In Vitro and *in Vivo* Transport of Zidovudine (AZT) Across the Blood–Brain Barrier and the Effect of Transport Inhibitors

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The transport of the antiviral nucleoside analogue zidovudine (3'-azido-3'-deoxythymidine; AZT) into the central nervous system (CNS) was characterized *in vitro* and *in vivo*. The *in vitro* model consisted of primary cultures of isolated bovine capillary endothelial cells. The transport rate of AZT across the monolayer, expressed as endothelial permeability *P*, was determined following luminal and abluminal administration. *P* did not differ between the two administration sites (luminal, 1.65 ± 0.44 cm/min/10^3; abluminal, 1.63 ± 0.28 cm/min/10^3). The transport of AZT across the endothelial cell monolayer was found to be concentration independent in the range between 0.4 and 50 μg/mL. AZT transport was not affected by pretreatment of the cells with either metabolic inhibitors (DODG and DODG/NaN_3) or probenecid. This suggests that AZT passes the monolayer mainly by passive diffusion. The *in vivo* transport of AZT across the blood–brain barrier and the blood–CSF barrier was studied in male Wistar rats after coadministration of potential inhibitors of active transport of AZT: probenecid (organic anion transport) and thymidine (nucleoside transport). Intracerebroventricular and intravenous coadministration of probenecid caused a significant ($P < 0.001$) increase in the CSF/plasma concentration ratio compared to the control phase, indicating that the organic anion carrier is involved in AZT transport from CSF to blood. Since there was no effect of probenecid on the transport of AZT *in vitro*, it is suggested that this carrier is located at the choroid plexus. Coadministration of thymidine did not affect the CSF/plasma concentration ratio, suggesting that a nucleoside carrier system is not involved in AZT transport into or out of the CNS.

KEY WORDS: azidothymidine (AZT); central nervous system; blood–brain barrier; brain; cerebrospinal fluid; transport; acquired immunodeficiency syndrome (AIDS).

INTRODUCTION

Zidovudine, 3'-azido-3'deoxythymidine (azidothymidine; AZT)[3] was shown to inhibit human immunodeficiency virus (HIV) *in vitro* (1) and to induce immunologic, virologic, and neurologic improvements in HIV-I-infected patients (2). Furthermore, it increases the life duration of patients with acquired immunodeficiency syndrome (AIDS) (3). HIV can enter and replicate in the central nervous system (CNS) (4), causing several neurological disorders. This occurs in about 40% of AIDS patients (5), the most frequent of which is the AIDS–dementia complex, which is characterized by several motor, cognitive, and behavioral impairments (6). AIDS–dementia complex often appears in the late stage of an HIV infection (7). Furthermore, the infected brain may serve as a reservoir for the virus, from which the periphery may continuously be reinfected (4). Therefore, it is very important that an anti-HIV agent passes the blood–brain barrier (BBB) and achieves effective concentrations in the brain.

AZT has been shown to reduce the incidence and symptoms of AIDS–dementia complex (8). AZT was found to penetrate into the cerebrospinal fluid (CSF) (2), but its brain concentrations are very low (9). In principle it should be possible to achieve higher concentrations by giving higher doses of AZT, but since AZT has also toxic effects, particularly bone marrow suppression (3,10), this is not really possible in practice. A more appropriate approach would be site-specific enhancement of the CNS levels of AZT. This should lead to increased efficacy in patients with AIDS–dementia complex without further increases in toxicity. Detailed knowledge of the transport pathways of AZT into and out of the CNS is required for the development of strategies to enhance brain availability.

Our group has developed two models to characterize the transport of drugs across the BBB: an *in vitro* model which consists of primary cultures of bovine brain endothelial cells (11) and an *in vivo* rat model with simultaneous collection of CSF and blood (12). The cell culture model provides information on the transport rate and mechanism of drugs across the BBB alone, whereas in the *in vivo* model the availability of drugs in the CSF can be studied as a function of drug passage across the BBB (capillary endothelium), the blood–CSF barrier (mainly the choroid plexus epithelium), and the CSF–brain barrier (ependymal cells) in conjunction with total-body pharmacokinetics.

The aim of this investigation was to use both models to understand better the transport pathways of AZT between blood, CSF, and brain tissue. Special emphasis is given to carrier-mediated transport and its direction. Specific (probenecid, thymidine) and nonspecific inhibitors [2-desoxy-D-glucose (DODG), sodium azide] were used to identify potential carrier systems.

MATERIALS AND METHODS

Drugs

AZT (3'-azido-3'deoxythymidine; zidovudine) was a gift from Burroughs Wellcome Co. (Research Triangle Park, NC). Probenecid, thymidine, and sodium azide were ob-

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[3] Abbreviations used: AIDS, acquired immunodeficiency syndrome; AZT, azidothymidine; BBB, blood–brain barrier; CCM, cell culture medium; CNS, central nervous system; CSF, cerebrospinal fluid; DODG, 2-desoxy-D-glucose; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; icv, intracerebroventricular; iv, intravenous; LDL, low-density lipoprotein; MEM, minimal essential medium; TEER, transendothelial electrical resistance.

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tained from Sigma (St. Louis, MO). DODG was purchased from Merck AG (Darmstadt, Germany).

In vitro Model

With the *in vitro* model, the transport of AZT across bovine cerebrovascular endothelial cell monolayers grown on collagen-coated filter membranes (Transwell-Col) was investigated.

Isolation Procedure

To isolate cerebrovascular endothelial cells from freshly obtained bovine brains, a modified procedure of Audus and Borchardt ((13) was used, as described by Van Bree *et al.* (11).

Cell Culturing Procedure

For the initiation of cell cultures, Transwell-Col filters with a surface area of 4.71 cm^2 (Costar Corp., Cambridge, MA) were placed on a plate with six wells (Costar Corp.) and coated with a 0.01 mg/mL collagen solution in 0.1% (v/v) acetic acid. The filter was then placed under ammonia vapor to cross-link the collagen matrix and sterilized under UV light for at least 1 h. Thirty minutes before seeding, the filters were coated with 0.04 mg/mL fibronectin (Boehringer Mannheim B.V., Almere, The Netherlands). Aliquots of the endothelial cell suspension were thawed and washed twice with 30 mL cell culture medium (CCM), containing 45% (v/v) F-12 nutrient mixture, 45% (v/v) MEM, 10% (v/v) horse serum, 50 m*M* HEPES, 13 m*M* $NaHCO_3$, 100 μg/mL streptomycin, 100 μg/mL penicillin G, and 2.5 μg/mL amphotericin B. On the luminal side of the filter, 1.5 mL of the cell suspension in CCM was added. The density of the cell suspension depended on the batch of isolated cells used and varied from 2.2 to 2.9 million cells per filter. In the abluminal compartment, 2.5 mL CCM was added. The cells were cultivated at 37°C and 5% carbon dioxide until a confluent monolayer was obtained, within 8 to 11 days. Every second day the medium was renewed, and the cell cultures were checked by phase-contrast microscopy. Only confluent monolayers were used for transport experiments. To determine the quality of each monolayer and to compare batches of endothelial cells, the transendothelial electrical resistance (TEER) was measured 2 hr before the experiment, using the method of Van Bree *et al.* (14). The cells were characterized by Ac-LDL uptake and γ-glutamyl transpeptidase activity (H. E. de Vries, personal communication).

Transport Experiments

All transport experiments were performed in cell culture medium at 37°C. AZT was administered in the donor compartment (on either the luminal or the abluminal side of the endothelial monolayer). The transport rate of AZT was determined after luminal administration of 0.4, 2, 10, and 50 μg/mL and after abluminal administration of 2 μg/mL. For the inhibition experiments, the transport rate of 2 μg/mL AZT was determined after a 3-hr pretreatment with either 25 m*M* DODG [metabolic inhibition (15)], 25 m*M* DODG/1 m*M* sodium azide [inhibition of the oxidative phosphorylation (16)], or 20 μg/mL probenecid [inhibition of the organic anion carrier system (17)].

At 15, 30, 45, 60, 75, and 90 min after the onset of the experiment 200-μL samples were taken from the acceptor side and replaced with 200 μL of prewarmed cell culture medium. The concentrations in the acceptor chamber were corrected for sampling. Immediately after addition of AZT and at the end of the experiment, 50-μL samples were taken from the donor compartment. At the end of the transport experiment the endothelial cell monolayer was microscopically evaluated for its integrity.

Data Analysis

The amount of AZT transported across the endothelial cell monolayer was expressed as the cumulative cleared volume of the donor compartment. The method of Cooper *et al.* (18) was used, as modified by Van Bree *et al.* (11). The clearance across the endothelial cell monolayer was calculated by means of linear regression of the cumulative cleared volume versus time by using a custom-made APL plus program (STSC Inc., Rockville, MD). The clearance was corrected for the control clearance across the filter membrane alone and expressed as endothelial permeability P_{BBB}, according to Eq. (1):

$$P_{BBB} = \frac{Cl_{Test} \cdot Cl_{Control}}{A \cdot (Cl_{Control} - Cl_{Test})} \quad (1)$$

where

P_{BBB} = permeability of the endothelial cell monolayer (cm/min)
Cl_{Test} = clearance across cell monolayer and filter membrane (μL/min)
$Cl_{Control}$ = clearance across the filter membrane alone (μL/min)
A = surface area (4.71 cm^2)

In vivo Model

The *in vivo* model comprised simultaneous collection of CSF and plasma in male Wistar rats. Cannulas were implanted in the cisterna magna (for CSF sampling), the lateral ventricle (for intracerebroventricular infusion), the femoral artery (for blood sampling), and the jugular vein (for intravenous infusion). The penetration of AZT into the CSF was studied after coadministration of potential inhibitors (probenecid and thymidine) of active transport of AZT. A three-phase study design was chosen to determine the direction of the carrier system.

Animals and Surgery

The methods of Bouman *et al.* (19) and Van Bree *et al.* (12) were performed for the cannulation of the animals, as described by Van Bree *et al.* (12).

Drug Administration and Sample Collection

For intravenous (iv) administration, the cannula in the jugular vein was connected to a motor-driven syringe pump (Braun-Melsungen A.G., Melsungen, Germany). During the

first 5 min of the experiment, AZT (1 mg/mL) was given as a bolus infusion at a rate of 100 μL/min to reach steady state rapidly. The steady-state concentration was fixed at 2 μg/mL in plasma. For the remaining 6 hr, AZT was given as a constant-rate infusion (20 μL/min). For intracerebroventricular (icv) administration the lateral ventricle cannula was connected to a motor-driven syringe pump (Harvard, Plato B.V., Diemen, The Netherlands). The solution was infused at a constant rate of 1.25 μL/min. AZT administration was performed in three phases: the control phase A (without coadministration), phase B (with coadministration of the inhibitor icv), and phase C (with coadministration of the inhibitor icv and iv).

In phase A, AZT was given intravenously and artificial CSF [mock: 140 m*M* NaCl, 3.0 m*M* KCl, 2.5 m*M* $CaCl_2$, 1 m*M* $MgCL_2$, 1.2 m*M* Na_2HPO_4, 0.27 m*M* NaH_2PO_4, and 7.2 m*M* glucose, pH 7.2 (20)] was given intracerebroventricularly without an inhibitor (control phase). Blood samples (about 200 μL) were drawn at 0 (blank), 5, 15, 30, 45, 60, 75, and 90 min following start of drug infusion. CSF samples (ca. 20 μL) were drawn from the cisterna magna cannula with glass capillaries at 0 (blank), 15, 30, 45, 60, 75, and 90 min after the onset of the experiment.

At the beginning of phase B, the infusion solution for icv administration was replaced with a solution containing 2 mg/mL inhibitor (probenecid or thymidine) in artificial CSF. Plasma and CSF samples were drawn at 2, 2.25, 2.5, 2.75, and 3 hr after the onset of the experiment. At the beginning of phase C, the iv infusion syringe was replaced with a syringe containing a solution of 1 mg/mL AZT and 5 mg/mL inhibitor. Blood and CSF samples were drawn at 3.5, 4, 4.5, 5, 5.5, and 6 hr after the onset of the experiment.

Plasma was obtained after centrifugation of the heparinized blood samples. The weight of each CSF sample was determined before freezing. Plasma and CSF samples were stored at −30°C until analysis. At the end of the experiment, the position of the cannula in the lateral ventricle was verified by injection of 5 μL of a 0.5% Evans Blue solution in saline. Then the rat was decapitated, the skull opened, and the brain removed and dissected by a paramedian coronal section. Blue discoloration of the entire ventricular system indicated a correct cannula position.

Data Analysis

The mean CSF/plasma steady-state concentration ratio and the total plasma clearance of AZT were determined for the three different phases of each experiment separately. Total plasma clearance (CL_{tot}) was calculated, according to

$$Cl_{tot} = \frac{R_{inf}}{C_{SS}} \quad (2)$$

where

R_{inf} = infusion rate
C_{SS} = steady-state concentration

Drug Analysis

CCM, plasma, and CSF samples were analyzed for AZT using a reversed-phase HPLC assay modified from Kupferschmidt and Schmid (21). The HPLC system consisted of a pump (Waters, Milford, MA), an autoinjector (Promis, Spark Holland B.V., Emmen, The Netherlands), a Spherisorb S3ODS2 C18 packed column (Phase Separations Ltd., Queensferry, UK) with a length of 10 cm and an inner diameter of 4.6 mm, and a UV absorbance detector (UV-spectroflow 757, ABI Analytical Kratos Division, Ramsey, at an operating wavelength of 267 nm. The mobile phase consisted of 15% acetonitrile and 85% 0.12 *M* phosphate buffer, pH 6.2, containing 14.40 m*M* triethylamine. The flow rate was 1 mL/min. Chromatographic data were recorded and processed using a Shimadzu C-R3A integrator (Shimadzu Corp., Kyoto, Japan). The sample preparation was done as follows: 50 μL of a phenacetin solution (1.6 μg/mL) was added to all samples as internal standard. Subsequently, the samples were deproteinated with 500 μL acetonitrile and centrifuged, and the acetonitrile was evaporated in a vacuum evaporator (Vacuum Vortex, Buechler, Fort Lee, NJ). Fifty microliters of the residue was injected into the HPLC system. The CSF samples were vortexed and centrifuged and the supernatant was directly injected into the HPLC system.

The between-day precision of the HPLC assay was determined by measuring the same biological plasma sample with each run. The coefficient of variation was found to be 7.4%.

Statistics

The statistical analysis of the *in vitro* data was performed by one-way analysis of variance (ANOVA) to detect significant differences in clearance and endothelial permeability among different transport experiments. The *in vivo* data were analyzed using a two-way ANOVA to detect significant differences in the CSF/plasma steady-state concentration ratios and plasma clearances among the three different phases of each experiment. The homogeneity of variances was assessed by Bartlett's test. The level of significance was set to $P < 0.05$. The S-STAT program of the SIPHAR pharmacokinetic modelling software package (SIMED S.A., Creteil, France) was used for all statistical tests.

RESULTS

In Vitro Model

The clearance and permeability values of AZT after an initial luminal and abluminal concentration of 2 μg/mL are given in Table I. There are no significant differences in test clearance and permeability among both administration sites. The TEER of the monolayers varied from 10 to 31 $\Omega \cdot cm^2$, due to different batches of endothelial cells. Only monolayers with similar TEER values were used for comparison (Table I, II, and III). The amount of AZT transported in 90 min after luminal administration of 0.4, 2, 10, and 50 μg/mL is plotted versus the initial concentration in Fig. 1. There is no significant difference in endothelial permeability for the different concentrations used. Linear regression analysis showed a highly significant correlation ($P < 0.001$) between the initial concentration and the amount transported, indicating that the transport rate of AZT across the monolayer is concentration independent in this concentration range.

Table I. *In Vitro* Transport Parameters of AZT After Luminal and Abluminal Administration and the TEER Value of the Monolayers Used (Mean ± SD; $n = 3$)

Site of administration	$Cl_{control}$ (μL/min)	Cl_{Test} (mL/min)	P_{BBB} (cm/min/10^3)	TEER ($\Omega \cdot cm^2$)
AZT				
Luminal	12.90 ± 0.64	4.80 ± 0.80	1.65 ± 0.44	10.19 ± 1.42
Abluminal	11.52 ± 1.08	4.59 ± 0.48	1.63 ± 0.28	10.98 ± 2.45

Two metabolic inhibitors were used to inhibit energy-dependent processes: DODG and sodium azide (NaN_3). Table II shows that AZT permeability did not differ significantly after a 3-hr pretreatment of the endothelial cell monolayers with 25 m*M* DODG or a combination of 25 m*M* DODG and 1 m*M* NaN_3.

The effect of probenecid on the endothelial permeability of AZT was studied after luminal and abluminal administration of AZT. Because of the high affinity of penicillin G for the organic anion carrier (22), the cell culture medium did not contain penicillin G in this series of experiments. A 3-hr pretreatment of the endothelial cell monolayers had no significant effect on AZT clearance either after luminal or after abluminal administration of 2 μg/mL AZT (Table III).

In Vivo Model

To assess the effect of the experimental conditions (change of syringes, effect of the time of day) on CSF penetration, a control study was carried out without administration of an inhibitor but with saline. In Fig. 2 the result of this experiment is shown. The experimental conditions had no significant effect on steady-state plasma and CSF concentration of AZT. The CSF/plasma steady-state concentration ratios were 0.24, 0.28, and 0.24 for phases A, B, and C, respectively ($n = 3$).

The effect of probenecid on the concentration of AZT in CSF and plasma was studied after icv and icv/iv coadministration of probenecid in six rats (see Fig. 3). Table IV shows the pharmacokinetic parameters of this experiment. The CSF/plasma steady-state concentration ratio in phase B (0.45 ± 0.22) and C (0.43 ± 0.19) differed significantly from that in phase A (0.25 ± 0.14; $P < 0.001$). The plasma clearance in phase C (4.35 ± 1.49 μL/min) was significantly decreased ($P < 0.001$).

The effect of thymidine on AZT transport was studied using the same study design. There was no significant effect of thymidine on the CSF/plasma concentration ratio of AZT. A small, but significant decrease in plasma clearance ($P < 0.01$) was observed in phase C (Table V). Only five rats were used for calculation of the pharmacokinetic parameters, because the data for one rat were not complete due to a blocked CSF sampling cannula after 2.75 hr.

Table II. The Effect of a 3-hr Pretreatment with Metabolic Inhibitors (DODG and DODG/NaN_3) on the *in Vitro* Transport Parameters of AZT (Mean Values; $n = 2$)

Pretreatment	Cl_{Test} (μL/min)	P_{BBB} (cm/min/10^3)	TEER ($\Omega \cdot cm^2$)
No pretreatment	3.66	1.09	18.89
25 m*M* DODG	3.33	0.95	17.15
25 m*M* DODG + 1 m*M* NaN_3	4.12	1.29	20.87

DISCUSSION

The transport of AZT across the capillary endothelium was characterized in our *in vitro* cell culture model. After luminal and abluminal administration, the permeability of the endothelial cell monolayer to AZT was not different, indicating that the transport of AZT across the monolayer is symmetrical. Hence it is very unlikely that an asymmetrical carrier system is involved in the transport of AZT across the BBB. Compared to fluorescein sodium, a marker compound for paracellular permeability, AZT exhibits a two- to threefold higher clearance and permeability. Van Bree *et al.* (11) determined the endothelial permeability of fluorescein sodium in the same model ($P_{BBB} = 0.51 \times 10^{-3}$ cm/min). Since AZT and fluorescein have a similar molecular weight (267 and 376, respectively), this result suggests that AZT passes the monolayer not only paracellularly, but also via the transcellular route. This is in contrast to the results of Terasaki and Pardridge (23), who found in anesthetized rats that the first-pass extraction of AZT is not different from that of the extracellular space marker sucrose. This difference may be due to the short exposure time of 15 sec in that study underestimating the brain extraction of slowly penetrating drugs. The higher endothelial permeability of AZT compared to fluorescein might be partly explained by its higher lipid solubility. The difference in octanol/buffer partition coefficients [1.26 and 0.62 for AZT and fluorescein, respectively (5,24)] suggests that AZT might be able to pass the monolayer also by passive transcellular diffusion.

In the range of 0.4 to 50 μg/mL, the transport of AZT is concentration independent. This concentration range in-

Table III. The Effect of a 3-hr Pretreatment with 20 μg/mL Probenecid on the *in Vitro* Transport Parameters of AZT (Mean ± SD; *n*, Number of Monolayers Used)

Pretreatment[a]	*n*	Cl_{Test} (μL/min)	P_{BBB} (cm/min/10^3)	TEER ($\Omega \cdot cm^2$)
No pretreatment (lum)	3	5.29 ± 0.92	1.95 ± 0.59	24.46 ± 2.74
Probenecid (lum)	2	4.83	1.64	23.79
No pretreatment (abl)	5	4.28 ± 1.15	1.53 ± 0.65	28.86 ± 8.20
Probenecid (abl)	4	3.68 ± 0.46	1.16 ± 0.21	30.83 ± 6.12

[a] Lum, luminal; abl, abluminal.

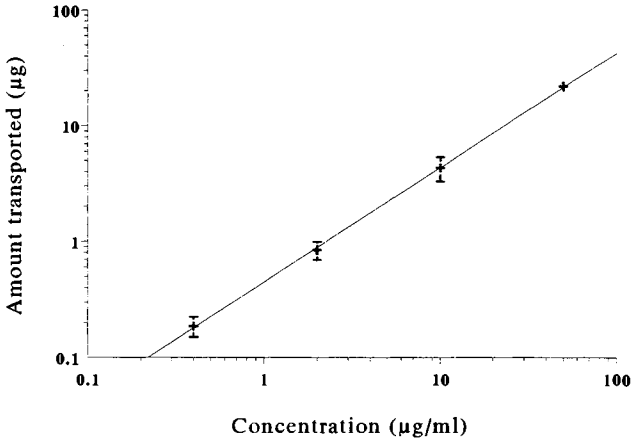

Fig. 1. Concentration independence of AZT transport *in vitro*. Each point represents the mean ± SD of three different experiments.

cludes the effective concentration of AZT. Between 1 and 5 µ*M* (0.27–1.34 µg/mL) AZT inhibits the HIV infection of T lymphocytes *in vitro* (1). The concentration independence of AZT between 0.4 and 50 µg/mL indicates that no active transport mechanism is involved in the transport of AZT across the BBB *in vitro*. This result was confirmed by the inhibition experiments. When the transport experiments were performed after preincubation with DODG or DODG/NaN_3, no significant change in endothelial permeability of AZT was found, suggesting the absence of energy-dependent processes.

Moreover, probenecid did not influence the transport of AZT. Although the existence of the organic anion carrier in the cerebral capillaries was proven (24), AZT does probably not use this carrier to cross the BBB *in vitro*. On the other hand, it cannot be excluded that this carrier is not present in our endothelial cell monolayers.

In our *in vivo* model, AZT reached the CSF rapidly by passing the BBB and/or the blood–CSF barrier. The steady-state concentration of AZT in plasma was fixed at about 2 µg/mL to have comparable conditions *in vitro* and *in vivo*.

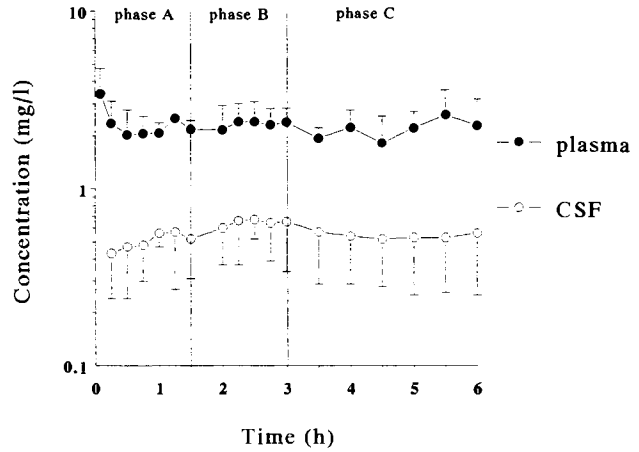

Fig. 2. The effect of the experimental conditions on the steady-state plasma and CSF concentration of AZT (mean ± SD; $n = 3$) following iv infusion of AZT. First 5 min, 100 µL/min; remaining 6 hr, 20 µL/min of AZT (1 mg/mL).

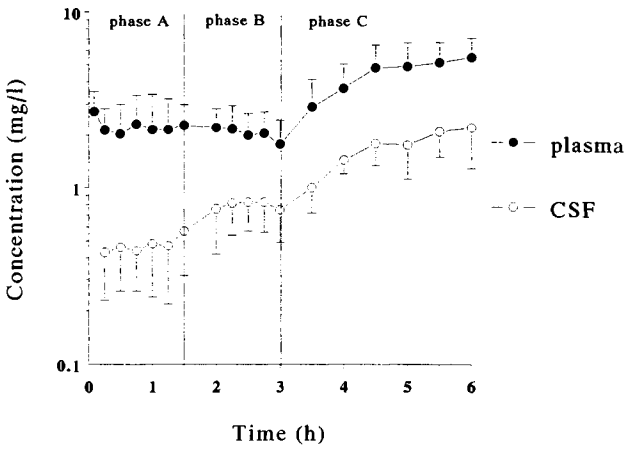

Fig. 3. The effect of probenecid on the steady-state plasma and CSF concentration of AZT (mean ± SD; $n = 6$). Phase A: a constant-rate iv infusion of 6.3 mg/kg/hr AZT alone; phase B, with 0.4 mg/kg/hr probenecid icv; phase C, 0.4 mg/kg/hr probenecid icv plus 31.5 mg/kg/hr probenecid iv.

Steady state was reached about 30 min after the beginning of the intravenous infusion. The pharmacokinetic parameters of AZT during constant-rate infusion are in agreement with data obtained after a single bolus dose of 42.5 mg/kg AZT iv in the same animal model (Heijligers-Feijen *et al.*, unpublished data). Galinsky *et al.* (9) found similar pharmacokinetic parameters in male Sprague Dawley rats. They reported a total clearance of 12.4 mL/min and a mean CSF/plasma ratio of 0.15. In rabbits a ratio of 0.18 was found. In humans a wide range has been reported in the CSF/plasma concentration ratio, between 15 and 135%, with a mean of about 60% (25). This high variability of CSF penetration in humans may be due to the heterogeneity of the study population (AIDS patients in different stages and severities of the HIV infection).

The experimental conditions, changing of the syringes before phases B and C, time of day did not affect the steady-state concentration of AZT in plasma and CSF. Probenecid almost doubled the CSF/plasma steady-state concentration ratio after icv administration. These results show that AZT is actively transported from CSF to blood by the organic anion carrier, since probenecid is known to inhibit this carrier system competitively. The organic anion carrier is probably located at the choroid plexus, because there was no effect of

Table IV. Pharmacokinetic Parameters of AZT After a Constant-Rate iv Infusion of 6.3 mg/kg/hr AZT Alone (Phase A), with 0.4 mg/kg/hr Probenecid icv (phase B), and 0.4 mg/kg/hr Probenecid icv Plus 31.5 mg/kg/hr Probenecid iv (Phase C) (Mean ± SD; $n = 6$)

Inhibitor	Phase	CL_p (µL/min)	$C_{ss_{CSF}}/C_{ss_{plasma}}$
AZT (control)	A	10.43 ± 3.90	0.25 ± 0.14
AZT + probenecid (icv)	B	10.99 ± 3.82	0.45 ± 0.22*
AZT + probenecid (icv + iv)	C	4.35 ± 1.49*	0.43 ± 0.19*

* $P < 0.001$.

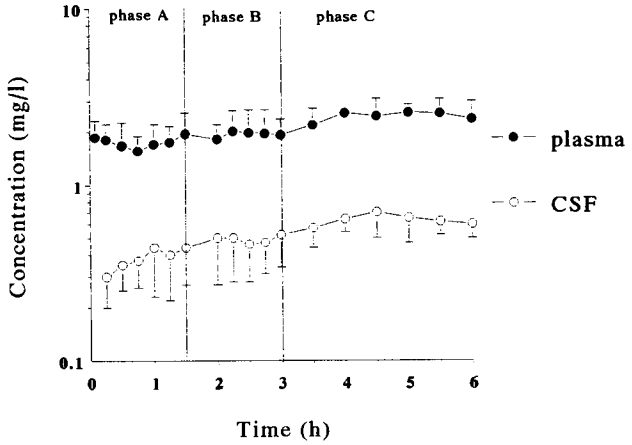

Fig. 4. The effect of thymidine on the steady-state plasma and CSF concentration of AZT (mean ± SD: $n = 5$). Phase A, a constant-rate iv infusion of 6.3 mg/kg/hr AZT alone; phase B, with 0.4 mg/kg/hr thymidine icv; phase C, with 0.4 mg/kg/hr thimidine icv and 31.6 mg/kg/hr iv.

probenecid on the endothelial permeability of AZT *in vitro* even when administered at the abluminal site. The existence of the organic anion carrier at the choroid plexus and the effect of probenecid on this carrier system have been reported previously by Spector *et al.* (17). During simultaneous iv administration of probenecid, the plasma and CSF concentration of AZT both increased, but the steady-state CSF/plasma concentration ratio remained unchanged. This indicates that the organic anion carrier transports AZT only from CSF to blood, and not from blood to CSF, otherwise the CSF/plasma ratio in phase C would have decreased compared to that in phase B. The increase in the steady-state plasma level of AZT in phase C is the result of a reduced total-plasma clearance due to the iv coadministration of probenecid. Sawchuk *et al.* (26) found an inhibition of metabolic and renal clearance of AZT after iv coadministration with probenecid in rabbits, thereby increasing the CSF and plasma concentration. This interaction is caused by competitive inhibition of the organic anion carrier in the kidney, but also by competition of the glucuronidation. In humans, about 65% of the oral administered dose is excreted in the urine as AZT-glucuronide (2,27). In rats, however, only 5–10% is excreted as AZT-glucuronide and about 70% is excreted unchanged in the urine (28). Because of this high contribution of renal clearance in rats, the effect of iv-administered probenecid in our study is probably due mainly to a competition in the kidney, resulting in a reduced plasma clearance of 42%.

Table V. Pharmacokinetic Parameters of AZT After a Constant-Rate iv Infusion of 6.3 mg/kg/hr AZT Alone (Phase A), with 0.4 mg/kg/hr Thymidine icv (Phase B), and with 0.4 mg/kg/hr Thymidine icv and 31.6 mg/kg/hr iv (Phase C) (Mean ± SD; $n = 5$)

Inhibitor	Phase	Cl_p (μL/min)	$C_{ss_{CSF}}/C_{ss_{plasma}}$
AZT (control)	A	12.21 ± 3.18	0.23 ± 0.04
AZT + thymidine (icv)	B	10.89 ± 3.06	0.25 ± 0.04
AZT + thymidine (icv + iv)	C	8.04 ± 1.31*	0.26 ± 0.05

* $P < 0.01$.

Hedaya *et al.* (29) and Sawchuk *et al.* (26) have reported a similar effect of probenecid on the CSF penetration of AZT after iv coadministration in rabbits. They found a comparable increase (56%) in the CSF/plasma concentration ratio. However, from their study it could not be decided whether AZT is transported by the organic anion carrier only out of the CSF or also, to a certain extent, into the CSF. With our three-phase study design and the *in vitro* experiments, we could show that this carrier is located only at the CSF-facing membrane. With probenecid it is thus possible to increase the concentration of AZT in the CSF and thereby enhance the CNS exposure, without increasing the systemic concentration. It is not known yet whether this also leads to an increased brain tissue concentration of AZT.

Intracerebral microdialysis would be a suitable technique for estimating the extracellular brain concentration of AZT (20). Nevertheless caution should be taken with the clinical use of this combination. Clinical studies showed an unacceptably high incidence of adverse reactions (skin rash, fever) thought to be related to the use of a combination of AZT with probenecid (T. Blaschke, personal communication). Petty *et al.* reported adverse reactions (erythematous eruption) after a combination therapy of AZT with probenecid. In four of eight patients the administration of probenecid had to be stopped (30). Therefore, it would be worthwhile to look for other compounds with an inhibitory effect on the organic anion carrier at the choroid plexus. An interesting alternative would be the betalactam antibiotics, because it is reported that they are transported from CSF to blood via the organic anion carrier system as well (22).

Intracerebroventricularly and intravenously administered thymidine did not affect the concentration of AZT in CSF. A small reduction (35%) in plasma clearance in phase C was observed, probably due to a competition in metabolic and/or renal clearance. Thymidine is structurally related to azidothymidine and may, therefore, be a potential competitor of AZT transport if AZT enters and/or leaves the CNS using a nucleoside carrier. From the literature (24,31,32) it is known that thymidine is transported into the CNS via a nucleoside carrier at the choroid plexus that differs from the carrier that transports nucleosides back to the blood. Our results, however, suggest that neither of these transport systems is involved in the CSF transport of AZT. Our *in vitro* data with BBB endothelial cell cultures confirm what has been reported by Cornford and Oldendorf (33).

In conclusion, AZT exhibits symmetrical and concentration-independent transport across bovine endothelial cell monolayers *in vitro*. There is no active mechanism involved in AZT transport as shown by specific and unspecific inhibition experiments, but the calculated permeability suggests that AZT can pass the BBB via the transcellular route. Since AZT is relatively nonpolar, it may penetrate through the endothelial cells by passive diffusion. Accordingly, structural modification of the AZT molecule increasing its lipophilicity may enhance transcellular diffusion and increase CNS availability. *In vivo* the involvement of the organic anion carrier in AZT elimination from the CSF could be shown. The three-phase study design clearly indicated that this car-

rier is asymmetrical and localized at the CSF side. The organic anion carrier is probably located only at the blood–CSF barrier (the choroid plexus), since probenecid had no effect on the BBB *in vitro*. In contrast, AZT has no affinity to the nucleoside carrier at the choroid plexus.

Our results suggest that the CSF and eventually the CNS brain exposure of AZT can be increased by the coadministration of drugs with a high affinity to the organic anion carrier system. Drugs with an affinity to the different nucleoside carrier systems will probably have no effect on the disposition of AZT in the CNS.

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