Pharmacokinetics of 3'-Fluoro-3'-Deoxythymidine and 3'-Deoxy-2',3'-Didehydrothymidine in Rhesus Monkeys

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3'-Fluoro-3'-deoxythymidine and 3'-deoxy-2',3'-didehydrothymidine are nucleoside analogs which inhibit human and simian immunodeficiency virus in vitro. The pharmacokinetic properties of these compounds in rhesus monkeys after intravenous, oral, and subcutaneous administration of the drug were compared. Half-lives, total clearances, and steady-state volumes of distribution for the two drugs were determined. The half-lives for the drugs by the different routes were between 0.58 and 1.4 h. Oral bioavailability of 3'-deoxy-2',3'-didehydrothymidine was incomplete, with an average of $42\% \pm 15\%$ of the dose reaching the systemic circulation. Absorption of 3'-fluoro-3'-deoxythymidine after oral administration was variable, with bioavailability ranging from 21 to 95%. Bioavailability after subcutaneous administration ranged from 59 to 77% for 3'-deoxy-2',3'-didehydrothymidine and from 52 to 59% for 3'-fluoro-3'-deoxythymidine. The ratio of concentrations in cerebrospinal fluid and serum for the drugs was about 0.15 at 1 h after drug administration and was independent of the route of administration, suggesting that a nucleoside carrier-mediated process is involved in the transport of these compounds to the central nervous system. Because of the similar metabolism of nucleoside analogs in monkeys and humans, the potential glucuronide formation was assessed. Whereas the glucuronide of 3'-fluoro-3'-deoxythymidine was readily detected in urine, the amount of 3'-deoxy-2',3'-didehydrothymidine glucuronidated was small or not detectable in one-half of the urine samples. Pharmacokinetic parameters for the two drugs were similar to each other and analogous to those for 3'-azido-3'-deoxythymidine in monkeys, suggesting that the same dose and scheduling of the drug can be used for all three compounds in prophylactic and therapeutic efficacy drug studies in rhesus monkeys.

Several 2',3'-dideoxynucleoside analogs have been shown to have potent in vitro antiviral activity against human immunodeficiency virus type 1. Among the most potent are compounds either with a modified 2',3'-dideoxyribose moiety containing a 3'-azido or 3'-fluoro function or with unsaturation at the 2',3' position of the nucleoside (1, 3-6, 13-15, 19, 20, 22, 23, 25, 27, 28). A well-studied compound in this class is 3'-azido-3'-deoxythymidine (AZT), a compound that is approved for the treatment of acquired immunodeficiency syndrome and human immunodeficiency virus type 1 infections (10, 23). AZT can be administered orally (p.o.), intravenously (i.v.), and subcutaneously, and extensive pharmacokinetic investigations in mammals have been performed with this compound (2, 7, 8, 18). Much less is known about the pharmacokinetic characteristics of 3'-fluoro-3'-deoxythymidine (FDT, FLT, FddT, FT) and 3'-deoxy-2',3'-didehydrothymidine (D4T), potent antiviral agents that are currently being evaluated in advanced preclinical and early clinical trials, respectively (1, 3-6, 13-15, 19, 20). Both FDT and D4T are phosphorylated to the corresponding 5'-triphosphates, resulting in powerful competitive inhibitors of human immunodeficiency virus type 1 reverse transcriptase (6, 11, 23). Inhibition of this enzyme is thought to be the main mechanism for the antiviral effect of these compounds.

The objectives of the present investigation were to define the single-dose pharmacokinetic parameters of FDT and D4T in rhesus monkeys after i.v., p.o., and subcutaneous

administration. The determination of these parameters is essential as a prelude to studies of monkeys infected with simian immunodeficiency virus and for comparison of these drugs with AZT, since FDT and D4T inhibit this virus in vitro at submicromolar concentrations (R. F. Schinazi, B. H. Arnold, and C. K. Chu, Program Abstr. Int. Symp. Basic Clin. Approaches Virus Chemother., abstr. no. T 48, 1988). From these studies, the initial dosages and routes of administration of the drugs for additional studies of primates can be selected. In addition, the potential formation of the glucuronide derivatives of FDT and D4T can be ascertained, since it is well established that AZT can be inactivated to its 5'-O-glucuronide form in both monkeys and humans and thus its bioavailability can be reduced (7, 8).

MATERIALS AND METHODS

Chemicals. D4T and FDT were provided by the Developmental Therapeutic Branch, AIDS Program, National Institutes of Health, Rockville, Md. For i.v. and subcutaneous administration, D4T and FDT were dissolved in phosphate-buffered saline, pH 7.4. For p.o. administration, nucleosides were dissolved in water. Acetonitrile, high-pressure liquid chromatography (HPLC) grade, and all other chemicals, analytical grade, were obtained from J. T. Baker (Phillipsburg, N.J.). β -Glucuronidase was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Experimental design. Young adult male rhesus monkeys (*Macaca mulatta*) weighing 3 to 6 kg were used for the pharmacokinetic studies. The animals were maintained at

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the Yerkes Regional Primate Research Center of Emory University, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care, in accordance with guidelines established by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health.

The disposition of D4T and FDT was characterized after i.v. and p.o. administration. Three monkeys (RDP1, RKO1, and AP19) were used to study D4T pharmacokinetics, and three additional monkeys (RFL1, AP31, and AP21) were used to examine FDT pharmacokinetics. Monkeys were administered 60 mg of D4T and FDT per kg of body weight i.v. (6 to 7 ml), and after a 3-week washout period, they received 60 mg/kg p.o. in a total volume of 40 to 45 ml by gastric intubation. Three control animals were administered phosphate-buffered saline without drug. Blood samples were taken prior to and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 24 h after drug administration. Animals were maintained under anesthetics for the first 4 h after drug administration. A single cerebrospinal fluid (CSF) sample was taken from all monkeys at 1 h after drug administration, and urine was collected by cystocentesis at 0.5 and 2 h after dosing. Serum, CSF, and urine samples were frozen at -20°C until analysis.

Absorption of D4T and FDT after subcutaneous drug administration was also assessed. Two monkeys each received 33.3 mg of D4T (RBS1 and RTU1) or FDT (RHZ1 and RCZ1) per kg subcutaneously. Blood samples were obtained prior to and at 0.5, 1, 1.5, 2, 3, 4, and 6 h after drug administration. Serum was frozen at -20°C until analysis.

Analytical methodology. D4T and FDT concentrations in biological fluids were determined by HPLC. To measure nucleoside concentrations in serum, a 100- μl serum sample, 10 μl of FDT or D4T (50 $\mu\text{g}/\text{ml}$) as internal standard, and 100 μl of 2 M perchloric acid as a protein precipitant were added to polypropylene microcentrifuge tubes (400 μl). Previous studies had indicated that perchloric acid did not affect the stability of either D4T or FDT. The contents of the tubes were thoroughly mixed for 30 s, and the tubes were centrifuged at $2,000 \times g$ for 5 min. Supernatant (15 to 150 μl) was injected onto the HPLC column. For drug concentrations in CSF, 100 μl of CSF was added to 20 μl of internal standard and 80 μl of water. A 100- μl sample was then injected onto the HPLC column. The presence of unchanged D4T and FDT in urine was ascertained by diluting 50- μl urine samples and 100- μl internal standard to 1 ml with water and injecting 50 μl onto the HPLC column.

The concentrations of D4T and FDT glucuronide in urine were determined after the glucuronate was hydrolyzed with β -glucuronidase (21). To 100 μl of urine were added 15 μl of 0.12 N acetic acid, 100 μl of β -glucuronidase (500 U/ml in water), and 35 μl of phosphate buffer, pH 5.8. The solution was mixed and incubated at 37°C in a shaker water bath for 12 h. To a 50- μl sample of hydrolyzed urine, 100 μl of internal standard and 850 μl of water were added. Then, 50 μl was injected onto the HPLC column. Glucuronide concentration was calculated as the difference between nucleoside concentrations measured before and after hydrolysis with β -glucuronidase.

Chromatography was performed on an Alltech Hypersil octadecanoylsulfate column (4.6 by 150 mm; particle diameter, 5 μm) with an isocratic mobile phase of 4% acetonitrile in 40 mM sodium acetate, pH 7.0, at a flow rate of 2 ml/min. Compounds were detected at a UV wavelength of 283 nm with a detector range setting of 0.005 absorbance units, full scale. The retention times for D4T and FDT were 5.7 and 10.8 min, respectively.

The D4T and FDT standards, ranging from 0.1 to 100 $\mu\text{g}/\text{ml}$, were prepared in blank monkey plasma and urine. Standard curve slopes and intercepts were generated by weighted (1/y) NONLIN least-squares regression (24). The assay was linear in the range of 0.1 to 100 $\mu\text{g}/\text{ml}$, and the lower limit of quantitation was 0.1 $\mu\text{g}/\text{ml}$ (10 ng). Extraction recoveries of D4T and FDT were 92 and 94%, respectively. The intra- and interday coefficients of variation for the assay were less than 10% for both drugs at all drug concentrations.

Pharmacokinetics. Area-moment analysis was used to calculate pharmacokinetic parameters for D4T and FDT. Area under the plasma concentration-time curve (AUC) and first nonnormalized moment were determined by Lagrange polynomial interpolation and by integration from time zero to the last measured sample time (26), with extrapolation to time infinity by using the terminal slope generated by weighted (1/y) NONLIN least-squares regression (24). Total clearance was calculated from $\text{dose}_{i.v.}/\text{AUC}_{i.v.}$, steady-state volume of distribution was calculated from $(\text{dose}_{i.v.} \times \text{AUMC}_{i.v.})/(\text{AUC}_{i.v.})^2$ (where $\text{AUMC}_{i.v.}$ is the area under the curve of the first nonnormalized moment after i.v. administration), and half-life was calculated from $0.693/l_z$, where l_z is the terminal disposition slope. Bioavailability after p.o. and subcutaneous drug administration was calculated from $(\text{AUC}_{p.o.,s.c.} \times \text{dose}_{i.v.})/(\text{AUC}_{i.v.} \times \text{dose}_{p.o.,s.c.})$, where $\text{AUC}_{p.o.,s.c.}$ is area under the curve after oral or subcutaneous administration. When drug concentration curves were rising at the end of the sample collection period after p.o. administration, as seen with monkey RKO1 after treatment with D4T and monkey AP31 after treatment with FDT, extrapolation of $\text{AUC}_{p.o.}$ to time infinity was calculated by using the terminal slope obtained after i.v. drug administration. This calculation most likely results in an underestimation of p.o. bioavailability for these monkeys. Calculation of bioavailability based on AUCs determined from time zero to 6 h yielded bioavailability values virtually identical to those based on total AUCs.

Partition coefficient determination. The drugs (1 mg of each per ml) were dissolved in water, and then octanol (1 ml) was added. The compound partitioned between the two solvents after being shaken for 2 h. The drug concentrations in the different phases were determined by HPLC, as described above.

RESULTS

Serum D4T concentration-time profiles after i.v. and p.o. administration of 60 mg of D4T per kg to three monkeys are illustrated in Fig. 1. D4T concentrations in CSF 1 h after drug administration are also depicted in this figure. Serum D4T concentrations declined rapidly after i.v. injection, with a D4T half-life of 1.1 ± 0.28 h (mean $\pm$ standard deviation). No drug was detected in any of the 24-h plasma samples. Pharmacokinetic parameters for D4T after i.v. and p.o. administration are presented in Table 1. Total clearance averaged 0.69 ± 0.15 liters/h per kg, and the steady-state volume of distribution was 1.03 ± 0.08 liters/kg. Peak serum D4T concentrations after p.o. administration were achieved within 1 h in two monkeys; however, absorption was erratic in one monkey, with D4T concentrations increasing from 2 to 6 h. The p.o. bioavailability was incomplete, with an average of $42\% \pm 15\%$ of the dose reaching the systemic circulation. The CSF/D4T concentration ratio in serum in all monkeys averaged 0.15 ± 0.04 and was independent of the route of administration.

Concentrations of FDT in serum after p.o. and i.v. admin-

FIG. 1. D4T concentrations in three rhesus monkeys after administration of 60 mg of D4T per kg. Concentrations were measured in CSF after i.v. (○) and p.o. (□) administration and also in serum after i.v. (●) and p.o. (■) administration.

istration of 60 mg/kg are illustrated in Fig. 2. CSF FDT concentrations measured 1 h after drug administration are also shown. Pharmacokinetic parameters for FDT in rhesus monkeys are presented in Table 2. Total clearance averaged 0.88 ± 0.11 liters/h per kg, and the steady-state volume of distribution was 0.90 ± 0.05 liters/kg. Absorption of FDT after p.o. administration was variable, with bioavailability ranging from 21 to 95%. The time to reach peak FDT concentrations in serum was also variable, with the peak occurring between 15 min and 1.5 h. The CSF/FDT concentration ratio in serum was independent of the route of administration, averaging 0.14 ± 0.03 .

Figure 3 illustrates concentrations of D4T and FDT in serum after subcutaneous administration of 33.3 mg of D4T or FDT per kg to rhesus monkeys. Peak nucleoside concentrations were achieved within 1 h for both D4T and FDT. Bioavailability after subcutaneous administration ranged from 0.59 to 0.77 for D4T and from 0.52 to 0.59 for FDT. Similarly, the half-life ranged from 0.8 to 0.87 h for D4T and from 0.58 to 0.80 h for FDT.

Drug concentrations in urine and drug-glucuronide concentrations were measured from samples obtained at 0.5 and 2 h after drug administration to qualitatively determine if the nucleosides were excreted unchanged or as a glucuronide

TABLE 1. Pharmacokinetic parameters after i.v. and p.o. administration of 60 mg of D4T per kg to rhesus monkeys^a

Monkey and route	AUC (mg · h/liter)	CL _T (liters/h per kg)	V _{ss} (liters/kg)	t _{1/2} (h)	F	Ratio of concn in CSF/serum
RDP-1						
i.v.	108.1	0.55	0.94	1.4		0.12
p.o.	54.0			1.1	0.50	0.09
RKO-1						
i.v.	90.0	0.67	1.07	1.1		0.18
p.o.	22.6			1.1	0.25	0.19
AP-19						
i.v.	71.7	0.84	1.09	0.84		0.15
p.o.	37.3			0.88	0.52	0.18

^a Abbreviations: CL_T, total clearance; V_{ss}, steady-state volume of distribution; t_{1/2}, half-life; F, bioavailability.

metabolite. Concentrations in urine varied considerably because of the different routes of administration and the various volumes of urine collected. The molar ratios of glucuronide metabolite/unchanged drug for D4T and FDT were 0.65 ± 0.97 and 1.45 ± 0.96 , respectively. The concentration of D4T glucuronide metabolite in urine was highly variable, with no glucuronide detected in one-half of the urine samples. This high variability could not be explained by route of administration or time of sample collection.

The octanol-water partition coefficients were 0.11 for D4T, 0.68 for FDT, and 1.1 for AZT. The value we determined for D4T is identical to the octanol-water partition coefficient of 1.1 reported previously by Collins et al. (9).

For all the animals treated with a single dose of the drugs by the different routes, hemogram and blood chemistry values at 24 h after drug administration did not exhibit any significant changes from those of untreated control monkeys.

DISCUSSION

The thymidine analogs D4T and FDT are structurally similar to AZT, differing only at the 2' and 3' positions of the ribose. While AZT is characterized by an azido group at the 3' position, FDT has a fluoro group at this position and D4T possesses a double bond between the 2' and 3' carbon atoms of the sugar. The pharmacokinetics of AZT have been previously described after administration to rhesus (8) and cynomolgus monkeys (P. De Miranda, T. C. Burnette, and S. S. Good, 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 378, 1987). Total clearance values of 0.9 and 1.5 liters/h per kg have been reported for AZT in monkeys. The steady-state volume of distribution of AZT in monkeys is 1 liter/kg, and the half-life ranges from 0.5 to 1 h.

Pharmacokinetic parameters for D4T and FDT are similar to each other and to those for AZT in monkeys. This is not unexpected, since the three compounds are structurally similar. D4T and FDT are eliminated at a moderate rate, with total clearance values that are approximately one-half of hepatic and renal blood-flow rates (12). The nucleosides appear to be distributed intracellularly, at least in some tissues in the body, since their steady-state volume of distribution is greater than that of total body water. Results with mice and rats demonstrate an appreciable accumulation of AZT and ddC in kidney, pancreas, liver, and lung (17; F. D. Boudinot, S. S. Ibrahim, and B. A. Patel, unpublished results).

FIG. 2. FDT concentrations in three rhesus monkeys after administration of 60 mg of FDT per kg. Concentrations were measured in CSF after i.v. (○) and p.o. (□) administration and also in serum after i.v. (●) and p.o. (■) administration.

Bioavailability of D4T and FDT after p.o. administration was variable, ranging from 25 to 50% for D4T and from 21 to 95% for FDT. The rate of absorption was also quite variable for both nucleosides; the time to achieve observed maximum

TABLE 2. Pharmacokinetic parameters after i.v. and p.o. administration of 60 mg of FDT per kg to rhesus monkeys^a

Monkey and route	AUC (mg · h/liter)	CL _T (liters/h per kg)	V _{ss} (liters/kg)	t _{1/2} (h)	F	Ratio of concn in CSF/serum
RFL-1						
i.v.	72.9	0.82	0.94	0.81		0.11
p.o.	41.8			0.84	0.57	0.15
AP-31						
i.v.	60.3	1.00	0.85	0.77		0.15
p.o.	12.5			0.77	0.21	
AP-21						
i.v.	74.4	0.81	0.91	0.89		0.10
p.o.	70.7			1.2	0.95	0.18

^a Abbreviations: CL_T, total clearance; V_{ss}, steady-state volume of distribution; t_{1/2}, half-life; F, bioavailability.

FIG. 3. D4T and FDT concentrations in serum in rhesus monkeys after subcutaneous drug administration of 33.3 mg of drug per kg. D4T concentrations were measured in monkeys RBS-1 (■) and RTU-1 (□), and FDT concentrations were measured in monkeys RHZ-1 (●) and RCZ-1 (○).

levels in plasma occurred between 0.5 and 6 h after drug administration. This variability in the absorption of D4T and FDT may be due to differences in gastric emptying rate or erratic dissolution of precipitated drug in the gastrointestinal tract.

The second drug concentration peak observed at 6 h in monkeys RKO1 and AP31 after p.o. administration of D4T and FDT, respectively, could possibly be due to enterohepatic recycling. This, however, is unlikely, since the second peak was seen only in the two cases in which drug absorption was lowest. No indication of enterohepatic recycling was observed after any other route of administration or in any of the other monkeys. In addition, the second D4T peak observed with monkey RKO1 after p.o. administration was twofold greater than the first, an unlikely consequence of recycling.

Drug absorption was lowest when absorption rate was slowest (longer time to achieve maximum levels in plasma). Indeed, the extent of absorption in the monkeys with the slowest rate of absorption (RKO1 and AP31) may be underestimated, since it appears that absorption of drug was still occurring at the 6-h blood-sampling time in these animals. Incomplete and variable bioavailability may have been due to poor absorption of drug and extensive first-pass metabolism or both. The bioavailability of AZT in rhesus monkeys after p.o. administration of 60 mg/kg is virtually complete (90 to 100%) (8). Thus, first-pass metabolism of AZT in monkeys is negligible. Since the clearance (hepatic extraction) of D4T and FDT is similar to that of AZT, it is unlikely that the low bioavailability of these nucleosides is due to extensive first-pass metabolism. However, the possibility cannot be ruled out. The low and variable bioavailability of D4T and FDT can most likely be attributed to poor absorption from the gastrointestinal tract. The three nucleosides differ in their lipophilicity, with AZT being the most lipophilic and D4T being the least lipid soluble. The octanol-water partition coefficients determined were 0.11 for D4T, 0.68 for FDT, and 1.1 for AZT. There appears to be a correlation between extent of drug absorption and the octanol-water partition coefficient for D4T, FDT, and AZT.

D4T and FDT were well absorbed after subcutaneous administration. Peak drug concentrations in serum occurred within 1 h in all monkeys and averaged 14.9 µg/ml for D4T and 10.8 µg/ml for FDT after subcutaneous administration of 33.3 mg/kg. Peak D4T and FDT concentrations in serum

normalized for dose were similar after subcutaneous and p.o. administration, averaging 0.37 for the two compounds after both routes of administration. Bioavailability of each of the compounds was nearly identical after subcutaneous and p.o. administration. Thus, similar drug concentrations in serum can be obtained after either route of drug administration; however, variability in drug absorption was much lower after the subcutaneous route.

Penetration of anti-human immunodeficiency virus nucleosides into the central nervous system is a critical component of antiviral therapy. Both D4T and FDT penetrated into the CSF to the same extent, with drug concentration ratios for CSF/serum averaging 0.15 and 0.14 for D4T and FDT, respectively. Previous studies demonstrated a similar ratio of 0.13 for AZT in monkeys. Penetration of these nucleosides into the CSF appears to be independent of the lipophilicity of the drugs, suggesting that transport of the compounds may be via a carrier-mediated process. It also appears that substitution of the functional group at the 3' position on the sugar has little or no effect on the transport of the thymidine analogs into the central nervous system. These results support the observations of Collins et al. (9), who also demonstrated that nucleoside transport into the central nervous system is independent of lipophilicity and functional group substitution at the 3' position on the sugar.

When drug concentrations in CSF at a single time point after administration of a single dose are interpreted, it must be noted that the drug concentration ratio for CSF/serum may change with time. Collins et al. (9) showed that peak concentrations of several nucleosides in CSF were achieved within 1 h after i.v. drug administration. Plasma and CSF nucleoside concentrations then declined in parallel, yielding a constant ratio between concentrations in CSF and serum. In the present study, CSF samples were taken 1 h after drug administration, and thus the D4T and FDT concentration ratios for CSF/serum reported probably represent pseudoequilibrium values.

Glucuronidation of AZT is the primary mechanism of clearance of the drug in monkeys and humans; 60 to 80% of an administered dose is eliminated as the glucuronide metabolite (7, 8; De Miranda et al., 27th ICAAC). The presence of a glucuronide metabolite of D4T and FDT was examined indirectly by treatment of monkey urine samples with β -glucuronidase. The ratio of glucuronide metabolite/FDT of 1.45 is virtually identical to the ratio of 1.50 reported for AZT in monkeys (De Miranda et al., 27th ICAAC). However, a much smaller fraction of D4T was found in urine samples, indicating that another route of elimination predominates for this nucleoside. The results with D4T are not in complete agreement with those in the recent report by Kaul et al. (16), which indicated that the D4T glucuronide was not detected in urine from monkeys.

In summary, in monkeys the pharmacokinetics of D4T and FDT are similar to those of AZT. Both nucleosides penetrate into the CSF. Absorption of the nucleosides is variable after p.o. administration; however, D4T and FDT appear to be absorbed relatively well after subcutaneous administration. Irrespective of the route of administration, levels in serum above their *in vitro* human and simian immunodeficiency virus inhibitory concentrations are produced by these antiviral drugs.

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