as a brain-targeting CDS (Scheme 1). The sensitivity of the AZT-CDS to destruction in low pH conditions was examined by following its degradation in buffers of various pH. As illustrated in Fig. 1, AZT-CDS is efficiently destroyed at pH values above those that

Table 1
Comparison of brain and blood levels ($\mu g/g \pm SE$ or $\mu g/ml \pm SE$, respectively) of AZT after oral AZT administration or AZT-Q^+ and AZT after AZT-CDS administration using different suspending agents

Time (h)	Agent	AZT		AZT-CDS			
		AZT		AZT		AZT-Q^+	
		Blood	Brain	Blood	Brain	Blood	Brain
1	PEG400	5.4 ± 1.3	0.0	0.0	0.0	0.0	0.0
4	PEG400	2.0 ± 0.8	0.0	0.0	0.0	0.0	0.0
24	PEG400	0.0	0.0	0.0	0.0	0.0	0.0
1	CMC	14 ± 2.2	1.2 ± 0.2	0.54 ± 0.02	0.0	0.32 ± 0.10	0.20 ± 0.02
4	CMC	3.0 ± 1.0	0.35 ± 0.11	0.54 ± 0.03	0.0	0.08 ± 0.05	0.04 ± 0.02
24	CMC	0.0	0.0	0.0	0.0	0.0	0.0

The dose administered was 100 mg/kg AZT-CDS or equimolar AZT.

Table 2
Cmparison of brain and blood levels ($\mu g/g \pm$ SE or $\mu g/ml \pm$ SE, respectively) of AZT after intraduodenal AZT administration or AZT-Q^+ and AZT after AZT-CDS administration using DMSO at doses of either 100 or 200 mg/kg

Time (h)	Dose (mg/kg)	AZT		AZT-CDS			
		AZT		AZT		AZT-Q^+	
		Blood	Brain	Blood	Brain	Blood	Brain
1	100	28 ± 2.9	3.0 ± 0.3	1.5 ± 0.1	0.39 ± 0.01	0.87 ± 0.06	0.46 ± 0.01
4	100	2.1 ± .02	0.54 ± 0.11	0.73 ± 0.05	0.24 ± 0.06	0.40 ± 0.03	0.16 ± 0.10
24	100	0.0	0.0	0.0	0.0	0.0	0.0
1	200	45 ± 4.5	5.5 ± 0.6	3.1 ± 0.1	0.23 ± 0.05	2.5 ± 0.1	1.1 ± 0.01
4	200	2.0 ± 0.2	0.21 ± 0.09	0.47 ± 0.20	0.02 ± 0.01	0.0	0.0
24	200	0.0	0.0	0.0	0.0	0.0	0.0

may be encountered in the stomach. This is punctuated by the poor stability of the AZT-CDS in simulated gastric fluid ($t_{1/2} \ll 5$ s). These data confirm that the AZT-CDS will not be available if administered directly to the stomach due to the acid conditions found there, suggesting the possibility of enterically coated systems. In order to investigate the feasibility of using such systems, the intestinal uptake efficiency of the AZT-CDS was considered since, subsequent to intestinal delivery by a potential enteral system, the drug must dissolve, penetrate the intestinal wall and survive the first pass through the liver.

The first evaluations of intestinal uptake were performed using a DMSO vehicle which ensured complete dissolution of the drug product. AZT-CDS or AZT were administered via invasive administration into the duodenum at doses equimolar to 100 and 200 mg/kg AZT-CDS. AZT, when administered in this manner, was readily absorbed with 1.0 h blood levels exceeding 28 $\mu g/ml$ at the 100 mg/kg dose and 44 $\mu g/ml$ at the 200 mg/kg dose (Table 2). These high blood levels were associated with AZT penetration into the CNS with the higher doses producing a brain concentration of almost 5.5 $\mu g/g$ and the lower doses generating 3.0 $\mu g/g$ tissue concentrations. The AZT-CDS provided for very low blood and brain levels of AZT and AZT-Q^+ which were at the limit of detection. Given the proximity of the duodenum to the pyloric portion of the stomach and resulting pH variability, uptake of the AZT-CDS was screened in three additional intestinal regions distal to the duodenum including the jejunum, ileum and colo-caecum. All of these regions are more alkaline in nature than the duodenum and potentially more suitable for AZT-CDS absorption (Proudfoot, 1988; Thoma, 1989). Administration of a 50 mg/kg dose of the AZT-CDS in DMSO resulted in 1 h blood levels ranging from 0.74 $\mu g/ml$ subsequent to administration to the ileum to 2.36 $\mu g/ml$ after colo-caecum administration. Brain levels of AZT were at or below the limit of detection (Table 3).

Table 3
Comparison of brain and blood levels ($\mu g/g \pm$ SE or $\mu g/ml \pm$ SE, respectively) of AZT-CDS, AZT-Q^+ and AZT after AZT-CDS administration to various intestinal sites at 1 h post-dosing using a DMSO vehicle

Intestinal site	AZT		AZT-Q^+		AZT-CDS	
	Blood	Brain	Blood	Brain	Blood	Brain
Jejunum	1.5 ± 0.1	0.02 ± 0.01	0.0	0.0	0.04 ± 0.04	0.0
Ileum	0.74 ± 0.23	0.0	0.07 ± 0.07	0.0	0.0	0.0
Colo-caecum	2.4 ± 0.5	0.04 ± 0.03	0.15 ± 0.05	0.05 ± 0.05	0.0	0.0

The dose was 50 mg/kg AZT-CDS.

Table 4
Comparison of brain and blood levels ($\mu g/g \pm$ SE or $\mu g/ml \pm$ SE, respectively) of AZT-CDS, AZT-Q^+ and AZT after AZT-CDS administration to various intestinal sites at 1 h post-dosing using a liposome preparation

Intestinal site	AZT		AZT-Q^+		AZT-CDS	
	Blood	Brain	Blood	Brain	Blood	Brain
Jejunum	7.4 ± 0.4 [a]	0.60 ± 0.04	0.04 ± 0.04	0.04 ± 0.04	0.30 ± 0.10	0.04 ± 0.04
Ileum	1.8 ± 0.3	0.06 ± 0.01	0.23 ± 0.07	0.0	0.0	0.0
Colo-caecum	2.4 ± 0.4	0.13 ± 0.04	0.12 ± 0.05	0.05 ± 0.05	0.0	0.0

The dose was 50 mg/kg AZT-CDS.
[a] Blood levels after jejunal administration were higher than those produced after administration to the ileum or colo-caecum ($p < 0.05$).

Given the poor uptake of AZT-CDS from organic co-solvents, alternative formulations were considered including lipid-based systems and water-soluble modified β-cyclodextrins. These approaches have been reported to improve oral bioavailability specifically when drug absorption from a solid dosage form is disintegration or dissolution-limited. Drug complexes of both cyclodextrin and chemically modified cyclodextrins and carbamazepine, ketoprofen, dipyridamole and digoxin, to name only a few, dissolve more rapidly than the uncomplexed pharmaceuticals and the drug systems have higher apparent aqueous solubilities (Nakai et al., 1983; Uekama et al., 1981; Seo and Uekama, 1989; Stracciari et al., 1989). These properties are associated with improved drug transit through the intestinal wall and increased systemic blood levels. The increased rate of dissolution offered by cyclodextrin complexation has been modeled by Corrigan and Stanley (1981, 1982). Importantly, hydrophilic cyclodextrin derivatives are not orally available (Mesens et al., 1991). As reviewed by Aungst, 1993, lipid-based systems such as emulsions and liposomes can also be employed to enhanced enteral uptake through a variety of actions including improved solubilization, prolonged gastric residence times, enhanced membrane permeability via increased contact time and absorption via the lymphatics. Although controversial, liposomes have been suggested to improve oral delivery of various hydrolytically labile substances such as peptides (Spangler, 1990; Davis, 1992).

AZT-CDS was incorporated into lyophilizable liposomes at concentrations of 5–10 mg/ml and administered into various intestinal sites. The liposomes were composed of egg lecithin (80% phosphatidylcholine), cholesterol, an anti-oxidant, cryoprotectant and demonstrated particle sizes (> 95%) of less than 100 nm. Since the lipid solubility of the AZT-CDS is relatively low, it is likely that the AZT-CDS is entrapped in the

Table 5
Comparison of brain, blood and kidney levels ($\mu g/g \pm$ SE, $\mu g/ml \pm$ SE or $\mu g/g \pm$ SE, respectively) of AZT-CDS, AZT-Q^+ and AZT after AZT-CDS administration to the jejunum at 1 h post-dosing using a liposome preparation

Time (min)	AZT			AZT-Q^+		
	Blood	Brain	Kidney	Blood	Brain	Kidney
10	7.4 ± 1.5	0.47 ± 0.05	55 ± 14	3.7 ± 0.7	1.6 ± 0.1	3.2 ± 1.0
20	8.1 ± 0.5	0.48 ± 0.01	42 ± 0.4	5.1 ± 3.4	1.5 ± 0.1	3.9 ± 2.6
30	11 ± 1.0	0.74 ± 0.05	62 ± 3.9	2.0 ± 1.3	1.7 ± 0.2	3.9 ± 0.4
40	11 ± 1.5	0.70 ± 0.10	54 ± 6.3	1.1 ± 0.6	2.7 ± 0.3	8.7 ± 0.4
50	10 ± 0.01	0.76 ± 0.04	61 ± 5.9	10 ± 3.7	1.7 ± 0.4	9.5 ± 6.3
60	8.9 ± 0.4	0.70 ± 0.04	44 ± 1.1	12 ± 5.8	1.7 ± 1.1	10.7 ± 4.5

The dose was 50 mg/kg AZT-CDS.

phospholipid bilayer domains of the liposome particle where the AZT-CDS can be protected from oxidation. Unlike the results from DMSO administration, AZT-CDS administered in liposomes provided for significantly higher AZT levels in blood and brain and a clear preference for intestinal sites (Table 4). Thus, jejunal administration of a 50 mg/kg dose of the AZT-CDS in liposomes generated 1 h AZT blood levels of greater than 7.0 μg/ml while administration to the ileum and colo-caecum produced blood levels of less than 2.5 μg/ml. The 1 h level obtained after jejunal dosing compared favorably to C_{max} values of AZT obtained after i.v. administration of the AZT-CDS/liposome formulation (9.71 μg/ml at 40 mg/kg). Brain levels of AZT were also comparable to i.v. values (60 min i.v. AZT-CDS/liposome = 0.75 μg/g, 60 min intrajejunal AZT-CDS/liposome = 0.60 μg/ml). These data suggested that the jejunum may represent the best site of absorption for AZT-CDS and further studies were restricted to this anatomical locus.

A preliminary time course of AZT and AZT-Q^+ uptake after AZT-CDS was examined using the liposome formulation. Brain, blood and kidney were examined with results presented in Table 5. In the study, the AZT-CDS was administered at a dose of 50 mg/kg and samples were obtained every 10 min from the time of drug administration through 1.0 h. Blood levels of AZT were similar to those reported after earlier jejunal experiments although AZT-Q^+ levels tended to be higher consistent with AZT-CDS uptake, delivery and metabolism. Similarly, AZT levels in brain reproduced earlier experimental data but AZT-Q^+ levels were higher than those detected in preliminary liposome experiments. Kidney levels were significantly higher than blood levels consistent with the role of renal elimination in the disposition of AZT (Patel et al., 1989). Subsequent studies examined AZT produced in blood and brain after dosing with AZT-CDS incorporated into liposomes controlled with an AZT/liposome preparation. These experiments (at a 50 mg/kg dose of AZT-CDS or equimolar AZT) followed a longer time course (2 h) and results are presented in Figs. 2 and 3. Higher blood levels were detected after administration of

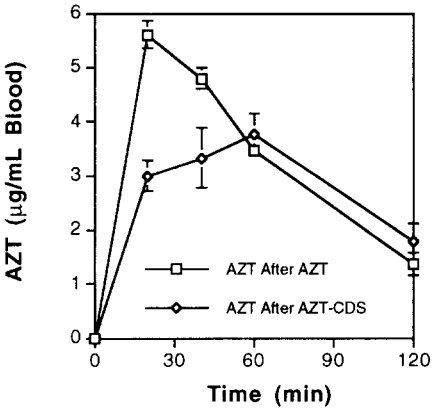


Fig. 2. AZT in blood as a function of time after intrajejunal administration of either AZT or AZT-CDS formulated in liposomes. The administered dose was 50 mg/kg AZT-CDS or equimolar AZT.

AZT as compared to AZT-CDS ($AUC_{120\ min}$ = 330 μg min ml^{-1} for AZT from AZT-CDS and 390 μg min ml^{-1} for AZT after AZT) while brain levels of AZT were higher after CDS treatment ($AUC_{120\ min}$ = 46 μg min ml^{-1} for AZT from AZT-CDS and 37 μg min ml^{-1} for AZT after AZT). These data indicate that the brain to blood ratio for AZT improved by almost 70% subsequent to AZT-CDS administration.

A second formulation that was examined in the above-described intrajejunal model was AZT-

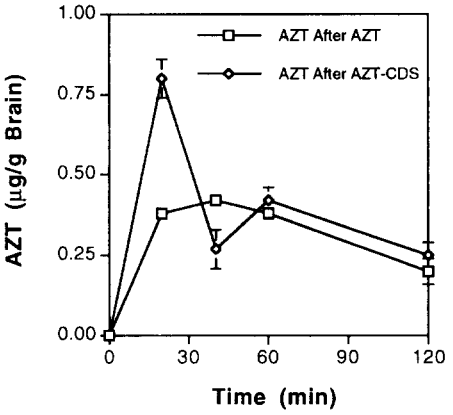


Fig. 3. AZT in brain as a function of time after intrajejunal administration of either AZT or AZT-CDS formulated in liposomes. The administered dose was 50 mg/kg AZT-CDS or equimolar AZT.

CDS complexed with either 2-hydroxypropyl-β-cyclodextrin (HPβCD) or dimethyl-β-cyclodextrin (DMβCD). As illustrated in Fig. 4, administration of a 50 mg/kg dose of either AZT-CDS or equimolar AZT in HPβCD provided for significant AZT blood levels with the CDS generating about half the blood levels of AZT compared to AZT dosing itself (AZT $C_{60\ min} = 11 \pm 1$ μg/ml after AZT dosing and 6.0 ± 0.3 μg/ml after equimolar AZT-CDS dosing). The DMβCD formulation gave a similar AZT profile (AZT $C_{60\ min} = 14 \pm 1.5$ μg/ml after AZT dosing and 7.1 ± 0.8 μg/ml after equimolar AZT-CDS dosing) but contributed higher AZT-Q^+ blood levels compared to the HPβCD vehicle (AZT-Q^+ $C_{60\ min} = 1.1 \pm 0.3$ μg/ml after AZT-CDS/HPβCD dosing and 3.9 ± 0.4 μg/ml after AZT-CDS/DMβCD administration). Low but detectable levels of the AZT-CDS were detected subsequent to dosing with both vehicles confirming intestinal uptake. Brain levels (Fig. 5) of AZT were lower at 1 h after AZT-CDS/HPβCD administration compared to AZT dosing and AZT-Q^+ could not be detected. The DMβCD vehicle generated increased AZT concentrations in brain compared to the HPβCD system and also provided for significant concentrations of the AZT-Q^+ in brain at 60 min. These data would suggest that the

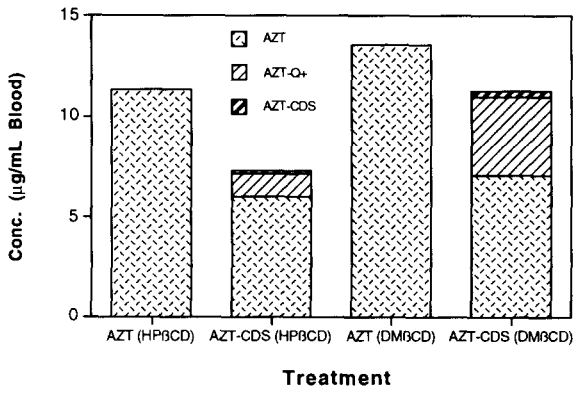


Fig. 4. Stacked bar graph illustrating AZT in blood at 1 h after intrajejunal administration of AZT or AZT-CDS, AZT-Q^+ and AZT after administration of AZT-CDS. In this study, AZT or the AZT-CDS was solubilized with either HPβCD and DMβCD and the dose was 50 mg/kg AZT-CDS or equimolar AZT.

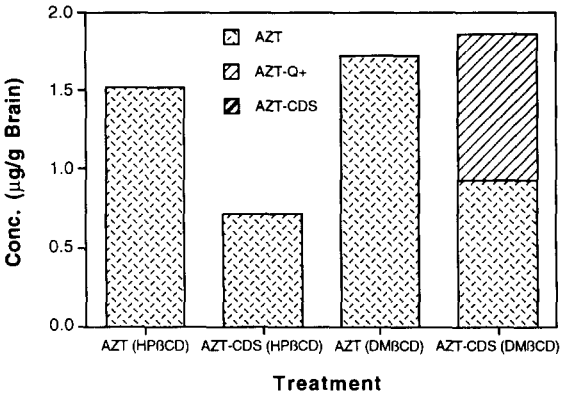


Fig. 5. Stacked bar graph illustrating AZT in brain at 1 h after intrajejunal administration of AZT or AZT-CDS, AZT-Q^+ and AZT after administration of AZT-CDS. In this study, AZT or the AZT-CDS was solubilized with either HPβCD and DMβCD and the dose was 50 mg/kg AZT-CDS or equimolar AZT.

methylated cyclodextrin is a better delivery adjunct for the AZT-CDS than is HPβCD. While such use of methylated cyclodextrins has been documented, for example, to improve rectal delivery, the mechanism of this permeation enhancement may be associated with cellular perturbation (Uekama et al., 1985; Kikuchi and Uekama, 1988). Methylated cyclodextrins, unlike their hydroxylated counterparts, possess considerable surface activity and exert detergent-like effects (Yoshida et al., 1988). These properties cause DMβCD to be irritating to mucous membranes and hemolytic. While such effects may be more apparent in the static model examined and therefore of less concern in the eventual indication (oral dosing), irritation is often highly correlated with improved drug uptake for permeation enhancers. For this and other reasons, further efforts have concentrated on HPβCD which has been shown to be parenterally and orally safe and non-irritating to mucous membranes (Brewster et al., 1990; Mesens et al., 1991).

While intrajejunal administration suggested that significant uptake of the AZT-CDS did occur, the studies did not address the possibility of metabolism associated with penetration through the intestinal wall. To examine this component of

Table 6
Concentration of AZT or AZT-CDS in the perfusate at perfusion initiation ($t = 0$), at 30 min and at perfusion termination ($t = 60$ min)

Time (min)	Perfusate concentration (μg/ml) $\pm$ SE	
	AZT-CDS [a]	AZT
0	428 $\pm$ 24	196 $\pm$ 28
30	389 $\pm$ 13	185 $\pm$ 25
60	375 $\pm$ 12	183 $\pm$ 10

[a] AZT-Q$^+$ and AZT concentrations in the AZT-CDS perfusate were constant (AZT-Q$^+$ = 22 $\pm$ 2 μg/ml) over the infusion time course.

the uptake scheme, an dynamic in situ single intestinal perfusion model was developed in the rat. The collected data (Table 6 and Fig. 6) suggest significant absorption of the AZT-CDS as indicated by the pre-and post-intestinal concentrations. The AZT-CDS was detected in mesenteric blood at concentrations of almost 3 μg/ml. Importantly, this may reflect an underestimate for CDS influx since cyclodextrin complexation in situ may reduce intestinal mucosal flux (Nakanishi et al., 1989). Post-liver blood also contained the AZT-CDS but at concentrations approx. 50% lower that those in mesenteric blood (Fig. 6). The difference suggests a significant first-pass effect but one which is practically acceptable.

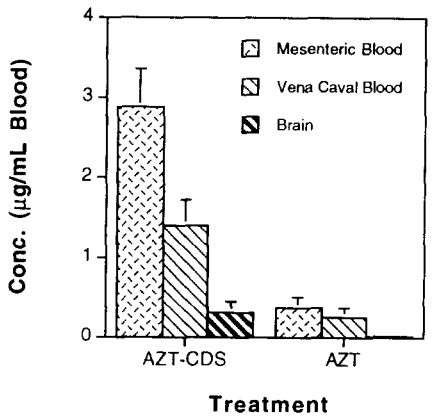


Fig. 6. Mesenteric or vena caval blood or brain concentration of either AZT-CDS or AZT after a 1 h jejunal perfusion of AZT-CDS or AZT, respectively. Drugs were solubilized in HPβCD in a borate buffer.

Another administration route which was considered was rectal dosing. This route has the potential advantage of avoiding the first-pass effects because of the vascular architecture of the rectum. As reviewed by De Boer et al. (1982), the distal portion of the rectum is drained by the inferior and middle rectal veins which direct blood to the general circulatory system. The vasculature more proximal to the rectum includes the superior rectal veins which drain blood to the portal system and hence to the liver. An appropriate placement of a drug delivery device may therefore improve bioavailability to shunting drug into the general circulation thereby avoiding the metabolic potential of a direct pass through the liver. In the rat, a variety of reports suggest that drug placement 2 cm proximal to the rectum exposes the drug to uptake from the distal rectal vein which empties into the inferior vena cava (De Boer et al., 1982; Kamiya et al., 1982). While AZT-CDS uptake colonically is potentially accessible, the literature suggests that rectal uptake of AZT is likely to be poor. Park and Mitra (1992) using a reperfusion model, found only limited AZT absorption from the perfusate and uptake of AZT from the colon was the lowest of any intestinal section examined. Importantly, the site of reperfusion in these studies encompassed the entire colon, from the colo-caecal junction to the rectum which may obscure any area of selectively high absorption.

In any case, prototype devices containing either AZT or the AZT-CDS were administered to rats. Blood and brain were then assayed over a 4 h time course. As shown in Fig. 7, administration of AZT in this manner provided for significant and sustained blood and brain levels of AZT while peripheral organs like the liver demonstrated rapid disposition of AZT. Blood levels tended to be more persistent that those generated by i.v. administration and brain levels of AZT generated by this dosing technique were significantly higher than those generated after i.v. administration. The generated brain to blood ratio AZT after rectal dosing was approx. 0.3, i.e., 30%. This value is above those ratios produced after i.v. bolus administrations of AZT but similar to those obtained after constant i.v. infusions

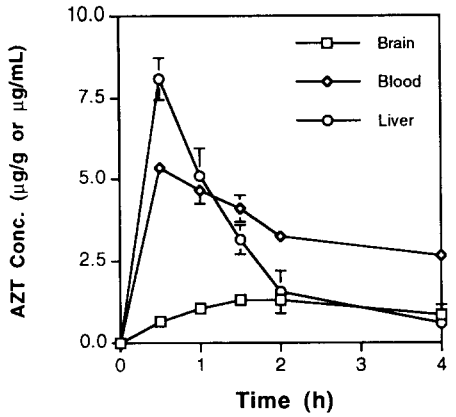


Fig. 7. AZT in blood, brain and liver as a function of time after rectal administration of AZT using a polybase suppository. In this study, a dose of 36 mg/kg AZT (equimolar to 50 mg/kg AZT-CDS) was used.

suggesting that the rectal administration of AZT may provide for constant drug release (Gallo et al., 1989). As illustrated in Fig. 8, administration of the AZT-CDS by this route provided for significant uptake of the AZT-CDS as indicated by blood levels approaching 3 μg/ml. On the other hand, brain levels of AZT were lower after the CDS than those obtained after rectal AZT dosing.

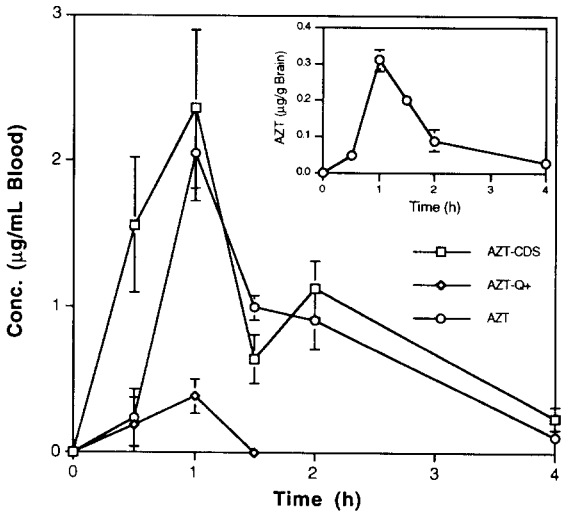


Fig. 8. AZT-CDS, AZT-Q^+ or AZT in blood at various times after rectal administration of a 50 mg/kg dose of AZT-CDS in a suppository prepared from polybase. The inset gives brain levels of AZT after AZT-CDS administration (AZT-Q^+ and AZT-CDS were not detected).

In conclusion, these data suggest that while the AZT-CDS is not amenable to simple oral dosing due to the acidic environment of the stomach but that if the CDS is supplied to the intestine, significant uptake, distribution and metabolism to AZT may occur. Data suggest that the jejunum is the optimal site of drug administration and that direct injection to this site results in significant brain and blood uptake of AZT. Perfusion studies indicate that AZT-CDS does penetrate the intestinal wall intact and is partially metabolized at the liver. Importantly, the extent of metabolism is manageable and would not, in and of itself, preclude further development. Other evaluations indicate that AZT-CDS can also penetrate the colon although the profile of drug distribution and metabolism is not improved over AZT administration. This information intimates that the fabrication of enterically coated tablets may allow for successful oral delivery of the AZT-CDS.

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