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Review

TOXICOKINETICS OF 2′,3′-DIDEHYDRO-3′-DEOXYTHYMIDINE, STAVUDINE (D4T)

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ABSTRACT:

The toxicokinetic profile of D4T was assessed by conducting in vivo and in vitro studies. In the various studies, the i.v. and oral doses ranged from 12.5 to 600 and 5 to 2000 mg/kg, respectively. D4T was rapidly absorbed with an absolute oral bioavailability ranging from 77 to 100% in various species. The steady-state volume of distribution of D4T ranged from 0.50 to 1.12 liters/kg; radioactivity was distributed in all tissues, with the highest concentrations in the organs of excretion, liver and kidneys. D4T was eliminated from the body with a half-life of 0.30 to 1.23 h. Urinary recovery of unchanged drug was species-dependent and ranged from approximately 37 to 86%. In the mass balance studies, the recovery of total radioactivity at 96 h in rats and monkeys was approximately 85% and 50%, respectively; fecal recovery was <1.5% and approximately 14% was recovered as $^{14}CO_2$ in expired air in rats. The in vitro protein binding of D4T was negligible (<10%) and D4T did not induce cytochrome P-450 in rats or monkeys. D4T was metabolized to thymine and polar metabolites by the S9 and liver slices in vitro. Significant interspecies correlations were found for total body clearance, steady state of volume of distribution, and $T_{1/2}$ and species body weight. The multiples of exposure observed at the various no-effect doses in the drug safety evaluation studies (10× – 1102×) affirm that adequate doses of D4T were administered to laboratory animals to discern potential human risk.

Stavudine, 2′,3′-didehydro-3′-deoxythymidine (D4T)[1] (Zerit, Bristol-Myers Squibb, Princeton, NJ) is a thymidine nucleoside analog (Fig. 1) approved for the treatment of HIV infection (Lea and Faulds, 1996). Like other members of this class of antiretrovirals (Arts et al., 1996), its purported active metabolite, D4T-5′-triphosphate (Balzarini et al., 1989), is an inhibitor of the HIV reverse transcriptase and acts as a chain terminator during DNA synthesis (Huang et al., 1992). In its development as an oral anti-HIV agent, a battery of nonclinical studies were conducted to delineate the toxicologic and toxicokinetic profile of D4T. The toxicologic profile of D4T has been previously reported (Schilling et al., 1995). The objectives of the present studies were to investigate the influence of dose on the toxicokinetics of D4T in the rat and monkey after i.v. or oral administration, to characterize its tissue distribution properties, to elucidate the pathways of elimination, and to assess multiples of human exposure for safety in humans. The secondary purpose of these investigations was to compare the key toxicokinetic parameters with those reported for other anti-HIV nucleoside analogs. In addition, results from this study were combined with previously reported pharmacokinetic data after D4T administration to various species to provide a basis for interspecies scale-up of the disposition characteristics of the drug in humans.

Materials and Methods

Chemicals and Reagents. D4T (lot C88G574, C88D555, C88K154, C89D069, C89H146, C89L218, 1174007, and R11192), [2-^{14}C]D4T (lot 27239-10-1), and [4-^{14}C]D4T (lot 32773-4-1), radiolabel at positions 2 and 4 in the thymine base, respectively, were obtained from Bristol-Myers Squibb Co. (Syracuse, NY). [*methyl*-^{14}C]thymidine (CFA0.532, lot 86) and [2-^{14}C]thymine (T-9019, lot 108F9257-2) were purchased from Amersham North America (Arlington Heights, IL) and Sigma Chemical Company, (St. Louis, MO) respectively. The purity of the compounds was greater than 97%. The specific activities of the radiolabeled D4T compounds were 38.3 and 30.4 μCi/mg, respectively, and that of [*methyl*-^{14}C]thymidine and [2-^{14}C]thymine was 238 μCi/mg. Dulbecco's phosphate-buffered saline (D-8662), Dulbecco's modified Eagle's medium (D-5536), and fetal bovine serum (F-2268) were obtained from Sigma Chemical Co. All other chemicals and reagents were of analytical grade or better and were used as supplied.

Animals. Male and female Crl:CD-1 (ICR) BR mice were obtained from Charles River Labs., Inc. (Portage, MI); male and female Sprague-Dawley rats were obtained from Charles River Labs., Inc., Harlan Sprague-Dawley Inc. (Frederick, MD), and Taconic Farms (Germantown, NY); male and female New Zealand rabbits were obtained from Hazelton Labs., Inc. (Denver, PA); male and female cynomolgus monkeys were obtained from Charles River Research Primate Center (Port Washington, NY) and Buckshire Cooperation (Perkasie, PA). All animals were housed in individual stainless steel cages under standard conditions (12-h light/dark cycle) with free access to food and water, except for the monkeys on toxicology studies, which were fed twice daily. In some studies, animals were fasted overnight before dosing with D4T.

Formulations.

Intravenous Formulations. D4T was formulated for i.v. administration as sterile solutions in 0.9% sodium chloride injection (USP) at concentrations

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[1] Abbreviations used are: LSS, liquid scintillation spectrophotometry; CYP450, cytochrome P-450; HPLC, high-performance liquid chromatography; AUC, area under the concentration-time curve; CL, total body clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; V_{ss}, steady-state volume of distribution; C_{max}, peak concentration; T_{max}, time of peak concentration; UR, urinary recovery; D4T, stavudine; DDC, lamivudinr; DDI, didanosine; ZDV, zidovudine.

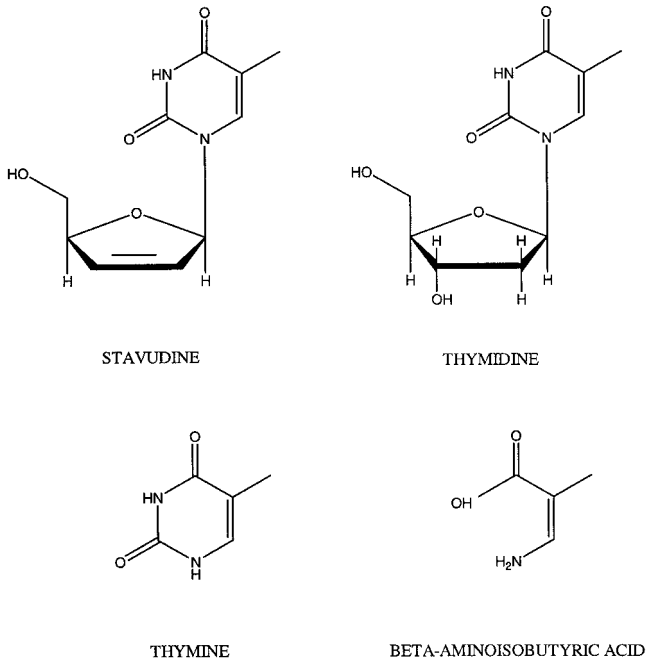

FIG. 1. *Chemical structures of D4T, thymidine, thymine, and β-aminoisobutyric acid.*

ranging from 0.5 to 60 mg/ml. When using ^{14}C-labeled material, the specific activity of the dosing solution ranged from 19 to 23 μCi/ml. The i.v. formulations were sterilized by filtration through a sterile 0.22-μm filter and used within 4 h of preparation.

Oral Formulations. For oral administration, D4T was formulated as solutions in sterile water, as suspensions in 0.5% sodium carboxymethylcellulose, and in ground rodent chow. The concentrations of D4T in the solution, suspension, and dietary formulations ranged from 0.5 to 66 mg/ml, 20 to 250 mg/ml, and 0.475 to 52.9 mg/g, respectively. When using ^{14}C-labeled material, the specific activity of the dosing solutions ranged from 10 to 48 μCi/ml. The oral solution and suspension formulations were used within 4 h of preparation. The stability, potency, and specific activity of D4T was confirmed in all formulations.

Toxicokinetic Studies in Rats and Monkeys. Six groups of five male rats (body weight range of 345–456 g) received single i.v. or oral doses of 50, 150, and 600 mg/kg D4T. The i.v. and the oral solution doses were administered in the tail vein and by gavage, respectively. All animals were anesthetized with ether, and a cannula was placed in the right external jugular vein 48 h before dosing. Three groups of two or three male and female monkeys (body weight range of 1.5–6.0 kg) received single i.v. or oral solution doses of D4T. The i.v. (12.5, 50, and 200 mg/kg) and oral (30, 100, and 300 mg/kg) doses were administered in the saphenous vein and by gavage, respectively. All animals were fasted overnight and for the first 4 h of the study. Blood samples (ca. 0.3 ml) in the rat studies were obtained at 0 (predose), 0.1, 0.3, 0.6, 1, 2, 4, 6, 8, 12, and 24 h postdose via the jugular cannula and placed in EDTA tubes; rat urine was cage-collected over three periods: 0 to 6 h, 6 to 12 h, and 12 to 24 h. Rat urine samples were collected in containers surrounded by a jacketed ethylene glycol cooling system maintained at about 0°C; previous studies indicated that D4T was stable in rat urine for 24 h at 4°C. Blood samples (ca. 1.5 ml) in the monkey studies were obtained at 0 (predose), 0.1 (i.v. only), 0.5, 1, 2, 4, and 6 h postdose. Blood samples were processed immediately for plasma by centrifugation at 1000*g* for 10 min. Plasma and urine samples were frozen and stored at −20°C until analysis for unchanged drug. Unless specified, the procedure for blood sample handling in all studies was as described above.

Mass Balance Studies in Rats. Five male rats (body weight range of 371–460 g) received single i.v. or oral doses of 50 mg/kg (200 μCi/kg) of [2-^{14}C]D4T. The i.v. and the oral solution doses were administered in the rat tail vein and by gavage, respectively. The animals were fasted overnight and for the first 4 h of the study. In the rat study, urine and feces were cage-collected over four periods: 0 to 24 h, 24 to 48 h, 48 to 72 h, and 72 to 96 h. In the monkey study, urine and feces were collected at 12-h intervals for 1 month. Samples were frozen and stored at −20°C until analysis for unchanged drug and total radioactivity.

General Toxicology Studies in Rats and Monkeys. Groups of 5 or 10 male and 5 or 10 females rats (body weight range of 151–275 g) were administered oral solution doses of 0 (control), 100, 300, and 600 mg/kg/day. Groups of three or five male and female monkeys (weight range 1.5–4.0 kg) were administered oral solution doses of 0 (control), 60, 200, and 600 mg/kg/day. All doses were equally divided into two daily administrations separated by approximately 4 to 6 h apart. The duration of treatment ranged from 1 month up to 1 year. Blood samples were collected at 0.5 h (rat) or 1.0 h (monkey) after the morning dose on day 1 and at 1, 3, 6, and 12 months. Samples in the rat studies were collected under CO_2 anesthesia after which the animals were exsanguinated. Plasma samples were frozen and stored at −20°C until analysis for unchanged drug.

Effect on Cytochrome P-450 Enzymes in Rats and Monkeys. This study was part of the general toxicology studies as described above. After 3 months of dosing, whole livers were immediately excised at necropsy, rinsed with ice-cold isotonic KCl-phosphate buffer (pH 7.4), blotted dry, weighed, and immediately frozen in liquid nitrogen. Liver samples were stored at −70°C until analysis of cytochrome P-450 (CYP450).

Carcinogenicity Studies in Mice and Rats. Three groups of 15 male and 15 female mice or rats (dosing period = 1 month) and three groups of six male and six female mice or rats (dosing period =12 months) were offered D4T mixed with ground rodent chow to achieve target daily doses of 80, 400, and 2000 mg/kg/day. Body weights of mice and rats ranged from 23 to 38 and 175 to 479 g, respectively. Blood samples were collected from randomly selected animals of each gender within each dose group at 2, 6, 10, 16, and 24 h after the onset of the dark cycle after 1 month; after 12 months, blood samples were collected at 2 and 6 h after the onset of the dark cycle. Samples (ca. 0.5 ml) were collected by cardiac puncture under CO_2 anesthesia, after which the animals were euthanatized with CO_2 (mice) or from the tail vein (rat). Blood samples were processed immediately, and plasma samples were frozen and stored at −20°C until analysis of D4T.

Teratology Studies in Rats and Rabbits. D4T was administered orally to groups of four dams (body weight range of 165–195 g) or groups of three female rabbits (body weight ca. 3 kg) from the 6th to the 14th or 16th day of gestation at total daily doses of 50, 250, and 1000 (rat) or 60, 150, 300, and 600 (rabbit) mg/kg/day. On gestation day 14 (rat) or 6 and 16 (rabbit), 0.5-h blood samples were collected and plasma was assayed for D4T.

Tissue Distribution Study in Rats. Male and female rats, body weight range of 241 to 277 g and 194 to 235 g, respectively, received a single oral solution dose of 5 mg/kg (150 μCi/kg) of [4-^{14}C]D4T. Three rats of each gender were sacrificed by exsanguination under CO_2 anesthesia at 0.5, 3, 6, 24, and 48 h after dose administration. Selected tissues (see Table 4) were removed from the remaining three rats. The tissues were excised, rinsed with sterile normal saline, blotted dry, and weighed. All samples were frozen and stored at −20°C until analysis for total radioactivity.

$^{14}CO_2$ Excretion Study in Rats. Two groups of three male rats (body weight range of 227–249 g) were given a single oral solution dose of 5 mg/kg (150 μCi/kg) of [2-^{14}C]D4T or [4-^{14}C]D4T. Immediately after dosing, the animals were placed in glass metabolism cages for the collection of $^{14}CO_2$. The metabolism cage was equipped with two traps in series, each containing 110 ml of Carbosorb (Packard Instrument Co., Meriden, CT). Expired $^{14}CO_2$ was trapped in 0 to 24 h and 24 to 48 h intervals. Nine milliliters of Carbosorb was neutralized with 11 ml of Permafluor (Packard Instrument Co.) and the radioactivity was determined by liquid scintillation spectrophotometry (LSS).

Maternal-Fetal Transfer Study in Rats. Twelve pregnant rats (body weight range of 260–305 g) on their 18th day of gestation were used in this study. The animals received a single oral solution dose of 300 mg/kg (46 μCi/kg) of [2-^{14}C]D4T after an overnight fast. Three rats were sacrificed by CO_2 anesthesia at 1, 4, 8, and 24 h after dosing. Plasma, amniotic fluid, whole fetus, and placenta were harvested, frozen, and stored at −20°C until analysis for total radioactivity.

Milk Secretion Study in Rats. Twelve primiparous rats (body weight range of 201–238 g) on day 7 of lactation were used in this study. The animals received a single oral solution dose of 100 mg/kg (150 μCi/kg) of [4-^{14}C]D4T

after an overnight fast. The pups were removed from the rats 2 h before sample collection. Intramuscular oxytocin, 2 units/rat (Wyeth-Ayerst Laboratories, Philadelphia, PA), was administered 1 h before breast milk collection to stimulate breast milk flow. Samples of breast milk and blood were obtained from 3 dams per time point at 1, 4, 8, and 24 h after dosing. Milk was obtained by applying intermittent suction with a milk collection device (McBurney et al., 1964). The milk samples were diluted with water (4- to 21-fold). After milking, blood samples were obtained immediately by exsanguination (cardiac puncture) under CO_2 anesthesia. Plasma and milk samples were frozen and stored at −20°C until analysis for total radioactivity.

Fertility and Reproduction Study in Rats. This study was conducted as described under general toxicology study, except that groups of four female rats (171–201 g) received D4T 14 days before mating, during mating, and through gestation day 14. On gestation day 14, blood samples were collected from four dams at 0.5 h, and plasma was assayed for unchanged drug.

Neurotoxicity Study in Rabbits. In this two-part study, groups of three male rabbits (weight range 3.0−4.5 kg) received 600, 1500, and 3750 mg/kg/day D4T as two equally divided doses approximately 6 h apart in the first part of the study. The doses were given by oral gavage in 0.5% carboxymethylcellulose suspension for 4 weeks. On the last day of the study (day 30), the total daily dose was given as a single dose. Serial blood samples were collected for 24 h after the first dose of the day on study days 16 and 30. Blood samples for D4T trough levels were collected just before the first dose of the day on study days 2, 3, 9, 16, 17, 23, and 30. In the second part of the study, two groups of five male rabbits were dosed with 750 and 1500 mg/kg/day for 24 weeks. A single blood sample was collected 1 h after dosing on day 1, at weeks 10 and 20. Plasma samples were assayed for unchanged drug.

In Vitro Studies.

Protein binding study. The in vitro protein binding of [2-^{14}C]D4T was investigated in fresh rat, monkey, and human sera by the ultrafiltration technique (Gaver et al., 1987). Three concentrations of [2-^{14}C]D4T, 0.1, 1, and 10 μg/ml, were prepared in triplicate in serum, and 1.0-ml sample aliquots were subjected to ultrafiltration with 10,000 daltons molecular weight cutoff Centricon 10 filtration units (Amicon Co., Danvers, MA). The filtration units were centrifuged at 25°C for 20 min at 1000*g* to generate 80 to 110 μl of protein-free ultrafiltrate. The radioactivity in the serum and ultrafiltrate samples (50-μl aliquots) was measured by LSS. The above described procedure was used to determine the nonspecific binding of D4T by spiking the compound in Plasmalyte A, pH 7.4 (Travenol, Co., Deerfield, IL).

Red blood cell (RBC) uptake study. The in vitro RBC distribution of [4-^{14}C]D4T was investigated in rat, monkey, and human EDTA whole blood by the incubation technique (Gaver et al., 1987). Three concentrations of [2-^{14}C]D4T, 0.1, 1, and 10 μg/ml in triplicate, were prepared in freshly drawn whole blood. Hematocrit of the individual samples was measured and recorded. Blood samples (1.0 ml) were incubated at 37°C for 60 min. At the end of the incubation period, blood samples were centrifuged to obtain plasma. Aliquots (100 μl) of plasma samples were assayed for radioactivity by LSS.

Metabolism study. The metabolism of D4T was investigated using liver S9 fractions and intact liver slices of rats, monkeys, and humans. The liver S9 fractions and intact liver slices were prepared by previously described methods (Powis et al., 1989). The incubation was started by the addition of [4-^{14}C]D4T to give a drug concentration of 100 μM. [*methyl*-^{14}C]thymidine (100 μM) was used as a positive control. The flasks were incubated with shaking at 37°C in a water bath. S9 fraction incubations were sampled at 0.5, 1, 2, and 4 (human only) h. Rat and human liver slices were incubated for 2 and 6 h, at which time the media were decanted from the slice tissue. Incubations were terminated by placing the samples immediately on ice. All samples were stored at −20°C until radiochromatograghic analysis. Preliminary experiments demonstrated the stability of the two compounds in the media.

Analytical Methodologies.

Assay of D4T in plasma and urine. Plasma samples were analyzed for unchanged D4T by a previously reported high-performance liquid chromatography/UV (HPLC/UV) assay method (Kaul et al., 1989). The method of Janiszewski et al. (1992) was used to quantitate D4T in urine samples. The standard curve ranges for the plasma and urine assay methods were 0.1 to 100 and 0.5 to 100 μg/ml, respectively. Data acquisition and processing was done by a previously described method (Farmen et al., 1987).

Determination of total radioactivity. Samples of plasma, urine, amniotic fluid, milk, and ultrafiltrate from the protein binding studies (0.1 ml in duplicate) were mixed with 10 ml of Ready-Safe scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA) and directly assayed by LSS. Aliquots of homogenized fecal samples ($\leq$ 0.5 g in duplicate) were placed in combustion boats, allowed to dry at ambient conditions, and combusted in a model OX300 biological oxidizer (R. J. Harvey Instrument Co., Hillsdale, NJ), and the resulting $^{14}CO_2$ was trapped and counted in 15 ml of ^{14}C cocktail (R. J. Harvey Instrument Co.). [2-^{14}C] or [4-^{14}C]D4T standards in fecal homogenates (100–100,000 dpm per sample) prepared and combusted with study samples yielded combustion recoveries of $>$ 94%. Tissues and fluids from the rat tissue distribution study were solubilized directly for LSS in Soluene-350 (5 ml/g of tissue; Packard Instrument Co.) or homogenized before solubilization. The digested samples (0.2 g) were bleached with 1 ml of 20% solution of benzoyl peroxide in toluene, neutralized with 0.1 ml of a mixture of a saturated solution of sodium pyruvate in methanol/glacial acetic acid/methanol (4:3:1 v/v/v), and mixed with 15 ml of cocktail for LSS. Whole rat carcasses were ground in a meat grinder and processed as described above.

Total protein and CYP450 assays. Total protein concentrations were measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Microsomal CYP450 content was measured by the method of Omura and Sato (1965) using an extinction coefficient for CYP450 of 100 mM/cm.

Metabolite profiling procedure. The metabolite profiles in different matrices from studies using radiolabeled D4T were determined by gradient HPLC analysis of 50- to 100-μl samples. Before HPLC, samples were centrifuged at 2000*g* for 10 min and clarified by ultrafiltration. The HPLC system was equipped with two model 510 pumps (Waters Associates, Inc., Milford, MA), a WISP model 710B autosampler (Waters Associates, Inc.), and an Apex ODS C-18 column (5 μ, 4.6 $\times$ 250 mm; Jones Chromatography, Littleton, CO). After injection of the ultrafiltrate sample, the column was eluted with a mobile phase at a flow rate of 1 ml/min for 45 min using a gradient system. The composition of the mobile phase changed from 0% methanol to 50% methanol in phosphate buffer (50 mM KH_2PO_4, pH 3.5) in 35 min, and returned to 0% methanol in 45 min. The eluate was collected in 1.0-ml fractions and total radioactivity was determined as described above. Preliminary experiments showed that unchanged D4T eluted as a single peak at approximately 24 to 25 min. The HPLC recovery of the injected radioactivity was $>$97%.

The presence of potential metabolites in the in vitro metabolism studies of D4T and thymidine was determined by reverse-phase gradient HPLC using radioactive flow detection. The S9 fraction and liver media samples were prepared for HPLC analysis by the addition of 3 volumes of methanol. The samples were vortex-mixed and centrifuged to remove precipitated proteins. The supernatant was removed and dried under a steady stream of nitrogen, reconstituted with 200 μl of 0.01 M ammonium acetate buffer (pH 5.0). An aliquot of the reconstituted sample (100 μl) was injected onto the HPLC system described which used a Zorbax ODS column (5 μ, 4.6 $\times$ 250 mm; Mac-Mod Analytical, Inc., Chadds Ford, PA). The composition of the mobile phase changed from 0% methanol to 30% methanol in ammonium acetate buffer (0.01 M, pH 5.0) in 20 min followed by a linear gradient for 3 min to 100% methanol. The system remained at 100% methanol for 2 min, after which it was re-equilibrated for 15 min with 100% ammonium acetate buffer. The HPLC eluate was mixed on-line with 3 volumes of liquid scintillation cocktail (Flo-Scint II, Radiometric Instruments, Tampa, FL) for the detection of radioactivity by the continuous flow radioactivity monitor (FLO-ONE, Radiometric Instruments). Preliminary experiments indicated that thymine, thymidine, and D4T eluted as single peaks at approximately 9 to 10, 13 to 14, and 17 to 18 min, respectively.

LSS. Radioactivities in samples from different matrices were determined with a Beckman LS9000 liquid scintillation spectrophotometer (Beckman Instruments, Inc.) equipped with an automatic data reduction system. Samples were counted for 2% 2-Σ error or 10 to 90 min. All counts of radioactivity were corrected for background and compensation for sample quenching was made by an automatic external standard method. Where appropriate, the levels of radioactivity were expressed as dpm, μg-eq/ml, μg-eq/g, or percentage of the administered radioactive dose.

Data Analyses.

Pharmacokinetics. Plasma concentration versus time data was analyzed by a noncompartmental method (Gibaldi and Perrier, 1982). Using no weighting factor, the terminal slope (k_{el}) was determined by log-linear regression of

TABLE 1

Pharmacokinetic parameters for stavudine after i.v. administration in various laboratory animals (N = 3–5)

Species	Dose	Mean (S.D.) Pharmacokinetic Parameter Value[a]					
		$T_{1/2}$	CL	CL_R	CL_{NR}	V_{ss}	UR
	(mg/kg)	*(h)*	*(ml/min/kg)*	*(ml/min/kg)*	*(ml/min/kg)*	*(l/kg)*	*(%)*
Mouse[b]	25	0.30	28.7	ND	ND	0.76	ND
Rat	50	0.43 (0.06)	24.0 (2.73)[e]	19.1 (2.75)[f]	4.86 (1.24)	0.70 (0.08)[h]	79.2 (5.4)
	150	0.56 (0.13)	16.0 (2.93)[e]	11.6 (2.47)	4.45 (0.66)	0.51 (0.10)	71.9 (4.0)
	600	1.02 (0.38)[d]	10.0 (0.92)[e]	8.31 (1.51)	1.69 (1.12)[g]	0.50 (0.05)	83.1 (11.8)
Rabbit[c]	10	0.85 (0.10)	23.4 (3.6)	8.82 (3.9)	ND	1.12 (0.13)	36.0 (8.0)
Monkey	12.5	0.74 (0.06)	14.5 (2.2)	ND	ND	0.92 (0.07)	ND
	50	0.76 (0.05)	14.2 (4.7)	ND	ND	0.92 (0.23)	ND
	200	0.91 (0.18)	10.7 (1.5)	ND	ND	0.83 (0.05)	ND

[a] $T_{1/2}$, terminal half-life; CL, total body clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; V_{ss}, steady state volume of distribution; UR, urinary recovery of unchanged drug; ND, not determined.
[b] Data from reference Russell et al., 1990, using a composite curve, $N = 3$ animals/time point.
[c] Data from reference Wong and Swachuk, 1991.
[d] Statistically different from the 50 and 150 mg/kg dose groups, $p < .05$.
[e] Statistically different between dose groups, $p < .05$.
[f] Statistically different from the 150 and 600 mg/kg dose groups, $p < .05$.
[g] Statistically different from the 50 and 150 mg/kg dose groups, $p < .05$.
[h] Statistically different from the 150 and 600 mg/kg dose groups, $p < .05$.

at least the final three data points which yielded a minimum mean square error. The following pharmacokinetic parameters were determined by using previously reported equations (Gibaldi and Perrier, 1982): Area under the concentration-time curve (AUC) (0 to ∞ α5 or 0 to t, where t is the last quantifiable time point), $T_{1/2,}$ total body clearance (CL), renal clearance (CL_R), nonrenal clearance (CL_{NR}), and steady-state volume of distribution (V_{ss}). peak concentration (C_{max}), time of peak concentration (T_{max}), and urinary recovery of unchanged drug (UR) were the observed values from the tabulated data. All pharmacokinetic parameters for D4T were derived from the HPLC/UV assay for unchanged drug. Oral bioavailability (F) was calculated as $[(AUC_{0-\infty})_{po} \cdot D_{i.v.}]/[(AUC_{0-\infty})_{i.v.} \cdot D_{po}] \times 100$. The extent of absorption (EA) in the rat was calculated from the ratio of the urinary recovery of total radioactivity after p.o. (UR_{po}) and i.v. doses ($UR_{i.v.}$).

Multiples of exposure. The multiples of human exposure in laboratory animals in the various toxicology studies were calculated by taking the ratio of C_{max} of D4T in animals to the C_{max} at the therapeutic dose in humans and/or the ratio of AUC (daily exposure) in animals to the AUC in humans. The mean steady-state C_{max} (0.657 μg/ml) and AUC in a 12-h dosing interval (1.176 μg·h/ml) in humans were obtained from the literature for the 0.5 mg/kg b.i.d. regimen (Kaul et al., 1992). Because D4T exhibits linear kinetics in humans over the dose range of 0.67 to 4.0 mg/kg (Dudley et al., 1992), the AUC value was multiplied by 2 to obtain the total daily exposure in humans at the clinical dose of 1 mg/kg/day. Mean C_{max} and AUC data were obtained from the various pharmacokinetic and toxicology studies. If the verification of exposure in toxicology studies was based on a single 0.5- or 1.0-h plasma D4T concentration, then this concentration was considered to be an apparent C_{max} for D4T.

Interspecies scaling. Intravenous pharmacokinetic parameters, CL, V_{ss}, and $T_{1/2}$, were taken from the literature for mice (Russell et al., 1990), rats (present study; Boudinot et al., 1991), rabbits (Wong and Swachuk, 1991), monkeys (present study, Schinazi et al., 1990; Kaul and Dandekar, 1993), and humans (Dudley et al., 1992), and correlations between pharmacokinetic parameters and species body weight were generated by allometric relationships (Boxenbaum, 1982).

Statistics. Pharmacokinetic parameters were analyzed in the context of one-way analysis of variance using SAS (Statistical Analysis System, 1985). If the effect of dose, period or gender was statistically significant, then Tukey's method of multiple comparison was used to compare group means (Gill, 1978). A p value of < 0.05 was considered significant. For interspecies scaling, the correlations between pharmacokinetic parameter and species body weight were analyzed by linear least-squares regression analysis of logarithmically transformed data. Statistical significance of correlations were examined with the Student's t test.

Results

Intravenous Pharmacokinetics. The pharmacokinetic parameters of D4T after various doses to laboratory animals are presented in Table 1. D4T was rapidly distributed and eliminated from the rat and monkey plasma in an apparent biexponential fashion (Figs. 2 and 3) with a terminal $T_{1/2}$ of $\leq$1.0 h. The pharmacokinetic data in rats indicated a dose-dependent relationship for D4T. Previously reported data showed that D4T was eliminated from mouse and rabbit plasma with a $T_{1/2}$ of 0.30 and 0.85 h, respectively (Russell et al., 1990; Wong and Swachuk, 1991). The urinary recovery of unchanged drug ranged from 36.0 to 83.1% and was highest in the rat.

Oral Pharmacokinetics. Plasma D4T concentration-time profiles after oral administration of various doses to rats and monkeys are illustrated in Figs. 2 and 3, respectively. Peak plasma D4T concentrations after oral administration were achieved within 2 h. $AUC_{0-\infty}$ increased disproportionately with dose, indicating dose-dependent pharmacokinetics of D4T in the rats and monkeys. The oral bioavailability was complete in the rodents, with 100% of the dose reaching systemic circulation. The oral bioavailability in the monkey was 77%. The oral bioavailability at higher doses was not estimated because of dose-dependent pharmacokinetics in rats and monkeys. After oral administration, $T_{1/2}$, CL_R, and UR were comparable to the corresponding values measured after i.v. administration (Table 2).

Mass Balance. The cumulative percentages of dose excreted in the urine as unchanged drug and total radioactivity are shown in Table 3. In the 0 to 96 h urine, 85.7% and 84.7% of the dose were recovered in the urine as total radioactivity after i.v. and oral administration of a 50 mg/kg dose of [2-^{14}C]D4T to rats, respectively; mean recoveries of unchanged drug accounted for 88.2 and 86.5%, respectively. In monkeys, 50.5 and 39.8% of the dose was excreted as total radioactivity in the 0 to 96 h urine after i.v. and oral administration of a 25-mg/kg dose of [4-^{14}C]D4T; the mean recoveries of D4T were 44.4% and 37.4%, respectively. Fecal excretion of total radioactivity in rats and monkeys was $<$1.5% of the administered dose. In the monkeys, no radioactivity was recovered in the urine or feces beyond 96 h. The extent of absorption was 100% in the rat; because of incomplete recovery of total radioactivity, the extent of absorption was not determined in the monkey. The fraction appearing in the monkey urine as metabolites was 6.4 and 13.7% after oral and i.v. administration, respectively.

Tissue Distribution. There appeared to be no gross differences in the tissue concentrations of radioactivity between male and female rats. Therefore, concentrations of total radioactivity in tissues at various times (from male rats only, except for ovaries and uterus from females) after oral administration of [4-^{14}C]D4T at a 5-mg/kg dose

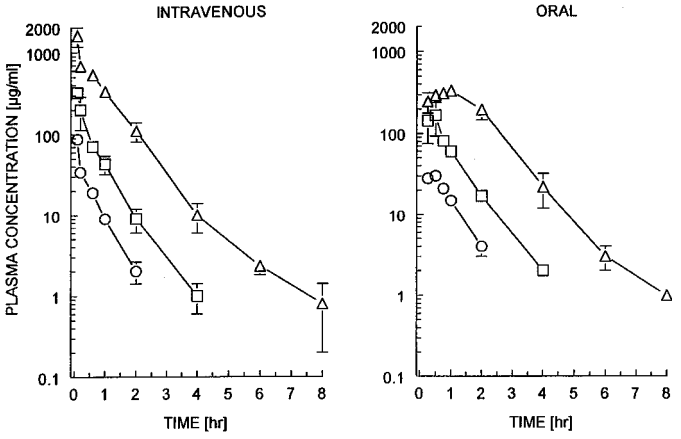

FIG. 2. *Mean ± S.D. plasma concentrations after i.v. and oral administration of D4T to rats.*

○, 50 mg/kg; □, 150 mg/kg; △, 600 mg/kg; $N = 5$/dose group.

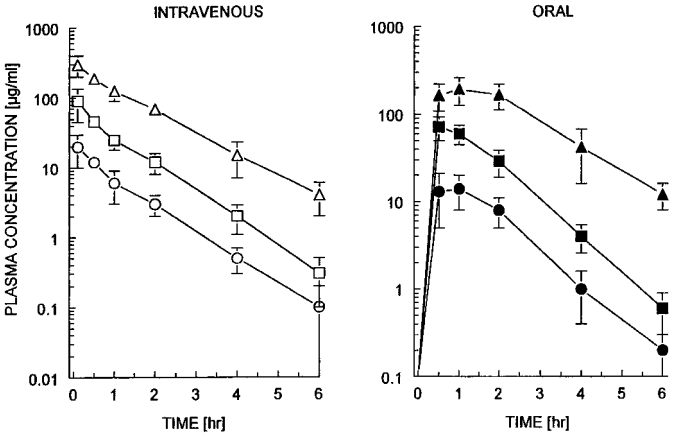

FIG. 3. *Mean ± S.D. plasma concentrations after i.v. and oral administration of D4T to monkeys.*

i.v.: ○, 12.5 mg/kg; □, 50 mg/kg; △, 200 mg/kg; $N = 2$/gender/dose group. Oral: ●, 30 mg/kg; ■, 100 mg/kg; ▲, 300 mg/kg; $N = 3$/gender/dose group.

are summarized in Table 4. The highest concentrations of radioactivity were observed at 0.5 h after dosing in majority of the tissues. After the 0.5 h time point, concentrations of total radioactivity were higher in the following tissues than in plasma: liver, kidney, stomach, and small and large intestine. In 24 to 48 h, concentrations in all tissues had decreased by ≥99% of the concentrations observed at 0.5 h after dosing. Approximately 1% of the dose remained in the carcasses of male and female rats at 48 h.

In the rat maternal-fetal transfer study, the C_{max} in maternal plasma, fetal tissue, and placental tissue was observed at 1 h and were 210, 127, and 123 μg-eq/ml, respectively, after oral administration of a 300 dose of [2-^{14}C]D4T (Fig. 4); the corresponding value for the amniotic fluid was 28.8 μg-eq/ml and occurred at 4 h after dose. The AUC values over a 24-h period were 510, 351, 319, and 300 μg-eq·h/ml for maternal plasma, fetal tissue, placental tissue, and amniotic fluid, respectively. The AUC values for the latter three tissues were quite similar and relative to maternal plasma, the percentage exposure of these tissues to [4-^{14}C]D4T ranged from 59 to 69%.

Figure 4 depicts the composite profiles of radioactivity in maternal plasma and milk of rats after oral administration of a 100-mg/kg doses of [4-^{14}C]D4T. The apparent mean C_{max} of radioactivity in maternal plasma and milk were observed at 1 h and were 44.7 and 15.5 μg-eq/ml, respectively. At 24 h, the concentrations of radioactivity in plasma and milk were <0.4% of the 1-h concentrations. The milk/plasma concentration ratio at 1 h was approximately 0.3; however, at 4, 8, and 24 h, the concentrations of radioactivity in milk were 1.2- to 8-fold greater than in plasma.

Verification of Exposure in Toxicology Studies. Figures 5 and 6 depict the 0.5-h and 1.0-h D4T plasma concentrations (apparent C_{max}) in rats and monkeys, respectively, in the general toxicology studies spanning 12 months. The results for the 1-and 3-month oral toxicity studies indicated that apparent C_{max} in male rats was significantly lower compared with female rats in the 600-mg/kg/day dose group. The mean S. D. apparent $\mathbf{C}_{max}$ at 1 month was 154 (52.5) and 224 (26.8) μg/ml for male and female rats, respectively; the corresponding values at 3 months were 173 (30.2) and 242 (35.3) μg/ml, respectively. No such differences were observed at 6 and 12 months. In the monkeys, there were no gender differences in the apparent C_{max} values; however, at all dose levels, the apparent C_{max} values were significantly greater at 1 month [range, 14.1 (1.7) to 160 (69.2) μg/ml] compared with the concentrations observed at the subsequent sampling months [range, 6.4 (1.9) to 64.8 (34.3) μg/ml]. The apparent D4T C_{max} data in the fertility/reproduction and teratology studies conducted using pregnant rats and rabbits and in the neurotoxicity study in rabbits revealed dose-related exposures to D4T (Table 5).

The composite D4T plasma concentration-time profiles in the carcinogenicity studies in mice and rats after 1 month of dietary administration are depicted in Fig. 7. The exposure data are presented in Table 5. The C_{max} and AUC data suggest dose-related exposure to D4T and no marked difference between gender in either study. In the mouse carcinogenicity study, the 2- [6-] h plasma D4T concentrations (pooled across gender) after 12 months of dosing in mice were 1.3 (0.5) [1.2 (0.5)], 7.1 (2.1) [6.0 (1.2)], and 25.7 (8.4) [37.4 (10.0)] μg/ml for the 80-, 400-, and 2000-mg/kg/day dose groups, respectively; these levels were reasonably comparable to the levels observed at 1 month {1.2 (0.4) [1.6 (0.9)], 5.1 (0.8) [5.0 (1.8)], and 30.0 (15.0) [30.6 (7.8)], respectively}. In the rat carcinogenicity study, the 2- [6-] h plasma D4T concentrations pooled across gender after 12 months of dosing in rats were 4.5 (1.7) [5.2 (2.5)], 33.7 (15.0) [25.5 (5.2)], and 106 (20.0) [124 (20.0)] μg/ml for the 100-, 600-, and 2000-mg/kg/day dose groups, respectively; these levels were reasonably comparable to the levels observed at 1 month {3.8 (1.0) [4.0 (1.3)], 26.8 (9.9) [19.7 (6.5)], and 155 (30.0) [149 (34.0)], respectively}.

Effects on CYP450 Enzymes. In rats, there were no significant effects of gender or dose on the CYP450 levels after 3 months of repeated administration of D4T (Table 6). There were no significant effect of gender on the CYP450 levels in the monkey. CYP450 levels were significantly greater for the 60-mg/kg/day dose compared with the control group; however, the CYP450 levels for the 200- and 600-mg/kg/day dose groups did differ from the control group (Table 6). Because the only measurement was for total CYP450, it is impossible to tell whether selective isozymes were induced.

Protein Binding and RBC Uptake. Over a concentration range of 0.01 to 10 μg/ml, the mean (S.D.) nonspecific binding of radioactivity to the ultrafiltration cone was 9.7% (1.7). The extent of serum protein binding did not exceed nonspecific binding. Therefore, the extent of serum protein binding of D4T in fresh rat, monkey, and human sera was negligible (<10%). The uptake of D4T by RBCs was independent of species and concentration; the uptake of D4T, as a percentage of nominal blood concentration, was 39.9 (1.8) %, 40.5 (2.9) %, 37.1 (0.9) % in fresh rat, monkey, and human whole blood, respectively.

Metabolism of D4T. HPLC elution profiles for samples collected at the final time point after [4-^{14}C]D4T incubation with rat, monkey, or human liver S9 fraction and rat or human liver slices are shown in Fig. 8. The rat 120-min liver S9 fraction contained mostly parent

TABLE 2

Pharmacokinetic parameters for stavudine after oral administration in various laboratory animals (N = 3 to 6)

Species	Dose	Mean (SD) Pharmacokinetic Parameter Value[a]						
		C_{max}	T_{max}	$AUC_{0-\infty}$	$T_{1/2}$	CL_R	UR	F
	(mg/kg)	*μg/ml*	*(h)*	*(μg · hr/ml)*	*(h)*	*(ml/min/kg)*	*(%)*	*(%)*
Mouse[b]	25	23.0	0.08	ND	0.30	ND	ND	98
Rat	50	30.9 (1.79)	0.50 (0.25, 0.50)	33.5 (3.51)	0.56 (0.08)	21.0 (2.54)[e]	82.4 (2.8)	102
	150	166 (73.6)	0.50 (0.25, 0.50)	155 (37.3)	0.67 (0.09)	12.0 (3.98)	72.6 (13.3)	ND
	600	336 (31.4)	1.00 (1.00, 1.00)[c]	739 (95.9)	1.23 (0.86)	12.0 (2.13)	84.8 (6.7)	ND
Monkey	30	16.1 (4.93)	1.00 (0.50, 2.00)	27.0 (7.50)	0.69 (0.06)	ND	ND	77
	100	77.3 (18.0)	0.50 (0.50, 2.00)	121 (17.9)	0.70 (0.07)	ND	ND	ND
	300	207 (64.8)	1.00 (1.00, 2.00)	554 (148)	1.10 (0.30)[d]	ND	ND	ND

[a] C_{max}, peak concentration in plasma; T_{max}, time at which C_{max} occurred; $AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; $T_{1/2}$, terminal half-life; CL_R, renal clearance; UR, urinary recovery of unchanged drug; F, oral bioavailability; ND, not determined; median (minimum, maximum) values reported for T_{max}.
[b] Data from reference Russell et al., 1990, using a composite curve, N = 3 animals/time point.
[c] Statistically different from the 50 and 150 mg/kg dose groups, $p < .05$.
[d] Statistically different from the 30 and 100 mg/kg dose groups, $p < .05$.
[e] Statistically different from the 150 and 600 mg/kg dose groups, $p < .05$.

TABLE 3

Mean (SD) cumulative urinary recovery of unchanged stavudine and total radioactivity following administration of a 50 mg/kg dose of [2-^{14}C] stavudine to male rats (N = 5) and 25 mg/kg dose of [4-^{14}C] stavudine to female monkeys (N = 3)

Route[a]	Time Interval	Mean (SD) Cumulative Urinary Recovery (% of Dose)			
		Rat		Monkey[b]	
		Unchanged stavudine	Total radioactivity	Unchanged stavudine	Total radioactivity
i.v.	0–24	86.8 (2.7)	84.1 (2.8)	43.9 (6.3)	49.5 (5.0)
	0–48	87.9 (3.1)	85.2 (2.9)	44.4 (6.6)	50.3 (5.4)
	0–72	88.2 (3.1)	85.6 (2.9)	44.4 (6.6)	50.4 (5.3)
	0–96	88.2 (3.1)	85.7 (3.0)	44.4 (6.6)	50.5 (5.4)
p.o.	0–24	85.0 (3.6)	83.0 (3.7)	36.9 (5.8)	37.9 (1.5)
	0–48	86.1 (3.1)	84.1 (3.1)	37.4 (5.8)	39.6 (3.1)
	0–72	86.4 (2.8)	84.5 (2.9)	37.4 (5.8)	39.8 (3.1)
	0–96	86.5 (2.7)	84.7 (2.7)	37.4 (5.8)	39.8 (3.1)

[a] IV, intravenous; PO, oral.
[b] From reference Cretton et al., 1993.

compound and a minor, very polar unidentified peak which represented 10% of the radioactivity. In the monkey liver S9 fraction, 63%, 13%, and 23% of the radioactivity in the 120-min sample was associated with the parent compound, thymine, and the polar peak, respectively; the human 240-min S9 sample contained mostly D4T, with only 1 and 13% of the radioactivity associated with thymine and the polar peak, respectively (Fig. 8A). Using rat liver slices, only parent compound was observed after 6-h incubation; a small amount of metabolism was observed in the 6-h human liver slice sample, with 2 and 7% of the radioactivity associated with thymine and the polar peak (Fig. 8B). In contrast, [*methyl*-^{14}C]thymidine was rapidly metabolized by the liver S9 (Fig. 8C) and liver slice preparations (Fig. 8D).

After a single 5-mg/kg oral dose of [2-^{14}C]D4T ([4-^{14}C]D4T) to rats, the radioactivity expired as $^{14}CO_2$ accounted for 18.1 (0.4)% [14.2 (1.1)%] and 0.2 (0.2)% [0.5 (0.2)%] of the dose in 0- to 24- and 24- to 48-h intervals, respectively.

Radiochromatograms of the metabolic profiles of whole blood, plasma, urine, cerebrospinal and amniotic fluids, and brain and fetal homogenates showed a single major peak (accounting for >90% of the radioactivity), which eluted at the retention time of D4T. In the brain homogenate, two minor peaks were observed at the retention time of 5 and 15 min, the identity of which could not be elucidated.

Interspecies Scaling. Allometric equations showed that the pharmacokinetic parameters, $CL = 1.10*W^{0.84}$, $R^2 = 0.98$, $p = .0001$; $V_{ss} = 0.81*W^{0.96}$, $R^2 = 0.99$, $p = .0001$; and $T_{1/2} = 0.62*W^{0.18}$, $R^2 = 0.89$, $p = .0014$, significantly correlated with body weight (Fig. 9).

Discussion

The absorption, distribution, metabolism, and excretion of D4T was carried out in a series of in vivo and in vitro studies employing the species used for toxicologic assessment: the mouse, rat, rabbit, and cynomolgus monkey. The toxicokinetic studies demonstrated that intact D4T was rapidly absorbed after oral administration. The extent of absorption was more or less complete, and the absolute oral bioavailability of D4T in the rat and monkey was in concurrence with that reported in the mouse (Russell et al., 1990). The $T_{1/2}$ values are in excellent agreement with previously reported values in rats and monkeys (Schinazi et al., 1990; Boudinot et al., 1991; Cretton et al., 1993; Kaul and Dandekar, 1993). Although carrier-mediated intestinal transport of D4T has been reported (Waclawski and Sinko, 1996), saturation of the absorption process was not observed at toxicologic doses because in the rat (monkey), for oral doses in 1:3:12 (1:3:10) proportion, the mean $AUC_{0-\infty}$ values for D4T were in the ratio of 1:5:22 (1:4:21). However, these results are partly confounded by the dose-dependent pharmacokinetics observed in the rats and monkeys.

The V_{ss} in the various species indicates that D4T is distributed in extravascular space. Results of tissue distribution studies with radiolabeled D4T in the rat support this observation. D4T is distributed in all tissues of the rat, including brain, cerebrospinal fluid, placenta, fetus, and milk, with highest concentrations in the organs of excretion, liver and kidneys. There was almost complete elimination of administered radioactivity within 48 h postdose. D4T has been reported to permeate a variety of cells, such as erythrocytes, by nonfacilitated diffusion (August et al., 1990). The RBC binding studies indicate that D4T was diffusible into RBCs by nonfacilitated diffusion because a 100-fold increase in D4T concentration had no effect on RBC permeation. The serum protein binding of D4T is negligible. The aforementioned results suggest that D4T can reach virus in tissues and blood cells and D4T will not lead to drug–drug interactions resulting from displacement of protein-bound drug.

The clearance of D4T proceeds by both renal and nonrenal elimination pathways. The CL_R of D4T is greater than endogenous creatinine clearance (Davies and Morris, 1993), indicating that in addition to glomerular filtration, D4T also undergoes active renal tubular secretion. The nonrenal clearance of D4T may in part be due to metabolism via the thymidine catabolic pathway, a process which is similar across most species, because thymine and β-aminoisobutyric acid were formed from D4T in rat and human liver homogenates and

TABLE 4

Mean (SD) concentrations of total radioactivity in tissues after administration of a 5 mg/kg oral dose of [4-^{14}C] stavudine to male rats (N = 3/gender)

Tissue	Mean (SD) Tissue Concentrations at Various Times (μg-eq/g)				
	0.5 h	3 h	6 h	24 h	48 h
Adrenal Gland	2.72 (0.70)	0.20 (0.10)	0.07 (0.04)	0.01 (0.00)	0.02 (0.00)
Aqueous Humor	0.87 (0.18)	0.30 (0.02)	0.05 (0.01)	0[c]	0
Blood[a]	2.61 (0.14)	0.18 (0.02)	0.03 (0.00)	0.01 (0.00)	0.01 (0.00)
Bone	0.16 (0.06)	0.07 (0.01)	0.02 (0.00)	0	0
Bone Marrow	2.42 (0.17)	0.26 (0.01)	0.06 (0.01)	0.02 (0.00)	0.02 (0.00)
Brain	0.30 (0.08)	0.12 (0.01)	0.04 (0.00)	0	0
CSF[a]	0.55 (0.12)	0.11 (0.04)	0.02 (0.00)	0	0
Eyes	0.98 (0.25)	0.08 (0.14)	0.06 (0.01)	0.04 (0.03)	0.01 (0.00)
Fat	1.83 (1.65)	0.13 (0.00)	0.06 (0.07)	0	0
Heart	3.07 (0.97)	0.17 (0.04)	0.03 (0.00)	0	0
Kidney	10.6 (1.91)	1.16 (0.82)	0.11 (0.02)	0.01 (0.00)	0.01 (0.00)
Large Intestine	2.47 (0.35)	0.49 (0.12)	1.49 (0.09)	0.06 (0.01)	0.03 (0.00)
Liver	5.31 (2.03)	0.29 (0.04)	0.09 (0.01)	0.04 (0.02)	0.04 (0.01)
Lung	2.99 (0.95)	0.20 (0.03)	0.03 (0.01)	0.01 (0.00)	0.01 (0.00)
Lymph Nodes	2.13 (0.28)	0.16 (0.04)	0.03 (0.01)	0.01 (0.01)	0.01 (0.00)
Ovaries[b]	2.55 (0.56)	0.21 (0.07)	0.04 (0.01)	0.01 (0.00)	0
Pancreas	3.89 (2.21)	0.27 (0.09)	0.11 (0.07)	0.01 (0.00)	0.01 (0.00)
Pituitary Gland	4.19 (1.43)	0.15 (0.04)	0.04 (0.01)	0.01 (0.00)	0.01 (0.00)
Plasma[a]	3.18 (0.15)	0.22 (0.03)	0.05 (0.01)	0.01 (0.00)	0.01 (0.00)
Salivary Gland	2.56 (0.46)	0.22 (0.04)	0.07 (0.01)	0.01 (0.00)	0.01 (0.00)
Skeletal Muscle	1.91 (0.42)	0.29 (0.13)	0.03 (0.02)	0	0
Skin	2.06 (0.23)	0.18 (0.04)	0.03 (0.01)	0.01 (0.00)	0.01 (0.00)
Small Intestine	14.5 (2.03)	1.16 (0.00)	0.18 (0.06)	0.02 (0.01)	0.01 (0.00)
Spleen	2.55 (0.19)	0.19 (0.04)	0.05 (0.02)	0.01 (0.00)	0.01 (0.00)
Stomach	26.3 (22.1)	0.41 (0.17)	0.11 (0.02)	0.01 (0.00)	0.01 (0.00)
Testes	0.78 (0.13)	0.55 (0.08)	0.12 (0.02)	0.01 (0.00)	0.01 (0.00)
Thymus	2.42 (0.35)	0.21 (0.06)	0.04 (0.01)	0.02 (0.01)	0.02 (0.00)
Thyroid	2.80 (1.07)	0.18 (0.03)	0.04 (0.01)	0	0
Tongue	2.56 (0.49)	0.20 (0.02)	0.05 (0.01)	0.01 (0.00)	0.01 (0.00)
Trachea	2.00 (0.31)	0.18 (0.08)	0.03 (0.01)	0.01 (0.00)]	0.01 (0.00)
Urinary Bladder	20.0 (11.7)	31.3 (25.8)	1.95 (2.58)	0.01 (0.00)]	0.01 (0.00)
Uterus[b]	3.00 (0.43)	0.21 (0.10)	0.03 (0.01)	0.01 (0.00)	0
Vena Cava	3.92 (1.90)	0.82 (0.71)	0.11 (0.11)	0	0

[a] Concentrations are expressed as μg-eq/ml.
[b] In females only.
[c] Not detectable; counts were <60 dpm/sample and counting error was >20%.

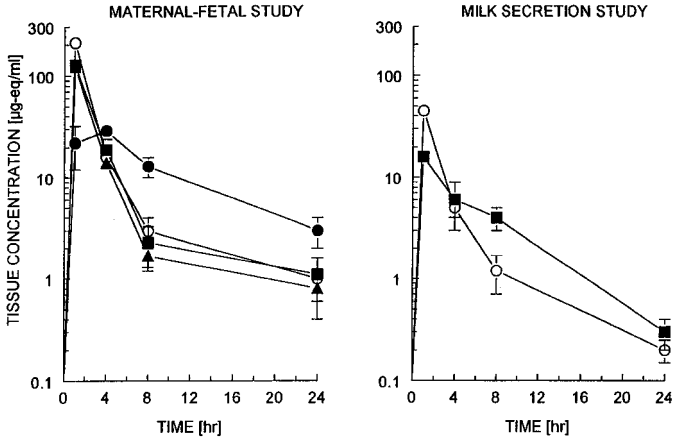

FIG. 4. *Mean ± S.D. tissue concentrations of radioactivity after oral administration of radiolabeled D4T to rats.*

Maternal-fetal study: dose, 300 mg/kg (46 μCi/kg) of [2-^{14}C]D4T; ○, maternal plasma; ■, fetal tissue; ▲, placental tissue; ●, amniotic fluid; N = 3 animals/time point. Milk secretion study: dose, 100 mg/kg (150 μCi/kg) of [4-^{14}C]D4T; ○, maternal plasma; ■, milk; N = 3 animals/time point.

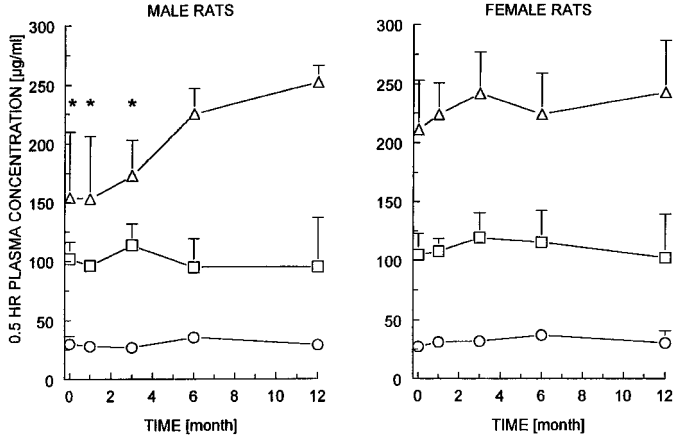

FIG. 5. *Mean (S.D.) 0.5-h plasma concentrations after oral administration of D4T to male and female rats in the general toxicology studies.*

The total daily dose was administered as two equally divided doses 4 to 6 h apart. ○, 100 mg/kg/day; □, 300 mg/kg/day; △, 600 mg/kg/day; N = 5/gender/dose group; [asterisk], plasma concentrations in male rats were significantly different compared with female rats ($p < .05$).

$^{14}CO_2$ was recovered from expired air in rats. Fecal excretion, and by inference biliary excretion, does not contribute significantly to the elimination of D4T as fecal excretion averaged <1.5%.

An extensive battery of toxicology and special studies were conducted in laboratory animals with D4T. In the 1-month repeat-dose toxicity study in rats, increased liver weights were observed in the male rats at the 300- and 600-mg/kg/day dose levels (Schilling et al., 1995). To determine whether the increase in liver weight was due to induction of the CYP450 enzymes, rat and monkey liver samples were analyzed for CYP450 and total protein in the 3-month repeat-dose toxicity studies. In general, CYP450 levels were not significantly different from control animals, suggesting that perturbation of

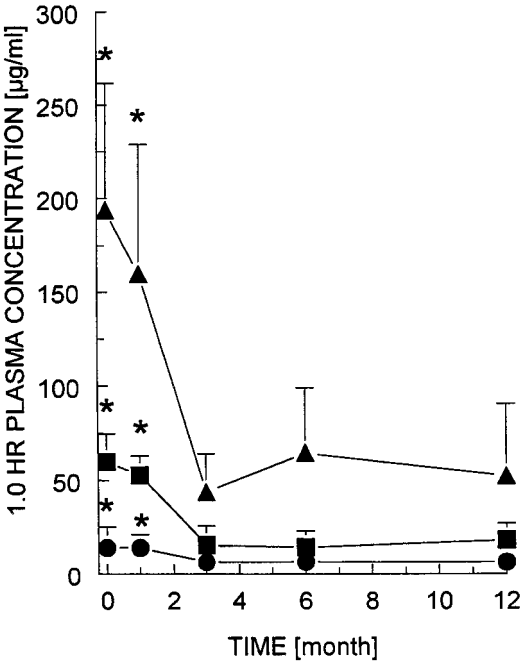

FIG. 6. *Mean (S.D.) 1.0-h plasma concentrations after oral administration of D4T to monkeys in the general toxicology studies.*

The total daily dose was administered as two equally divided doses 4 to 6 h apart. ●, 60 mg/kg/day; ■, 200 mg/kg/day; ▲, 600 mg/kg/day; *N* = 3/gender/dose group; [asterisk], the day 1 and 1-month plasma concentrations were significantly different compared with the 3, 6, and 12 month concentrations ($p < .05$).

TABLE 5

Stavudine exposures in selected toxicology studies in mice, rats, and rabbits

Type of Study	Species	Dose[a]	Mean (SD) Parameter Value	
			C_{max}[b]	AUC[c]
		(mg/kg/day)	*(µg/ml)*	*(µg · h/ml)*
Fertility and reproduction	Rat	100	30.5 (3.4)	ND[d]
	(*N* = 4)	300	89.3 (7.6)	ND
		600	142 (41.7)	ND
Teratology	Rat	50	16.2 (0.7)	ND
	(*N* = 4)	250	93.6 (12.4)	ND
		1000	262 (80.2)	ND
	Rabbit	60	11.5 (2.3)	ND
	(*N* = 3)	300	26.3 (16.9)	ND
		600	120 (34.5)	ND
Neurotoxicity (part 1)	Rabbit	600	140 (19.9)	1245 (737)
	(*N* = 3)	1500	427 (1.4)	3126 (129)
Neurotoxicity (part 2)	Rabbit	750	289 (65.7)	ND
	(*N* = 5)	1500	724 (133)	ND
Carcinogenicity[e]	Mouse	80	1.64	29.9
	(*N* = 3)	400	5.05	92.1
		2000	31.9	587
	Rat	100	4.07	79.2
	(*N* = 3)	600	26.8	394
		2000	155	1723

[a] Doses were administered by gavage approximately 4 to 6 h apart.
[b] Except for the neurotoxicity study, C_{max} was sampled at 0.5 h; in the neurotoxicity study, C_{max} occurred at 1.0 h.
[c] Area under the curve in a 24-h interval.
[d] ND, not determined.
[e] Standard deviations are missing because composite plasma profiles were obtained; C_{max} occurred between 2 and 10 h.

CYP450 was not responsible for the increase in liver weight. Indeed, lipid containing hepatocellular vacuolation correlated with increase in liver weight (Schilling et al., 1995). These findings tend to suggest an "adaptive" rather than a toxic response to D4T (Schulte-Hermann, 1974). The multiples of exposures observed in the drug safety evaluation studies indicate a wide safety margin and confirmed that adequate doses of D4T were administered to laboratory animals to discern potential risk in humans (Table 7). Intriguingly, peripheral neuropathy, the major side effect associated with the clinical use of D4T (Lea and Faulds, 1996), was not observed in the drug safety evaluation studies in the laboratory animals in spite of the enormous multiples of human exposure. This difference is attributed to the HIV infection in humans (Simpson and Tagliati, 1995), whereas, the toxicology studies were carried out in disease-free laboratory animals.

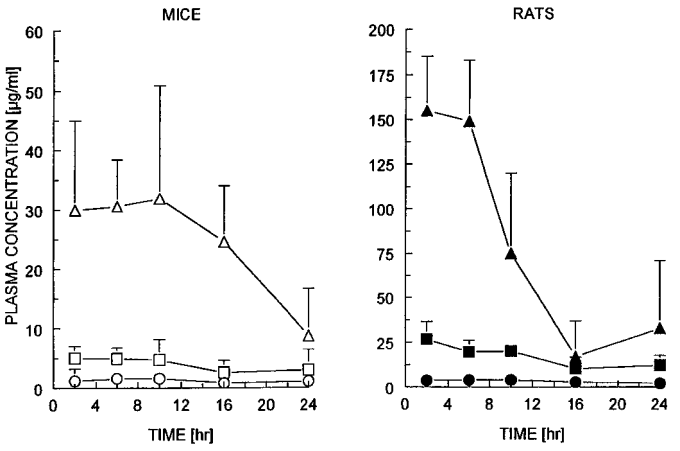

FIG. 7. *Mean (S.D.) plasma concentrations after one month of dietary administration of D4T to mice and rats in the carcinogenicity studies.*

Mouse study: ○, 80 mg/kg/day; □, 400 mg/kg/day; △, 2000 mg/kg/day; N = 3/gender/time point. Rat study: ●, 100 mg/kg/day; ■, 600 mg/kg/day; ▲, 2000 mg/kg/day; N = 3/gender/time point.

TABLE 6

Mean (SD) CYP-450 levels in rats and monkeys (N = 3/gender)

Species	Dose	Mean (SD) CYP450 Levels[a]
	(mg/kg/day)	*(nmol/mg protein)*
Rat	Control[b]	0.440 (0.084)
	100	0.444 (0.049)
	300	0.415 (0.108)
	600	0.397 (0.084)
Monkey	Control	0.791 (0.072)
	60	0.977 (0.084)[c]
	200	0.807 (0.083)
	600	0.688 (0.121)

[a] CYP-450 levels were not significant between gender.
[b] Control animals were administered sterile water.
[c] $p < 0.05$ compared with control.

In the general toxicology studies in rats and monkeys, a number of interesting observations were noted. Although there were no gender differences in the plasma D4T concentrations in the monkeys, the 0.5-h plasma D4T concentrations in the male and female rats were significantly different up to 3 months (Fig. 5). Albeit the reason for this gender difference is unclear, beyond 3 months, no significant differences were observed between male and female rats. In the studies with the monkeys, the 1-h plasma D4T concentrations up to 1 month were significantly greater (2- to 4-fold) than the values observed in the 3- to 12- month period (Fig. 6). In the 1-month study, D4T was administered orally to monkeys after an overnight fast; whereas, in the 3- and 12-month studies, the animals were fed about 2 h before dosing. This appeared to be the only exceptional difference in study conduct and suggests that food may influence oral absorption of D4T. Indeed, pharmacokinetic data in humans corroborate the finding in the monkey that the absorption rate of D4T is influenced by food intake (Lea and Faulds, 1996). Based on a recent report which

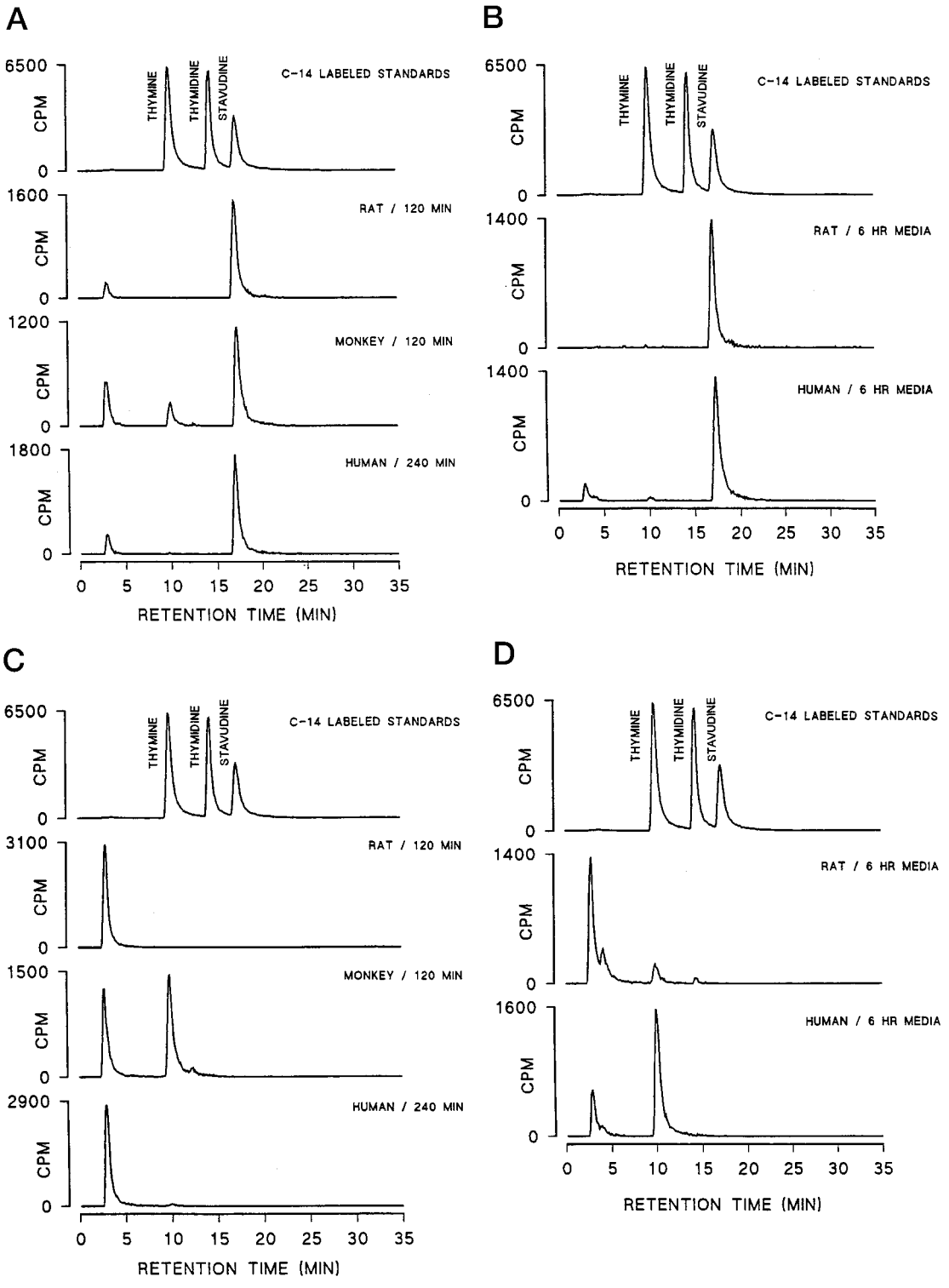

FIG. 8. *Radiochromatograms after incubation of 100 μM [4-^{14}C]D4T with S9 fraction from rat, monkey, and human liver (A), 100 μM [4-^{14}C]D4T with liver slices from rat and human liver (B), 100 μM [methyl-^{14}C]thymidine with S9 fraction from rat, monkey, and human liver (C), and 100 μM [methyl-^{14}C]thymidine with liver slices from rat and human liver (D).*

characterized AUCs by the sparse sampling approach (Pai et al., 1996), one criticism of our investigation to verify exposure in some of the drug safety evaluation studies could be that D4T concentration at a single time point was used to verify exposure of laboratory animals. The selection of the single time point (0.5 and 1.0 h in rats and monkeys, respectively), which was approximately the time of the peak plasma D4T concentration, was based on the complete plasma concentration-time profile after single oral doses. Moreover, as shown in Fig. 10, the relationship between the 0.5- (1.0) h concentration and D4T $AUC_{0-\S}$ in the rats (monkeys) suggests that this concentration is a reasonable surrogate for AUC.

The key pharmacokinetic parameters of the anti-HIV nucleoside

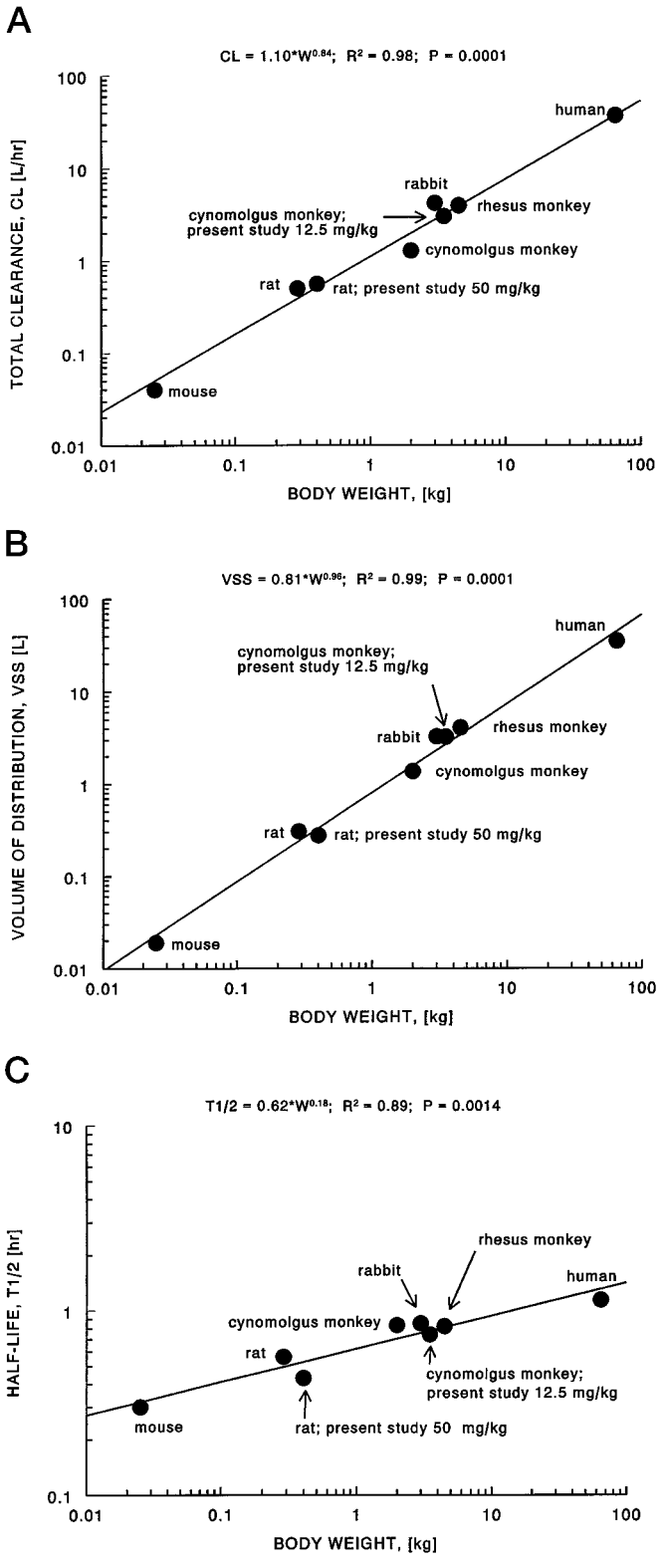

FIG. 9. *Interspecies correlations among CL (A), V_{ss} (B), and half-life, $T_{1/2}$ (C) of D4T and species body weight.*

analogues (Russell and Klunk, 1989; Ibrahim and Boudinot, 1989; Patel et al., 1990; Qian et al., 1991; Kaul et al., 1991; Knupp et al., 1991; Wientjes and Au, 1992; van Leeuwen et al., 1992; Hussey et al., 1994) in various mammalian species are compared in Table 8. Although the rest of the compounds are pyrimidine-type of nucleoside

TABLE 7

Multiples of human exposure to stavudine of the animals in the toxicology studies

Study	Species	Dose	Multiples of Human Exposure
		(mg/kg/day)	
12 mo	Rat	100[a]	50×
		300	155×
		600	358×
	Monkey	60[a]	10×
		200	24×
		600	67×
Fertility and reproduction	Rat	100	46×
		300[a]	135×
		600	216×
Teratology	Rat	50	24×
		250[a]	143×
		1000	399×
	Rabbit	60	18×
		300	82×
		600[a]	183×
Maternal-fetal	Rat Fetus	300[a]	146×
Neurotoxicity	Rabbit	750	440×
		1500[a]	1102×
Carcinogenicity	Mouse	80	13×
		400[b]	39×
		2000	250×
	Rat	100	34×
		600[b]	168×
		2000	732×

[a] No-effect dose level from reference Schilling et al., 1995.
[b] No-effect dose in carcinogenicity studies; data on file.

analogs, didanosine (DDI) is a purine nucleoside analog. All of these drugs have some common features. Qualitatively, they are rapidly cleared from the body by renal excretion of unchanged drug and metabolism. In general, the CL of these compounds is relatively high in rodents in comparison to humans, which is in accordance with the principles of allometry. The V_{ss} of the nucleoside analogues suggests that these molecules distribute in total body water, which is consistent with their low protein binding and hydrophilicity. However, the extent of unchanged drug recovered in urine is dissimilar in the various species and between the nucleoside analogues. The percentage of D4T and zidovudine (ZDV) dose recovered in rodent urine is approximately 2- to 3-fold higher than in human urine. Although the urinary recovery of zalcitabine (DDC) is relatively constant across species, the urinary recovery of unchanged lamivudine (3TC) and DDI tend to increase as one goes from lower to higher species. These data indicate that different elimination mechanisms are responsible for the above-mentioned quantitative differences.

The interspecies scaling of pharmacokinetics has been successfully utilized for ZDV (Patel et al., 1990), DDC (Ibrahim and Boudinot, 1989), and 3TC and DDI (Hussey et al., 1994). Using the simple allometric equation from this study, the predicted values for clearance, volume of distribution, and half-life of D4T for a 70-kg human were 9.29 ml/min/kg, 0.68 liters/kg, and 1.3 h, respectively; these values deviated by +14, +28, and +18% from the observed values, respectively, suggesting a reasonably accurate prediction of data in humans using interspecies scaling. Although data in several species were used for interspecies scaling, a recent report suggested that, for a reliable prediction, data in three or more species are needed for clearance and two or more species are required for volume of distribution (Mahmood and Balian, 1996). This approach was investigated with D4T data from mouse, rat, and monkey. When two species (mouse and monkey) were used for interspecies scaling, the predicted values for CL (10.2 ml/min/kg), V_{ss} (1.01 liters/kg), and $T_{1/2}$ (1.2 h) for a 70-kg human deviated from the observed values by +25, +94, and +5%,

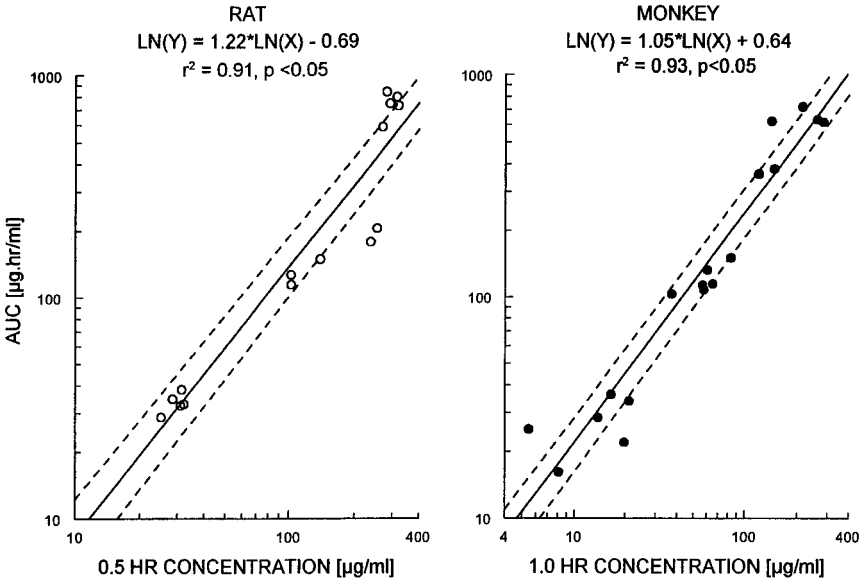

FIG. 10. *The relationship between the 0.5- (1.0-) h concentration and AUC in rats (monkeys).*

Solid lines, linear regression lines and dashed lines, approximate 95% confidence bands.

TABLE 8

Key pharmacokinetic parameters for nucleoside analogues obtained in various mammalian species

Parameter	Species[b]	Nucleoside Analogue[a]				
		D4T	ZDV	DDC	3TC	DDI
CL *(ml/min/kg)*	Mouse	28.7	22.7	25.0	ND	75.0
	Rat	24.0	47.0	25.2	20.0	66.0
	Monkey	14.5	26.2	7.60	12.7	12.3
	Dog	ND[c]	13.9	ND	6.06	22.7
	Human	8.17	26.7	6.33	5.41	13.5
V_{ss} *(l/kg)*	Mouse	0.76	0.73	0.59	ND	0.90
	Rat	0.70	1.61	1.54	2.70	0.99
	Monkey	0.92	1.08	0.87	1.16	0.92
	Dog	ND	1.00	ND	0.87	0.95
	Human	0.53	1.40	0.54	1.30	0.81
$T_{1/2}$ *(h)*	Mouse	0.3	1.1	1.5	ND	0.1
	Rat	0.4	1.0	1.0	1.6[d]	0.6
	Monkey	0.7	0.5	1.8	1.4	1.2
	Dog	ND	1.0	ND	1.7[d]	1.1
	Human	1.1	1.1	1.2	2.5	1.2
UR *(%)*	Mouse	ND	70	64	ND	ND
	Rat	79	72	49	ND	28
	Monkey	44	40	68	46	19
	Dog	ND	65	ND	ND	46
	Human	43	19	75	71	50

[a] D4T, stavudine; ZDV, zidovudine; DDC, zalcitabine; 3TC, lamivudine; DDI, didanosine.
[b] Data from references Hussey et al., 1994; Ibrahim and Boudinot, 1989; Kaul et al., 1991; Knupp et al., 1991; 1992; Patel et al., 1990; Qian et al., 1991; Russell et al., 1990; Russell and Klunk, 1989; van Leeuwen et al., 1992; Wientjes and Au, 1992.
[c] ND, not determined.
[d] Estimated from clearance and volume terms.

respectively; inclusion of data from a third species (rat), resulted in an improvement in the predicted values, which deviated by +34, +51, and 0%, respectively. These results suggest that for D4T, data from three or more species are needed to reliably predict the pharmacokinetic parameters in humans.

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