ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1991, p. 801–807
0066-4804/91/050801-07$02.00/0

Vol. 35, No. 5

Pharmacokinetics of 3′-Azido-3′-Deoxythymidine and Its Catabolites and Interactions with Probenecid in Rhesus Monkeys

ERIKA M. CRETTON,[1] RAYMOND F. SCHINAZI,[2,3,4] HAROLD M. McCLURE,[4,5] DANIEL C. ANDERSON,[4] AND JEAN-PIERRE SOMMADOSSI[1,4]*

Department of Pharmacology, Center for AIDS Research, Comprehensive Cancer Center, Division of Clinical Pharmacology, University of Alabama at Birmingham, Birmingham, Alabama 35294[1]; Veterans Affairs Medical Center, Decatur, Georgia 30033[2]; and Department of Pediatrics, Laboratory of Biochemical Pharmacology, Emory University School of Medicine,[3] and Yerkes Regional Primate Research Center[4] and Department of Pathology,[5] Emory University, Atlanta, Georgia 30322

Received 29 October 1990/Accepted 11 February 1991

The pharmacokinetics and metabolism of 3′-azido-3′-deoxythymidine (AZT) were investigated in rhesus monkeys after subcutaneous administration of 33.3 mg of AZT per kg of body weight alone or in the presence of 100 mg of probenecid per kg. In addition to unchanged drug, two catabolites, 5′-*O*-glucuronide (GAZT) and 3′-amino-3′-deoxythymidine (AMT), were detected in plasma within 30 min. GAZT exhibited a kinetic profile similar to that of AZT, with an elimination half-life of approximately 1 h, while AMT was more variable, with an apparent half-life of 1.6 ± 1.5 h. Approximately 90% of the total administered dose was recovered in urine within 24 h as AZT, GAZT, AMT, and the 5′-*O*-glucuronide of AMT. AZT and AMT demonstrated similar cerebrospinal fluid (CSF) penetration 1 h after AZT treatment, while GAZT poorly crossed the blood-brain barrier. Concomitant administration of probenecid greatly altered the pharmacokinetics of AZT, GAZT, and AMT, resulting in prolongation of their apparent elimination half-lives, increased concentrations in plasma, and marked reduction in renal clearances. In addition, the CSF/plasma concentration ratios for AZT and its catabolites were greatly increased, suggesting that probenecid inhibits efflux of AZT and its catabolites from CSF to plasma. The substantial levels of AMT in plasma suggest that this catabolite affects the pharmacodynamic properties of AZT in relation to its activity against human immunodeficiency virus replication and cytotoxicity to host cells. Enhanced AMT levels in plasma in the presence of probenecid may decrease the therapeutic efficacy of the AZT-probenecid combination.

3′-Azido-3′-deoxythymidine (AZT) is currently the only drug clinically approved for the treatment of AIDS. Studies in humans have shown that AZT is primarily eliminated as a 5′-*O*-glucuronide (GAZT), with 60 to 80% of the administered dose being detected in urine as this metabolite (3, 18). Although the pharmacokinetics of unchanged AZT have been studied extensively in both humans (3, 8, 23, 24, 29) and various animal species, including nonhuman primates (2, 4, 11), an understanding of the in vivo disposition of AZT catabolites is limited. Significantly, recent in vitro studies by our group have demonstrated that, in addition to GAZT, two previously unidentified AZT catabolites, 3′-amino-3′-deoxythymidine (AMT) and its glucuronide (GAMT), are formed in isolated hepatocytes and human liver microsomes (10). These novel AZT catabolites were demonstrated to form through enzymatic reduction of the 3′-azido group of AZT and GAZT. Of note, AMT was not a substrate for uridine 5′-diphosphoglucuronyltransferase (UDPGT). In addition, AMT was demonstrated to be at least five- to sevenfold more toxic for CFU granulocyte-macrophage and burst-forming unit erythroid than AZT (10), which would suggest that this metabolite plays a role in the AZT-induced myelosuppression observed in patients treated with AZT (33, 37, 38). De Miranda and colleagues (12) recently reported that AMT was a major fecal catabolite of AZT in rats, suggesting that the intestinal microflora might convert AZT to AMT. However, in that study, determination of AMT concentration in plasma or serum was not assessed. The importance of this catabolic pathway which leads to AMT formation is further suggested by the possible formation of this metabolite in the human placenta (25, 34), although an observed unknown polar chromatographic peak with a retention time similar to that of AMT has not yet been identified. Our recent demonstration of the formation of novel AZT catabolites by using in vitro systems, including a potentially toxic catabolite, emphasizes the need to characterize their in vivo formation and elimination.

AZT is rapidly eliminated, with an apparent elimination half-life ($t_{1/2}$) of approximately 1 h in humans and various animal species (2, 4, 8, 11, 21, 23, 24, 29). Although the frequency of dose administration (every 4 h) is based on the intracellular $t_{1/2}$ of the active triphosphate derivative, modulating agents which may decrease the degradation of the unchanged drug are being investigated to maintain adequate AZT concentrations in plasma. In that context, probenecid, an inhibitor of renal tubular secretion of organic acids and formation of a number of acyl and ester glucuronides (1, 36), has been evaluated for its ability to decrease the extensive degradation of AZT to GAZT. This combination could reduce the frequency of AZT dosing and the cost associated with AZT therapy. Recent studies in humans and animals have shown that probenecid substantially alters the pharmacokinetics of both AZT and GAZT (13, 19, 22), resulting in a significant increase in the mean levels in plasma, area under the plasma-concentration time curve (AUC), and $t_{1/2}$ values for AZT and GAZT. These data suggest that probenecid affects the glucuronidation of AZT and also alters the renal elimination of both AZT and GAZT through inhibition of their tubular secretion in renal proximal tubules. Of note,

* Corresponding author.

cutaneous side effects may be observed in some patients treated with AZT in combination with probenecid, although the significance of these clinical findings is not known (14, 31).

The purpose of this study was to provide a detailed analysis of the in vivo catabolic disposition of AZT, particularly in relation to the formation of AMT and GAMT. The alteration of the pharmacokinetics and catabolism of AZT by probenecid was also investigated to evaluate the importance of AMT formation as a route of AZT elimination once glucuronidation has been inhibited. Although direct extrapolation of animal studies to the clinical situation should be made with caution, it should be emphasized that these investigations have been performed in rhesus monkeys, an animal model which exhibits pharmacokinetics and metabolism of AZT similar to those of humans (4, 11) when compared with other animals (11, 17, 19).

MATERIALS AND METHODS

Chemicals. [5-^{3}H]AZT (10 Ci/mmol) was obtained from Moravek Biochemicals, Inc. (Brea, Calif.), and was 99% pure as ascertained by the high-performance liquid chromatography (HPLC) technique described below. Nonlabeled AZT was synthesized in our laboratory by the procedure of Lin and Prusoff (27), and its chemical purity, confirmed by spectral and HPLC analysis, was greater than 99%. Probenecid was obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were reagent grade.

Experimental design. Rhesus monkeys (*Macaca mulatta*) were used for the catabolic and pharmacokinetic studies. The animals were maintained at the Yerkes Regional Primate Research Center of Emory University in accordance with guidelines established by the Animal Welfare Act and the NIH *Guide for the Care and Use of Laboratory Animals*. The Yerkes Center is fully accredited by the American Association for Accommodation of Laboratory Animal Care.

Young adult rhesus monkeys weighing approximately 5 to 6 kg were used for all studies. Three animals (RHZ1, RKO1, and AP31) received one subcutaneous dose of 33.3 mg of AZT per kg of body weight dissolved in phosphate-buffered saline, pH 7.4, and warmed at 37°C to facilitate dissolution, and two animals (ROU1 and RKO1) received one subcutaneous dose of 33.3 mg of AZT per kg immediately followed by a subcutaneous dose of 100 mg of probenecid per kg dissolved in a diluted sodium hydroxide solution (0.1 N) and titrated to neutral pH with hydrochloric acid. This procedure did not alter the chemical characteristics of this compound. To facilitate the subsequent measurement of AZT and its catabolites in biological fluids of treated animals, a tracer amount of 200 μCi of [5-^{3}H]AZT was mixed with the "cold" AZT dose to be administered. A 1-week washout period separated the first and second doses (AZT and probenecid combination) when the same animal, RKO1, was used. Samples (2 ml) of blood were collected in heparinized tubes at 0, 0.25, 0.50, 1, 2, 4, 6, 8, and 24 h after injection. When possible, urine was also collected at similar time points by cystocentesis. A single cerebrospinal fluid (CSF) sample was taken from four animals 1 h after drug administration. Plasma, CSF, and urine samples were frozen at −20°C until analysis.

Analytical methodology. Samples (1 to 2 ml) of CSF, plasma, or urine were passed through a 0.45-μm-pore-size Acro LC 13 filter (Gelman Sciences, Ann Arbor, Mich.), and 100- to 250-μl samples were analyzed by HPLC by using a Hewlett-Packard model 1050 liquid chromatograph equipped with a manual injector and a fixed wavelength spectrophotometer. Reversed-phase chromatography was performed by using a Hypersil ODS 5-μm column (Jones Chromatography, Littleton, Colo.). Elution was performed at 1 ml/min with 25 mM phosphoric acid (pH 7.2) and a 35-min linear gradient of acetonitrile from 0 to 30% starting at the time of injection. Column temperature was maintained at 25°C, and A_{254} was recorded. Eluent from the column was directed via a low dead-volume connection line into a model 2112 Redirac fraction collector (LKB Instruments, Rockville, Md.), and timed fractions of 0.5 ml were collected into miniscintillation vials over 35 min. After adding 5 ml of Budget-Solve scintillation fluor (Research Products International Corp., Mount Prospect, Ill.), radioactivity was measured by using a Beckman model 5801 liquid scintillation counter equipped with an automatic quench correction program. Under these conditions, AZT and its catabolites, GAZT, AMT, and GAMT, eluted at 21 to 22 min, 12 to 13 min, 9 to 10 min, and 7 to 8 min, respectively. Intra- and interday percentage coefficients of variation were less than 5% for AZT and GAZT and less than 10% for AMT and GAMT, at concentrations between 0.1 and 100 μg/ml. Standard curves for AZT, GAZT, and AMT were linear, with $r^2 \geq 0.99$. The detection limits were 0.1 μg/ml for AZT and AMT and 0.2 μg/ml for GAZT and GAMT.

Pharmacokinetic analysis. The pharmacokinetic parameters of AZT and its catabolites, GAZT, AMT, and GAMT, were estimated by model-independent methods by using a SIPHAR/Base program (16). The AUC was calculated by the trapezoidal rule with extrapolation to time infinity by using the terminal disposition slope (K) generated by weighted nonlinear least-squares regression (28), with the weighted factor set as the reciprocal of the calculated concentration squared. Elimination $t_{1/2}$ values of unchanged drug and catabolites (GAZT, AMT, and GAMT) were calculated from 0.693/K. The total plasma clearance (CL) of AZT was calculated by dividing the dose by the AUC. Renal clearance (CL_R) of AZT and catabolites was determined by dividing the amount of drug or catabolites excreted in urine during a 6-h period (for animals which received AZT alone) or an 8-h period (for animals which received AZT and probenecid in combination) by the AUC corresponding to the same time period. The peak plasma concentration (C_{max}) values and time-to-peak plasma concentration (T_{max}) values were observed experimental values.

RESULTS

Kinetics of unchanged drug and catabolites in plasma. Plasma concentration-time profiles of AZT and its catabolites after subcutaneous administration of 33.3 mg of AZT per kg alone or in the presence of 100 mg of probenecid per kg in representative monkeys are illustrated in Fig. 1A and B. Unchanged AZT and GAZT exhibited similar kinetic profiles, while AMT levels in plasma were more variable. In all monkeys, GAZT and AMT were detected within 30 min after drug administration, while GAMT was not observed at any time point in the presence or absence of probenecid. After administration of AZT alone, C_{max} values of AZT, GAZT, and AMT were 8.87 ± 1.40, 12.34 ± 4.75, and 0.80 ± 0.31 μg/ml, respectively. The T_{max} value of AZT was similar to those of its catabolites (Table 1). In the presence of probenecid, AZT and AMT C_{max} values remained unchanged while GAZT C_{max} values increased approximately twofold, averaging 21.62 ± 8.54 μg/ml. Although the T_{max}

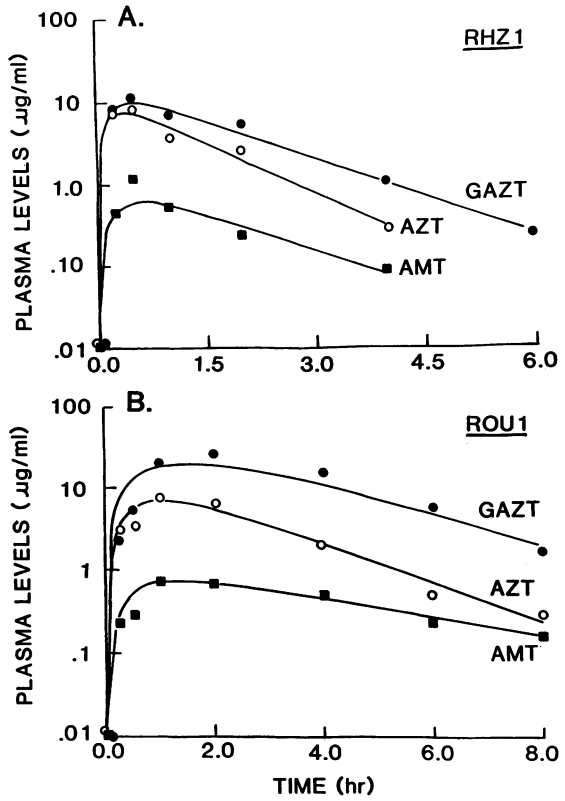

FIG. 1. Plasma concentration-time profiles of AZT (○), GAZT (●), and AMT (■) in a representative monkey after subcutaneous administration of 33.3 mg of AZT per kg (A) or 33.3 mg of AZT per kg in the presence of 100 mg of probenecid per kg (B). Symbols represent the experimental concentrations in plasma of AZT and its metabolites, while solid lines represent computer-fitted lines generated by using a SIPHAR/Base program (16).

values of AZT and its catabolites were slightly increased after probenecid treatment, these values were not substantially different from those obtained after AZT treatment alone. No drug or catabolites were detected in any of the 24-h plasma samples. The AZT and AMT CSF/plasma concentration ratios, obtained 1 h post-AZT treatment, were in the same range, with values of 0.30 ± 0.17 and 0.40 ± 0.04, respectively, whereas GAZT poorly crossed the blood-brain barrier, with a 10-fold-lower mean ratio than those of AZT and AMT (Table 1). After probenecid administration, CSF/plasma concentration ratios of AZT and its catabolites were substantially increased (Table 1). In particular, AZT values were at least twofold higher, and a 40% increase was also observed for AMT, suggesting that probenecid may substantially increase the transport of 2',3'-dideoxynucleosides across the blood-brain barrier. Pharmacokinetic parameters for AZT and its catabolites, GAZT and AMT, are presented in Table 1.

After administration of AZT alone, the average apparent elimination $t_{1/2}$ values of AZT and GAZT were in the same range, with values of 0.8 ± 0.1 and 0.9 ± 0.2 h, respectively. The apparent elimination $t_{1/2}$ of AMT was comparable to those of AZT and GAZT in two monkeys but was substantially prolonged in one animal, with a value of 3.33 h (Table 1). Concomitant administration of probenecid substantially increased the mean terminal $t_{1/2}$ values of AZT and its catabolites, GAZT and AMT (Table 2). Similarly, the AUC values of AZT increased approximately twofold from 14.9 to 33.2 μg · h/ml, leading to a corresponding decrease for total plasma clearance. AUC values of GAZT and AMT were also enhanced, but to a greater magnitude, i.e., approximately four- to fivefold higher than those calculated in animals who received AZT alone.

Urinary excretion of AZT and its catabolites. Figures 2A and B illustrate the urinary excretion of AZT and its catabolites in representative monkeys after subcutaneous administration of 33.3 mg of AZT per kg alone (RHZ1) or in the

TABLE 1. Pharmacokinetic parameters after subcutaneous administration of 33.3 mg of AZT per kg alone or in the presence of 100 mg of probenecid per kg in rhesus monkeys

Treatment	Monkey	Compound	C_{max} (μg/ml)	T_{max} (h)	AUC (μg · h/ml)	CL (ml/h/kg)	Apparent $t_{1/2}$ (h)	CSF/plasma ratio (1 h)
AZT	RHZ1	AZT	8.38	0.50	12.58	2,650	0.76	0.42
		GAZT	11.34	0.50	21.87		0.98	0.06
		AMT	1.13	0.50	1.44		0.83	0.36
	RKO1	AZT	10.45	0.50	17.29	1,930	0.86	NA[a]
		GAZT	8.18	1.00	16.82		1.15	NA
		AMT	0.51	0.50	2.39		3.33	NA
	AP31	AZT	7.79	1.00	14.90	2,230	0.75	0.18
		GAZT	17.52	1.00	31.73		0.69	0.02
		AMT	0.77	1.00	0.99		0.66	0.42
	Mean (SD)	AZT	8.9 (1.4)	0.7 (0.3)	14.9 (2.4)	2,300 (300)	0.8 (0.1)	0.30 (0.17)
		GAZT	12.3 (4.7)	0.8 (0.3)	23.5 (7.6)		0.9 (0.2)	0.04 (0.02)
		AMT	0.8 (0.3)	0.7 (0.1)	1.6 (0.7)		1.6 (1.5)	0.39 (0.04)
AZT/probenecid	ROU1	AZT	7.79	1.00	23.26	1,430	0.92	0.84
		GAZT	17.52	2.00	106.78		1.33	0.11
		AMT	0.77	1.00	3.80		2.03	0.51
	RKO1	AZT	8.68	1.00	43.12	770	2.64	0.67
		GAZT	15.58	1.00	117.35		3.13	0.16
		AMT	0.58	1.00	9.20		9.10	0.59
	Mean	AZT	8.00	1.00	33.20	1,050	1.78	0.76
		GAZT	21.60	1.50	112.10		2.23	0.13
		AMT	0.70	1.00	6.50		5.56	0.55

[a] NA, not available.

TABLE 2. Renal clearance and urinary excretion of AZT and its metabolites after subcutaneous administration of 33.3 mg of AZT per kg alone or in the presence of 100 mg of probenecid per kg in rhesus monkeys[a]

Treatment	Monkey	Compound excreted	Amt (% of total dose)	CL_R (ml/h/kg)	Catabolite/AZT urinary excretion ratio
AZT	RHZ1	AZT	23.8	99.31	
		GAZT	58.4	84.61	0.85
		AMT	2.1	85.81	0.86
		GAMT	0.6	NE[a]	NE
	RKO1	AZT	23.7	42.91	
		GAZT	65.3	98.75	2.30
		AMT	0.9	17.46	0.41
		GAMT	0.1	NE	NE
	AP31	AZT	32.8	72.78	
		GAZT	55.0	109.52	1.50
		AMT	1.2	76.21	1.05
		GAMT	0.6	NE	NE
	Mean (SD)	AZT	26.8 (5.2)	71.7 (28.2)	
		GAZT	59.6 (5.3)	97.6 (12.5)	1.36 (0.6)
		AMT	1.4 (0.6)	59.8 (37.0)	0.83 (0.3)
		GAMT	0.4 (0.3)	NE	NE
AZT/probenecid	ROU1	AZT	20.6	39.77	
		GAZT	66.2	58.41	1.47
		AMT	1.2	28.45	0.71
		GAMT	NE	NE	NE
	RKO1	AZT	17.4	15.02	
		GAZT	71.2	20.97	1.40
		AMT	1.0	3.61	0.24
		GAMT	0.3	NE	NE
	Mean	AZT	19.0	27.4	
		GAZT	68.7	39.7	1.45
		AMT	1.1	16.0	0.58
		GAMT	0.3	NE	NE

[a] NE, not evaluable.

presence of 100 mg of probenecid per kg (ROU1). Excretion of AZT, GAZT, and AMT was rapid, with most occurring between 1 and 6 h post-AZT administration, while excretion of unchanged drug and catabolites was delayed in the presence of probenecid, with the largest amounts being eliminated between 4 and 8 h post-drug administration. Of note, GAMT, a catabolite which was not observed in plasma, was detected in urine at 1 h post-drug administration and followed an excretion pattern similar to those observed for the other catabolites. Urinary recovery data with CL_R values of AZT and its catabolites are presented in Table 2. After administration of AZT, in the presence or absence of probenecid, the total urinary recovery of the parent drug and its catabolites was essentially complete, with approximately 90% of the dose being excreted within 24 h. The excreted amount of unchanged drug and each catabolite did not vary substantially whether probenecid was administered in combination with AZT or AZT was administered alone. GAZT represented the major catabolite in all urine fractions, accounting for as much as 60 to 70% of the administered dose of AZT alone or in the presence of probenecid, while AMT and GAMT amounts recovered in urine were minimal, representing 1 to 2% and 0.3 to 0.7%, respectively. The mean CL_R values of unchanged drug and its catabolites, GAZT and AMT, were markedly decreased by as much as two- to fourfold after probenecid administration; however, concurrent administration of 100 mg of probenecid per kg with 33.3 mg of AZT per kg in monkeys did not alter the urinary ratio of GAZT/AZT or AMT/AZT.

DISCUSSION

Pharmacokinetic and metabolic studies of AZT were undertaken in monkeys on the basis of a postulated metabolic pathway from our recent in vitro studies which included the conversion of AZT to GAZT by UDPGT and the reduction of AZT and GAZT to previously unrecognized 3′-amino derivatives, AMT and GAMT (Fig. 3). In the present study, the in vivo formation and disposition of these AZT catabolites were evaluated in monkeys through the administration of a radiolabeled tracer amount of AZT which permitted direct HPLC analysis with a limit of sensitivity of 0.1 to 0.2 μg/ml for unchanged drug and all catabolites by using a specific activity of 1.6 mCi/mmol, thus avoiding extensive extraction steps which may have resulted in the partial or complete loss of putative metabolites. The pharmacokinetic data (apparent $t_{1/2}$ and CL) of unchanged AZT in the present study are in agreement with previous reports on AZT disposition characteristics after intravenous administration in primate models (2, 16) and in humans (3, 8, 21, 23) and are consistent with the observed rapid and nearly complete AZT absorption after subcutaneous administration (2). Although GAZT kinetics have been reported in humans (3, 24), none of the studies in primates assessed the disposition of this metabolite in plasma or serum. As observed in patients after administration of AZT alone, the AUC of GAZT was greater than that of the parent drug, and plasma concentration profiles decayed in parallel with those of the unchanged drug. AMT, a novel metabolite of AZT recently identified by our group, was present in the plasma of each monkey at

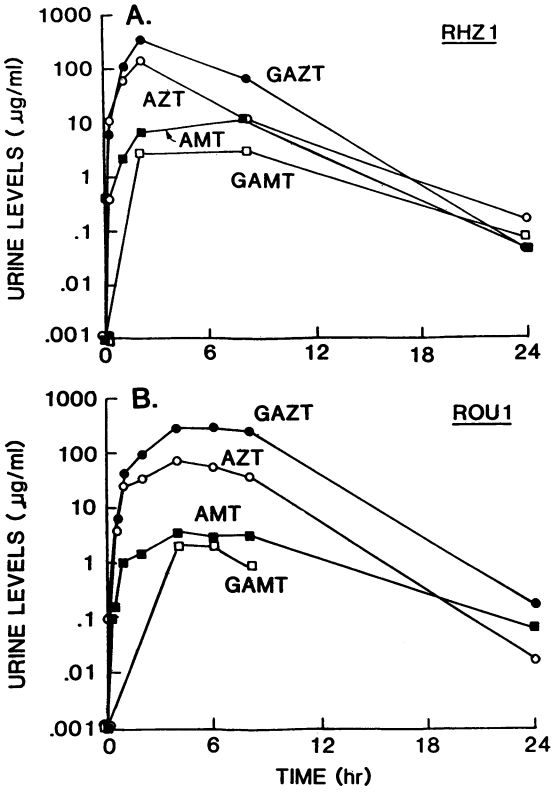

FIG. 2. Urinary excretion profiles of AZT (○), GAZT (●), AMT (■), and GAMT (□) in a representative monkey after subcutaneous administration of 33.3 mg of AZT per kg (A) or 33.3 mg of AZT per kg in the presence of 100 mg of probenecid per kg (B).

multiple time points. The important variation of AMT $t_{1/2}$ does not permit evaluation at the present time on whether the elimination of AMT is limited by its formation, as was observed with GAZT. The importance of the substantial AMT levels in plasma is further emphasized by our recent report that AMT may itself have toxic properties towards such highly proliferative host cells as human bone marrow cells and thus potentially contributes to the myelosuppressive effects of AZT (33, 37, 38). The mechanism of AMT toxicity remains to be elucidated; however, it may be explained in part by a low K_i value of 3.3 μM for DNA polymerase alpha (26). Although AMT has been reported to be inactive against human immunodeficiency virus (HIV) replication in various cell systems (6, 20), the in vivo formation of this catabolite raises the possibility of competitive interaction with AZT phosphorylation, leading to a decreased anti-HIV activity of AZT in patients.

GAMT was not detected in plasma, possibly because either GAMT levels in plasma were below the limit of sensitivity (0.2 μg/ml) or that the rate of elimination of this AZT catabolite is far greater than its rate of formation.

Urinary excretion represents the major pathway of elimination of unchanged AZT and metabolite(s), although major differences between humans and various animal species have been observed regarding the percentage of GAZT recovered in urine (3, 11, 19, 24), probably reflecting a different rate of glucuronidation at the hepatic site (9, 32). Previous studies have reported that GAZT was probably a major metabolite in monkeys, as it was demonstrated to be in humans, but incomplete recovery of the administered dose did not permit a detailed assessment of the urinary excretion of AZT and its metabolites (4, 11). In our study, 90% of the total administered dose was recovered in urine within 24 h, and the excretion pattern of individual metabolites during this interval was clarified. Of note, the urinary excretion ratio of GAZT/AZT, with a mean value of 1.36,

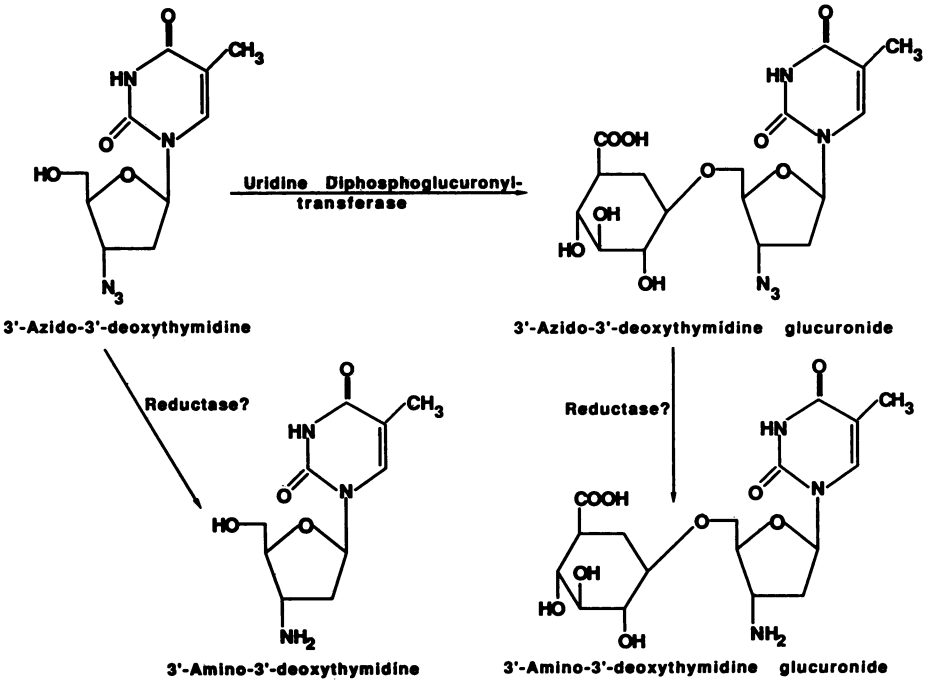

FIG. 3. Proposed metabolic pathways of AZT.

was in agreement with published reports in monkeys and humans (4, 11, 13), while AMT was eliminated at a slower rate than AZT, with a mean ratio of 0.83.

Penetration of anti-HIV drugs into the central nervous system is crucial to antiretroviral treatment of AIDS. In our study, after administration of AZT alone, the AZT CSF/plasma concentration ratio, determined 1 h post-drug administration, averaged 0.30 ± 0.17, similar to previously reported values in monkeys (4, 7) and humans (21). In contrast, the GAZT CSF/plasma concentration ratio was low, averaging 0.04 ± 0.02, indicating that GAZT penetration into the central nervous system is minimal, possibly because of the large carbohydrate moiety of this catabolite. Although AMT is a more polar thymidine derivative than AZT, the AMT CSF/plasma concentration ratio approximately equaled that of AZT, averaging 0.40 ± 0.04 (Table 1). When drug concentrations in CSF at a single time point after administration of a single dose are interpreted, it should be emphasized that the ratio of CSF to plasma concentration is subject to the effects of system hysteresis and thus may change with time. Notably, similar ratios obtained at the same time period have been reported with other thymidine analogs, including 2′,3′-dideoxythymidine (7), 3′-fluoro-3′-deoxythymidine, and 3′-deoxy-2′,3′-didehydrothymidine (35). Our data reinforce the hypothesis of Collins et al. (7) that penetration of thymidine dideoxynucleoside analogs into the CSF is independent of their lipophilicity and of the functional group substitution at the 3′ position on the sugar. Possible involvement of a carrier-mediated process has also been suggested by Collins et al. for the transport (influx and efflux) of pyrimidine dideoxynucleosides into the central nervous system (7). More recently, Hedaya and Sawchuck (19) reported that combined administration in rabbits of AZT and probenecid, an inhibitor of active transport of weak acids, resulted in a significant increase in the AZT CSF/plasma concentration ratio, possibly because of the inhibition of CSF-to-plasma transport of AZT by probenecid.

In our study, the presence of probenecid substantially increased the AZT CSF/plasma concentration ratio by approximately threefold. Of particular importance, both GAZT and AMT CSF/plasma concentration ratios were also markedly enhanced. These data suggest that existence of a specific pyrimidine dideoxynucleoside transport system from CSF to plasma is quite unlikely and that most probably these compounds utilize a more general active transport system which has been well described for various weak acids (15, 30). Consistent with this hypothesis, Chatton et al. (5) recently demonstrated that AZT is transported by an anion transport system.

The effects of probenecid on AZT disposition and metabolism in monkeys were examined to extend our knowledge of this drug-drug interaction, especially as related to the effects of probenecid on the formation and elimination of AMT in a situation where the glucuronidation metabolic pathway of AZT is being inhibited. The AZT-probenecid interaction has been studied in AIDS patients, with the demonstration of decreased AZT glucuronidation associated with a reduction of AZT and GAZT renal elimination by inhibitory effects of probenecid (13, 22, 31). These effects resulted primarily in an extended apparent $t_{1/2}$ of AZT and a twofold decrease of the CL. Similar studies were conducted in rabbits (19); however, no metabolism of AZT was observed, thus limiting the significance of these investigations that used an animal model with a different disposition of AZT than in humans. Alterations of the pharmacokinetic parameters of AZT and GAZT in monkeys by using a relevant combination ratio of 1:3 of AZT to probenecid in an attempt to mimic the observed interactions in humans are in agreement with the clinical studies (13, 22).

The AUC values of AZT and GAZT in monkeys were markedly increased in the presence of probenecid, with a twofold decrease of the AZT CL similar to that in humans. Prolongation of the mean $t_{1/2}$ of AZT and GAZT in monkeys was in the same range as that observed in humans, reflecting a similar inhibition of their CL_R. Although probenecid did not affect the GAZT/AZT urinary excretion ratio, the limited impact of the CL_R, accounting for less than 5 to 10% of the CL, still suggests that inhibition of AZT glucuronidation by probenecid is also a predominant factor in monkeys. Of interest, the AUC and apparent $t_{1/2}$ for AMT were also markedly increased in the presence of probenecid. This increase may be a consequence of inhibition of renal tubular secretion of AMT by probenecid, as was observed for AZT and GAZT. However, the elevation of AMT levels in plasma might also reflect an increased formation of this metabolite due to increased AZT levels in plasma in the presence of probenecid. The mean AMT/AZT urinary excretion ratio appears to decrease in the presence of probenecid, suggesting that probenecid might affect conversion of AZT to AMT. This is probably an artifact since in vitro studies with intact liver cells demonstrated that AMT formation was not reduced by probenecid but was rather enhanced as a consequence of higher AZT concentrations resulting from inhibition of the glucuronidation pathway (8a).

In summary, the results of the present study provide the first comprehensive analysis of formation and excretion of AZT metabolites in an appropriate animal model. Of particular note, the substantial levels of AMT detected in plasma suggest that AMT may affect the pharmacodynamic properties of AZT as related to its activity against HIV replication and cytotoxicity to host cells. Since the disposition and metabolism of AZT in monkeys have been reported by several groups to be similar to those seen in humans, AMT formation in AIDS patients undergoing AZT therapy is likely to occur and should be investigated. In addition, the coadministration of probenecid was demonstrated to substantially alter not only the kinetics of AZT and GAZT but of AMT as well, which may lower the therapeutic efficacy of AZT.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants HL-42125 and AI-25784 to J.-P.S., RR00165 to H.M.M., and AI-26055 to R.F.S., all from the National Institutes of Health. R.F.S. also received support from the Department of Veterans Affairs. J.-P.S. is a recipient of a Junior Faculty Research Award from the American Cancer Society.

We thank Ellen Lockwood and the staff at the Yerkes Regional Primate Research Center for assisting with the monkey experiments and Janna Stockinger for preparing and editing the manuscript.

REFERENCES

1. **Abernethy, D. R., D. J. Greenblatt, B. Ameer, and R. I. Shader.** 1985. Probenecid impairment of acetaminophen and lorazepam clearance: direct inhibition of ether glucuronide formation. J. Pharmacol. Exp. Ther. **234**:345–346.
2. **Balis, F. M., C. McCully, L. Gough, P. A. Pizzo, and D. G. Poplack.** 1989. Pharmacokinetics of subcutaneous azidothymidine in rhesus monkeys. Antimicrob. Agents Chemother. **33**: 810–812.
3. **Blum, M. R., S. H. T. Liao, S. S. Good, and P. De Miranda.** 1988. Pharmacokinetics and bioavailability of zidovudine in humans. Am. J. Med. **85**:189–194.

4. **Boudinot, F. D., R. F. Schinazi, T. M. Gallo, H. M. McClure, D. C. Anderson, K. J. Doshi, P. C. Kambhampthi, and C. K. Chu.** 1990. 3'-Azido-3'-deoxyuridine (AzddU): comparative pharmacokinetics with 3'-azido-3'-deoxythymidine (AZT) in monkeys. AIDS Res. Hum. Retroviruses **6**:219–228.
5. **Chatton, J.-Y., M. Odone, K. Besseghir, and F. Roch-Ramel.** 1990. Renal secretion of 3-azido-3'-deoxythymidine by the rat. J. Pharmacol. Exp. Ther. **255**:140–145.
6. **Chu, L. K., R. F. Schinazi, M. K. Ahn, G. V. Ullas, and Z. P. Gu.** 1989. Structure-activity relationships of pyrimidine nucleosides as antiviral agents for human immunodeficiency virus type 1 in peripheral blood mononuclear cells. J. Med. Chem. **39**:612–617.
7. **Collins, J. M., R. W. Klecker, J. A. Kelly, J. S. Roth, C. L. McCully, F. M. Balis, and D. G. Poplack.** 1988. Pyrimidine dideoxynucleosides: selectivity of penetration into cerebrospinal fluid. J. Pharmacol. Exp. Ther. **245**:466–470.
8. **Collins, J. M., and J. D. Unadkat.** 1989. Clinical pharmacokinetics of zidovudine: an overview of current data. Clin. Pharmacokinet. **17**:1–9.

8a. **Cretton, E., and J.-P. Sommadossi.** Unpublished results.

9. **Cretton, E. M., D. V. Waterhaus, R. J. Bevan, and J. P. Sommadossi.** 1990. Glucuronidation of 3'-azido-3'-deoxythymidine by rat and human liver microsomes. Drug Metab. Dispos. **18**:369–372.
10. **Cretton, E. M., M.-Y. Xie, R. J. Bevan, N. M. Goudgaon, R. F. Schinazi, and J. P. Sommadossi.** 1991. Catabolism of 3'-azido-3'-deoxythymidine in hepatocytes and liver microsomes with evidence of formation of 3'-amino-3'-deoxythymidine, a highly toxic catabolite for human bone marrow cells. Mol. Pharmacol. **39**:258–266.
11. **De Miranda, P., T. C. Burnette, and S. S. Good.** 1987. Disposition and pharmacokinetics of the antiviral drug 3'-azido-3'-deoxythymidine (Retrovir) in monkeys and rats. Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1471.
12. **De Miranda, P., T. C. Burnette, and S. S. Good.** 1990. Tissue distribution and metabolic disposition of zidovudine in rats. Drug Metab. Dispos. **18**:315–320.
13. **De Miranda, P., S. S. Good, R. Yarchoan, R. V. Thomas, M. R. Blum, E. M. Charles, and S. Broder.** 1989. Alteration of zidovudine pharmacokinetics by probenecid in patients with AIDS or AIDS-related complex. Clin. Pharmacol. Ther. **45**:473–475.
14. **Duckworth, A. S., G. W. Duckworth, G. Henderson, and G. Contreras.** 1990. Zidovudine with probenecid. Lancet **336**:441.
15. **Fishman, R. A.** 1966. Blood-brain and CSF barriers to penecillin and related organic acids. Arch. Neurol. **15**:113–124.
16. **Gomeni, R., and C. Gomeni.** 1978. Interactive graphic package for pharmacokinetic analysis. Comput. Biomed. Res. **11**:345.
17. **Good, S. S., D. T. Durach, and P. De Miranda.** 1986. Biotransformation in various species and in humans of 3'-azido-3'-deoxythymidine, a potential agent for treatment of AIDS. Fed. Proc. **45**:444.
18. **Good, S. S., C. S. Koble, R. Crouch, R. L. Johnson, J. L. Rideout, and P. De Miranda.** 1990. Isolation and characterization of an ether glucuronide of zidovudine, a major metabolite in monkeys and humans. Drug Metab. Dispos. **18**:321–326.
19. **Hedeya, M. A., and R. J. Sawchuck.** 1989. Effect of probenecid on the renal and nonrenal clearances of zidovudine and its distribution into cerebrospinal fluid in the rabbit. J. Pharm. Sci. **78**:716–722.
20. **Kedar, P. S., J. Abbotts, T. Kovacs, K. Lesiak, P. Torvence, and S. H. Wilson.** 1990. Mechanism of HIV reverse transcriptase: enzyme-primer interaction as revealed through studies of a dNTP analogue, 3'-azido-dTTP. Biochemistry **29**:3603–3611.
21. **Klecker, R. W., J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers.** 1987. Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido-3'-deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin. Pharmacol. Ther. **41**:407–412.
22. **Kornhauser, D. M., G. W. Hendrix, L. J. Nerhood, B. G. Petty, A. S. Woods, J. G. Bartlett, and P. S. Lietman.** 1989. Probenecid and zidovudine metabolism. Lancet **ii**:473–475.
23. **Langtry, H. D., and D. M. Campoli-Richards.** 1989. Zidovudine: a review of pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. Drugs **37**:408–450.
24. **Laskin, O. L., P. De Miranda, and M. R. Blum.** 1989. Azidothymidine steady-state pharmacokinetics in patients with AIDS and AIDS-related complex. J. Infect. Dis. **159**:745–747.
25. **Liebes, L., S. Mendoza, D. Wilson, and J. Dancis.** 1990. Transfer of zidovudine (AZT) by human placenta. J. Infect. Dis. **161**:203–207.
26. **Lin, T. S., P. H. Fischer, and W. H. Prusoff.** 1982. Effect of 3'-amino-3'-deoxythymidine on L1210 and P388 leukemia in mice. Biochem. Pharmacol. **31**:125–128.
27. **Lin, T. S., and W. H. Prusoff.** 1978. Synthesis and biological activity of several amino analogues of thymidine. J. Med. Chem. **21**:109–112.
28. **Marquardt, D. W.** 1963. An algorithm for least squares estimation of nonlinear parameters. J. Soc. Indust. Appl. Math. **11**:431–441.
29. **Morse, G. D., A. Portmore, J. Olson, C. Taylor, C. Plank, and R. C. Reichman.** 1990. Multiple-dose pharmacokinetics of oral zidovudine in hemophilia patients with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **34**:394–397.
30. **Neff, N. H., T. N. Tozer, and B. B. Brodie.** 1967. Application of steady state kinetics to studies of the transfer of 5-hydroxyinoleacetic acid from brain to plasma. J. Pharmacol. Exp. Ther. **158**:214–218.
31. **Petty, B. G., D. M. Kornhauser, and P. S. Lietman.** 1990. Zidovudine with probenecid: a warning. Lancet **335**:1044–1045.
32. **Restar, A., and T. Spector.** 1989. Glucuronidation of 3'-azido-3'-deoxythymidine: human and rat enzyme specificity. Biochem. Pharmacol. **38**:1389–1393.
33. **Richman, D. D., M. A. Fischl, M. H. Grieco, M. S. Gottlieb, D. A. Volberding, O. L. Laskin, J. M. Leedom, S. E. Groopman, D. Miduan, M. S. Hirsch, G. G. Jackson, D. T. Durack, S. Nusinoss-Lehrmann, and AZT Collaborative Working Group.** 1987. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind placebo-controlled trial. N. Engl. J. Med. **317**:192–197.
34. **Schenker, S., R. F. Johnson, T. S. King, R. S. Schenken, and G. I. Henderson.** 1990. Azidothymidine (Zidovudine) transport by the human placenta. Am. J. Med. Sci. **299**:16–20.
35. **Schinazi, R. F., F. D. Boudinot, K. J. Doshi, and H. M. McClure.** 1990. Pharmacokinetics of 3'-fluoro-3'-deoxythymidine and 3'-deoxy-2',3'-didehydrothymidine in rhesus monkeys. Antimicrob. Agents Chemother. **34**:1214–1219.
36. **Smith, P. C., P. N. Lanjendijk, J. A. Bosso, and L. Z. Benet.** 1988. Effect of probenecid on the formation and elimination of acyl glucuronides: studies with zomepirac. Clin. Pharmacol. Ther. **38**:121–127.
37. **Sommadossi, J.-P., and R. Carlisle.** 1987. Toxicity of 3'-azido-3'-deoxythymidine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine in normal human hematopoietic progenitor cells in vitro. Antimicrob. Agents Chemother. **31**:452–545.
38. **Surbone, A. R., R. Yarchoan, N. McAfee, M. R. Blum, M. Maha, J. Allain, R. V. Thomas, H. Mitsuya, S. N. Lehrman, H. Kessler, C. E. Myers, and S. Broder.** 1988. Treatments of the acquired immunodeficiency. Ann. Intern. Med. **108**:534–540.