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NOTES

Pharmacokinetics of 3′-Fluoro-3′-Deoxythymidine and 3′-Deoxy-2′,3′-Didehydrothymidine in Rats

F. DOUGLAS BOUDINOT,[1]* SALLY G. SMITH,[1] ERIC D. FUNDERBURG,[1] AND RAYMOND F. SCHINAZI[2,3]

Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, Georgia 30602[1]; Veterans Affairs Medical Center, Decatur, Georgia 30033[2]; and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322[3]

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Concentrations of 3′-fluoro-3′-deoxythymidine (FDT) and 3′-deoxy-2′,3′-didehydrothymidine (D4T) in plasma declined in a biexponential fashion. Total clearance of D4T (1.75 ± 0.22 liters/h/kg; mean ± standard deviation) was significantly greater than that of FDT (1.19 ± 0.19 liters/h/kg) owing to greater renal and nonrenal clearances of the former. Steady-state volumes of distribution of FDT (1.20 ± 0.12 liters/kg) and D4T (1.07 ± 0.15 liters/kg) were similar.

The search for antiretroviral agents for the treatment of AIDS has yielded a number of compounds with selective in vitro antiviral activity. Compounds that inhibit human immunodeficiency virus type 1 reverse transcriptase, especially nucleoside analogs containing a 3′-azido,3′-fluoro or unsaturation, have proven to be particularly potent and selective. Among these are 3′-fluoro-3′-deoxythymidine (FDT; FLT) and 3′-deoxy-2′,3′-didehydrothymidine (D4T), nucleoside analogs shown to have potent activity against human immunodeficiency virus type 1 in culture (1–5, 10–12, 15, 16). Structurally, these compounds are quite similar to 3′-azido-3′-deoxythymidine (AZT; zidovudine), the only antiviral agent currently approved for the treatment of AIDS. The purpose of the present investigation was to characterize the dispositions of FDT and D4T in an animal model, the rat.

Adult male Sprague-Dawley rats (Charles River, Wilmington, Mass.) weighing 250 to 325 g were used for the study. External jugular vein cannulas were implanted under light ether anesthesia the day before the experiment, and the rats were fasted overnight. FDT and D4T were provided by the Developmental Therapeutic Branch, AIDS Program, National Institutes of Health, Rockville, Md. FDT or D4T, dissolved in physiologic saline (7 to 8 mg/ml), was administered intravenously at a dose of 25 mg/kg of body weight through the jugular vein cannula, and the rats were placed in a metabolism cage. Six rats were studied for each drug. Blood samples (0.3 ml) were collected at selected times from the cannula into heparinized tubes. Preliminary studies showed no adsorption of the nucleosides to the cannulas. Blood was replaced with normal saline. Blood samples were immediately centrifuged, and plasma was frozen at −20°C until analysis. Urine samples were collected at 6-h intervals for 24 h and frozen until analysis. Preliminary studies demonstrated the stability of the nucleosides in urine. No drug was detected in urine from the last sample collection interval; therefore, the total amount of unchanged nucleoside excreted in urine was determined.

FDT and D4T concentrations in plasma and urine were determined by high-performance liquid chromatography (HPLC). D4T served as the internal standard for measuring FDT concentrations, and FDT was used as the internal standard for determining D4T concentrations. To measure nucleoside concentrations in plasma, 100 μl of plasma sample, 50 μl of internal standard (20 μg/ml), and 100 μl of 2 M perchloric acid as a protein precipitant were thoroughly mixed in polypropylene microcentrifuge tubes. The tubes were centrifuged at 2,000 × *g* for 5 min. Supernatant (20 to 200 μl) was injected onto the HPLC. The HPLC separation was achieved with a C_{18} reversed-phase column (4.6 mm [inner diameter] by 15 cm; 5-μm particle size; Alltech Associates, Deerfield, Ill.) using a mobile phase of 4% acetonitrile in 0.04 M sodium acetate, pH 7.0, at a flow rate of 2.0 ml/min. Compounds were quantitated at a UV wavelength of 260 nm with a detector range setting of 0.005 absorbance units, full scale.

Urine samples were diluted in the ratio of 1:100 with HPLC water. Internal standard (100 μl) was mixed with 100 μl of diluted urine sample, and 100 μl of the sample was injected onto the HPLC. The potential formation of glucuronide metabolites of FDT and D4T was ascertained in urine by hydrolysis with β-glucuronidase as previously described (19).

Calibration standards ranging from 0.1 to 100 μg/ml for FDT and from 0.05 to 100 μg/ml for D4T were prepared in blank rat plasma and urine. Sample FDT and D4T concentrations were determined from the slope of calibration plots of the peak area ratio of the drug: internal standard versus standard nucleoside concentrations. A weighting factor of $1/y$ used for regression analysis yielded a normal distribution of residuals around the fitted calibration line. The retention times for D4T and FDT were 5.7 and 10.8 min, respectively. Standard curves were linear in the range of 0.1 to 100 μg/ml for FDT and 0.05 to 100 μg/ml for D4T. The limits of quantitation were 0.1 μg/ml for FDT and 0.05 μg/ml for D4T. The intra- and interday coefficients of variation for the assay were less than 10% for all concentrations of both drugs. The extraction recoveries of FDT and D4T were 94 and 92%, respectively.

The dispositions of FDT and D4T are described in terms of

* Corresponding author.

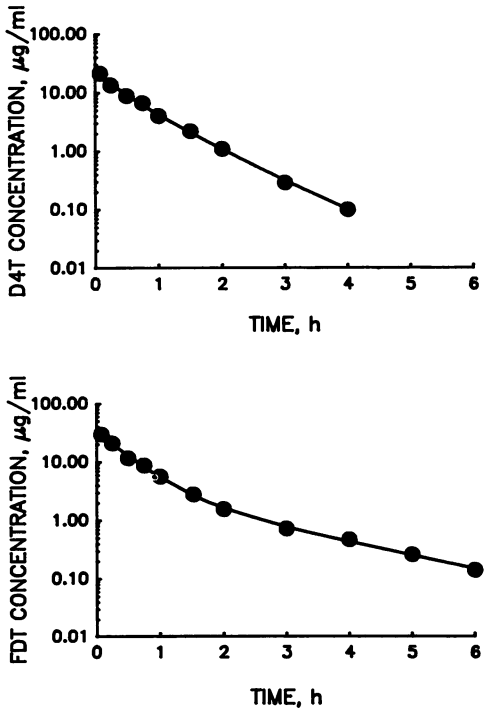

FIG. 1. Concentrations of D4T and FDT in plasma after intravenous administration of 25 mg/kg to rats.

the SHAM (slope, height, area, moment) properties of the curves (13). The F test (7) and lack of systematic deviations around fitted disposition curves were used to select exponential equations. Concentrations of FDT and D4T in plasma were best characterized by a biexponential decline; thus, a biexponential function was fitted to plasma nucleoside concentrations (C) as a function of time by NONLIN least-squares regression (17). Reciprocal C values were found to be acceptable as weighting factors for generating a normal distribution of weighted residuals in NONLIN. Pharmacokinetic parameters were calculated by standard SHAM analysis equations (13). Statistical analysis comparing the pharmacokinetic parameters of FDT and D4T was performed using the t test.

Representative plasma nucleoside concentration-versus-time curves after intravenous administration of 25 mg of FDT and D4T per kg to rats are illustrated in Fig. 1. The initial and terminal-phase half-lives for FDT were 0.34 ± 0.07 h (mean ± standard deviation) and 1.37 ± 0.32 h, respectively. The corresponding half-lives for D4T were 0.26 ± 0.26 and 0.56 ± 0.16 h. The longer terminal-phase half-life of FDT relative to that of D4T was statistically significant.

The pharmacokinetic parameters of FDT and D4T are presented in Table 1. The steady-state volumes of distribution and the volumes of the central compartment were similar for both compounds (Table 1). Total clearance, renal clearance, and nonrenal clearance of FDT were significantly lower than those of D4T. Virtually all of the administered dose of FDT was excreted unchanged in the urine, with 98.8% ± 8.1% of the dose being excreted by the kidneys. Renal excretion was also the primary route of elimination of D4T, accounting for 78.7% ± 6.7% of total clearance of the nucleoside.

Renal clearance of both nucleosides was comparable to renal plasma flow (1.5 liters/h/kg) in rats (9), indicating that a highly efficient active renal tubular secretion is involved in the excretion of FDT and D4T. Differences in the renal clearances of these nucleosides can be attributed to variances in the transport capacity, to affinity of the active secretion mechanism, or to differences in degree of passive tubular reabsorption. Previous studies have suggested that AZT does not undergo tubular reabsorption in rats (18). Since AZT is more lipophilic (octanol:water partition coefficient [k_p] = 1.1) than either FDT (k_p = 0.68) or D4T (k_p = 0.11) (19), it is unlikely that these nucleosides are reabsorbed. Thus, the difference in renal clearances of these nucleosides is most probably related to the active tubular secretion mechanism.

Nonrenal clearance accounted for approximately 20% of total clearance of D4T, while virtually all of FDT was excreted unchanged in urine. No glucuronide metabolites of either FDT or D4T were detected in urine samples. Interestingly, Kaul et al. (14) also reported the absence of glucuronide or other metabolites in the urine of rats receiving D4T.

The anti-human immunodeficiency virus thymidine analogs FDT and D4T are structurally quite similar to AZT, differing only at the 2′ and 3′ positions of the ribose moiety. AZT and FDT have an azido group and a fluoro group, respectively, at the 3′ position of the sugar, while D4T possesses a double bond between the 2′ and 3′ carbon atoms of the sugar. The dispositions of these nucleosides in rats are generally similar; however, differences do exist, particularly with the elimination of the compounds. All three nucleosides appear to be distributed intracellularly, since their steady-state volumes of distribution, which range from 1.1 to 1.5 liters/kg, are greater than the total body water (0.7 liter/kg) of rats (9). Appreciable accumulation of AZT has been shown in the kidney, liver, lung, and pancreas in rats (6).

The structural differences of FDT, D4T, and AZT result in small but statistically significant differences in the routes and rates of elimination of the compounds. Renal excretion is the major route of elimination of the thymidine analogs, accounting for 72, 79, and 99% of the total clearances of AZT, D4T, and FDT, respectively. While FDT is renally excreted to the greatest degree, it has the lowest renal clearance. The renal clearance of D4T is similar to that of AZT (1.1 to 1.4 liters/h/kg) (18, 20). Nonrenal clearance of AZT (0.4 to 0.98

TABLE 1. Pharmacokinetic parameters for FDT and D4T after intravenous administration of 25 mg/kg to rats[a]

Agent	AUC (mg · h/liter)	CL_T (liters/h/kg)	CL_R (liters/h/kg)	CL_{NR} (liter/h/kg)	V_{ss} (liters/kg)	V_1 (liter/kg)	$t_{1/2}$ (h)	MRT (h)
FDT	21.47 (3.10)*	1.19 (0.19)*	1.14 (0.17)*	0.05 (0.05)*	1.20 (0.12)	0.81 (0.12)	1.37 (0.32)*	1.03 (0.16)*
D4T	14.47 (1.83)*	1.75 (0.22)*	1.37 (0.12)*	0.38 (0.16)*	1.07 (0.15)	0.78 (0.31)	0.56 (0.16)*	0.62 (0.11)*

[a] Abbreviations: AUC, area under the plasma concentration-time curve; CL_T, total clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; V_{ss}, steady-state volume of distribution; V_1, volume of the central compartment; $t_{1/2}$, half-life; MRT, mean residence time. Values are means (standard deviation) for six rats. *, Statistically significant difference ($P < 0.05$).

liter/h/kg) is greater than that of either D4T or FDT. Biotransformation of AZT to 3′-amino-3′-deoxythymidine with subsequent biliary excretion of the metabolite accounts for at least part of the nonrenal clearance of AZT (8). The mechanism of nonrenal elimination of D4T in rats remains to be determined, while nonrenal clearance of FDT is of minor consequence.

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