Adamantane as a Brain-Directed Drug Carrier for Poorly Absorbed Drug. 2. AZT Derivatives Conjugated with the 1-Adamantane Moiety

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Abstract □ Five AZT (azidothymidine) prodrugs conjugated with the 1-adamantane moiety via an ester bond were synthesized to improve the transport of AZT into the central nervous system (CNS). In *in vitro* degradation studies with rat and human plasma, it was demonstrated that the prodrugs were degradated enzymatically and converted quantitatively to their parent drug, AZT. As assessed by octanol–buffer partitioning, the prodrugs were much more lipophilic than AZT and were expected to penetrate the blood–brain barrier (BBB) readily. In *in vivo* studies, in which the prodrugs were administered intravenously to rat, the prodrugs in brain tissue were detected at 7–18 times higher concentrations than AZT in spite of the negligible amount of the prodrug in the cerebrospinal fluid. These results indicate that the introduction to AZT of the 1-adamantane moiety results in the enhancement of the BBB penetration. This pharmaceutical approach would be beneficial for the efficient treatment of the CNS infection by human immunodeficiency virus.

Acquired immune deficiency syndrome (AIDS) is a malignant disease, caused by human immunodeficiency virus (HIV). The typical symptoms of AIDS, opportunistic infections such as pneumocystic carinii pneumonia and Kaposi's sarcoma, are well known. Besides, recently neurological dysfunction associated with the infection of the central nervous system (CNS) by HIV is getting more attention and is recognized as a serious problem in clinical fields. Actually, about 60% of patients with AIDS develop neurological complications including cognitive deficits, autism, incontinence, and degeneration of locomotor functions,[1-4] and these encephalopathies are the leading causes of death of patients after pneumonia.[5] The high incidence of this complications seems to be attributable to the property of HIV that has a tropism for the CNS.[6-8]

AZT (azidothymidine; zidovudine; 3′-azido-3′-deoxythymidine) is widely used in the clinic for the treatment of AIDS and AIDS-related complex (ARC). This drug is readily transported through the blood–cerebrospinal fluid barrier (BCSFB) and is detected at high concentrations in the cerebrospinal fluid (CSF) after administration.[9-11] However, transport of AZT into the brain parenchyma from the CSF, in which HIV exists in a high concentration, is minimal because the rate of bulk flow and arachnoid villi absorption of CSF is log orders faster than the diffusion kinetics. Considering the disadvantage of the BCSFB transport, the BBB transport systems which allows the direct access to the brain parenchyma becomes more attractive for AZT. However, the BBB restricts the entry of many drugs into the CNS by its tight junction, and actually, AZT cannot pass the BBB.[12]

In order to overcome this problem, some studies have been reported that attempted to enhance the penetration of AZT through the BBB via a pharmaceutical approach.[13,14]

A few years ago, we had interest in the physicochemical properties of adamantane. Adamantane is a tricyclodecane cage-shaped compound, and some aminoadamantane derivatives have a high solubility in *n*-heptane and in water and are likely to sublime. 1-Aminoadamantane hydrochloride (Amantadine hydrochloride) has been orally administered for the treatment of Parkinson's disease and for the prophylaxis of influenza infection. The mechanism for this antiviral action is explained by the adamantane moiety perhaps being incorporated efficiently into the cell membrane and the cationized amino group located on the surface of the cell membrane changing the net charge of the cell surface to prevent infulenza virus from entering into the cell.[15] The 1-aminoadamantane analogue memantine (1-amino-3,5-dimethyladamantane), an antiparkinsonism drug, has been reported to be distributed to the brain and liver after systemic administration.[16] These physicochemical and pharmacological properties of 1-aminoadamantane and its analogues are assumed to be due to the characteristic structure of the adamantane moiety. Therefore, we supposed that the adamantane moiety would be useful as a brain-directed drug carrier for drugs poorly absorbed into the CNS. In these last few years, we have been modifying some drugs by conjugating them with the adamantane moiety.[17] In the present study, we synthesized five derivatives of AZT by conjugating them to the adamantane moiety and evaluated their abilities to penetrate the BBB.

Experimental Section

Materials—AZT was purchased from Sigma Chemical Co. (St. Louis, MO) and used as supplied.

Synthesis of Prodrugs I–V—Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. FAB mass spectra were obtained with a JEOL JMS-D 300 mass spectrometer. FTIR spectra were obtained with a Perkin-Elmer 1720 spectrometer. ^{1}H NMR spectra were taken on a JEOL JNM-FX 200 (200 MHz) spectrometer and chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard.

(*1*) *General Procedure for the Preparation of 1-Adamantanylcarbonyl Amino Acids*—Glycine (Gly), β-alanine (β-Ala), and γ-aminobutyric acid (GABA) were acylated with 1-adamantanecarbonyl chloride under Schotten–Baumann conditions. The purity of each compound was ascertained by TLC and FABMS. Also, satisfactory elemental analysis (C, H, N) was obtained for each compound. Adamantanylcarbonyl-Gly: yield 73%; mp 200–203 °C. Adamantanylcarbonyl-β-Ala: yield 85%; mp 187–192 °C. Adamantanylcarbonyl-GABA: yield 84%; mp 163–166 °C.

(2) *Typical Procedure for the Esterification of AZT—Preparation of Prodrug* (*I*)—1-Adamantanecarboxylic acid (135 mg, 0.75 mmol) was dissolved in distilled DMF (10 mL), and to this ice-chilled solution were added AZT (100 mg, 0.375 mmol), dicyclohexylcarbodiimide (162 mg, 0.79 mmol), and 4-(dimethylamino)pyridine (68.5 mg, 0.56 mmol). The reaction mixture was stirred for 48 h at 4 °C, then the formed urea-derivative was removed by filtration. The solvent was removed by evaporation *in vacuo*, and the residue was purified by silica gel column chromatography using $CHCl_3$ as an eluent. The fractions containing the desired compound were pooled, the solvent was removed by evaporation, and the residue was dried over KOH pellets *in vacuo* to yield **I**. The compound exhibited a single spot on TLC and the structure was supported by the ^{1}H NMR and FT-IR.

Determination of Partition Coefficient (PC)—1-Octanol and pH 7.0 phosphate buffer (0.01 M) were saturated with each other prior to

® Abstract published in *Advance ACS Abstracts*, December 15, 1993.

0022-3549/94/1200-481$04.50/0

use. AZT and its prodrugs (I–V) were each dissolved in 10 mL of presaturated 1-octanol at a concentration of ca. 500 μg/mL and mixed with 5 mL of pH 7.0 phosphate buffer. The mixture was vigorously shaken for 60 min with a mechanical shaker and then was centrifuged at 3000 rpm. The concentrations of each compound in each phase were determined by HPLC.

In addition, the partition coefficients in a 1-butanol–buffer system were studied following the same procedure as described above.

The partition coefficient was calculated as the ratio of the drug concentration in the octanol phase to that in the buffer phase.

***In vitro* Degradation Studies**—The *in vitro* degradation profile of AZT and prodrugs I–V in rat and human plasma were investigated. Each test compound was dissolved in methanol at a concentration of 0.8 mM to prepare the stock solutions. Ten microliters of the stock solution was spiked with 200 μL of freshly separated rat or human plasma and the mixture was then incubated at 37 °C under shading with aluminum foil. At predetermined time intervals, the intact compound remaining and the AZT produced from each prodrug in each mixture was extracted with 2 mL of ethyl acetate–diethyl ether mixture (50:50, v/v) by shaking for 10 min. After centrifugation at 3000 rpm for 5 min, the organic phase was transferred to a 10-mL glass tube and evaporated by a nitrogen gas stream at 40 °C. The residue was later reconstituted in 200 μL of methanol and injected onto the HPLC.

For comparison, the degradation study for AZT-C18 reported by Kawaguchi et al.[18] was performed in the same way.

The above extraction was done according to the method of Unadkat et al.[19]

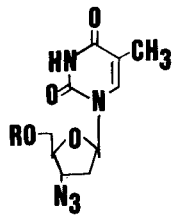
***In Vivo* Studies**—Wistar strain male rats weighing 180–220 g were used for the experiments. The test compounds (AZT, prodrugs I and II and AZT-C18) dissolved in propylene glycol were intravenously administered to rats with a dose of 18.7 μM/kg into the tail vein. Control animals received propylene glycol only. Before administration and at 15 and 30 min and 1, 2, and 4 h after administration, the rats were sacrificed under ether anesthesia, and then blood and whole brain were taken immediately. Blood was collected from the abdominal aorta. On the other hand, for AZT and II, a CSF sample was collected through a cisterna magna on a conscious rat at the corresponding time as described above. Plasma separated from blood, the excised brain, and the CSF were stored at −20 °C until analysis.

The analysis of AZT and its prodrugs in plasma was performed followed by the same method as described in *In Vitro* Degradation Studies. For analysis in brain, the method of Chu et al.[20] was adopted. That is, the frozen brain was thawed at room temperature and then rinsed, blotted dry, and weighed. A brain homogenate was prepared in a 1:1 (g/mL) ratio with ice-cold, pH 7.4, isotonic phosphate buffer by using a microhomogenizer. A 500-μL aliquot of the homogenate was transferred to a new tube and mixed with 2 mL of acetonitrile for extraction and deproteinization by vortex mixing for 30 s. To the mixture was added 500 mg of sodium chloride and the whole was then centrifuged for 5 min at 10 000 rpm and 0 °C. One milliliter of the supernatant was separated and dried at 25 °C under a nitrogen gas stream. The residue was reconstituted with 100 μL of mobile phase and was injected onto an HPLC. For CSF, sample was injected directly onto the analytical column without clean up.

Analytical Methods—The amounts of AZT and its prodrugs in each sample were determined by HPLC. The HPLC apparatus was an Hitachi (Hitachi Seisakusho, Tokyo, Japan) HPLC Model 655 consisting of a UV detector (655A-21), a pump control unit (655A-11), and an integrator (655-61). The chromatographic conditions were as follows: column, 150 mm × 4.6 mm i.d., Wakosil 5C18 (Wako Pure Chemical Ind.); column temperature, 40 °C; flow rate, 1.0 mL/min; UV detection, 265 nm. Mobile phase was comprised of acetonitrile and 0.1% acetic acid. The ratios of acetonitrile and 0.1% acetic acid were 15:85 for AZT, 50:50 for prodrugs III–V, 60:40 for prodrugs I and II, and 95:5 for AZT-C18, respectively. For analysis of AZT in rat brain, alternatively, a 250 mm × 6 mm i.d. Wakosil 5C18 column was used to improve the resolution.

Results and Discussion

Five derivatives of AZT conjugated with a lipophilic adamantane-derived moiety via an ester linkage were prepared (Figure 1, Table 1). Two of them (prodrugs I and II) were directly O-acylated with 1-adamantanecarboxylic acid or 1-adamantaneacetic acid, respectively, by dicyclohexylcarbodiimide (DCC) in the presence of 4-(dimethylamino)pyridine (DMAP).[21,22]



R	Compound
H-	AZT
CO-	Prodrug (I)
CH_2-CO-	Prodrug (II)
CO-NHCH_2-CO-	Prodrug (III)
CO-NH$(CH_2)_2$-CO-	Prodrug(IV)
CO-NH$(CH_2)_3$-CO-	Prodrug (V)
$CH_3(CH_2)_{16}$-CO-	AZT-C18

Figure 1—AZT and its prodrugs.

Table 1—Physical Properties of Prodrugs I–V

Compd	mp (°C)	Yield (%)	Elemental Analysis
I	140–142	65	$C_{21}H_{27}N_5O_5$ Calcd: C, 58.7; H, 6.3; N, 16.3 Found: C, 59.1; H, 6.6; N, 16.1
II	95–99	72	$C_{22}H_{29}N_5O_5$ Calcd: C, 59.6; H, 6.6; N, 15.8 Found: C, 59.7; H, 7.0; N, 15.3
III	95–98	76	$C_{23}H_{30}N_6O_6 \cdot {}^1/_4H_2O$ Calcd: C, 56.3; H, 6.3; N, 17.1 Found: C, 56.7; H, 6.3; N, 16.6
IV	92–95	77	$C_{24}H_{32}N_6O_6 \cdot {}^1/_4H_2O$ Calcd: C, 57.1; H, 6.5; N, 16.6 Found: C, 57.4; H, 6.8; N, 16.1
V	95–97	83	$C_{25}H_{34}N_6O_6 \cdot {}^1/_4H_2O$ Calcd: C, 57.8; H, 6.7; N, 16.2 Found: C, 57.6; H, 7.0; N, 15.8

Others (III–V) were similarly O-acylated with 1-adamantanylcarbonyl amino acids containing various lengths of methylene chains [$NH(CH_2)_nCO$, n = 1–3], which served as a spacer between AZT and the adamantane-derived moiety.

Table 2 shows the log PC of AZT and its prodrugs (I–V) in octanol–buffer and butanol–buffer systems. I and II in the octanol phase were not measurable by means of HPLC. This might be due to ester exchange of the side chain including the adamantane moiety for octanol. Compared to AZT, any prodrugs had fairly high log PC values. The introduction of the adamantane moiety results in an increase in lipophilicity as expected. On the basis of the log PC values of each derivative in butanol–buffer I and II should possess a log PC of around 2.4 in the octanol–buffer system. Considering that compounds with log

Table 2—log PC and Susceptibility to Enzymatic Hydrolysis of AZT and Its Prodrugs

compd	log PC: in octanol–buffer (pH 7)	log PC: in butanol–buffer (pH 7)	Enzymatic Hydrolysis Rate Constant (h^{-1}): in rat plasma	Enzymatic Hydrolysis Rate Constant (h^{-1}): in human plasma
AZT	0.048	0.44	*a*	*a*
I	*b*	2.48	>1.39	0.35
II	*b*	2.52	0.21	*a*
III	2.10	2.29	>1.39	*b*
IV	2.14	2.33	>1.39	*b*
V	2.47	2.52	>1.39	*b*

[a] No degradation was observed up to 4 h. [b] Not tested.

Table 3—Drug Concentrations in Brain and Plasma of Rats after Iv Administration of AZT, Prodrugs I and II, and AZT-C18 (dose, 18.7 μM/kg)[a]

Time after Administration	Concentration of Compound (nmol/g of brain or mL of plasma): AZT	I	AZT from I	II	AZT from II	AZT-C18	AZT from AZT-C18
			Brain				
15 min	0.97	2.49	4.34	17.14	1.12	0.03	0.22
30 min	0.67	0.63	2.43	3.68	0.88	0.01	0.20
1 h	0.26	0.07	0.67	1.15	0.69	0	0.17
2 h	0.11	0	0.04	0	0.39	0.05	0.03
4 h	0	0	0	0	0.08	0	0
			Plasma				
15 min	6.92	0	13.10	0.70	4.75	0.19	3.59
30 min	5.58	0	8.31	1.80	5.35	0.13	4.23
1 h	2.06	0	3.18	0.23	2.92	0.06	2.02
2 h	0.30	0	0.71	0.09	0.60	0.02	0.41
4 h	0.04	0	0.15	0.07	0.30	0	0

[a] Each value represents the mean from three rats.

PC values of 1.5–2.5 in the octanol–water system can be permeable through the BBB,[23] II–V having a log PC value of ca. 2.4 should be readily transported across the BBB into the CNS.

The enzymatic hydrolysis rate constants of AZT and prodrug II–V in rat and human plasma are also summarized in Table 2. The degradation for I and II was a pseudo-first-order reaction, and the hydrolysis rate constants can be calculated from the half-lives. I, III–V, and AZT-C18 were quickly converted into the corresponding parent drug AZT, within 30 min in rat plasma. Whereas, II was less degradable than the rest of the prodrugs and showed more resistant to enzymes in body fluid. The decomposition of AZT was not observed under the present experimental conditions. In the *in vitro* degradation study in human plasma, the rate of the degradations for I and II was much slower than that in rat plasma and II remained 100% intact even in 4 h in human plasma.

The difference in susceptibility to enzymatic hydrolysis between I and II seems to be explainable by the works of Chang and Lee[24] and Kawaguchi et al.[18] in which it was reported that there existed a carbon atom number in the ester side chain which optimized hydrolysis. However, this theory could not be applied to III–V, which incorporated amino acid as the spacer between AZT and the adamantane moiety. The higher resistance against plasma esterases, as well as the appropriate lipophilicity, is a very important property of a prodrug in order to deliver AZT to the brain in the form of the prodrug. With this point, we assumed that II was the most promising prodrug of AZT among I–V and conducted the following *in vivo* studies in rats, mainly focusing on II.

Table 4—Drug Concentrations in CSF of Rats after Iv Administration of AZT and Prodrug II (dose, 18.7 μM/kg)[a]

Time after Administration	Concentration of Compound (nmol/mL of CSF): AZT	II	AZT from IV
	CSF		
15 min	4.37	0.22	1.14
30 min	1.59	0	1.30
1 h	1.53	0	0.69
2 h	0.54	0	0.40
4 h	0.34	0	0.23

[a] Each value represents the mean from three rats.

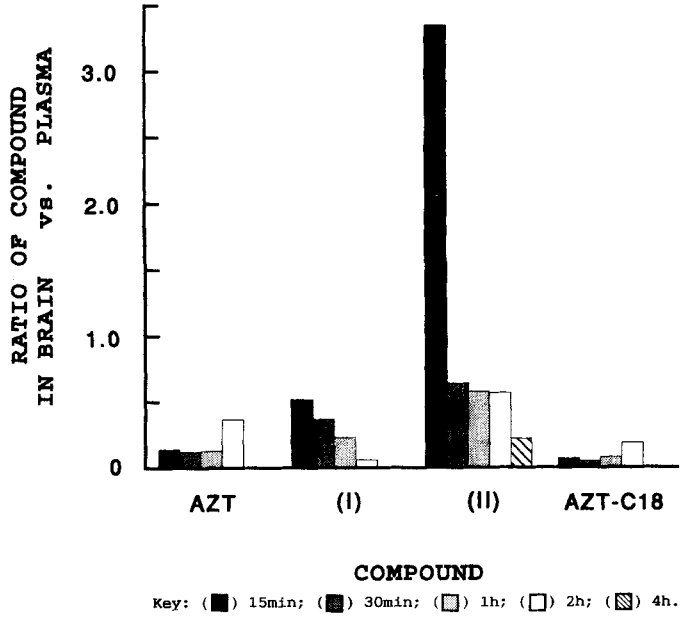


Figure 2—Time courses of brain:plasma ratio for AZT, prodrugs I and II and AZT-C18 after iv administration of a dose of 18.7 μmol/kg. Each value represents the mean of three rats.

Prior to *in vitro* studies, we have confirmed that prodrugs I–V in plasma were degradable only by enzymatic hydrolysis, by performing the stability study in aqueous solutions, without plasma esterases, ranging from pH 2 to 9 for up to 4 h at 37 °C.

Finally, the results of *in vivo* studies are summarized in Tables 3 and 4. Table 3 shows the concentrations of AZT, I, II, and AZT-C18 in brain and plasma. In order to manifest whether the drug levels in brain in Table 3 are attributable to the penetration through the BBB or through the BCSFB, we performed an assay of AZT and II in CSF (Table 4). The AZT concentration in CSF was high, as reported previously,[9–11] whereas the drug levels of II in CFS were very low and much less compared to that in brain besides. These results suggest that the contribution of BCSFB to the transport of II into the CNS seems to be very small. It can be concluded that the introduction of the adamantane moiety to AZT changes the physicochemical properties of AZT and the delivery of prodrug II into the brain is mainly attributable to the BBB pathway. In Table 3, each of the drug concentrations in the brain peaked at the first sampling point (15 min) and their maximal levels were significantly different. That is, in the case of prodrug I, the value was 7 times higher than that obtained when AZT itself was administered. II was more permeable through the BBB than I and the maximum amount attained in the brain was 18 times higher. The distribution to brain at high

concentration of **II** may be due to its higher resistance to enzymatic hydrolysis relative to **I**.

Moreover, it is noteworthy that concentrations of **I** and **II** were fairly high in brain compared to that in plasma. As shown Figure 2, the mean brain:plasma drug concentration ratio at 15 min following AZT administration was 0.14. Whereas, for prodrugs **I** and **II**, mean brain:plasma ratios were 0.52 and 3.35, respectively. The improvement of transport to brain of prodrug **I** and **II** seems to be mainly due to the greater lipophilicity relative to AZT. However, considering that memantine indicated a high CNS affinity and that amantadine has a high affinity to the cell membrane,[15,16] the possibility remains that the adamantane moiety might have a tropism for the CNS and this property might enhance the prodrugs' penetration of the BBB compared to the parent drug. The mechanism for the uptake of these prodrugs into the CNS is not still clear. We are going to carry out further experiments to explore this mechanism. AZT-C18 did not permeate as much as we expected and the value was nearly equal to that of AZT, in spite of the considerably high log PC value of AZT-C18. It is supposed that this poor permeability of AZT-C18 was caused by the its excessive lipophilicity beyond the proper range for permeability.[23]

In conclusion, the above results imply that the adamantane moiety is a useful brain-directed carrier for poorly absorbed drugs. We expect the prodrug approach in the present study to bring sufficient effects to the treatment of the HIV infection of the CNS.

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