Pharmacokinetic Evaluation of Drug Interactions with Anti-Human Immunotrophic Virus (HIV) Drugs. III. 2′,3′-Dideoxycytidine (ddC) and Zidovudine in Monkeys

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A pharmacokinetic evaluation of a potential drug interaction between zidovudine (AZT) and dideoxycytidine (ddC) was conducted in monkeys. Each of six animals received 20 mg/kg of AZT intragastrically in the absence and presence of an intravenous steady-state dosage regimen of ddC. The regimen was designed to produce steady-state ddC plasma concentration of 1.77 μg/ml for 30 min. Plasma and urine samples were analyzed for AZT, its major glucuronide metabolite, GAZT, and ddC by HPLC techniques. Pharmacokinetic parameters for AZT and GAZT were calculated by noncompartmental methods. The mean apparent clearance of AZT was 1.40 and 1.78 L/hr/kg in the absence and presence of ddC, respectively. The mean AUC for GAZT was 36.39 μg-hr/ml in the absence of ddC and 28.81 μg-hr/ml in the presence of ddC. No statistical differences were found in these and other pharmacokinetic parameters in the absence and presence of ddC. The absence of an effect on AZT's pharmacokinetics by ddC is attributed to the primary metabolic and renal elimination pathways for AZT and ddC, respectively. The results of this study provide a rational basis to design combined AZT–ddC treatment regimens in AIDS patients.

KEY WORDS: anti-human immunotrophic virus (HIV) nucleosides; drug interactions; pharmacokinetics; monkeys.

INTRODUCTION

A variety of 2′,3′-dideoxynucleosides, a family of DNA chain terminators, is being examined as potential anti-HIV agents. 3′-Azido-3′-deoxythymidine (zidovudine, AZT, ZDV) has been approved for clinical use against the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC), whereas 2′,3′-dideoxycytidine (ddC), the most potent on a molar basis *in vitro* (1), has reached Phase III clinical trials.

Although AZT and ddC have demonstrated clinical efficacy as single drugs, drug-related toxicities at higher doses (2,3) and the emergence of AZT-resistant HIV-1 variant (4) suggest the need for alternate chemotherapeutic approaches. Concurrent administration of AZT and ddC for AIDS and ARC has been suggested recently by Meng *et al.* (5), and this combination is now in Phase III clinical trials. Potential advantages of this combination are as follows. First, AZT and ddC can exert an additive or synergistic inhibitory effect on HIV replication (6). Second, the different toxicity profiles of AZT and ddC, significant myelosuppression for AZT (2), and painful peripheral neuropathy for ddC (3) may reduce dose-related toxicities if each agent is coadministered at a lower dose than doses used in single-drug regimens. Third, AZT-resistant HIV isolates were sensitive to ddC (4). Therefore, combination chemotherapy of AZT and ddC is a potential means to enhance efficacy, decrease drug toxicity, and reduce the development of HIV resistance to chemotherapy.

The pharmacokinetics of AZT and ddC have been characterized in numerous species as single-drug administrations (7–10). Among animals studied, monkeys serve as the most suitable model for human pharmacokinetics. In both man and monkey, AZT undergoes extensive metabolism to a glucuronide metabolite (GAZT,GZDV) (11), whereas ddC is primarily excreted unchanged. Since there is no pharmacokinetic data following combined AZT and ddC administrations, the current study was designed to evaluate ddC's effect on AZT pharmacokinetics in monkeys.

MATERIALS AND METHODS

Chemicals

Zidovudine (AZT) and 3′-azido-3′-deoxy-5′-*O*-β-D-glucopyranuronosylthymidine (GAZT) were provided by Burroughs Wellcome, Co. (Research Triangle Park, NC). 2′,3′-Dideoxycytidine (ddC) was provided by Dr. Karl Flora of the National Cancer Institute (Bethesda, MD). The internal standard, 3′-azido-2′,3′-dideoxyuridine (AZddU), was obtained from Dr. David Chu, College of Pharmacy, University of Georgia, and 2′,3′-dideoxyinosine (ddI) was provided by the Bristol-Myers Squibb Co. (Wallingford, CT).

HPLC-grade acetonitrile and methanol were purchased from J. T. Baker (Phillipsburg, NJ). All other chemicals were of reagent grade or better.

Experimental Design

Animals. Six adult male monkeys (*Macaca fascicularis*) weighing from 4.2 to 5.0 kg (4.45 ± 0.29 kg) were used for the pharmacokinetic studies. The animals were housed and studied in the Behavioral Research Laboratory, University of Georgia. All animals were inspected by a veterinarian and had clinical lab tests completed to ensure the animals were normal and able to participate in the study.

First AZT Administration. Each animal was fasted for 12 hr prior to receiving AZT and for 4 hr after dosing. Monkeys were administered 10 mg/kg of ketamine intramuscularly (im) followed by 20 mg/kg of AZT, prepared in sterile water, intragastrically (ig). Immediately following dosing, the animals were placed in individual metabolism cages to facilitate the collection of urine. Blood samples were collected by venipuncture at 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, and 10 hr after AZT administration, placed in heparinized microcentrifuge tubes, and then centrifuged to yield plasma. Other

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than the initial dose of ketamine that facilitated oral dosing, no other agents were used to impair the animals. At blood sampling times, animals were transferred from the metabolism cages to a blood collection device. Animals were trained to undergo this procedure and required only mild to moderate restraint. Animals did urinate during the blood collection procedure, and thus, complete urine collections could not be obtained for all animals. The total volume of urine collected at the end of 10 hr was measured. Plasma and urine samples were stored at −20°C until analysis. At the end of the 10-hr sampling period, the animals were placed in regular cages for a minimum of 38 hr prior to administration of ddC and the second AZT dose.

Second AZT Administration with ddC. After a 38-hr washout period, each animal was administered 10 mg/kg of ketamine im followed by 20 mg/kg of AZT ig as described before for the first dose of AZT. Approximately 10 min after the AZT dose, 1.72 mg/kg of ddC, prepared in sterile normal saline, was given intravenously (iv) as a loading dose, followed by a constant-rate iv infusion of 10.9 μg/min/kg of ddC over 30 min via an infusion pump (Model 22, Harvard Apparatus, Inc., MA). The 10-min lag period between AZT and ddC administrations facilitated animal dosing techniques and allowed some AZT to be absorbed prior to ddC administration. This would enable AZT to be present at higher plasma concentrations during the steady-state ddC regimen and, thus, enhance the likelihood of detecting an interaction. All blood and urine collections and processing and storage of samples were conducted as described for the first AZT dose.

Analytical Methodology

AZT and GAZT concentrations in plasma and urine were determined by a high-performance liquid chromatography (HPLC) method as described previously (12). Briefly, 200-μl plasma samples containing the internal standard (AZddU) were processed by a solid-phase extraction procedure and then analyzed by a reversed phase HPLC method. The mobile phase consisted of 8% (v:v) acetonitrile:water (pH 2.5), and the UV detector was set at 267 nm. This method provided a lower limit of quantitation in plasma of 100 ng/ml and analyte recoveries of greater than 70% for both AZT and GAZT. Aliquots of urine were filtered, diluted, and then injected directly onto the HPLC system. The HPLC methods showed satisfactory precision and accuracy with interday coefficients of variation and percentage biases of less than 14%.

ddC Analysis in Plasma. To 100 μl of plasma, 10 μl of internal standard (ddI, 100 μg/ml) and 90 μl of deionized-distilled water were added and vortexed for 30 sec. The 200-μl sample was loaded onto an Amicon Centrifree Micropartition System (Amicon Corp., Danver, MA) and centrifuged at 1165*g* for 25 min. A 100-μl aliquot of the ultrafiltrate was injected onto the HPLC system. For ddC HPLC procedures, intraday percentage coefficients of variation were 7.6% or less, and percentage biases were 13.7% or less at low (50 ng/ml), medium (1 μg/ml), and high (20 μg/ml) concentrations. The lower limit of quantitation in monkey plasma was 50 ng/ml. Mean extraction recoveries of ddC and ddI were 93.8 and 101.3%, respectively.

Separation of ddC and ddI was attained on a 150 × 46-mm ODS analytical column (Hypersil, Alltech Assoc., Deerfield, IL) preceded by a guard column filled with 30- to 40-μm RP-18 perisorb pellicular material (Upchurch Scientific, Inc., WA). The mobile phase, pumped at 1.5 ml/min, consisted of 3% (v:v) acetonitrile:20 m*M* Na_2HPO_4 in water, pH 7.0. ddC and ddI eluted at 4.98 and 11.96 min, respectively, and were detected at 273 nm.

Sample peak height ratios of AZT, GAZT, or ddC and internal standards were used to calculate concentrations from regression equation obtained from standards prepared in blank monkey plasma or urine for each set of analyses. Standard curves prepared for AZT and GAZT in monkey plasma were linear over a concentration range from 0.1 to 50 μg/ml with $r^2 \geq 0.998$. Standard curves of ddC in monkey plasma were linear over a concentration range from 0.1 to 20 μg/ml with $r^2 \geq 0.999$.

Data Analysis

Noncompartmental analysis was used to calculate pharmacokinetic parameters for AZT and GAZT. For each animal and AZT administration, the area under the plasma concentration–time curve (AUC) was determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time with extrapolation to time infinity using the least-squares terminal slopes (13).

Since absolute bioavailability (*F*) cannot be determined from only oral data, total systemic clearance is expressed as the apparent total clearance (CL_t/F). CL_t/F was calculated as dose/AUC, and the elimination half-life ($t_{1/2}$) was equal to $0.693/K$. *K* is the terminal disposition rate constant. Complete urine collections were not attained for any animal in both the absence and the presence of ddC. Fractional urine collections were attempted but too few were obtained to allow estimation of total amounts of drug in the urine. Therefore, reliable estimates of AZT and GAZT's renal clearance or the percentage of the dose excreted unchanged in the urine in the absence and presence of ddC could not be made. Determination of the urinary GAZT/AZT concentration ratio was made since this parameter is not influenced by complete urine collections.

The AUC and the half-life associated with the terminal

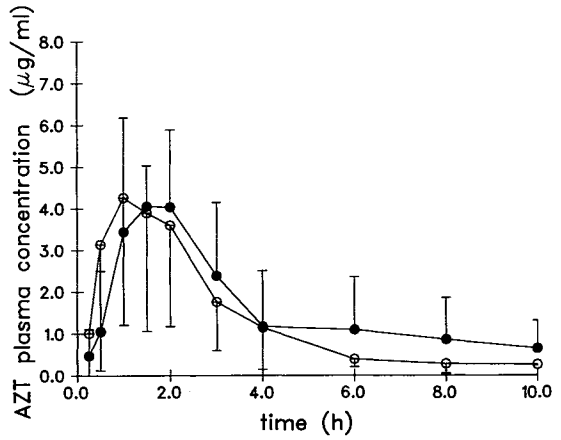

Fig. 1. AZT plasma concentrations following 20 mg/kg of AZT ig in the absence (●) and presence (○) of ddC in monkeys. Each data point represents the mean and standard deviation.

Table I. Pharmacokinetic Parameters of AZT in Monkeys Following ig Administration of 20 mg/kg of AZT in the Absence and Presence of ddC

Drug	Animal	C_{max} (μg/ml)	t_{max} (h)	AUC (μg-hr/ml)	$t_{1/2}$ (hr)	CL_t/F (L/hr/kg)	GAZT/AZT urinary ratio
AZT	A	6.45	2.0	20.96	1.80	0.95	3.01
	P	6.08	1.02	12.56	1.19	1.59	5.91
	T	4.79	1.02	9.63	1.25	2.08	2.00
	H_1	6.60	0.95	26.34	0.77	0.76	4.63
	D	3.89	2.05	9.45	1.32	2.12	8.35
	H_2	5.22	3.03	22.37	1.63	0.89	2.04
	Mean	5.50	1.68	16.89	1.33	1.40	4.32
	SD	1.06	0.83	7.25	0.36	0.61	2.50
AZT/ddC	A	5.62	1.50	17.31	2.0	1.16	2.50
	P	4.67	0.67	10.94	1.58	1.83	2.69
	T	3.37	0.68	9.92	1.97	2.02	0.05
	H_1	8.21	0.48	15.92	1.86	1.26	3.68
	D	3.57	1.98	5.68	1.44	3.52	6.14
	H_2	8.40	1.52	22.67	1.24	0.88	1.66
	Mean	5.64	1.14	13.74	1.68	1.78	1.79
	SD	2.22	0.61	6.08	0.31	0.96	1.46

phase of GAZT's concentration–time curve were calculated for GAZT. Observed t_{max}, time of the maximum concentration, and C_{max}, the maximum concentration, were recorded for AZT and GAZT in both the absence and the presence of ddC.

A paired t test was used to determine significant differences in the measured parameter in the absence and presence of ddC. A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

AZT and ddC primary elimination routes are different. AZT is rapidly metabolized by glucuronidation and 60% of an intravenous dose is excreted as GAZT (8). ddC is excreted predominantly in the urine as unchanged parent compound (74%), and only a minor urinary metabolite (2',3'-ddurd) is detected in monkey urine (9). Unlike probenecid, an inhibitor of glucuronidation, which does interfere with the renal excretion and metabolic elimination of AZT (14–16), ddC was not expected to have a large effect on the disposition of AZT.

Figure 1 shows AZT mean plasma concentration–time profiles after oral administration of 20 mg/kg AZT in the absence and presence of ddC. Pharmacokinetic parameters for AZT administered alone and with ddC are presented in Table I. There were no statistical differences in any of the parameters listed in Table I between the two treatments. It was noted that there were erratic concentration–time profiles with double peaks in two animals, A and H_1. This phenomenon was not related to ddC because it appeared both in the absence and in the presence of ddC. It may represent individual differences in absorption (i.e., delayed) and/or elimination (i.e., enterohepatic cycling). Miranda *et al.* reported that 7% of the AZT dose was excreted in the bile of rats within 3 hr, and it consisted predominantly of GAZT (10). Moreover, GAZT could be converted to AZT by β-D-glucuronidase from *Escherichia coli* (11), one of the bacterial flora in the intestinal tract. Thus, it is possible that biliary secretion followed by gut conversion of GAZT to AZT and subsequent absorption occurred in animals A and H_1.

GAZT plasma concentration–time profiles after ig AZT alone and concurrently with ddC were similar (Fig. 2). Mean pharmacokinetic parameters for GAZT (Table II) were also not significantly different in the absence and presence of ddC.

Interpretation of the effect of ddC, or as yet unidentified metabolites, on renal excretion of AZT and GAZT is difficult since complete urine collections were not obtained. The GAZT/AZT urinary ratio, a parameter unaffected by incomplete urine collection, was reduced from 4.32 ± 2.50 to 1.79 ± 1.46 in the presence of ddC, but these values were not statistically different. The reduced GAZT/AZT ratio would suggest that ddC may interfere with GAZT's renal elimination by active tubular secretion.

The ddC administration regimen, based on the previous study by Kelley *et al.* (9), was chosen to produce steady-state plasma concentrations of approximately 1 μg/ml. The

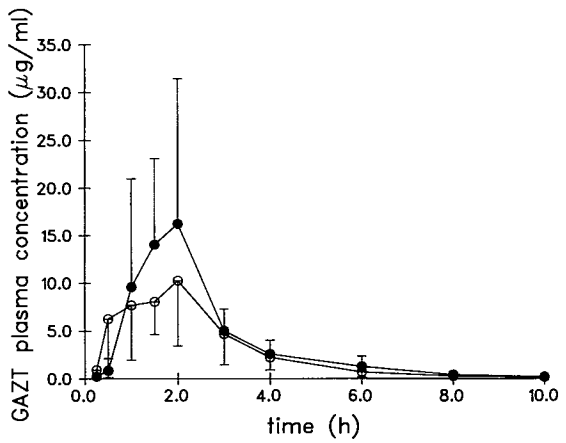

Fig. 2. GAZT plasma concentrations following 20 mg/kg of AZT ig in the absence (●) and presence (○) of ddC in monkeys. Each data point represents the mean and standard deviation.

Table II. Pharmacokinetic Parameters of GAZT in Monkeys Following ig Administration of 20 mg/kg of AZT in the Absence and Presence of ddC

Drug	Animal	C_{max} (μg/ml)	t_{max} (hr)	AUC (μg-hr/ml)	$t_{1/2}$ (hr)
AZT	A	10.04	2.0	25.13	1.56
	P	34.46	2.07	67.73	0.74
	T	12.72	1.53	20.73	1.09
	H_1	14.02	0.95	25.53	0.81
	D	36.79	2.05	56.42	1.25
	H_2	6.40	3.02	24.77	1.28
	Mean	19.07	1.94	36.39	1.12
	SD	13.11	0.69	20.28	0.31
AZT/ddC	A	7.19	2.00	17.99	1.31
	P	14.91	2.05	43.59	1.19
	T	8.77	1.00	22.06	1.82
	H_1	15.44	0.48	30.15	1.34
	D	20.84	1.98	27.50	0.85
	H_2	11.05	2.02	31.55	1.21
	Mean	13.03	1.59	28.81	1.29
	SD	5.03	0.68	8.81	0.32

mean ddC plasma concentration was 1.77 μg/ml at the end of ddC infusion. At 3 hr following ddC administration, the mean ddC plasma concentration declined to 0.25 μg/ml, and at 8 to 10 hr it was near the lower limit of quantitation. Thus, it could be assumed that during most of the 10-hr study period, ddC plasma concentrations ranged from 0.05 to 1.77 μg/ml and, therefore, exceeded *in vitro* concentrations necessary for complete inhibition of HIV (1). Additional ddC plasma concentration measurements were not possible due to limited blood sample volumes.

In conclusion, the results indicate that ddC does not alter the pharmacokinetics of AZT and GAZT. This result is attributed to the primary metabolic and renal elimination pathways for AZT and ddC, respectively. Implications from this study suggest that combination chemotherapeutic regimens of AZT and ddC in AIDS patients may be designed without concern for an alteration in AZT's pharmacokinetics by ddC.

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