Efflux of Zidovudine and 2',3'-Dideoxyinosine Out of the Cerebrospinal Fluid When Administered Alone and in Combination to *Macaca nemestrina*

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To determine if there is active efflux of zidovudine (ZDV) and 2',3'-dideoxyinosine (ddI) out of the cerebrospinal fluid (CSF), and if this efflux is saturable, we investigated the steady-state CSF/plasma concentration ratio of the two drugs when administered alone or in combination. Constant-rate infusions of ZDV, ddI or both were administered to seven macaques (*Macaca nemestrina*) through a chronic venous catheter for a minimum of 28 hr. Antipyrine, a marker of passive diffusion, was coinfused in all experiments. Blood (5 mL) and CSF samples (0.5–1 mL) were collected by venous and lumbar/thoracic punctures, respectively, at 24 and 28 hr after beginning the infusion. When ZDV and ddI were administered alone, the steady-state CSF/plasma concentration ratios were significantly different from unity (ZDV, 0.20 ± 0.08; ddI, 0.09 ± 0.04) and were independent of the plasma concentration ($P > 0.05$). In contrast, the CSF/plasma concentration ratio of antipyrine (0.82 ± 0.19) was close but significantly smaller than unity ($P > 0.05$). The CSF/plasma concentration ratios after simultaneous administration of ZDV and ddI were not significantly different ($P > 0.05$) from those obtained after administration of the drugs alone. These results suggest that ZDV and ddI are actively transported out of the CSF; however, within the concentration range studied, this efflux is neither saturable nor mutually competitive. Concomitant administration of ZDV and ddI did not produce a systemic interaction in the animals, indicating that the pharmacokinetics of either drug is unaffected by the presence of the other.

KEY WORDS: zidovudine; 2',3'-dideoxyinosine; cerebrospinal fluid; choroid plexus; active transport; drug interaction; AIDS; *Macaca nemestrina*.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is frequently complicated by diseases of the central nervous system. Approximately 40–50% of all adults and 70–80% of children with AIDS have clinical neurological dysfunction (AIDS–dementia complex) (1,2). Virological and morphological studies have demonstrated that HIV directly invades the brain and induces meningitis as well as encephalitis (1).

Zidovudine (ZDV; 3'-azido-2',3'-deoxythymidine) and 2',3'-dideoxyinosine (ddI; didanosine) are nucleoside analogue, of thymidine and inosine, respectively. AIDS patients receiving ZDV treatment show a significant improvement in neurological abnormalities (2). This clinical improvement is associated with a decrease of HIV p24 antigen in the cerebrospinal fluid (2). Similarly, improvement in HIV-associated cognitive dysfunction has been observed in patients treated with high doses of ddI (3).

ZDV administration to AIDS patients is associated with the development of HIV strains resistant to the drug (4) and with myelosuppression, which appears to be dose dependent (5). In contrast, the main toxic effect of ddI is painful peripheral neuropathy, which also appears to be dose dependent, and pancreatitis (6). *In vitro* studies have shown that ddI has activity against strains of HIV resistant to ZDV and causes minimal toxic effects on bone marrow progenitor cells (6). It has been demonstrated that patients infected with ZDV-resistant HIV respond to ddI treatment in a majority of cases (7). Patients receiving zidovudine, 600 mg daily for 16 weeks or more, are likely to respond favorably to changing the treatment from ZDV to ddI (8). Combining ZDV and ddI, at reduced doses, may increase the efficacy and diminish the overall toxicity of these drugs. It is currently not known whether combination therapy of ZDV and ddI leads to a pharmacokinetic interaction between the drugs. Such an interaction, if clinically relevant, would call for an alteration in the dosing regimen of the drugs when administered in combination.

A drug that freely diffuses in and out of the brain is expected to have an unbound cerebrospinal fluid (CSF)/plasma concentration ratio at steady state or pseudo-equilibrium which is close to unity, assuming that the bulk flow of CSF is small relative to the diffusion clearances of drug across the blood–brain and blood–CSF barriers. However, both ZDV and ddI have CSF/plasma concentration ratios that are significantly less than unity. A steady-state ZDV CSF/plasma concentration ratio of 0.24 ± 0.09 has been observed in pediatric patients (9), and an average non-steady-state ddI CSF/plasma concentration ratio of 0.21 ± 0.03 has been observed in adult subjects (3). Although the mechanistic basis for the deviation of these ratios from unity is not clearly defined, these data suggest that active transport processes may be responsible for facilitating the transfer of drugs out of the central nervous system.

A drug can enter the brain and the CSF via the blood–brain barrier, located in the cerebral endothelial capillaries, or via the blood–CSF barrier, located in the choroid plexus (10,11). Several transport systems for endogenous compounds have been identified across these biological barriers. It is conceivable that ZDV and ddI are capable of binding to the carrier proteins that exist across the blood–brain barrier or at the choroid plexus and undergo active transport into or out of the brain compartment. Previous studies in rats (12) and rabbits (13) indicated that the choroid plexus is the major site for the transport of ZDV into and out of the CSF. Studies performed with ZDV in rabbits (13,14) and rhesus monkeys (15) and with ddI in rats (16) further demonstrated that probenecid produced an increase in the concentration of the dideoxynucleosides in the CSF. Probenecid is an inhibitor of the organic anion transporter at the choroid plexus and is known to compete with other drugs for an active efflux carrier system at this barrier (17). These results suggest that

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ZDV and ddI may bind to the same active carrier system in the choroid plexus and that concurrent administration of ZDV and ddI could result in competitive inhibition of the transport of these drugs across the barrier. Such mutual competition could result in increased CSF/plasma concentration ratios of both drugs, which ultimately could enhance the neuropharmacological efficacy at the same systemic concentration.

The objective of this investigation was, first, to determine whether there is active efflux of ZDV and ddI out of the CSF at steady state in *Macaca nemestrina*. Next, we determined if the active efflux is mutually competitive and if the participating active efflux carrier system is saturable. Finally, we addressed the question whether ZDV and ddI, when administered simultaneously, produce an interaction in the systemic clearance of either drug.

MATERIALS AND METHODS

Selection and Preparation of Study Animals

Seven healthy adult macaques (*Macaca nemestrima;* five females and two males; 3.2–7.5 kg) were studied. Each animal had a chronic catheter placed in the femoral vein, under halothane anesthesia, at least 5 days prior to beginning the study. The catheter was exteriorized between the shoulder blades after subcutaneous tunneling (18) and placed in a swivel that enabled access to the catheter from outside the cage. This arrangement allowed the animals to move around freely in the cage while receiving the drug infusions. The catheter was perfused continuously (15–20 mL/hr) with 0.9% sterile saline solution to maintain its patency. Each animal was housed individually and was allowed access to food and water ad libitum. Twice a month, between experiments, the animals received supplemental injections of 100 mg iron-dextran and 1 mL vitamin B-complex solution (Combiplex-B Injectable, Fermenta Animal Health Company).

Drug Administration and Sampling of Biological Fluids

Each animal participated in seven experiments. In each experiment, the animals received a constant-rate infusion of ZDV or ddI, or both, for a minimum of 28 hr. Antipyrine, a marker of passive diffusion into the central nervous system, was infused at a rate of 2.7 mg/kg/hr in all experiments. A Walkmed 420 (Medfusion Incorporated) or a Sigma volumetric pump Model 6000 was used to administer drug solutions, and a Gilson Minipuls 3 pump was used to administer saline. Consecutive experiments were separated by at least 48 hr to allow sufficient time for recovery and washout. The rates of infusions of ZDV and ddI for each experiment are summarized in Table I. The high infusion rates (24 mg/kg/hr) were limited by the solubility of the drugs in saline and by the maximal acceptable fluid infusion rate to the animals. A femoral vein blood sample (8 mL) and a lumbar/thoracic CSF sample (0.5–1.0 mL) were obtained simultaneously, under ketamine (10 mg/kg iv) sedation, at 24 and 28 hr after beginning the infusion of the drugs. The red blood cell count in CSF samples was determined with a hemocytometer, and samples that appeared contaminated with blood were discarded. The plasma and CSF samples were stored at −20°C until analysis.

Table I. Infusion Rates of ZDV and ddI During the Dosing Regimens

Dosing regimen	Infusion rate (mg/kg/hr) ZVD	ddI
A. ZDV low	1.2	—
B. ZDV high	24.0	—
C. ddI low	—	1.2
D. ddI high	—	24.0
E. ZDV low + ddI low	1.2	1.2
F. ZDV low + ddI high	1.2	24.0
G. ZDV high + ddI low	24.0	1.2

Chemical Analysis

The concentration of ZDV in the plasma and CSF was determined by fluorescence polarization immunoassay (FPIA), using the Abbott TDx fluorescence polarization analyzer (19), or by a modification of a high-performance liquid chromatography (HPLC) method previously developed in our laboratory (20). The concentration of ddI and antipyrine in the plasma and CSF was determined by the same HPLC method (20). The binding of a ddI to plasma proteins, as determined by ultrafiltration (Centrifree, Amicon Corp., Danvers, MA), was found to be negligible.

Data Analysis

For each experiment, the CSF/plasma concentration ratio was computed for ZDV and/or ddI, respectively. Clearance values were computed as a ratio of the rate of infusion and the steady-state plasma concentration. The results from each experiment were analyzed using a paired *t* test. In cases where no significant differences were observed in the ratios obtained from the low and high infusion rate of a drug alone, the results were pooled and compared, using an unpaired *t* test, with the data obtained from simultaneous administration of drugs.

RESULTS

The average (±SD) red blood cell count in CSF was 4×10^5 ($\pm 4.3 \times 10^5$) cells/mL, which is less than 0.01% of the cell count in blood. This indicated that the CSF samples were not significantly contaminated with blood. The mean concentrations of antipyrine in plasma and CSF were 5.07 ± 1.02 and 3.99 ± 0.54 μg/mL, respectively. The elimination half-lives for ZDV and ddI in *Macaca nemestrina* are approximately 38 and 100 min, respectively. To ensure steady-state concentrations in both plasma and CSF compartments at the time of sampling, the duration of the experiments was conservatively fixed to 28 hr. Indeed, at 24 and 28 hr, both plasma and CSF concentrations appeared to be at steady state, as demonstrated by the small mean percentage difference (within the range of analytical errors) between the concentrations at these times (plasma, −0.93 ± 28.56%; CSF, −2.58 ± 36.49%).

The mean (±SD) CSF/plasma concentration ratio of antipyrine was 0.82 ± 0.19. The clearance of antipyrine was 9.01 ± 1.94 mL/min/kg. The mean concentration of ZDV and ddI in plasma and CSF, the CSF/plasma concentration ratios, and the clearance values of ZDV and ddI during the various dosing regimens (A through G) are presented in Tables II and III, respectively.

Paired *t*-test analysis of the ratios from the respective low and high infusion rates of ZDV and ddI showed that the CSF/plasma concentration ratios for both drugs were constant over the dose range studied ($P > 0.05$; Table III). Thus, in subsequent analyses, the low and high concentration ratios for each drug were pooled to increase the power of our statistical analyses. Combining the data for the low and high dosing regimens resulted in overall mean CSF/plasma concentration ratios of 0.20 ± 0.08 for ZDV and 0.09 ± 0.04 for ddI (Fig. 1). The mean CSF/plasma concentration ratios for ZDV and ddI were significantly different from each other ($P < 0.001$).

When ZDV was coadministered at a low infusion rate with a low (regimen E) or a high (regimen F) infusion rate of ddI, the CSF/plasma concentration ratios of ZDV were found to be 0.27 ± 0.09 and 0.25 ± 0.04, respectively (Fig. 1). The high infusion rate of ZDV combined with the low infusion rate of ddI (regimen G) resulted in a mean ZDV CSF/plasma concentration ratio of 0.19 ± 0.08 (Fig. 1). None of these ratios were significantly different from the mean CSF/plasma concentration ratio of 0.20 ± 0.08 obtained during infusion of ZDV alone ($P > 0.05$). Similarly, when ddI at low infusion rate was coadministered with a low (regimen E) or a high (regimen G) infusion rate of ZDV, the CSF/plasma concentration ratios of ddI were 0.12 ± 0.04 and 0.09 ± 0.05, respectively (Fig. 1). The high infusion rate of ddI combined with a low infusion rate of ZDV (regimen F) resulted in a mean ddI CSF/plasma concentration ratio of 0.10 ± 0.03. These ratios were not significantly different from the mean CSF/plasma concentration ratio of 0.09 ± 0.04 obtained during infusion of ddI alone ($P > 0.05$).

No significant differences ($P > 0.05$) were observed between the clearances obtained at the low and high infusion rates of ZDV (regimens A and B). The overall mean clearance of ZDV administered alone was 35.41 ± 8.47 mL/min/kg. In contrast, the clearance of ddI increased with an increase in the infusion rate [23.42 ± 7.45 (regimen C) to 31.18 ± 4.45 (regimen D); $P < 0.02$]. Concurrent administration of ZDV and ddI (regimens E, F, and G) produced no significant change ($P > 0.05$) in the clearance of either drug.

DISCUSSION

As indicated in the Introduction, considerable evidence is available to indicate that the choroid plexus is the predominant site of transfer of ZDV and ddI into and out of the CSF in the rat and rabbit. Based on these data, the following pharmacokinetic model can be proposed (Fig. 2). We assume that the choroid plexus is the main site of transfer of drug between the blood and the CSF and that no barrier exists between the CSF and the brain compartment. That is, we assume that the drugs passively diffuse between the CSF and the extracellular and intracellular fluids in the brain. However, even if a substantial amount of drug should enter the central nervous system at the blood–brain barrier and not at the choroid plexus, the concentration of the unbound drugs in the CSF and in the brain should be equal at steady state. Therefore, excluding the blood–brain barrier and the brain compartment in our model does not detract from the generality of the model. We further assume that drugs can be cleared from the CSF due to bulk flow of CSF. As fresh CSF (containing a zero concentration of drug) is produced by the choroid plexus, the existing CSF is displaced into the venous blood (11).

The rate of change of amount drug in the CSF is

$$\frac{dA_2}{dt} = (CL_{12})\, C_1 - (CL_{21} + CL_{bf})\, C_2 \qquad (1)$$

where CL_{12} and CL_{21} represent the distribution clearances of drug across the choroid plexus and CL_{bf} represents the clearance of the drug from the CSF due to bulk flow.

At steady state, $dA_2/dt = 0$, therefore

$$(CL_{12})\, C_{1ss} = (CL_{21} + CL_{bf})\, C_{2ss}$$

or

$$\frac{C_{2ss}}{C_{1ss}} = \frac{CL_{12}}{(CL_{21} + CL_{bf})} \qquad (2)$$

Thus, the steady-state CSF/plasma concentration ratio is dependent upon the relative magnitudes of the blood–CSF distribution clearances, as well as the bulk flow from the CSF to the blood. Provided that the clearance of drug out of the CSF due to bulk flow is insignificant, and in the absence of active transport across the blood–CSF barrier, the steady-state CSF/plasma concentration of the unbound drug should theoretically approach unity. To provide support for this thesis, we deliberately included antipyrine, a highly permeable and

Table II. Mean (±SD) Concentrations of ZDV and ddI in Plasma and CSF During the Dosing Regimens

	ZDV C_{ss} (μg/mL)		ddI C_{ss} (μg/mL)	
Dosing regimen	Plasma	CSF	Plasma	CSF
A. ZDV low	0.58 ± 0.17	0.13 ± 0.05	—	—
B. ZDV high	10.77 ± 2.17	1.88 ± 0.97	—	—
C. ddI low	—	—	0.86 ± 0.25	0.09 ± 0.04
D. ddI high	—	—	13.06 ± 1.88	1.14 ± 0.27
E. ZDV low + ddI low	0.58 ± 0.16	0.14 ± 0.02	0.89 ± 0.28	0.11 ± 0.04
F. ZDV low + ddI high	0.53 ± 0.11	0.13 ± 0.02	11.27 ± 2.01	1.23 ± 0.39
G. ZDV high + ddI low	11.16 ± 3.30	2.17 ± 1.14	0.90 ± 0.19	0.08 ± 0.03

Table III. Mean (±SD) CSF/Plasma Concentration Ratios and Systemic Clearance Values for ZDV and ddI During the Dosing Regimens

Dosing regimen	CSF/plasma conc. ratio		Clearance (mL/min/kg)	
	ZDV	ddI	ZDV	ddI
A. ZDV low	0.22 ± 0.08	—	33.40 ± 7.87	—
B. ZDV high	0.17 ± 0.07	—	38.69 ± 8.91	—
C. ddI low	—	0.10 ± 0.05	—	23.42 ± 7.45
D. ddI high	—	0.09 ± 0.02	—	31.18 ± 4.45
E. ZDV low + ddI low	0.27 ± 0.09	0.12 ± 0.04	35.46 ± 9.89	24.76 ± 7.51
F. ZDV low + ddI high	0.25 ± 0.04	0.10 ± 0.03	39.03 ± 8.87	36.81 ± 8.67
G. ZDV high + ddI low	0.19 ± 0.08	0.09 ± 0.05	37.83 ± 7.89	23.13 ± 5.36

not protein-bound passive diffusion marker in our studies. That the ratio of antipyrine is close to but significantly smaller than unity (0.82 ± 0.19; $P < 0.05$) suggests that antipyrine enters and exits the CSF by passive diffusion and that the clearance of antipyrine out of the CSF due to bulk flow is small compared with the blood–CSF distribution clearances.

In contrast, ZDV and ddI, which both display negligible plasma protein binding in *Macaca nemestrina* (20), had CSF/plasma concentration ratios of 0.2 and 0.09, respectively. This observation can be explained by one of the following two hypotheses. First, there is active transport of both ZDV and ddI out of the CSF, and this active efflux results in a drug concentration gradient between the CSF and the blood. This hypothesis is supported by the fact that probenecid, a potent inhibitor of anion transport, has been found to increase the CSF/plasma concentration ratio of ZDV in both the rabbit and the rhesus monkey (13–15) and of ddI in the rat (16).

Second, the clearance of ZDV and ddI from the CSF by metabolism or bulk flow may be greater than their respective blood–CSF distribution clearances, resulting in CSF/plasma concentration ratios less than unity. Under the passive diffusion scenario, CL_{12} and CL_{21} should be equal for a drug, but the magnitude of these clearances may differ between drugs. Extensive metabolism of at least ZDV in the central nervous system is unlikely since the major metabolite of ZDV, the glucuronide, was found not to be present in the CSF in *Macaca nemestrina* (21). Assuming that the bulk flow is unaffected by the administration of the drugs, the bulk flow clearance of ZDV, ddI, and antipyrine should be equal. Therefore, any differences in the CSF/plasma concentration ratios between the drugs are determined by the magnitude of the distribution clearances into and out of the CSF relative to that of the bulk flow [Eq. (2)].

If lipophilicity is the determining factor for the passive entry of the drugs into the CSF, the drugs' blood–CSF distribution clearance should be proportional to their octanol/water partition coefficient (10). Given this scenario, antipyrine would have to be 20 and 50 times more lipophilic than ZDV and ddI, respectively, to account for the observed differences in the CSF/plasma concentration ratios. However, the octanol/water partition coefficient of antipyrine (P = 1.6) (10) is almost equal to the partition coefficient of ZDV (P = 1.26) (22) and only 20-fold greater than the partition coefficient of ddI (P = 0.07) (23). Hence, it is not likely that diffusion processes alone can explain the observed differences in the CSF/plasma concentration ratios. That the lipophilicity of these dideoxynucleosides does not correlate with their CSF/plasma concentration ratios (24) lends support to the notion that mechanisms other than diffusion govern this ratio. Studies in the rat have demonstrated that the efflux rate of ddI from the CSF is more than 50-fold greater than that for influx (16), suggesting that the efflux, and not the influx, is an active process.

Although our study does not definitively prove active efflux out of the CSF in *Macaca nemestrina,* our data and those of others (13–15) support this conclusion. Our finding that the CSF/plasma concentration ratios of ZDV and ddI at steady state are significantly different from each other ($P <$

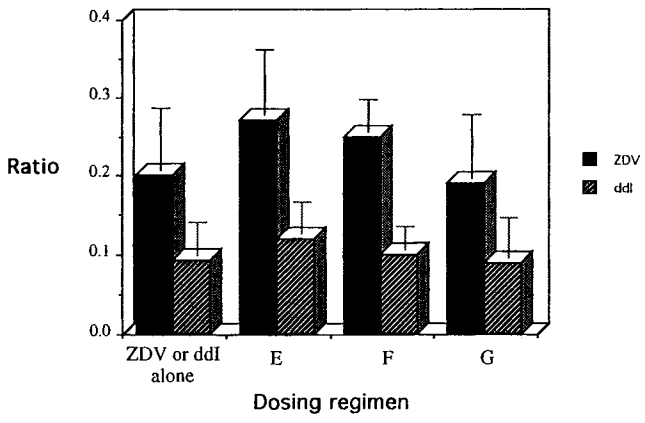

Fig. 1. CSF/plasma concentration ratio of ZDV and ddI when administered alone and in combination.

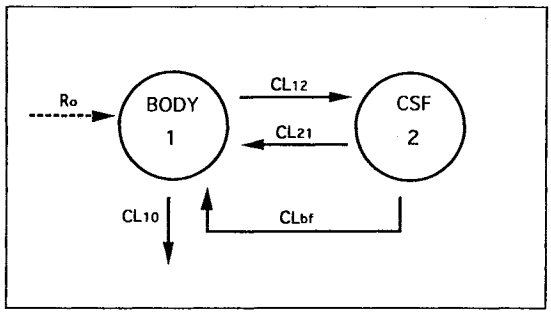

Fig. 2. Pharmacokinetic model.

0.001) suggests that the two drugs have an unequal binding affinity for a common efflux carrier system, that separate carrier systems exist for ZDV and ddI, or that the bulk flow clearance relative to the active efflux clearance is greater for ddI.

Despite a 20-fold increase in infusion rate, we found no concentration-dependent changes in the CSF/plasma concentration ratio at steady state for either drug. Thus, the carrier(s) is not saturable over a wide concentration range, indicating that it belongs to a class of high-capacity transport systems. This observation is also supported by the lack of change in the CSF/plasma concentration ratios of ZDV and ddI after simultaneous administration when compared with that obtained after administration of the drugs alone ($P > 0.05$). That is, the efflux of the drugs from the CSF is not mutually competitive. However, this result does not exclude the possibility that the two drugs may be transported by the same carrier system.

The identity of the carrier system responsible for the active efflux from the CSF is unknown. However, circumstantial evidence detailed below points toward the anion transport system. Concurrent administration of probenecid with ZDV or ddI significantly increases the CSF/plasma concentration ratio of ZDV in rabbits (13,14) and rhesus monkeys (15) and that of ddI in rats (16). The increase in the CSF/plasma concentration ratio is correlated with the plasma concentration of probenecid (13). Probenecid is a known inhibitor of the organic anion carrier system at the blood–CSF barrier and in the renal tubules (14). ZDV has been shown to have an affinity to the anion carrier system in the rat renal basolateral membrane vesicles (25), and its tubular secretion can be inhibited by probenecid. Thus, ZDV may be a substrate for the anion transporter at the choroid plexus. In contrast, ZDV or ddI does not have a high affinity for the nucleoside transporters present in peripheral blood cells (22,23). Furthermore, ZDV and other synthetic nucleosides are unable to inhibit the uptake of endogenous nucleosides across isolated rabbit choroid plexus (26), suggesting that the drugs are poor substrates for the nucleoside transporters in the blood–CSF barrier.

The steady-state clearance of ZDV when administered alone (regimens A and B) was constant over the dosage range studied, suggesting that ZDV follows linear pharmacokinetics. In contrast, the clearance of ddI increased significantly with increasing infusion rate, indicating that the systemic clearance of ddI is nonlinear in the concentration range studied. This is in contrast to studies in the rat (27), where ddI clearance decreased on increasing the dose. The reason for this difference between animal species is as yet unknown. Since we did not measure the renal clearance of ddI at the two infusion rates used, it is presently unclear what mechanism(s) is responsible for the increase. No changes in the clearances were apparent during simultaneous administration of ZDV and ddI (regimens E–G), indicating that the pharmacokinetics of either drug is unaffected by the presence of the other. Hence, concomitant administration of ZDV and ddI did not produce a systemic interaction in the animals. Similar results have been obtained in rats (28), but human data are currently unavailable.

Previous studies in our laboratory have demonstrated that the majority of an iv ZDV dose in *Macaca nemestrina* is recovered in urine as either the 5′-glucuronide metabolite, ZDVG (45%), or the unchanged drug (43%) (20). The metabolic pattern is similar in humans, where most of the iv dose is recovered as ZDVG (60%) or the unchanged drug (17–23%) (29). In contrast, an iv dose of ddI in *Macaca nemestrina* is excreted primarily unchanged in the urine (57%) (30), while a smaller fraction of the dose is recovered unchanged in the urine in humans (36%) (3).

The extent and nature of ddI metabolism in both *Macaca nemestrina* and humans are presently unknown, although it has been proposed that ddI is principally a substrate for the enzyme purine nucleoside phosphorylase. The absence of an interaction in the pharmacokinetics of ZDV and ddI during simultaneous administration is consistent with the fact that the two drugs follow different elimination pathways in *Macaca nemestrina*. Although small differences exist between human and nonhuman primates in the elimination of these drugs, based on our data, we predict that simultaneous administration of ZDV and ddI will not yield a pharmacokinetic interaction in humans.

In conclusion, our data indicate that ZDV and ddI are transported out of the CSF in *Macaca nemestrina* by a carrier system(s) which is not readily saturable. Further studies are ongoing in our laboratory to identify and characterize this transporter.

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