solution was basified by the addition of solid $NaHCO_3$. The product was extracted into EtOAc, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 95/5) to give the desired amino derivative **24** (110 mg, 80%): R_f (CH_2Cl_2/MeOH 95/5) = 0.48; ^1H NMR ($CDCl_3$) δ 5.45–5.30 (m, 2 H, CH=CH), 4.21 (s, 1 H, CH_2OCH), 3.80 (d, 1 H, J = 7.5, CH_2OCH), 3.61 (s, 3 H, OCH_3), 3.58–3.52 (m, 1 H, CH_2OCH), 2.66–2.54 (m, 2 H, CH_2NH), 2.46–2.03 (m, 9 H), 1.72–0.88 (m, 18 H); CIMS m/e 352 (M + 1).

[1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[(Hexylamino)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic Acid (25). The methyl ester **24** (70 mg, 0.2 mmol) was dissolved in THF (7 mL) and H_2O (1.4 mL) under a nitrogen atmosphere. A solution of 1 N LiOH (1.8 mL) was added, and the mixture was stirred at room temperature for 3.5 h. The mixture was acidified with solid $NaHSO_4$ to pH 6–6.5 and then diluted with CH_2Cl_2, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The crude product was chromatographed on activated neutral Al_2O_3, eluting first with MeOH and then with an 8/2 mixture of MeOH/H_2O. The MeOH/H_2O fractions were combined, concentrated (to about 4 mL), diluted with CH_2Cl_2, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The residue was triturated with cold petroleum ether to give acid derivative **25** (41 mg, 61%) as a white solid: R_f (CH_2Cl_2/MeOH 80/20) = 0.46; mp = 82–3 °C; ^1H NMR ($CDCl_3$) δ 5.45–5.30 (m, 2 H, CH=CH), 4.54 (s, 1 H, CH_2OCH), 3.79 (d, 1 H, J = 7.5, CH_2OCH), 3.56–3.26 (m, 1 H, CH_2OCH), 2.98–2.78 (m, 2 H, CH_2NH), 2.52–1.11 (m, 28 H); CIMS m/e 338 (M + 1). Anal. ($C_{20}H_{35}NO_3$) C, H, N.

Binding Inhibition Studies. Blood from healthy donors, who had denied having received medication for 10 days, was collected into 0.38% citrate–phosphate–dextrose–adenine buffer. Platelet rich plasma (PRP) prepared from this blood was purchased from the University of Illinois Blood Bank. [^{3}H]U46619 binding to washed human platelets was performed as previously described.[28-30] Briefly, the platelet suspensions (5–7 × 10^8 platelets/mL) were incubated 5 min with [^{3}H]U46619 (final concentration of 10 μM) in the presence of the amine **25** or 13-azaprostanoic acid at varying concentrations (2–250 μM). In order to prevent platelet activation, prostacyclin (final concentration 270 μM) was added 1 min prior to incubation. Nonspecific binding was assessed in a separate incubation in the presence of 10 μM unlabeled U46619. Specific binding was defined as total binding minus binding activity that could not be competed for by 10 μM of unlabeled U46619 and was 85% of total binding. After a 5-min incubation period, platelet suspensions were filtered rapidly under vacuum through Whatman GF/C filters and rinsed with 5 × 3 mL of ice-cold Tydrode-Hepes buffer. [^{3}H]U46619 activity on the filters was determined in a Beckman LS6800 liquid scintillation spectrometer. The results of these studies are summarized in Figure 2.

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Registry No. **5**, 31752-99-5; **6**, 142066-51-1; **7**, 142066-52-2; **8**, 142066-53-3; **9**, 142066-54-4; **10** (isomer 1), 142066-55-5; **10** (isomer 2), 142184-25-6; **10a** (isomer 1), 142066-56-6; **10a** (isomer 2), 142184-26-7; **10c**, 142066-57-7; **11**, 142066-58-8; **11a**, 142066-59-9; **11b**, 142102-19-0; **11c**, 142066-60-2; **11d**, 142066-61-3; **12**, 142066-62-4; **13**, 142066-63-5; **13a**, 142066-64-6; **13b**, 142066-65-7; **14**, 142066-66-8; **14a**, 142066-67-9; **15**, 142066-68-0; **16**, 142066-69-1; **17**, 142066-70-4; **18**, 142066-71-5; **19**, 142066-72-6; **20**, 142066-73-7; **21**, 142066-74-8; **22**, 142066-75-9; **23**, 142066-76-0; **24**, 142066-77-1; **25**, 142066-78-2; *N*-methyl-*S*-methylphenylsulfoximine, 30004-67-2; (4-carboxybutyl)triphenylphosphonium bromide, 17814-85-6; 4-phenylsemicarbazide, 537-47-3; hexylamine, 111-26-2.

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Improved Brain Delivery of AZT Using a Glycosyl Phosphotriester Prodrug

Abdelkader Namane,† Catherine Gouyette,† Marie-Paule Fillion,‡ Gilles Fillion,‡ and Tam Huynh-Dinh*,†

Unité de Chimie Organique, URA CNRS 487, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France, and Unité de Pharmacologie Neuro-Immuno-Endocrinienne, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. *Received February 10, 1992*

The concentration of AZT in mice plasma and brain was measured using HPLC after an ingestion of 20 mg/kg of AZT or the molar equivalent of hexadecyl 2-(α-D-mannopyranosidyl)ethyl 3′-azido-3′-deoxy-5′-thymidinyl phosphate **3**. The results demonstrated the promising qualities of the prodrug **3** which gave AZT-5′-phosphate as the main metabolite: the total concentration of AZT derivatives detected in brain presented a peak of 156 nmol/g (5 nmol/g for AZT) at 1 h; the half-life was about 24 h (1 h for AZT) with an AUC of 4366 nmol h/g as compared to 4 nmol h/g for AZT. The lipophilic properties of **3** were confirmed by its in vitro transport of inside synaptosomes. The derivative 2-(α-D-mannopyranosidyl)ethyl 3′-azido-3′-deoxy-5′-thymidinyl phosphate (**2**) provided also a good delivery of AZT to the central nervous system, with values intermediate between those of AZT and **3**.

3′-Azido-3′-deoxythymidine (AZT)[1] remains the only clinically approved drug against HIV infection[2-4] despite its undesirable side reactions[5] and the emergence of resistant HIV variants.[6] Its serious toxicity can be limited by lower doses[7] than those previously used, but this pro-

† Unité de Chimie Organique.
‡ Unité de Pharmacologie Neuro-Immuno-Endocrinienne.

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Table I.[a] AZT (nmol/mL) Detected in Mice Plasma after Oral Administration of 75 μmol/kg of AZT or Its Derivatives

compound	time (h)	AZT	AZT 5′-P	AZT phosphodiester 2	AZT phosphotriester 3	total AZT	AUC (nmol h/mL)
	0.5	26.6 ± 1.7	21.7 ± 0.7			48.3 ± 2.4	
	1	15.7 ± 1.1	27.5 ± 2.9			43.2 ± 4.0	
AZT	2	6.0 ± 2.2	13.1 ± 4.4			19.1 ± 6.6	90
(20 mg/kg)	4	4.4 ± 1.5	0			4.4 ± 1.5	
	8	0	0			0	
	24	0	0			0	
	48	0	0			0	
	0.5	31.6 ± 0.3	20.4 ± 3.8	4.5 ± 1.4		56.5 ± 5.5	
	1	18.3 ± 3.1	20.0 ± 2.0	5.2 ± 2.6		43.5 ± 7.7	
AZT	2	3.1 ± 1.6	15.0 ± 1.8	1.6 ± 1.3		19.7 ± 4.7	
phosphodiester 2	4	0	6.7 ± 1.2	0		6.7 ± 1.2	918
(41.63 mg/kg)	8	0	5.7 ± 2.1	0		5.7 ± 2.1	
	24	0	26.8 ± 0.9	0		26.8 ± 0.9	
	48	0	17.9 ± 3.0	0		17.9 ± 3.0	
	0.5	1.6 ± 0.7	12.1 ± 7.0	0	0	13.7 ± 7.7	
	1	1.3 ± 0.6	12.7 ± 5.1	0	0	14.0 ± 5.7	
AZT	2	1.4 ± 1.0	4.0 ± 3.7	0	0	5.4 ± 4.7	1035
phosphotriester 3	4	2.3 ± 2.0	3.0 ± 0.4	0	0	5.3 ± 2.4	
(58.33 mg/kg)	8	8.6 ± 7.5	17.7 ± 7.8	0	0	26.3 ± 15.3	
	24	0	28.5 ± 7.4	0	0	28.5 ± 7.4	
	48	0	14.2 ± 3.6	0	0	14.2 ± 3.6	

[a] In vivo experiment. HPLC determination of AZT and its derivatives in mice plasma.

tocol is not adequate for children and for cerebral infections[8] because low doses of AZT do not permit effective intracellular AZT concentrations. The intensive efforts to synthesize lipophilic[9-14] or brain-targeted[13,15,16] prodrugs of this nucleoside have not resulted so far in compounds with clear-cut therapeutic potency.

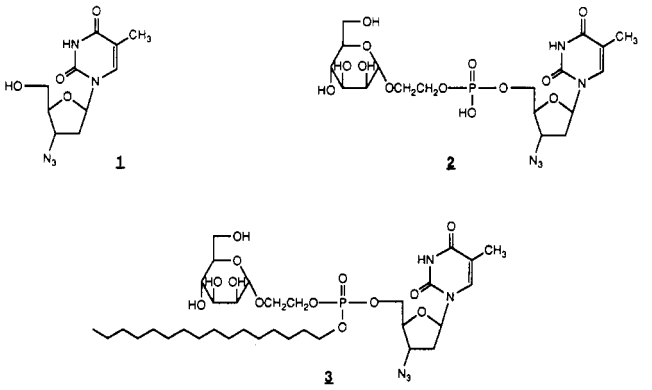

Figure 1. AZT derivatives.

We have recently described the synthesis of glycosyl phosphotriesters of AZT, an NMR transmembrane transport study, and their antiviral activity.[17] These

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compounds were synthesized as lipophilic prodrugs of the nucleoside which could be also hydrolyzed in situ into the 5′-nucleotide which was the first metabolite of AZT during its phosphorylation into the active 5′-triphosphate. A number of phosphate derivatives of nucleosides were also described by different authors.[9,10,18-21] As a pursuit of this work, we present here the cerebral bioavailability of a glycosyl phosphotriester of AZT in animal models.

Biological Results

On the basis of an NMR study with large unilamellar vesicles, we selected the glycosyl phosphate in the (mannopyranosidyl)ethyl series[17] and measured the cerebral and plasma concentrations of AZT (1) and AZT 5′-phosphate after oral administrations of AZT (reference), phosphodiester 2, and phosphotriester 3 derivatives of AZT,[17] from 0.5 to 48 h (Figure 1).

In order to avoid artifacts due to numerous extraction procedures or chemical treatments, the determination using HPLC was done on whole extracts of biological materials after a centrifugation and filtration step to discard insoluble materials.

All the figures given by HPLC referred to the concentration of unbound compounds in the biological extracts and were not corrected by a recovery factor which was determined as being in the 50–60% range by adding a known amount to brains or blood samples just after collection.

1. Plasma Concentration of AZT Derivatives. Before any in vivo experiment, we first checked the stability of AZT and its derivatives in the biological extracts upon standing. The control HPLC showed that hydrolysis in plasma was different according to the compound: AZT and phosphotriester 3 were recovered completely unchanged upon standing 40 h, with traces of AZT 5′-phosphate (~2%) detected from AZT; phosphodiester 2 was partially hydrolyzed into AZT and AZT 5′-phosphate (~10% in 10 h); AZT 5′-phosphate was completely hydrolyzed into AZT after 5 h (data not shown). Serial dilutions of samples were also done in order to determine the calibration curve.

After an ingestion of AZT, we observed in mice plasma a peak of AZT at 0.5 h (26 nmol/mL) which decreased rapidly. The concentration of AZT 5′-phosphate was of the same magnitude (27 nmol/mL), and its maximum level was shifted to 1 h. 4 h after ingestion, no AZT 5′-phosphate was detected. The total concentration of AZT and its 5′-phosphate ranged from 4 to 48 nmol/mL (Table I). It is surprising that AZT 5′-phosphate was found in significant concentration in the mouse plasma since it should result from the activity of cellular kinases and thus being produced inside the cells. We do not know if the presence of the monophosphate resulted from a metabolization by plasma enzymes or simply by a partial cell lysis during the experimental procedure; in the control experiments a small amount (~2%) of AZT 5′-phosphate was detected after incubation of AZT in plasma samples. To our knowledge, the observation of circulating AZT 5′-phosphate after an administration of AZT or its prodrugs[9-11,14] has not been reported.

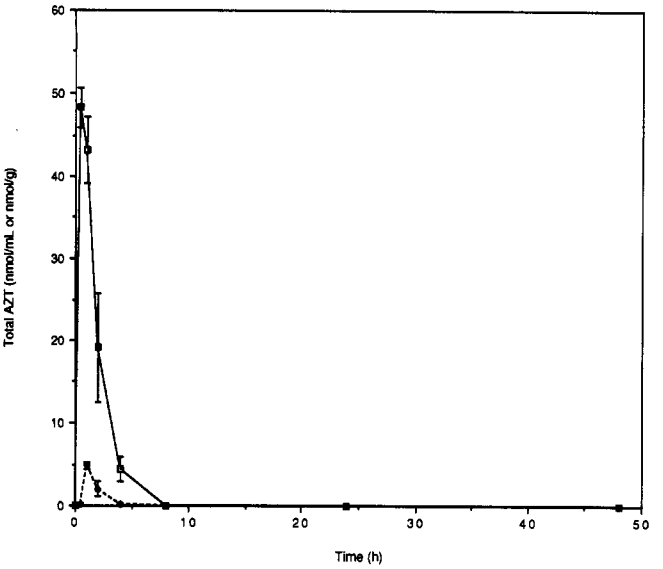

Figure 2. Total concentration of AZT derivatives from AZT in brain (---) and plasma (—).

With the ingestion of the phosphodiester 2, the two major compounds detected were also AZT (31 nmol/mL) and AZT 5′-monophosphate (20 nmol/mL). The main difference with the previous ingestion lay in the kinetics of formation of these two compounds: the peak level of AZT was at 0.5 h, with a total disappearance at 4 h, compared to a maximum level of AZT 5′-phosphate at 0.5–1 h which decreased slowly to 6 nmol/mL at 8 h and increased again up to 27 nmol/mL at 24 h. The complexity of the concentration curve of AZT 5′-phosphate could be explained by its different origins: the nucleotide may result from the partial hydrolysis of phosphodiester 2 or/and from the phosphorylation of AZT given by the total hydrolysis of 2; the kinetics parameters of the different enzymatic steps are unknown at the present state. Moreover, it is not excluded that compound 2 might bind to a protein compartment of the plasma and be released after 24 h. The mechanism involved in the late increase of unbound drug in the plasma is not yet clarified. A small amount of phosphodiester 2 (2–5 nmol/mL) was also detected up to 2 h. The total AZT derivatives arising from the ingestion of 2 was in the same order as that given by AZT, with the difference being in its long-lasting effect, since at 48 h, its value was 18 nmol/mL, with an area under curve (AUC) of 918 nmol h/mL to be compared to an AUC of 90 given by AZT.

With the phosphotriester 3, the AZT concentration remained very low (1–2 nmol/mL) throughout all the experiment; the concentration of AZT 5′-phosphate was similar to that given by the phosphodiester 2: one peak at 0.5–1 h, a slow decrease and again a rise after 8 h. No phosphodiester 2 was detected and phosphotriester 3 was also absent. The latter fact could be explained by the tight binding of this lipophilic prodrug to albumin or other lipoproteins occurring in plasma. It should be pointed out

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Table II.[a] AZT (nmol/g) Detected in Mice Brains after Oral Administration of 75 μmol/kg of AZT or Its Derivatives

compound	time (h)	AZT	AZT 5′-P	AZT phosphodiester 2	AZT phosphotriester 3	total AZT	AUC (nmol h/g)
	0.5	0.2 ± 0.1	0			0.2 ± 0.1	
	1	4.9 ± 0.4	0			4.9 ± 0.4	
AZT	2	2.0 ± 0.9	0			2.0 ± 0.9	4
(20 mg/kg)	4	0.2 ± 0.1	0			0.2 ± 0.1	
	8	0	0			0	
	24	0	0			0	
	48	0	0			0	
	0.5	1.8 ± 0.3	31.0 ± 1.6	5.8 ± 1.3		38.6 ± 3.2	
	1	1.9 ± 0.2	79.3 ± 9.0	16.5 ± 0.8		97.7 ± 10.0	
AZT	2	1.2 ± 0.3	19.8 ± 4.3	5.2 ± 1.3		26.2 ± 5.9	2625
phosphodiester 2	4	0.9 ± 0.2	59.3 ± 14.0	7.9 ± 1.3		68.1 ± 15.5	
(41.63 mg/kg)	8	0.9 ± 0.2	33.3 ± 7.1	8.9 ± 1.8		43.1 ± 9.1	
	24	0.8 ± 0.3	56.5 ± 7.9	4.5 ± 0.1		61.8 ± 8.3	
	48	0.8 ± 0.3	48.5 ± 13.1	2.5 ± 0.5		51.8 ± 13.9	
	0.5	1.5 ± 0.2	36.7 ± 5.9	0.3 ± 0.3	0	38.5 ± 6.4	
	1	1.5 ± 0.3	146.7 ± 12.5	8.5 ± 1.6	0	156.7 ± 14.4	
AZT	2	2.9 ± 0.5	132.7 ± 5.0	5.2 ± 0.9	0	140.8 ± 6.4	
phosphotriester 3	4	1.0 ± 0.3	113.7 ± 15.0	7.1 ± 2.1	0	121.8 ± 17.4	4366
(58.33 mg/kg)	8	2.0 ± 0.2	106.0 ± 15.5	5.3 ± 0.8	0	113.3 ± 16.5	
	24	1.4 ± 0.4	81.3 ± 21.2	5.2 ± 0.3	0	87.9 ± 21.9	
	48	1.1 ± 0.3	58.6 ± 12.9	3.8 ± 0.2	0	63.5 ± 13.4	

[a] In vivo experiment. HPLC determination of AZT and its derivatives in mice brain.

that if the total amount of AZT derivatives arising from phosphotriester 3 was lower (5–26 nmol/mL) than that given by AZT or phosphodiester 2, its AUC was of the same order (1035 nmol h/mL) as that given by phosphodiester 2. It appeared that, as expected, the prodrug 3 was rapidly metabolized into AZT and AZT 5′-phosphate and slowly released from its depot form or binding with lipoproteins or interfacial membranes.

2. Brain Concentration of AZT Derivatives. Opposite to the partial hydrolysis in plasma, all the AZT derivatives were not degraded in the cerebral extracts and were recovered unchanged by HPLC up to 40 h.

As shown in Table II, an oral dose of 20 mg/kg of AZT gave a peak level of 5 nmol/g of AZT after 1 h, with a total disappearance after 4 h. No trace of AZT 5′-phosphate was detected. The AUC is at 4 nmol h/g, to be compared to a value of 90 in plasma. These results were similar to those obtained by Chu et al.[16] Figure 2 shows the AZT concentrations in the two biological extracts, with the level of AZT in brain lower than in plasma.

With the administration of the same molar dose of phosphodiester 2, we detected the unchanged compound with a maximum peak of 16 nmol/g at 1 h and a small amount of AZT (in the range of 1–2 nmol/g) lasting up to 48 h. The main metabolite from the phosphodiester 2 is AZT 5′-monophosphate, with a main peak at 1 h (79 nmol/g) which decreased slowly, since at 48 h, 48 nmol/g were still detected. The drop of AZT 5′-phosphate at 2 h was similar to that observed in plasma, as the second peak at 24 h. In addition to the different kinetics of formation of AZT 5′-phosphate, the concentration of the nucleotide observed here was the result of two transport systems: the transport across the intestinal epithelia and the transport across the blood–brain barrier. The total amount of AZT and its derivatives ranged from 26 to 98 nmol/g of brain, with a very high AUC of 2625 nmol h/g. Figure 3 shows the total concentration of AZT derivatives arising from the phosphodiester 2; the striking fact was that the concentration in brain was now higher than that found in plasma. One could hypothesize that active transport has occurred with this derivative which was ionic and not lipophilic.[17]

With the phosphotriester 3, the ingested prodrug was not detected. This could be attributed, as in plasma, to

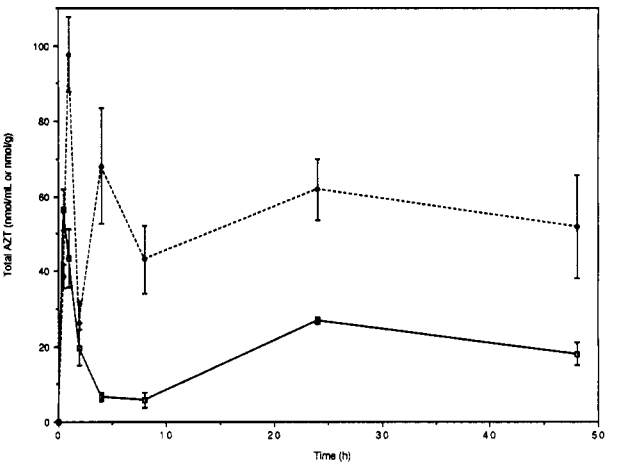

Figure 3. Total concentration of AZT derivatives from 2 in brain (- - -) and plasma (—).

efficient binding of this lipophilic derivative to a lipoprotein or cellular membranes. We detected its in vivo degradation into the phosphodiester 2 (0.3–8 nmol/g), AZT-5′ phosphate (36–147 nmol/g) which was again the major metabolite, and AZT (1–3 nmol/g). The total amount of AZT derivatives detected from phosphotriester 3 ranged from 38 to 156 nmol/g of brain with an AUC curve of 4366 nmol h/g. These figures were more than 100 times superior to those previously obtained with the dihydropyridine carrier system applied to nucleosides.[15,16,22] Figure 4 shows the same curve as observed with the phosphodiester 2: the phosphotriester 3 gave, as expected, a very higher concentration of AZT derivatives in brain than in plasma. This prodrug, besides being rapidly metabolized and slowly released, could be also very efficiently extracted from the plasma owing to its lipophilic properties.[17]

The comparison between plasma and cerebral concentrations of glycosyl phosphotriester 3 demonstrated that it possessed the two properties anticipated at the beginning

(22) Palomino, E.; Kessel, D.; Horwitz, J. P. A dihydropyridine carrier system for sustained delivery of 2′,3′-dideoxynucleosides to the brain. *J. Med. Chem.* **1989**, *32*, 622–625.

Table III.[a]

	external nucleoside (nmol/g)	total internal nucleoside (nmol/g)	R: internalization (%)
AZT	9750	2.5 ± 0.2	0.03
AZT 5′-phosphate	2000	1.1 ± 0.4	0.06
AZT phosphodiester 2	8000	8.3 ± 1.2	0.10
AZT phosphotriester 3	9100	567.8 ± 59.5	6.24

[a] In vitro experiment. HPLC determination of AZT and its derivative in synaptosomes.

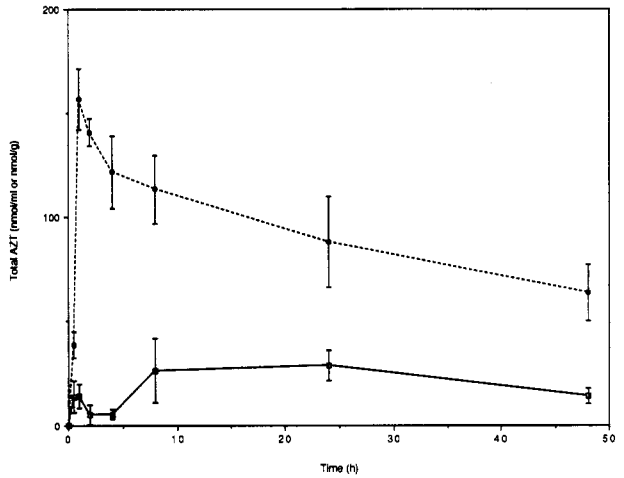

Figure 4. Total concentration of AZT derivatives from 3 in brain (- - -) and plasma (—).

of this work[23]: its lipophilic properties allowed transfer across the blood–brain barrier, and it was metabolized into AZT and especially into AZT-5′ monophosphate. Even 48 h after ingestion, the cerebral concentration of AZT derivatives was still 50 times higher than the minimum level needed for antiviral activity.[3]

3. Synaptosome Concentration of AZT Derivatives. Although the in vivo measurements were similar to published procedures,[14,15] the high values given by HPLC did not unambiguously prove the presence of AZT and its derivatives in the cerebral tissue. In order to confirm the blood–brain transfer (particularly to cancel out the effects of compounds adsorbed in the capillaries of the brain) and to distinguish between the two possible mechanisms involved in the transport of the phosphodiester (active transport) and the phosphotriester (lipophilic diffusion), we have performed an in vitro determination of AZT, AZT 5′-phosphate, and its derivatives 2 and 3 in purified extracts of cerebral tissue. Synaptosomes were incubated with 3 mM solutions of each compound for 15 min and washed, and the internal concentrations of the nucleoside were measured by HPLC. In each case, the compound incubated was found undegraded inside the synaptosomes. Table III shows that the internal concentrations of AZT from AZT, AZT 5′-phosphate, and phosphodiester 2 are in the range of 1 to 8 nmol/g. There was no significant difference between the nucleoside and its ionic derivatives: 0.03% yield of internalization from AZT, 0.06% for the 5′-phosphomonoester, and 0.10% for the phosphodiester. On the other hand, the phosphotriester 3 gave a very high yield (6.24%) of internal concentration at 567 nmol/g. Another experiment with an incubation time of 45 min gave very similar figures (data not shown). This result confirmed the NMR study[17] which demonstrated that only the phosphotriester 3 was detected inside large unilamellar vesicles, implying a transmembrane transport into the intravesicular interface.

Although both the phosphate derivatives 2 and 3 gave a high cerebral concentration of AZT in vivo, this simple experiment showed that two possible mechanisms may be involved, an active transport system similar to the translocation process of the glycosyl moiety (for 2 which is not lipophilic) and a very efficient diffusion due to the lipidic moiety of 3.[17]

Conclusion

The concept of a glycosyl phosphotriester based on the glucosyl dolichol phosphate[23] appears to be a very valuable transport system, compared to the brain-targeting Bodor's transport, although more experiments are needed to determine the distribution of AZT and its derivatives in other tissues and to clarify the exact transport mechanism. This system presents the additional advantages: (i) the main metabolite is the 5′-nucleotide which bypasses the first step of activation by cellular and viral kinases; (ii) the degradation products, a carbohydrate (glucose or mannose) and the lipidic alcohol (hexadecanol), are well-tolerated compounds; (iii) the solubility of the prodrug in water allows oral administration versus the intravenous injection of DMSO solutions in the previous work.[15,16,22]

The unexpected good delivery to central nervous system given by the glycosyl phosphodiester deserves by itself another independent study. This report also shows the potentiality of dosage of AZT derivatives using analytical HPLC on whole biological extracts.

In conclusion, this in vivo and in vitro study demonstrated that the glycosyl phosphotriester is a very effective transport system for brain targeting of AZT and appears promising as a potential prodrug for the clinical treatment of AIDS. This concept is under development for other nucleosides and pharmacologically active drugs.

Experimental Section

1. In Vivo Studies. Male mice (Swiss 18–20 g), six weeks old, were housed in a 12 h/12 h light/dark cycle at 22 °C with food and water ad libitum. Mice either received po (volume of gavage 500 μL/20 g mouse) the substance to be tested or the vehicle (NaCl 9%) corresponding to 20 mg/kg AZT, 41.63 mg/kg for phosphodiester 2, and 58.33 mg/kg for phosphotriester 3 in solution at 3 mM (1.5 μmol of AZT or its derivative/mouse).

Mice were sacrificed by decapitation 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, and 48 h after administration of the substance (treated mice) or vehicle (controls).

Tissue Preparation and Extraction Procedure. Mice brains were dissected out on ice and homogenized in acetonitrile–water (3:1) (5 mL per g of tissue) using an Ultraturax (30 s, 22000 rpm). Each homogenate was centrifuged in a glass tube (Corex) (6000g for 40 min) at 4 °C. The corresponding supernatant was collected, deep frozen, and lyophilized, and constituted the extract to be analyzed.

Three mice were used for each time point, measured in triplicates. Three series of measurements were done for each time point.

Plasma Sampling. Aortic blood samples (ca. 700–800 μL) were collected from mice in heparinized Ependorff tubes and gently agitated. After centrifugation (13000g for 10 min) the supernatant (plasma) was collected and stored frozen at −22 °C.

(23) Neumann, J.-M.; Hervé, M.; Debouzy, J.-C.; Iglesias-Guerra, F.; Gouyette, C.; Dupraz, B.; Huynh-Dinh, T. Synthesis and transmembrane transport studies by NMR of a glucosyl phospholipid of thymidine. J. Am. Chem. Soc. 1989, 111, 4270–4277.

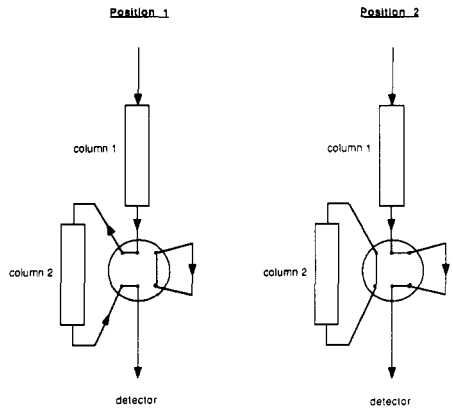

Figure 5. HPLC arrangement showing the two positions of column-switching valve.[25]

The blood collection was realized at different times after oral administration (25 mg/kg) of the various drugs (0.5, 1, 2, 4, 8, 24, and 48 h). The determination of the plasma contents in the studied compounds (AZT, **2**, and **3**) was done using the plasma of three mice for each experimental point, each time point being measured in triplicates.

2. In Vitro Assays. Mice brains (three) were dissected out on ice, and crude synaptosomes were prepared according to the method of Gray and Whittaker[24] with minor modifications. Briefly, cerebral tissue was homogenized using a Potter–Elvehjem homogenizer in sucrose solution (10%) buffered with Tris-HCl (5 mM, pH 7.4 at 0 °C) corresponding to 10 mL/g of fresh tissue. The homogenate was centrifuged at 3000*g* for 5 min to eliminate cell debris, nuclei, and blood vessels, and the resulting supernatant was centrifuged at 7000*g* for 30 min in Corex tubes to collect the crude mitochondrial fraction containing synaptosomes. The corresponding pellet was resuspended in the same Tris–sucrose medium (1.5 mL/g of brain tissue). This preparation was divided in three parts and was used to test the transport of AZT, AZT 5′-phosphate, **2**, and **3** at a concentration of 3 mM (2 mL). The incubation in the presence of these compounds was performed at 37 °C for 15 min. The reaction was stopped by dilution (10 times) with Tris–sucrose solution at 0 °C immediately followed by a centrifugation (30 min at 7000*g*). The resulting pellet was washed twice under the same conditions. The washed synaptosomal preparation was resuspended in acetonitrile–water solution (3:1) to extract the internal nucleoside. The extraction procedure lasted 30 min and was followed by a centrifugation (30 min, 7000*g*); the resulting supernatant was collected, deep frozen and lyophilized, and constituted the sample material to be analyzed.

3. HPLC Measurements. Instrumentation. The HPLC system was a Hewlett–Packard 1090 M equipped with a variable volume autoinjector, a six-port switching valve, a diode-array detector, and a HP 9153C station for the pumping system and data treatment. Analytical columns (15 cm × 4.6 mm) were packed with Nucleosil 5-C18 300-Å stationary phase (Macherey–Nagel). The instrument arrangement was that of Mathes et al.[25]: column 1 was used as a precolumn to separate the nucleosides from proteins of the biological samples. Column 2 was used to determine the concentration of AZT and its derivatives (Figure 5). An analytical cycle was of 46 min with a flow of 0.7 mL/min with the following chromatographic events as shown in Table IV. External standards (1–3 mg/mL) were used to determine the concentration of each nucleoside.

Table IV.

chromatographic event		time[c] (min)
column-switching valve to position 1		
eluent A[a] = 0%	eluent B[b] = 100%	0
sample injection		
column-switching valve to position 2		0.01
eluent A = 14%	eluent B = 86%	10
column-switching valve to position 1		10.50
eluent A = 14%	eluent B = 86%	20
eluent A = 100%	eluent B = 0%	25
end of run		
eluent A = 100%	eluent B = 0%	35
equilibration		46
eluent A = 0%	eluent B = 100%	

[a] Eluent A: methanol (Merck 6009). [b] Eluent B: triethylammonium acetate 10^{-2} M pH 7. [c] Retention time: AZT 30.0 min, AZT 5′-phosphate 17.6 min, **2** 23.0 min, and **3** 34.8 min.

Preparation of Samples. Lyophilized mice brain homogenates were dissolved with 800 μL of water, homogenized with a vortex, and divided in three Ultrafree-MC (Millipore) tubes which were centrifuged at 10000 rpm for 20 min to allow a filtration through 0.45 μm. The filtrates were pooled, homogenized with a vortex, and divided in three fractions of 250 μL for the autoinjector of the HP 1090M. For each analysis of 250 μL, two injections were done: (1) injection of 100 μL of the sample alone; (2) injection of 100 μL spiked with the standards (2 μg AZT, 3 μg AZT 5′-phosphate, 3 μg of **2**, and 3 μg of **3**). The quantification was performed by comparing the area of these two measurements with those obtained from a control (mice having ingested only the vehicle).

For the in vitro experiments, the HPLC conditions were those described precedently.[17]

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Registry No. 1, 30516-87-1; **2**, 127306-80-3; **3**, 127246-78-0; AZT-5′-phosphate, 29706-85-2.

(24) Gray, E. G.; Whittaker, V. P. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.* **1962**, *96*, 79–87.

(25) Mathes, L. E.; Muschik, G.; Demby, L.; Polas, P.; Mellini, D. W.; Issaq, H. J.; Sams, R. High-performance liquid chromatographic determination of 2′,3′-dideoxycytidine and 3′-azido-3′-deoxythymidine in plasma using a column-switching technique. *J. Chromatogr.* **1988**, *432*, 346–351.