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Pharmacokinetics of 2′,3′-Dideoxyinosine in Monkeys

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Comparison of the pharmacokinetic parameters obtained for dideoxyinosine (ddI) in monkeys with those obtained for humans indicates that the monkey is an appropriate animal model for ddI pharmacokinetics in humans. Following intravenous administration of 20 mg of ddI per kg of body weight and measurement of ddI in plasma and urine by high-performance liquid chromatography, pharmacokinetic parameters were determined by noncompartmental analysis. Total systemic clearance was 0.74 liters/h/kg, volume of distribution was 0.92 liters/kg, and the elimination half-life of ddI was 1.22 h. The pharmacokinetics of ddI in five monkeys were determined following intravenous administration of 20 mg/kg and were found to be comparable to those obtained in patients with AIDS.

2′,3′-Dideoxyinosine (ddI) is a purine nucleoside analog that suppresses the infectivity and the cytopathic effect of human immunodeficiency virus in vitro (8). Intracellular 5′-triphosphate metabolites of ddI mediate anti-human immunodeficiency virus activity by inhibition of viral reverse transcriptase and by their incorporation into DNA chains, resulting in chain termination (3, 8). The favorable in vitro activity-toxicity profile of ddI, as well as its long intracellular half-life of more than 12 h (1), has prompted a number of clinical trials of ddI as a potential anti-AIDS drug.

Pharmacokinetic studies of ddI have been completed in mice (11), in rats (2, 9), and in patients with AIDS (4, 5, 7, 12). Total systemic clearance and the elimination half-life in rodents were different from those in humans (elimination half-life ranged from 5 to 28 min in rodents and ranged from 38 min to 1.6 h in humans). The fractional urinary elimination and renal clearance of ddI have been reported in humans (7) but not in animals. Thus, at present there is not an abundance of pharmacokinetic data for ddI in animals, and of the species studied none appear to be suitable animal models for ddI pharmacokinetics in humans. Characterization of the pharmacokinetics of ddI in animals and hopefully, identification of an appropriate animal model will be useful in designing future studies, such as pharmacokinetic evaluations of drug interactions between anti-human immunodeficiency virus nucleosides. The current investigation was undertaken to determine the pharmacokinetics of ddI and to determine in what capacity monkeys would serve as an animal model for ddI pharmacokinetics.

ddI was kindly provided by the Bristol-Myers Squibb Co. (Wallingford, Conn.). Internal standard (3′-azido-2′,3′-dideoxyuridine [AZddU]) was provided by David Chu, University of Georgia.

Five adult male monkeys (*Macaca fascicularis*), each weighing from 4.09 to 5.34 kg, were used for the pharmacokinetic study, after receiving approval from the University of Georgia Animal Care and Use Committee. Each animal was administered 20 mg of ddI prepared in sterile normal saline per kg of body weight intravenously (i.v.) into a leg vein over 1 min. For ddI administration and at blood collection times, animals were transferred to a device that provided access to the leg vein without anesthesia. Immediately following dosing, each animal was placed in a metabolism cage to facilitate the collection of urine. Blood samples were collected by venipuncture at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min and in two animals at 480 min after ddI administration, placed in heparinized microcentrifuge tubes, and then centrifuged to yield plasma. The total volume of urine collected at the end of 360 or 480 min was measured. Plasma and urine samples were stored at −20°C until analyzed by high-pressure liquid chromatography (HPLC).

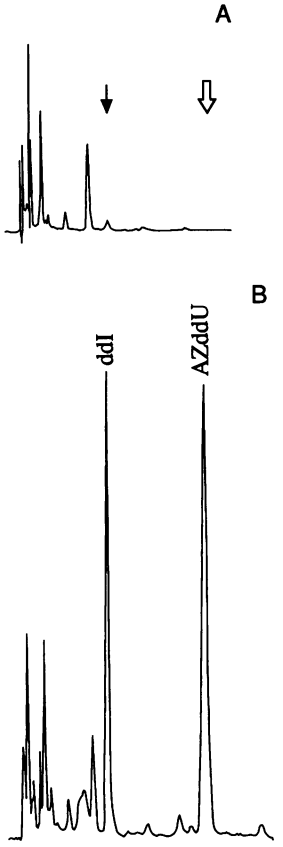

FIG. 1. Chromatograms obtained from HPLC analysis of ddI in a monkey plasma blank (A) and in a sample obtained from animal D1 3 h after i.v. administration of 20 mg of ddI per kg (B). Black and white arrows indicate retention times of ddI and AZddU, respectively.

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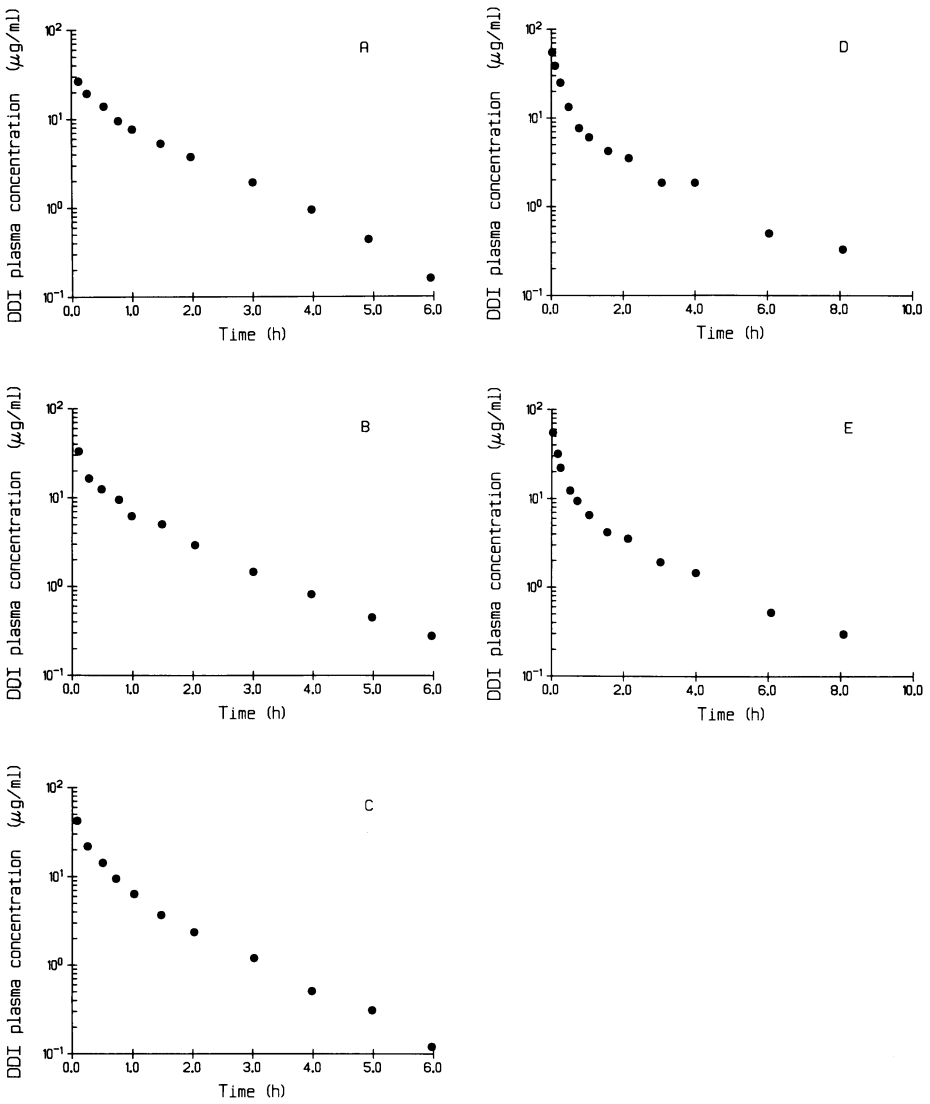

FIG. 2. Profile of ddI concentrations in plasma over time obtained after i.v. administration of 20 mg of ddI per kg to five monkeys: D1 (A), D2 (B), T1 (C), 01 (D), and A1 (E).

ddI was analyzed in plasma by solid-phase extraction and HPLC. To 100 μl of plasma, 10 μl of internal standard (AZddU, 30 μg/ml) solution was added and loaded onto an activated 1-ml Bond Elut C-18 cartridge (Analytichem International, Harbor City, Calif.). Each loaded cartridge was rinsed twice with 1 ml of deionized distilled water and then twice with 1 ml of methanol. Methanol eluents were evaporated to dryness under a stream of nitrogen at 50°C and reconstituted with 0.2 ml of the mobile phase. Aliquots were injected onto the HPLC system.

Urine was filtered through 0.22-μm-pore-size filters (Millex-GS; Millipore Products Division, Bedford, Mass.).

TABLE 1. Pharmacokinetic parameters of ddI obtained after i.v. administration (20 mg/kg) to monkeys[a]

Animal no.	AUC (μg · h/ml)	$t_{1/2}$ (h)	CL_T (liters/h/kg)	V_{ss} (liters/kg)	CL_R (liters/h/kg)	CL_{NR} (liters/h/kg)	% Dose in urine
D1	26.05	0.91	0.77	0.91	0.10	0.67	13.0
D2	24.65	1.08	0.81	0.96	0.03	0.79	3.7
T1	25.14	0.93	0.80	0.71	—[b]	—	—
O1	29.81	1.58	0.67	1.02	0.20	0.47	29.5
A1	31.18	1.59	0.64	0.98	0.20	0.44	31.1
Mean ± SD	27.37 ± 2.94	1.22 ± 0.34	0.74 ± 0.08	0.92 ± 0.12	0.13 ± 0.08	0.59 ± 0.17	19.33 ± 13.25

[a] $t_{1/2}$, elimination half-life; V_{ss}, volume of distribution at steady state; CL_R, renal clearance; CL_{NR}, nonrenal clearance.
[b] —, urinated during sample collection; renal elimination parameters not calculated.

Twenty microliters of filtered urine was added with 10 μl of AZddU solution (1 mg/ml) to a microcentrifuge tube and then diluted with 970 μl of deionized water to yield a 1:50 dilution. Twenty microliters of the final mixture was injected directly onto the HPLC system.

Separation of ddI and AZddU was attained on an octyldecyl silane analytical column (internal diameter, 150 by 4.6 mm) (Hypersil; Alltech Associates, Deerfield, Ill.) preceded by a guard column filled with 30- to 40-μm pellicular RP-18 Perisorb material (Upchurch Scientific, Inc., Oak Harbor, Wash.). The mobile phase, pumped at 2 ml/min, consisted of 4% (vol/vol) acetonitrile–30 mM Na_2HPO_4 in water, pH 7.0. All compounds were detected at 254 nm. Ratios of sample peak height of ddI to that of AZddU were used to calculate concentrations from regression equations obtained from standards prepared in blank monkey plasma or urine.

Area-moment analysis was used to calculate pharmacokinetic parameters of ddI. For each animal, area under the plasma concentration-time curve (AUC) and the first nonnormalized moment were determined by Lagrange polynomial interpolation and by integration from time zero to the last measured sample time with extrapolation to time infinity by using the least-squares terminal slope (10). The following pharmacokinetic parameters were estimated for ddI: CL_T = dose/AUC; V_{ss} = dose × AUMC/AUC^2; $t_{1/2} = 0.693/K$; $CL_R = (X_u)_0^{tn}/(AUC)_0^{tn}$; $CL_{NR} = CL_T - CL_R$ where CL_T is the systemic clearance, V_{ss} is the volume of distribution at steady state, AUMC is the area under the first moment of the concentration-time curve, $t_{1/2}$ is the elimination of half-life, K is the terminal disposition rate constant, CL_R is the renal clearance, tn is the terminal sample collection time and is equal to either 6 or 8 h, X_u is the amount of drug excreted in the urine from time zero to tn, and CL_{NR} is the nonrenal clearance.

Intraday and interday percent coefficients of variation and biases for the HPLC procedures were 10% or less. A representative chromatogram of ddI and AZddU in monkey plasma is shown in Fig. 1. Retention times of ddI and AZddU were approximately 5 and 10 min, respectively. Standard curves prepared for ddI in monkey plasma were linear over a concentration range from 100 ng/ml to 40 μg/ml, and the lower limit of quantitation was 100 ng/ml. Extraction recoveries of ddI and AZddU were 95.0 and 80.6%, respectively.

Plasma ddI concentration-time profiles after i.v. administration of 20 mg of ddI per kg to five monkeys are shown in Fig. 2. ddI concentrations in plasma declined rapidly, with a mean half-life (± SD) of 1.22 ± 0.34 h. Pharmacokinetic parameters for ddI are presented in Table 1. Total clearance averaged 0.74 ± 0.08 liters/h/kg, and the steady-state volume of distribution was 0.92 ± 0.12 liters/kg.

The percentages of the ddI dose excreted unchanged in urine varied from 3.7 to 31.1%, with renal clearances of ddI ranging from 0.03 to 0.20 liters/h/kg. Since the elimination half-life of ddI is approximately 1 h, the 6- and 8-h urine collection periods would allow greater than 98% of the maximum amount (i.e., X_u^∞) of ddI to be excreted. Therefore, renal clearance is essentially the same as that based upon a X_u^∞ value. The variability in the renal excretion parameters is attributed to one animal (D2), although reasons for this observation are not apparent. An average of 19.3% of the ddI dose was recovered as unchanged ddI in urine of four monkeys, suggesting that ddI is substantially metabolized.

The total clearance, volume of distribution at steady state, and elimination half-life values obtained were comparable to values reported for patients with AIDS. As determined by comparison with the data of Hartman et al. (7) which was collected for a ddI dose range of 0.2 to 6.4 mg/kg in humans, CL_T in monkeys is about 25% lower and volume of distribution at steady state in monkeys is essentially equal. At the high ddI doses (i.e., 3.2 and 6.4 mg/kg) in humans, total clearance was about 0.77 liters/h/kg, which agrees with the current data obtained at 20 mg/kg. Renal clearance is lower in monkeys than in humans (0.13 liters/h/kg in monkeys and 0.31 liters/h/kg in humans). The percentage of ddI excreted unchanged in the urine of humans is 36% (7), i.e., about 16% more than in monkeys. Pharmacokinetic analysis of the phase I ddI data (4, 5, 12) indicates a CL_T of 0.686 liters/h/kg, a volume of distribution at steady state of 0.73 liters/kg, and an elimination half-life of approximately 1.4 h (6). These values agree closely with those in this study. In conclusion, the monkey appears to be an acceptable model for ddI pharmacokinetics in humans, and investigations of drug interactions of ddI in monkeys would seem to be indicative of those in humans.

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