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Research Article

Modeling the Enhanced Uptake of Zidovudine (AZT) into Cerebrospinal Fluid. 1. Effect of Probenecid

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The kinetics of zidovudine (AZT) distribution into rabbit cerebrospinal fluid (CSF) were studied during continuous infusion of AZT and after iv bolus administration. The CSF/plasma steady-state AZT concentration ratio was 0.192 ± 0.003. That this ratio is less than unity, and the clearance from the CSF due to bulk flow is much smaller than the total CSF-to-plasma clearance, suggests active CSF-to-plasma transport of AZT. Probenecid coadministration significantly enhances AZT distribution into CSF when plasma and CSF concentrations of AZT are at steady state during continuous infusion of AZT or at a transient steady state after a single iv bolus dose administration. A linear pharmacokinetic model which describes the distribution of AZT into CSF and relates intercompartmental clearances between CSF and plasma was developed and was used to analyze the results. This analysis showed that probenecid enhances the distribution of AZT into the CSF by its effect on clearances between plasma and CSF. The CSF exit-rate constant for AZT estimated during probenecid coadministration was significantly different from controls. Probenecid coadministration resulted in a 36% reduction in the CSF-to-plasma transfer-rate constant. Reduction in the CSF to plasma clearance of AZT is probably due to the effect of probenecid on the active CSF-to-plasma transport of AZT. The model analysis also indicates that probenecid may have increased the plasma to CSF clearance of AZT. There was an increasing trend in the steady-state CSF/plasma AZT concentration ratio with increasing plasma probenecid concentrations. These results are consistent with probenecid competitively inhibiting the CSF-to-plasma transport of AZT.

KEY WORDS: zidovudine; cerebrospinal fluid distribution; pharmacokinetic modeling; intercompartmental clearances; probenecid; acquired immunodeficiency syndrome (AIDS).

INTRODUCTION

Distribution into the central nervous system (CNS) is essential for a variety of drugs, such as anticonvulsants, antidepressants, anesthetics, antibiotics, antivirals, and anticancer agents, to exert their pharmacological effects. The major obstacles for the distribution of drugs into the CNS are the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (blood–CSF barrier). Because of these barriers, created by the tight junctions between the capillary endothelial cells, the passive diffusion of hydrophilic drugs will be greatly restricted, although lipophilic drugs readily diffuse into the CNS. Specific active transport systems allow the transport of ions and essential hydrophilic nutrients across these barriers (1).

Studying the distribution kinetics across the BBB and the blood–CSF barrier is complicated by the relatively difficult experimental techniques necessary to obtain the needed data. In animal experiments, sampling of brain tissue usually limits analysis to one measurement per animal. In order to obtain sufficient data for kinetic studies, cerebrospinal fluid (CSF) sampling has been used.

The CSF is a primary source of information concerning drug distribution into the CNS in humans. This fluid, which occupies the subarachnoid space, is formed mainly in the choroid plexuses of the cerebral ventricles and is continuously drained via the arachnoid villi into the cerebral venous sinuses. It has been suggested that the CSF is an extension of the brain extracellular fluid (ECF), and drugs in the CSF can diffuse into the brain ECF (2). A good correlation between the CSF concentration and the brain tissue concentration for a number of drugs has also been demonstrated (3). A theoretical distributed model that relates the drug concentration in the plasma, CSF, and brain tissue has been described (4). Although this model does not consider active transport of drugs, the existence of specific active transport systems for anions, cations, and nucleosides into and from the CSF across the blood–CSF barrier is well established (5–8). These active transport systems are saturable, and substrates for the same system can compete for available transport sites. Because of the dependence of the distribution between CSF and brain tissue on the properties of each compound, the measured CSF drug concentrations and their relation to brain tissue concentrations should be interpreted carefully for individual drugs.

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Patients suffering from acquired immunodeficiency syndrome (AIDS) frequently suffer from neurological deficits. These abnormalities are due, at least in part, to the direct effect of the human immunodeficiency virus (HIV) on deep subcortical structures in the brain (9). This condition, known as HIV encephalopathy, is characterized by cognitive, motor, and behavioral dysfunction. In a recent report, it was estimated that one-third of AIDS patients show signs of neurological abnormalities at the time of diagnosis, with an increase to two-thirds with progression of the disease (9). Patients with AIDS-related complex (ARC) also develop neurological abnormalities. Because of these complications, effective anti-AIDS drugs should distribute adequately into the CNS.

Zidovudine (AZT) is currently the only antiviral drug effective in the treatment of AIDS (10–12). AZT is a thymidine derivative in which the hydroxyl group in the 3′ position is replaced by an azido group. It has been shown that AZT can penetrate into the cerebrospinal fluid (CSF) in AIDS patients, although it is not known if it gains access to brain parenchyma (13,14). Enhancing AZT distribution into the CNS may provide an avenue for more effective treatment of the AIDS-treated neurological deficit.

The main objectives of this study are to investigate the kinetics of distribution of AZT into rabbit CSF and to examine the effect of probenecid on this distribution process. A pharmacokinetic model that relates intercompartmental clearances between plasma and CSF was developed to analyze the results and to examine the mechanism by which probenecid enhances the distribution of AZT into rabbit CSF.

MATERIALS AND METHODS

Chemicals

We used AZT powder obtained from Burroughs Wellcome Co. to prepare solutions for iv administration and standard solutions for analysis. Probenecid, 3-chlorobenzoic acid, β-hydroxyethyl theophylline, and β-hydroxypropyl theophylline were supplied by Sigma Chemical Co.; acetonitrile, ether, and chloroform from Burdick and Jackson Laboratories; and ammonium phosphate monobasic, acetic acid, isopropyl alcohol, and methyl alcohol from Mallinckrodt, Inc. All solvents were HPLC grade.

Plasma and CSF Sample Collection

Male New Zealand White rabbits, weighing 3–3.5 kg, were obtained from Birchwood Farm Rabbitry. Plasma samples were obtained through a marginal ear vein catheter and CSF samples were obtained through a cisterna magna cannula as described previously (15). Samples were stored at −20°C until analysis.

Distribution of AZT into CSF During Continuous Infusion of AZT

Two groups of rabbits ($n = 4$, each) weighing 3.1 ± 0.22 kg (mean ± SD) were used in this experiment. The first group (control) received a loading dose of 1 mg/kg AZT iv followed by continuous infusion of 3 mg/hr AZT, continued throughout the experiment. After at least 8 hr of continuous AZT infusion, the rabbit was anesthetized and the cisterna magna cannula was placed. Simultaneous plasma and CSF samples were drawn at 30 min after the end of the surgery. An additional pair of CSF and plasma samples was obtained at 60 min whenever possible to ensure the attainment of steady state. The second group (probenecid treated) was treated exactly as the control group, but probenecid was coadministered with AZT as a 15-mg/kg loading dose followed by a 45-mg/hr continuous infusion administered throughout the experiment. Plasma and CSF samples were obtained as in the control group.

Distribution of AZT into CSF After iv Bolus Dose

In order to analyze the kinetics of distribution of AZT into rabbit CSF and to determine the mechanism by which probenecid enhances the distribution of AZT into rabbit CSF, a pharmacokinetic model was developed to describe the distribution of AZT between plasma and CSF. This model was used to analyze existing CSF and plasma data obtained as described previously (15). These data comprise multiple CSF and plasma samples obtained after a single bolus dose of AZT in control and probenecid-treated rabbits.

Sample Analysis

Plasma samples were analyzed for AZT using an HPLC method developed in our laboratory (16). CSF samples were analyzed for AZT, and plasma samples were further analyzed for probenecid by HPLC as described previously (15).

Pharmacokinetic Model

A linear pharmacokinetic model which describes the distribution of AZT into CSF and relates intercompartmental clearances between CSF and plasma was developed. The model assumptions are as follows.

(1) Drug transfer between the plasma and CSF obeys first-order kinetics.
(2) Only free (unbound) drug can transfer between plasma and CSF.
(3) Drug is not metabolized in the CSF.
(4) Intercompartmental clearances and the CSF bulk flow remain constant within an experiment.
(5) Drug in the CSF is homogeneously distributed.

The model, shown in Fig. 1, makes no assumptions

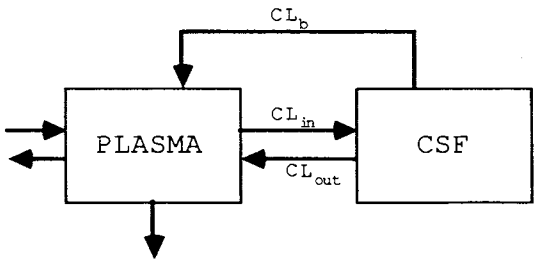

Fig. 1. A diagram representing the pharmacokinetic model. CL_{in} and CL_{out} are the intercompartmental clearances between plasma and CSF, and CL_b is the clearance of drug from CSF due to bulk flow.

about the general pharmacokinetics of AZT in the body. Distribution to other compartments is represented by reversible arrows connected to the central compartment and allows for the existence of a number of peripheral compartments.

A drug molecule can leave the CSF either by diffusion into the plasma across the blood–CSF barrier or by removal by bulk flow of CSF into the cerebral venous sinuses. The rate of change of the drug concentration in the CSF can be expressed by the following differential equation:

$$\frac{dC_f}{dt} = \frac{CL_{in}}{V_f} C_p - \frac{CL_{out}}{V_f} C_f - \frac{CL_b}{V_f} C_f \quad (1)$$

where

CL_{in} = the intercompartmental clearance from plasma to CSF
CL_{out} = the intercompartmental clearance from CSF to plasma
CL_b = the clearance of drug from the CSF due to bulk flow
C_p = the free drug concentration in plasma
C_f = the drug concentration in CSF
V_f = the volume of the CSF

Because of the minimal amount of protein in the CSF, all the drug in the CSF is present as free drug.

Integrating Eq. (1) from $t = 0$ to ∞ and rearranging, and because $C_f = 0$ at time 0 and ∞, we can obtain Eq. (2),

$$\frac{AUC_{CSF}}{AUC_{plasma}} = \frac{CL_{in}}{CL_{out} + CL_b} = R \quad (2)$$

where

AUC_{CSF} = area under the CSF concentration–time curve
AUC_{plasma} = area under the free plasma concentration–time curve

When both CSF and plasma concentrations of AZT are at steady state, e.g., during constant-rate infusion of AZT, the rate of change of the amount of drug in the CSF is equal to zero, and the following relationship can be obtained:

$$\frac{C_f^{ss}}{C_p^{ss}} = \frac{CL_{in}}{CL_{out} + CL_b} = R \quad (3)$$

After a single iv bolus dose administration, at the time when the concentration in the CSF is maximal (t_{max}), the rate of change of the amount of drug in the CSF is equal to zero. At this transient steady state, the drug CSF/plasma concentration ratio can be used to relate the intercompartmental clearances (17), i.e.,

$$\frac{C_f^{max}}{C_p^{t\ max}} = \frac{CL_{in}}{CL_{out} + CL_b} = R \quad (4)$$

Integrating Eq. (1) from $t = t_1$ to t_2 yields

$$\Delta C_f = \frac{CL_{in}}{V_f} \int_{t_1}^{t_2} C_p dt - \frac{CL_{out}}{V_f} \int_{t_1}^{t_2} C_f dt - \frac{CL_b}{V_f} \int_{t_1}^{t_2} C_f dt \quad (5)$$

Rearranging terms and substituting R ($CL_{out} + CL_b$) for CL_{in},

$$\Delta C_f = \frac{(CL_{out} + CL_b)}{V_f} [R \cdot AUC_{plasma}|_{t_1}^{t_2} - AUC_{CSF}|_{t_1}^{t_2}] \quad (6)$$

This is the equation of a straight line without intercept. Plotting the difference between consecutive CSF concentrations (ΔC_f) versus the quantity $[R \cdot AUC_{plasma}|_{t_1}^{t_2} - AUC_{CSF}|_{t_1}^{t_2}]$, a straight line is obtained with slope equal to the exit-rate constant from the CSF. The CSF exit-rate constant is the sum of the transfer-rate constant from the CSF to plasma and the turnover-rate constant of CSF by bulk flow. This analysis provides an estimate of the CSF exit-rate constant and insight regarding the mechanism by which the CSF distribution of drugs may be altered.

Although this model assumes first-order transfer rates between the plasma and CSF, it can be applied in the presence of a CSF to plasma saturable transport system, assuming that CL_{out} is independent of drug concentration in the observed range. Thus, linear kinetics will be obeyed, and CL_{out} will be the sum of clearances associated with passive diffusion across the blood–CSF barrier and the CSF-to-plasma active transport.

RESULTS

Distribution of AZT into CSF During Continuous Infusion of AZT

The terminal half-life of AZT in the rabbit is 1 hr, and slightly longer during probenecid coadministration (15,18). Therefore, an 8-hr AZT continuous infusion is sufficient to achieve steady state in the rabbit. AZT plasma and CSF concentrations measured at 30 and 60 min after the placement of the cisterna magna cannula in each of the rabbits confirmed the attainment of steady state. It has been shown that the unbound AZT fraction in the rabbit is >0.95 (15). Thus, total AZT plasma concentrations are approximately equal to free concentrations.

Because CSF samples in one of the rabbits in the control group were contaminated with blood, we excluded this rabbit from the analysis. A summary of the results is presented in Table I. AZT CSF/plasma concentration ratios at steady state in the control group were 0.192 ± 0.003 (mean ± SD). In the probenecid-treated group the steady-state probenecid concentrations were 206, 230, 180, and 293 μg/ml in the four rabbits, respectively. The steady-state AZT CSF/plasma concentration ratios in the probenecid-treated group, 0.299 ± 0.039, were significantly different from controls (Student's t test, $P < 0.005$). The enhancement of the steady-state AZT CSF/plasma concentration ratio, according to Eq. (3), results from the effect of probenecid on the intercompartmental clearances between plasma and CSF.

Distribution of AZT into CSF After iv Bolus Dose

Model-independent analysis of the results was described previously in detail (15). Briefly, probenecid coadministration caused a sevenfold increase in the AZT AUC_{CSF} in probenecid-treated rabbits compared with con-

Table I. The Distribution of AZT Between Rabbit CSF and Plasma During Continuous AZT Infusion

Control					Probenecid treatment				
Rabbit No.	Time (min)[a]	CSF conc. (μg/ml)	Plasma conc. (μg/ml)	CSF/plasma ratio[b]	Rabbit No.	Time (min)[a]	CSF conc. (μg/ml)	Plasma conc. (μg/ml)	CSF/plasma ratio[b]
1	30	0.123	0.631	0.195	4	30	0.318	0.975	0.336
	60	—	0.658			60	0.376	1.09	
2	30	0.107	0.559	0.191	5	30	0.474	1.79	0.261
	60	—	0.617			60	0.556	2.17	
3	30	0.179	0.878	0.189	6	30	0.466	1.39	0.329
	60	0.178	1.027			60	0.490	1.52	
					7	30	0.339	1.25	0.271
						60	—	1.32	
Mean				0.192					0.299*
SD				0.003					0.039

[a] Time after placement of the cisterna magna cannula.
[b] Mean ratio for each rabbit.
* Significantly different from control, Student's t test, $P < 0.005$.

trols. This overall increase is caused by probenecid's effect on the renal clearance of AZT as well as on the CSF/plasma distribution. The AUC_{CSF}/AUC_{plasma} ratio increased from 0.152 ± 0.023 in controls to 0.568 ± 0.222 in probenecid-treated rabbits. The CSF/plasma AZT concentration ratio during the postdistributive phase increased from 0.24 ± 0.05 in controls to 0.65 ± 0.12 in the probenecid-treated rabbits.

The pharmacokinetic model described above was used to analyze the results and to examine the mechanism by which probenecid enhances the distribution of AZT into the CSF. Since there was no evidence of any concentration dependence of AZT distribution into rabbit CSF in the range of concentrations observed and because the probenecid plasma concentrations were at steady state during the experiments, the model was applied to these data. The partial CSF AUCs were calculated over consecutive intervals from the actual CSF measured concentrations by the linear trapezoidal rule. The corresponding partial plasma AUCs were calculated from the interpolated plasma concentration–time data, also by the linear trapezoidal rule. ΔC_f was calculated as the difference between consecutive CSF concentrations. These are positive or negative values depending on whether the CSF concentration is increasing or decreasing.

The value of R can be estimated according to Eq. (2) from the ratio of AUC_{CSF}/AUC_{plasma} (from time 0 to ∞). In addition, R can be calculated at steady state during constant-rate infusion from the ratio of C_f^{ss}/C_p^{ss} and at the transient steady state after a single bolus dose from the ratio of $C_f^{t_{max}}/C_p^{t_{max}}$ according to Eqs. (3) and (4), respectively. A statistical test to compare the R values calculated for each of the six rabbits from both AUC_{CSF}/AUC_{plasma} and CSF/plasma concentration ratio at t_{max} (transient steady state) showed that there was no significant difference associated with the method of calculation (paired t test, $P = 0.67$).

Because error may be attributed to both x and y variables in Eq. (6), orthogonal linear regression was performed between ΔC_f and $[R \cdot AUC_{plasma}|_{t_1}^{t_2} - AUC_{CSF}|_{t_1}^{t_2}]$ (19). The slope of this line is the exit-rate constant from the CSF. Figures 2A and B show representative plots of this relationship in a control and a probenecid-treated rabbit, respectively. Table II represents a summary of the results obtained in the control and probenecid-treated rabbits, when R was calculated according to Eqs. (2) and (4). A statistical test was performed to compare the CSF exit-rate constants obtained from regression analysis of Eq. (6) where both methods of calculating R were employed. This comparison showed that there was no statistical difference between the CSF exit-rate constant estimates (paired t test, $P = 0.32$). In both cases,

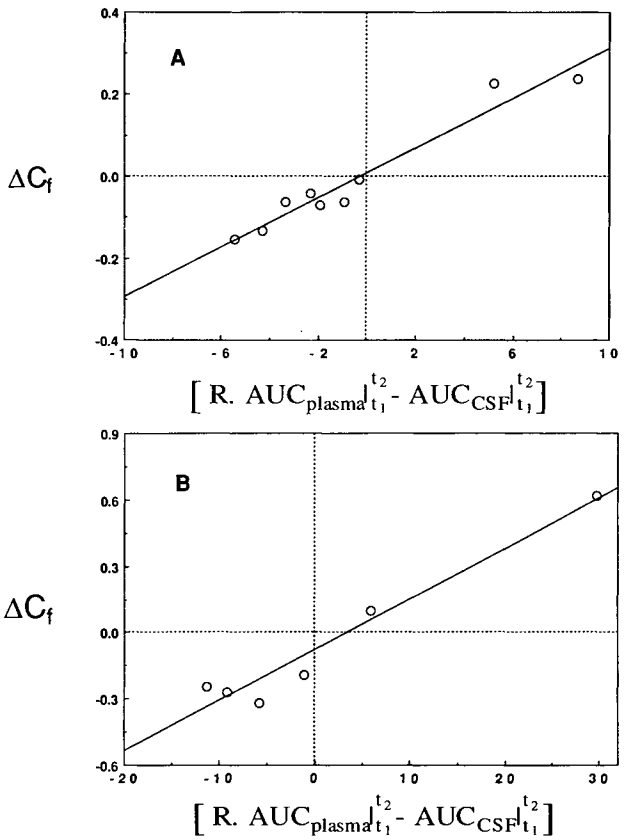

Fig. 2. A plot of ΔC_f versus $[R \cdot AUC_{plasma}|_{t_1}^{t_2} - AUC_{CSF}|_{t_1}^{t_2}]$ in (A) a control and (B) a probenecid-treated rabbit. R was calculated from the ratio of AZT $C_f^{t_{max}}/C_p^{t_{max}}$). The solid lines are the orthogonal regression lines.

Table II. CSF Exit-Rate Constant Estimated After a Single Bolus Dose of AZT in Control and Probenecid-Treated Rabbits

	(A) R calculated as AUC_{CSF}/AUC_{plasma}[a]				(B) R calculated as $C_f^{max}/C_p^{t_{max}}$[a]			
	Control		Probenecid treated		Control		Probenecid treated	
	R	CSF exit-rate constant (min^{-1})	R	CSF exit-rate constant (min^{-1})	R	CSF exit-rate constant (min^{-1})	R	CSF exit-rate constant (min^{-1})
	0.175	0.028	0.345	0.024	0.186	0.027	0.441	0.023
	0.152	0.030	0.570	0.022	0.148	0.030	0.433	0.022
	0.129	0.031	0.789	0.018	0.193	0.037	0.647	0.022
Mean	0.152	0.030	0.568*	0.021*	0.176	0.031	0.507*	0.022*
SD	0.023	0.002	0.222	0.003	0.024	0.005	0.121	0.001

[a] The R values calculated by the two methods for each of the rabbits were not statistically different (paired t test, $P = 0.67$).
* Significantly different from controls (Student's t test, $P < 0.05$).

the CSF exit-rate constants were statistically different in the probenecid-treated rabbits compared with controls (Student's t test, $P < 0.05$). There was, on average, a 28% reduction in the CSF exit-rate constant during probenecid coadministration. The exit-rate constant values obtained when R was calculated from the ratio of AZT $C_f^{max}/C_p^{t_{max}}$ were used in subsequent analysis and in the comparison of the results of the control and probenecid coadministration studies.

DISCUSSION

Studies in AIDS patients have shown that, after oral and iv administration of AZT, CSF-to-plasma concentration ratios ranged from 0.15 to 1.35 ($n = 6$) (13). These ratios were dependent on the sampling time relative to AZT administration, perhaps because of the time required to achieve pseudoequilibrium between AZT in plasma and CSF. The steady-state CSF/plasma ratio during iv infusion of AZT in children was found to be 0.24 ± 0.07 ($n = 21$) (14). Although it is not known whether AZT gains access to brain parenchyma in humans, several studies have shown that AZT administration to patients with AIDS-related neurological dysfunctions resulted in significant improvement of their neurological abnormalities (14,20–22).

AZT is a relatively nonpolar compound compared to its parent nucleoside, thymidine, with a partition coefficient (1-octanol:0.1 M sodium phosphate, pH 7) of 1.26, vs 0.064 for thymidine. This considerable increase in lipophilicity results from the replacement of the 3′-hydroxy group of thymidine with the azido group. After oral administration in AIDS patients, AZT is absorbed rapidly, achieving peak concentrations within 30 min (23). AZT permeates across the human erythrocyte cell membrane mainly by passive diffusion, and not via the nucleoside transport system (24). Although AZT does not undergo first-pass brain extraction after carotid artery injection in rats (25), it can be measured in rat brain (mean brain:serum concentration ratio, 0.227) during continuous AZT administration (26). These findings indicate that AZT diffuses through membranes passively.

For drugs that distribute across the blood–CSF barrier only by passive diffusion, the CSF/plasma free concentration ratio at steady state should approach unity but will be somewhat less because of the continuous removal of drug by CSF bulk flow. When this ratio is much different from unity and the clearance from CSF due to bulk flow is much less than the total CSF-to-plasma clearance, this suggests the presence of active transport in addition to passive diffusion. The CSF/plasma free concentration ratio at steady state will determine the direction of this transport. If the ratio is <1, this indicates CSF-to-plasma transport; if the ratio is >1, plasma-to-CSF transport is indicated (17).

AZT is not bound to protein in rabbit plasma or CSF. The rate constant associated with CSF bulk flow is much less than the overall CSF-to-plasma transfer-rate constant as described below, and its CSF/plasma steady-state concentration ratios in the control group are low (0.192 ± 0.003). These findings strongly suggest the presence of a CSF-to-plasma active transport. This relatively small ratio indicates that the active transport of this antiviral agent represents the main component of its CSF-to-plasma transfer. The increase in the AZT CSF/plasma steady-state concentration ratio during probenecid coadministration can be explained by the effect of probenecid on AZT intercompartmental clearances between CSF and plasma. This appears to be a result of inhibition by probenecid of the CSF-to-plasma active transport of AZT.

The existence of active transport systems for organic acids, bases, and nucleosides across the blood–CSF barrier has been well documented. These transport systems are analogous to the anionic and cationic excretory systems in the renal tubule (27). Probenecid has been found to block the transport of penicillin from CSF to plasma in dogs (28) and the efflux of 5-hydroxy-indoleacetic acid from rat brain (29). Probenecid also decreases the loss of *p*-aminohippurate from the CSF during ventriculocisternal perfusion (30) and inhibits the transport of penicillin and aminosalicylic acid from CSF to plasma in the rabbit (31). The effect of probenecid is attributed to competitive inhibition of the transport system responsible for active transport of weak acids from the CSF to blood. Probenecid inhibition of the renal excretion of AZT in the rabbit by competitive inhibition of its active secretion in the renal tubule has been shown (15,18). The enhanced distribution of AZT into rabbit CSF may result from a similar effect of probenecid on the CSF-to-plasma active transport of this drug.

The CSF exit-rate constant obtained in the model analysis represents the sum of the intercompartmental CSF-to-plasma transfer-rate constant and the rate constant asso-

ciated with CSF bulk flow. The CSF-to-plasma transfer-rate constant is comprised of a component representing the passive diffusion across the blood–CSF barrier and a component representing the CSF to plasma active transport, i.e.,

$$\text{CSF exit-rate constant } (K_{exit}) = K_{passive} + K_{active} + K_{bulk} \quad (7)$$

The average volume of rabbit CSF is 1.8 ml, and the rate of its formation is 0.01 ml/min (1). Thus, the fluid turnover-rate constant (K_{bulk}) is 0.0056 min^{-1}. This value can be subtracted from the calculated CSF exit-rate constant to obtain the CSF-to-plasma transfer-rate constant. Assuming that probenecid administration does not affect CSF bulk flow, the average AZT CSF-to-plasma transfer-rate constants are 0.025 and 0.016 min^{-1} in the control and probenecid-treated rabbits, respectively. This 36% reduction in the AZT CSF-to-plasma transfer-rate constant is probably due to the effect of probenecid on the active component of this transport.

By substitution of (CL_{out} + CL_b) by CL_{in}/R in Eq. (6), and rearrangement, an equation of a straight line is obtained with the slope equal to CL_{in}/V_f. Unlike the slope of the plot (Fig. 2) corresponding to Eq. (6), this hybrid constant does not represent a true plasma-to-CSF transfer-rate constant because it involves the volume of the receiving compartment. However, estimates for CL_{in} can be obtained if a value for CSF volume is assumed. When this analysis was performed, a significant increase in CL_{in}/V_f during probenecid treatment was observed when compared with control. This may have resulted from an increase in CL_{in} or a decrease in CSF volume caused by probenecid.

Estimates of CL_{out} can similarly be obtained from the CSF exit-rate constant determined from the slope of plots shown in Fig. 2. This calculation requires an assumed value of rabbit CSF volume and bulk flow and, further, assumes that probenecid has no effect on these physiological parameters. The average CSF exit clearance (passive + active + bulk) of AZT decreased from 0.056 ml/min during control experiments to 0.040 ml/min in probenecid-treated rabbits. By subtracting the CSF bulk flow from CSF exit clearance, we can estimate CL_{out}. Probenecid coadministration caused a decrease in CL_{out} from 0.046 to 0.030 ml/min. This decrease presumably resulted from the effect of probenecid on the active component of the CSF to plasma transport of AZT.

The observed increase in R during probenecid treatment may be explained equally by an increase in CL_{in} or a decrease in CL_{out}. Based upon the findings in earlier studies of treatment involving probenecid (28–31), the effect is likely to be on clearances across membranes. However, the calculation of K_{exit} from Eq. (6) does not involve any assumptions concerning probenecid's effect on clearances or volumes. The observed reduction in K_{exit} indicates an increased residence time of AZT in CSF, but from these data alone it is not possible to determine the mechanism of this effect, e.g., an increase in V_f or a decrease in CL_{out}.

The effect of probenecid on the CSF-to-plasma distribution of a number of drugs has been explained by competitive inhibition of the transport system responsible for active transport of weak acids from the CSF to blood (28,31). AZT CSF-to-plasma distribution was examined as a function of plasma probenecid concentrations. Because of the small volume of CSF samples we were not able to measure probenecid concentrations in the CSF. However, since probenecid was administered as a continuous infusion in all experiments, the probenecid plasma concentrations should be proportional to the CSF concentrations at steady state. The average steady-state probenecid plasma concentration in the probenecid-treated rabbits during the AZT infusion experiments was 227 ± 48 μg/ml, and during the AZT iv bolus dose experiments it was 349 ± 57 μg/ml. The AZT CSF/plasma concentration ratios determined at steady state [Eq. (3)] during continuous AZT infusion or at transient steady state [Eq. (4)] after a single iv dose were calculated. The AZT CSF/plasma concentration ratio in the two control groups (during AZT continuous infusion and at the transient steady state after a single iv dose) were 0.191 ± 0.004 and 0.175 ± 0.024, respectively. These two control groups were not statistically different (Student's t test, $P = 0.34$) and were used as one group in the analysis. Figure 3 shows the relationship between the AZT CSF/plasma concentration ratios at AZT steady state or transient steady state and the probenecid plasma concentrations. There was an increasing trend in the AZT CSF/plasma concentration ratio with increasing plasma probenecid concentrations. These results are consistent with competitive inhibition of CSF-to-plasma transport. Further studies are ongoing in our laboratory to characterize this interaction.

The enhancement of drug distribution into the CNS is a desirable goal for a wide variety of drugs that exert their pharmacological action in the CNS. Several strategies have been used to achieve this goal. Derivatives of AZT that might be sequestered in the CNS where they can release AZT have been investigated as a means of improving the delivery of AZT into the brain (32). The present work investigates a drug–drug interaction between AZT and probenecid that results in inhibition of the CSF-to-plasma transport of AZT and causes an increase in the CSF-to-plasma distribution ratio in the rabbit. Whether this enhancement of the distribution into CSF is accompanied by increased brain tissue uptake of AZT remains to be determined. Enhanced

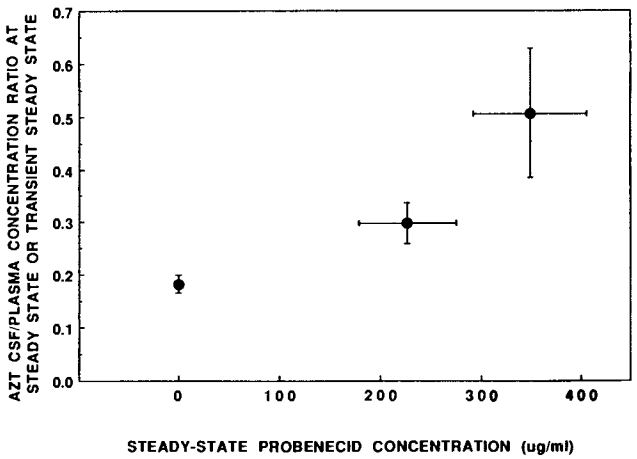

Fig. 3. A plot of the AZT CSF/plasma concentration ratios, at steady state (during continuous infusion) or at transient steady state (following iv bolus dose), as a function of probenecid plasma concentrations. Mean values (±SD) are plotted.

brain uptake of AZT in AIDS/ARC patients should result in more effective treatment of associated neurological deficits. In addition, this interaction may benefit patients who cannot tolerate usual doses of AZT because of side effects. This effect may enhance the normalization of their neurological dysfunction at reduced AZT doses.

In summary, we have shown that probenecid enhances the distribution of AZT into rabbit CSF. A pharmacokinetic model that relates intercompartmental clearances between CSF and plasma was developed to analyze the results. This analysis reveals that the enhanced distribution of AZT into rabbit CSF during probenecid coadministration is due in part to an effect of probenecid on the CSF exit clearance of AZT. This effect presumably results from competitive inhibition by probenecid of the CSF-to-plasma active transport of AZT. The analysis also suggests that CSF entry clearance may have increased or that the volume of CSF may have decreased during probenecid treatment.

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