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Pharmacokinetics of the Anti-Human Immunodeficiency Virus Nucleoside Analog Stavudine in Cynomolgus Monkeys

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Stavudine was administered (15 mg/kg of body weight) intravenously and orally to two monkeys in a randomized crossover study. Plasma and urine samples were analyzed for stavudine by high-performance liquid chromatography, and pharmacokinetic parameters were derived by a noncompartmental method. Total body clearance of stavudine was 0.64 liters/h/kg, with a steady-state volume of distribution of 0.68 liters/kg, a terminal half-life of 0.83 h, a urinary recovery of 44%, and an oral bioavailability of 80%. These values were reasonably similar to those reported for patients with AIDS or AIDS-related complex.

Zidovudine was the first nucleoside analog approved for the treatment of AIDS (28). The pharmacokinetics of zidovudine in humans (4, 8, 26, 27, 29) and various animal species (5, 11, 12, 25, 31) have been studied extensively. Studies with humans have shown that zidovudine is eliminated rapidly, with a terminal half-life ($t_{1/2}$) of approximately 1 h, and about 60 to 80% of a dose is cleared from the body primarily by metabolism to the 5′-*O*-glucuronide. Of the animals investigated, the monkey has been identified as an appropriate animal model for zidovudine pharmacokinetics in humans (3, 5, 10, 11, 18) compared with other animals (11, 18, 19, 37). Likewise, the monkey appears to be an acceptable model for didanosine pharmacokinetics in humans (33).

Of several dideoxynucleoside analogs with in vitro anti-human immunodeficiency virus activity, stavudine has emerged as a potent agent which is being evaluated currently in clinical trials for the treatment of human immunodeficiency virus infection (2, 14, 15, 32). The pharmacokinetics of stavudine in humans (13, 22, 24) and various animal species (6, 7, 35, 36, 38) have been reported previously. Studies with rodents have shown that the $t_{1/2}$ of stavudine ranged from 0.3 to 0.6 h, the urinary recovery was about 70 to 80%, and the oral bioavailability was in the range of 80 to 95%. Although the $t_{1/2}$ of stavudine in rhesus monkeys (0.9 to 1.4 h) is comparable to that in humans (13, 22, 24), interestingly, its absolute bioavailability is only 42% (versus 80 to 90% in humans) and the fractional urinary elimination of unchanged drug has not been reported. Therefore, the pharmacokinetics, oral bioavailability, and urinary excretion of stavudine in cynomolgus monkeys were evaluated to determine whether it is an appropriate model for stavudine pharmacokinetics in humans.

Two young adult male cynomolgus monkeys weighing 2.1 kg were used in this study, after approval from the Animal Care and Use Committee of International Research and Development Corp., Mattawan, Mich., was received. Each monkey received a single intravenous (i.v.) bolus dose (via the saphenous vein) or an oral dose (by a nasogastric tube) of 15 mg of stavudine per kg as a solution in sterile water in a randomized crossover study. There was a 1-week washout period between the doses. The oral dose was followed by ca. 10 ml of water to wash the test compound through the tube. The animals were made to fast overnight before dosing and during the period up to 3 h after dosing. The animals were given 20 ml of water (via a nasogastric tube) every hour for the next 6 h. During the experimental period (24 h), animals were placed in metabolism cages and primate restraining devices were used to facilitate blood sample collection.

Blood samples (1 ml) were collected from the femoral vein by venipuncture at 0 and 0.1 (i.v. only) h; 0.25, 0.5, and 0.75 (oral only) h; and 1, 2, 3, 4, 5, and 6 h after stavudine

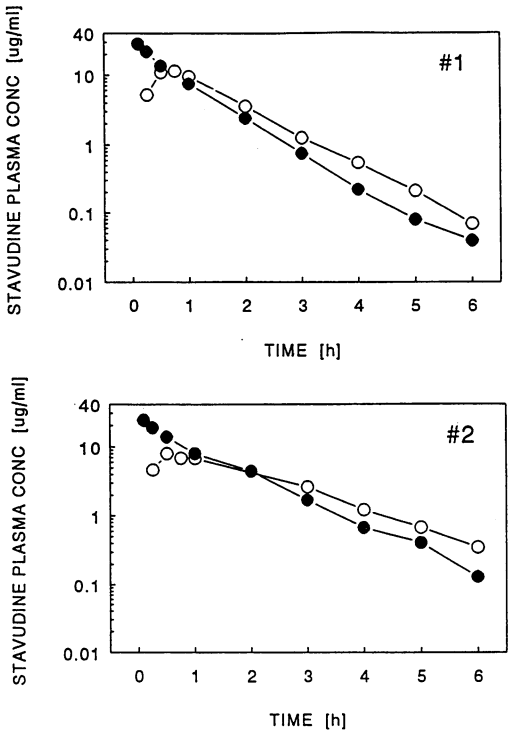

FIG. 1. Stavudine concentration in plasma-time profiles following i.v. (●) and oral (○) administration of a 15-mg/kg dose to monkeys 1 and 2.

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TABLE 1. Pharmacokinetic parameters of stavudine after i.v. administration of a 15-mg/kg dose to monkeys

Animal no.	AUC (μg · h/ml)	$t_{1/2}$ (h)	CL_T (liters/h/kg)	V_{ss} (liters/kg)	CL_R (liters/h/kg)	U_R (%)
1	22.29	0.83	0.67	0.61	0.27	39.6
2	24.71	0.83	0.61	0.75	0.29	48.1
Mean	23.50	0.83	0.64	0.68	0.28	43.9

administration. The samples were placed in tubes containing EDTA and then centrifuged to yield plasma. Urine samples were collected in 0- to 2-, 2- to 4-, 4- to 6-, 6- to 12-, and 12- to 24-h intervals. Urine was collected in polypropylene bottles on dry ice. At the end of each collection interval, the bladder was emptied by expression and the urine collection device was rinsed with 15 ml of water. Plasma and urine samples were stored at −20°C until analyzed by high-performance liquid chromatography (HPLC).

Stavudine concentrations in plasma and urine were determined by validated HPLC methods (21, 23). Plasma and urine samples were analyzed in one analytical session each. The within-day precision values for the quality control samples were less than 10% (coefficient of variation). The accuracy, calculated as (observed concentration/nominal concentration) × 100, of quality control samples was within 9%. Data acquisition and processing were done by a previously described method (16).

The pharmacokinetic parameters of stavudine were calculated by a noncompartmental method (17, 34). By using a weighting factor of 1, the terminal slope, K, was determined by log-linear regression of at least the terminal three datum points, which yielded a minimum mean square error. The following pharmacokinetic parameters were derived by previously reported equations (17, 34): terminal $t_{1/2}$, area under the concentration in plasma-time curve from time zero to infinity (AUC), total body clearance (CL_T), renal clearance (CL_R), steady-state volume of distribution (V_{ss}). The peak concentration in plasma, the time to peak concentration in plasma, and the amount of drug excreted in the urine as a percentage of the dose (U_R) were obtained directly from experimental observations. The oral bioavailability of stavudine was calculated as $F_{AUC} = AUC_{po}/AUC_{IV}$ and $F_{UR} = U_{R.po}/U_{R.iv}$, where po and iv represent the oral and i.v. routes of administration.

Stavudine concentrations in plasma-time profiles after i.v. and oral administration of a 15-mg/kg dose to two monkeys are depicted in Fig. 1. The pharmacokinetic parameters are listed in Tables 1 and 2. Following i.v. bolus administration, concentrations in plasma declined rapidly, with a mean terminal $t_{1/2}$ of 0.83 h. CL_T averaged 0.64 liters/h/kg, and V_{ss} was 0.68 liters/kg. The observed value for V_{ss} indicates that stavudine distributes in total body water (1). About 44% of CL_T was due to CL_R. The mean CL_R of stavudine is greater than creatinine clearance, 0.19 liters/h/kg (1), indicating that, in addition to glomerular filtration, stavudine also undergoes active renal tubular secretion. The urinary recovery of unchanged stavudine of only 44% after the i.v. dose suggests that the drug undergoes biliary excretion, is substantially metabolized, is susceptible to uptake by pyrimidine salvage pathways, or is converted to mono-, di-, and triphosphates (9, 20). For studies with [2-^{14}C]stavudine, Russell et al. (35) reported a urinary recovery of 51% and a lack of glucuronide or other metabolites. In contrast, Schinazi et al. (36) provided indirect evidence for the formation and excretion of a glucuronide metabolite in some urine samples.

Stavudine was rapidly absorbed following oral administration (mean time to peak concentration in plasma, 0.63 h) and declined with a mean $t_{1/2}$ of 0.92 h. The mean U_R and CL_R were in good agreement with the mean data after the i.v. dose. The mean absolute bioavailabilities based on AUC data, F_{AUC}, and urinary recovery data, F_{UR}, were 80 and 86%, respectively. The absolute bioavailability of stavudine differs from the previously reported value of 42% (36), possibly because of the different strains of monkeys used in the respective studies.

Stavudine pharmacokinetic data from clinical trials (13, 22, 24) indicate a CL_T of 0.49 liters/h/kg, a V_{ss} of 0.53 liters/kg, a $t_{1/2}$ ranging from 1.1 to 1.6 h, a urinary recovery of unchanged drug ranging from 34 to 43%, and an oral bioavailability of 80 to 90%. These values are in general agreement with those in the present study and support the thesis that the cynomolgus monkey appears to be an acceptable model for stavudine pharmacokinetics in humans.

The fact that in this initial study urinary recovery of unchanged stavudine accounted for only 44% of the administered dose caused us to investigate the metabolic fate of stavudine in cynomolgus monkeys. After an i.v. dose of 25 mg of [4-^{14}C]stavudine per kg to three monkeys, approximately 48 and 0.1% of the radioactivity administered were recovered in urine and feces, respectively, in 30 days. The urinary recovery of stavudine was identical to that reported in the initial study; in addition to unchanged drug, three metabolites were detected: thymine (1%), β-aminoisobutyric acid (2%), and an unidentified metabolite (1%). The sum of the three metabolites and unchanged drug accounted for virtually all of the radioactivity in the urine (36a). Although the fate of the remainder of stavudine in monkeys is unclear, it is speculated that, similar to thymidine (30), the unrecovered portion of the stavudine dose is salvaged and incorporated into endogenous macromolecules. Further work on metabolism and excretion is essential to elucidate fully the metabolic fate of stavudine in monkeys.

TABLE 2. Pharmacokinetic parameters of stavudine after oral administration of a 15-mg/kg dose to monkeys

Animal no.	C_{max}[a] (μg/ml)	T_{max}[b] (h)	$t_{1/2}$ (h)	AUC (μg · h/ml)	CL_R (liters/h/kg)	U_R (%)	Absolute bioavailability	
							F_{AUC}	F_{UR}
1	11.48	0.75	0.70	18.50	0.17	21.4	83.0	54.0
2	8.02	0.50	1.13	19.05	0.45	57.1	77.1	118.7
Mean	9.75	0.63	0.92	18.78	0.31	39.3	80.1	86.4

[a] C_{max}, peak concentration in plasma.
[b] T_{max}, time to peak concentration in plasma.

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