High-Performance Liquid Chromatographic Determination of 2′,3′-Didehydro-3′-deoxythymidine (d4T) in Human and Rabbit Plasma and Urine and Its Application to Pharmacokinetic Studies in the Rabbit

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A rapid and sensitive liquid chromatographic assay for 2′,3′-didehydro-3′-deoxythymidine (d4T) in plasma and urine is described. This assay uses thymidine oxetane (TO), a synthetic precursor of d4T, as internal standard. Sample preparation involves a simple extraction of plasma or urine with 5% isopropyl alcohol in methylene chloride. The method is specific and sensitive, allowing a linear response over a 2000-fold range of concentrations in human plasma (5 ng/ml to 10 μg/ml) and urine (50 ng/ml to 100 μg/ml). This assay, developed for human plasma and urine, is also applicable to rabbit samples with minor modification. Intravenous bolus doses of 10 mg/kg d4T to rabbits showed that the plasma concentration–time profile followed a biexponential decay. Estimates of the distribution and elimination half-lives were 6.7 ± 0.9 and 51 ± 6 min, respectively. The total-body and renal clearances were 23.4 ± 3.6 and 8.82 ± 3.9 ml/min · kg, respectively. That the renal clearance exceeds the glomerular filtration rate in the rabbit suggests that d4T is actively secreted in the renal tubule. The fraction excreted unchanged in the urine was 36 ± 8%. Similar results were obtained in the same rabbits at steady state during constant-rate intravenous infusion. Noncompartmental analysis estimates of the MRT and V_{dss} were 46 ± 5 min and 1.08 ± 0.13 L/kg, respectively.

KEY WORDS: 2′,3′-didehydro-3′-deoxythymidine (d4T); high-performance liquid chromatographic (HPLC) analysis; pharmacokinetics; rabbit model; renal secretion.

INTRODUCTION

2′,3′-Didehydro-3′-deoxythymidine (d4T or BMY-27857) is an unsaturated nucleoside analogue synthesized by Horwitz and co-workers (1). *In vitro* studies using various bioassays (2–6) demonstrated the potency of d4T to be comparable to that of 3′-azido-3′-deoxythymidine (zidovudine; AZT) in inhibiting human immunodeficiency virus (HIV). Acting at the level of reverse transcriptase, d4T effectively inhibited the cytopathic effects and expression of HIV-specific antigens of infected cells by blocking viral replication (2–4). Compared to AZT, the drug exhibits less toxicity as shown by its higher therapeutic index (2–5) in cell culture studies *in vitro*. In studies carried out in rat and monkey (7), no metabolites of d4T could be detected in the urine. The drug is currently undergoing clinical studies.

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Recently, a selective HPLC method using solid-phase extraction for the analysis of d4T in monkey and rat plasma was reported (7). The assay described here, which involves liquid phase extraction, not only provides for greater sensitivity in the analysis of this drug in human and rabbit plasma, but also permits the analysis of d4T in human and rabbit urine. This is significant since the renal clearance of this drug in the rabbit is approximately three times the glomerular filtration rate, indicating that renal excretion is an important route of elimination. The assay was fully validated in human plasma and urine and applied to preliminary pharmacokinetic studies in the rabbit.

MATERIALS AND METHODS

Chemicals

D4T, the reference compound (BMY-27857, Lot No. C88G574), and thymidine oxetane (TO), the internal standard (BMY-33644, Lot No. 25876-075), were gifts from Bristol-Myers Co., Syracuse, NY. Acetonitrile, isopropyl alcohol (Burdick and Jackson Laboratories, Inc., Muskegon, MI), methyl alcohol (EM Science, Gibbstown, NJ), and methylene chloride (Fisher Scientific, Fair Lawn, NJ) were HPLC grade. Ammonium phosphate monobasic (AR grade) was purchased from Mallinckrodt, Inc., St. Louis, MO.

Instrumentation

Analysis was performed using a high-pressure liquid chromatograph (Model 1090, Hewlett-Packard, Palo Alto, CA), equipped with an automatic injection system. Separation utilized a 15-cm × 4.6-mm (i.d.) prepacked C_{18} Supelcosil reversed-phase column with an average particle size of 5 μm (LC-18, Supelco Inc., Bellefonte, PA) attached to a 2-cm Supelguard LC-18 precolumn. The composition of the mobile phase is 10 m*M* monobasic ammonium phosphate (pH = 4.60) and acetonitrile (93.5:6.5, by volume), mixed on line. Flow rate is maintained at 0.75 ml/min. The column temperature is ambient, and the effluent is monitored at 264 nm (the λ_{max} of d4T at this mobile phase composition) using a variable-wavelength UV detector (Model SPD-6A, Shimadzu, Kyoto, Japan). Peak heights are measured with an electronic integrator (Model 3390, Hewlett–Packard).

Sample Preparation

D4T was dissolved in methanol to prepare stock solutions of 10 and 1 μg/ml. TO was dissolved in methanol to prepare a standard solution of 5 μg/ml. A 12-point standard curve was prepared by adding appropriate volumes of the d4T stock solutions into a series of 13-ml ground glass-stoppered centrifuge tubes (Kontes, Evanston, IL) in the amount of 0 (blank), 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, and 10 μg for the plasma. In another set of similar tubes, a urine standard curve of d4T is prepared in the same way. Twenty microliters of the internal standard solution was added to each tube in the plasma and urine standard curve (except the blank) and to each plasma and urine sample tube. The methanol was evaporated from the tubes at 45°C under reduced pressure in an evaporator (Evapo-Mix, Buchler Instruments, Fort Lee, NJ).

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One milliliter of blank human plasma (or 100 μl of blank urine diluted to 1 ml with distilled water) was added to each of the standard curve tubes, and 1 ml of the plasma sample (or 100 μl of urine sample diluted to 1 ml with distilled water) was added to the appropriate sample tube spiked with internal standard. Eight milliliters of the extracting solvent (5% isopropyl alcohol in methylene chloride) was added. Tubes were stoppered and shaken horizontally at 180 cycle/min on a mechanical shaker (Eberbach Corp., Ann Arbor, MI) for 10 min. After centrifugation at 750*g* (Damon, Needham Heights, MA), the supernate was aspirated and discarded. The organic layer was transferred to a set of clean 13-ml glass-stoppered centrifuge tubes and the solvent was evaporated at 45°C under reduced pressure. The residue was reconstituted in 100 μl of mobile phase, vortex-mixed, and transferred to a microvial for automatic injection. Twenty microliters of the reconstituted sample was injected, using the chromatographic conditions described above.

Calculations

The peak-height ratios of d4T to TO (internal standard) were used to construct the standard curve. Weighted least-squares linear regression (8) of the peak-height ratios as a function of the standard concentration was applied to each standard curve. Sample concentrations of d4T in plasma or urine were then determined from the respective regression equations.

Pharmacokinetic Studies in the Rabbit

Five male New Zealand White rabbits, weighing 3.1 ± 0.1 kg (Birchwood Farm Rabbitry, Birchwood, WI), received a single intravenous bolus dose of 10 mg/kg d4T dissolved in 3 ml of normal saline. The drug was administered over 2 min through a catheter inserted into the anterior vena cava via the marginal ear vein (9). Blood samples (0.5 ml) were collected over a period of 10 hr in heparinized tubes through a catheter inserted in the other ear. Plasma was obtained immediately by centrifugation. Urine samples were collected at timed intervals over 10 hr through a pediatric Foley catheter, which was inserted into the rabbit bladder via the urethra (9). Frequent irrigation of the bladder with normal saline maintained at 37°C was used toward the end of the timed interval to ensure complete recovery of bladder contents.

At the end of the intravenous bolus experiment, a constant-rate intravenous infusion of 3.0 mg/hr of d4T in four of the five rabbits was begun immediately and maintained for 10 hr to achieve steady state. Plasma samples were collected at the midpoint of the hourly urine collection interval for 6 additional hr. All urine and plasma samples were stored at −20°C until analysis. The method described above for the analysis of human samples was scaled appropriately to analyze 0.1 ml of rabbit plasma and 0.1 ml of diluted rabbit urine.

Pharmacokinetic Analysis

Compartmental analysis of d4T plasma concentration–time data obtained after i.v. bolus administration was performed on an IBM PS/2 microcomputer using PCNONLIN (10). The disposition of drug was fitted to a two-compartment pharmacokinetic model with elimination from the central compartment.

$$C_p = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha t} + \frac{X_0(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta t} \quad (1)$$

The plasma concentration–time data of each rabbit were fitted to Eq. (1) using the reciprocal of the observation squared as the weighting function. Pharmacokinetic parameters were obtained from the PCNONLIN output. Noncompartmental analysis was utilized to calculate V_{dss} and MRT (11). The fraction of the i.v. bolus dose excreted unchanged was calculated from the amount of d4T recovered in the urine. The renal clearance was estimated as the slope [Eq. (2)] of the plot of ΔAe_{t1}^{t2} versus ΔAUC_{t1}^{t2}.

$$\Delta Ae_{t1}^{t2} = Cl_r * \Delta AUC_{t1}^{t2} \quad (2)$$

In the analysis of the infusion data, steady state was assumed. Total-body clearance was calculated as the ratio of infusion rate (normalized to body weight) to steady-state plasma concentration. The renal clearance was obtained from the ratio of the urinary excretion rate to the midpoint steady-state plasma concentration of the respective urine collection interval. The fraction excreted unchanged was calculated for each hourly interval, as

$$fe = \frac{\Delta Ae/\Delta t}{k_0} \quad (3)$$

RESULTS AND DISCUSSION

Assay Validation

D4T and TO were extracted from human plasma and urine into a water-immiscible organic phase using a simple and rapid procedure. Because endogenous compounds were well separated from the drug and internal standard, no further cleanup of the sample was necessary.

Retention times of d4T and the internal standard were approximately 9.7 and 14.3 min, with coefficients of variation (within-run) of 0.47 and 0.55%, respectively. For routine analysis of human plasma or urine, the recommended run time is 24 min to avoid interference from late-eluting peaks. Typical chromatograms for blank human plasma and urine, with and without the addition of drug and internal standard, are shown in Fig. 1.

The analysis of rabbit plasma in this study was performed using a 19-min run time with 7% of acetonitrile in the mobile phase. Chromatograms obtained in the analysis of rabbit samples produced retention times for d4T and TO of approximately 8.7 and 12.7 min, with coefficients of variation (within run) of 0.18 and 0.16%, respectively. These retention times are very sensitive to minor changes in the concentration of acetonitrile in the mobile phase.

Sensitivity. Analytical sensitivity was established from human plasma standard curves analyzed on 5 days according to the method described by Oppenheimer *et al.* (8). The critical level was 0.00128 ± 0.00044 μg/ml. The detection limit was 0.00258 ± 0.00090 μg/ml, while the determination limit was calculated as 0.00670 ± 0.0024 μg/ml. Using the

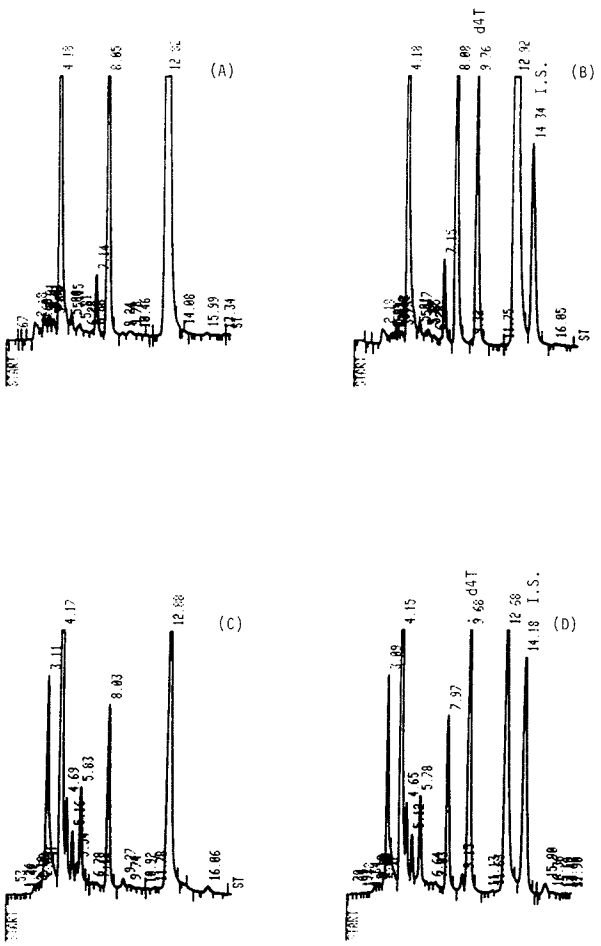

Fig. 1. Typical chromatograms in the analysis of d4T in human plasma and urine. (A) Blank human plasma; (B) blank human plasma spiked with d4T (0.2 μg/ml) and internal standard; (C) blank human urine; (D) blank human urine spiked with d4T (2.0 μg/ml) and internal standard.

same method, the critical level, detection limit, and determination limit for five human urine standard curves were 0.00610 ± 0.0022, 0.0122 ± 0.0045, and 0.0352 ± 0.015 μg/ml, respectively, utilizing 100 μl of urine (diluted to 1.0 ml) in the analysis.

Linearity and Precision. The peak-height ratios of drug to the internal standard obtained in both the plasma and the urine standard curves were linear despite a two thousand-fold range of concentrations. Within-day variation was assessed by simultaneously analyzing three different plasma and four different urine standard curves. Day-to-day variation was evaluated by preparing standard curves on each of 4 days over a period of 21 days. Table I shows that the range of coefficient of variation (within day) was 0.59–5.4% for plasma and 0.50–9.6% for urine, while the range of coefficient of variation (day to day) was 2.2–7.8% and 0.92–11% for plasma and urine, respectively.

The coefficient of variation for day-to-day precision was 1.4–6.2 and 0.80–11.0% for rabbit plasma (0.10–20 μg/ml) and urine (0.10–100 μg/ml), respectively, using 0.1 ml of sample.

Accuracy. Quality control in the analysis of d4T plasma samples (0.010–5.0 μg/ml) and urine samples (0.10–50 μg/ml) was evaluated by adding known amounts of drug to blank plasma or urine using an independently prepared standard solution. Quality-control aliquots were stored at −20°C until analysis. Accuracy, evaluated by assaying these samples over a 21-day period, ranged from 95.6 to 104% for plasma and 94.2–103% for urine, with coefficients of variation ranging from 2.6 to 9.6 and 1.4 to 11%, respectively.

Analytical Recovery. At concentrations of 0.020, 0.50, and 5.0 μg/ml of d4T, peak-height ratios of five extracted plasma samples were compared with the peak-height ratios of five unextracted samples. Internal standard was added to all samples just prior to injection. The mean percentage of recovery, without correction for volume loss during aspiration of the aqueous phase or transfer, was 46%. This modest

Table I. Analytical Precision for Assay of d4T in Human Plasma and Urine

d4T assay precision in plasma					d4T assay precision in urine				
	(A) Within run (n = 3)[a]		(B) Run to run (n = 4)[b]			(A) Within run (n = 4)[a]		(B) Run to run (n = 4)[b]	
Conc. (μg/ml)	Peak height ratio (mean ± SD)	CV (%)	Peak height ratio (mean ± SD)	CV (%)	Conc. (μg/ml)	Peak height ratio (mean ± SD)	CV (%)	Peak height ratio (mean ± SD)	CV (%)
0	0	—	0	—	0	0	—	0	—
0.005	0.0317 ± 0.0017	5.4	0.0408 ± 0.0032	7.8	0.050	0.0311 ± 0.0030	9.6	0.0329 ± 0.0020	6.1
0.010	0.0669 ± 0.0027	4.0	0.0772 ± 0.0026	3.4	0.100	0.0587 ± 0.0052	8.8	0.0676 ± 0.0072	11
0.020	0.130 ± 0.0032	2.5	0.148 ± 0.0048	3.2	0.200	0.123 ± 0.011	8.9	0.132 ± 0.0081	6.1
0.050	0.358 ± 0.014	3.9	0.345 ± 0.0075	2.2	0.500	0.330 ± 0.018	5.4	0.330 ± 0.010	3.0
0.100	0.684 ± 0.017	2.5	0.713 ± 0.026	3.6	1.000	0.654 ± 0.0033	0.50	0.667 ± 0.019	2.8
0.200	1.39 ± 0.017	1.2	1.44 ± 0.034	2.4	2.000	1.32 ± 0.013	0.98	1.35 ± 0.029	2.1
0.500	3.42 ± 0.040	1.2	3.34 ± 0.23	6.9	5.000	3.31 ± 0.035	1.0	3.42 ± 0.069	2.0
1.000	6.77 ± 0.040	0.59	7.11 ± 0.25	3.5	10.00	6.62 ± 0.046	0.69	6.70 ± 0.062	0.92
2.000	13.7 ± 0.12	0.88	14.4 ± 0.73	5.1	20.00	13.3 ± 0.22	1.6	13.5 ± 0.41	3.0
5.000	34.1 ± 0.52	1.5	35.0 ± 2.4	6.8	50.00	33.0 ± 0.45	1.4	34.4 ± 1.7	4.9
10.00	68.1 ± 0.59	0.87	68.7 ± 3.4	4.9	100.0	64.9 ± 1.4	2.2	68.5 ± 2.6	3.8
Slope	6.81 ± 0.033	0.48	6.95 ± 0.36	5.2	slope	0.659 ± 0.0075	1.1	0.684 ± 0.026	3.8

[a] Analyzed on the same day.
[b] Analyzed on 4 days.

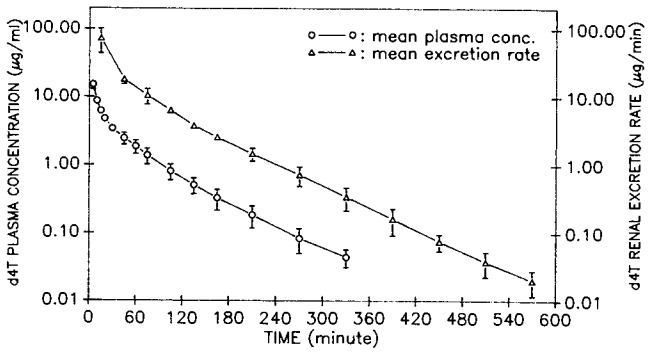

Fig. 2. Mean d4T plasma concentration–time profile (○——○), and urinary excretion rate–time profile (△——△) in rabbits receiving a single i.v. bolus of 10 mg/kg.

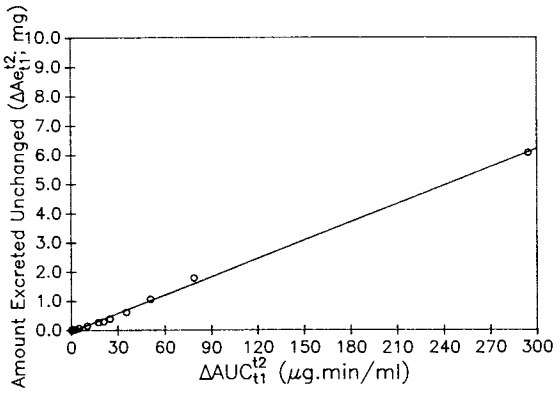

Fig. 3. Plot of ΔAe_{t1}^{t2} versus ΔAUC_{t1}^{t2} for rabbit 1.

recovery is due in part to the relatively high water solubility of the drug. Nevertheless, good analytical precision and accuracy were obtained, and recoveries over the range of concentrations used were not significantly different from one another ($P > 0.36$, by one-way ANOVA). Recovery of d4T in the urine analysis was not determined; however, a comparison of the slopes (urine vs plasma) of absolute peak-height versus concentration showed no statistical difference ($P > 0.51$), suggesting that the recoveries from plasma and urine are similar.

Pharmacokinetics of d4T in the Rabbit

In the 10 mg/kg d4T intravenous bolus study, both the plasma concentration–time and the urinary excretion rate–time curves declined biexponentially, and appeared proportional to each other in each animal, indicating concentration independent renal clearance. Figure 2 shows the mean of the plasma concentration–time and urinary excretion rate–time profiles. Plasma concentrations were measurable up to 5.5 hr. The distribution half-life was 6.7 ± 0.9 min and the elimination half-life was 51 ± 6 min, according to the two-compartment model. The fraction of drug excreted unchanged in urine was 36 ± 8%. Table II summarizes the mean pharmacokinetic parameters obtained from the studied rabbits based on compartmental analysis. Total-body clearance was 23.4 ± 3.6 ml/min · kg. Urine collection could not be performed in one rabbit because of catheter failure. Figure 3 shows a typical plot of the data according to Eq. (2). The renal clearance ($n = 4$), estimated as the slope of the regression line, was 8.82 ± 3.9 ml/min · kg. This high renal clearance of d4T exceeds the glomerular filtration rate in the rabbit (12), confirming the active secretion of d4T in the renal tubule. This observation is consistent with the results of previous studies utilizing similar nucleoside analogues in the rabbit and in normal humans (13,14). Estimation of the renal clearance as the ratio of the urinary excretion rate to the midpoint plasma concentration in each urine collection yielded comparable values.

Noncompartmental analysis of the same data provided estimates of MRT and V_{dss} of 46.3 ± 5 min and 1.08 ± 0.13 L/kg, respectively. The proportions of the AUC and AUMC not accounted for during the sampling period were only 0.46 and 4.0%, respectively, indicating that the error in this analysis is minimal. As a result, there is no significant difference between the V_{ss} and the V_{dss} ($P > 0.65$), suggesting that both the compartmental and the noncompartmental analyses are equally reliable in characterizing the volume of distribution of d4T at steady state.

The mean pharmacokinetic parameters determined at steady state are also summarized in Table III. During the constant-rate infusion, the fraction excreted unchanged in the urine was 39 ± 3%, while the measured total and renal clearances were 20.3 ± 5.3 and 7.85 ± 1.7 ml/min · kg, respectively ($n = 4$). These values were not statistically different from those determined following single intravenous

Table II. d4T Pharmacokinetic Parameters Obtained After a Single Intravenous Bolus in the Rabbit: Compartmental Analysis ($n = 5$)

	Parameter value	
	Mean	(SD)
V_c (L/kg)	0.496	(0.074)
V_{ss} (L/kg)	1.12	(0.13)
k_{10} (l/min)	0.0483	(0.0017)
k_{12} (l/min)	0.0400	(0.012)
k_{21} (l/min)	0.0310	(0.0048)
$t_{1/2}\alpha$ (min)	6.72	(0.94)
$t_{1/2}\beta$ (min)	51.2	(5.7)

Table III. d4T Pharmacokinetic Parameters in the Rabbit After a Single Intravenous Bolus and at Steady State: Noncompartmental Analysis

	i.v. bolus		Steady state	
	Mean	(SD)	Mean	(SD)
V_{dss} (L/kg)	1.08	(0.13)	—	
MRT (min)	46.3	(5.0)	—	
Cl_{tot} (ml/min · kg)	23.4	(3.6)	20.3	(5.3)[a]
Cl_r[b] (ml/min · kg)	8.82	(3.9)	7.85	(1.7)[a]
f_e[c]	0.36	(0.08)	0.39	(0.03)[a]

[a] No statistical difference from the corresponding values measured after i.v. bolus, $P > 0.05$.
[b] Estimated by Eq. (2).
[c] Estimated by the ratio of total amount of d4T excreted unchanged in urine to the amount administered.

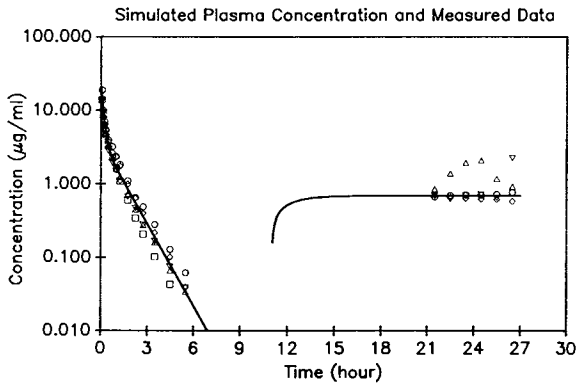

Fig. 4. Simulation of plasma concentration–time profile and data. (○) Rabbit 1; (△) rabbit 2; (□) rabbit 3; (▽) rabbit 4; (◇) rabbit 5; (——) simulation curve.

bolus administration in the same animals (paired *t* test), indicating that these parameters are time independent. Figure 4 shows the plasma concentration–time data following i.v. bolus and infusion administration of d4T, as well as the simulated profile based on the mean i.v. bolus pharmacokinetic parameters and the expected function during infusion. The observed agreement shows that in all but one animal the steady-state plasma concentrations of d4T were extremely well predicted.

In summary, a rapid and sensitive method for the assay of the nucleoside analogue d4T in the plasma and urine of humans and rabbits has been developed. The method permits the analysis of this compound over a range of concentrations expected when doses of 10 mg/kg are given intravenously to rabbits. A preliminary crossover study involving i.v. boluses and constant-rate infusions in this animal model showed that a significant proportion of the dose was excreted unchanged in the urine. The pharmacokinetics of d4T were well described by a linear two-compartment model, and both total and renal clearances were unchanged between the two modes of dosing. The renal clearance of d4T was approximately three times the glomerular filtration rate, strongly suggesting active secretion by the kidney.

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REFERENCES

1. J. P. Horwitz, J. Chua, M. A. Da Rooge, M. Noel, and I. L. Klundt. Nucleosides. IX. The formation of 2′,3′-unsaturated pyrimidine nucleosides via a novel β-elimination reaction. *J. Org. Chem.* 31:205–211 (1966).
2. M. M. Mansuri, J. E. Starrett, Jr., I. Ghazzouli, M. J. M. Hitchcock, R. Z. Sterzycki, V. Brankovan, T. Lin, E. M. August, W. H. Prusoff, J. Sommadossi, and J. C. Martin. 1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl) thymine. A highly potent and selective anti-HIV agent. *Proc. Natl. Acad. Sci. USA* 83:8333–8337 (1986).
3. T. Lin, R. F. Shinazi, and W. H. Prusoff. Potent and selective in vitro activity of 3′-deoxythymidin-2′-ene (3′-deoxy-2′,3′-didehydrothymidine) against human immunodeficiency virus. *Biochem. Pharmacol.* 36:2713–2718 (1987).
4. J. Balzarini, G. J. Kang, M. Dalal, P. Herdewijn, E. De Clercq, S. Broder, and D. G. Johns. The anti-HTLV-III (anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides: A comparison with their parental 2′,3′-dideoxyribonucleosides. *Mol. Pharmacol.* 32:162–167 (1987).
5. Y. Hamamoto, H. Nakashima, T. Matsui, A. Matsuda, T. Ueda, and N. Yamamoto. Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects, and replication of human immunodeficiency virus. *Antimicrob. Agents Chemother.* 31:907–910 (1987).
6. P. Herdewijn, J. Balzarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder, and H. Vanderhaeghe. 3′-Substituted 2′,3′-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. *J. Med. Chem.* 30:1270–1278 (1987).
7. S. Kaul, K. A. Dandekar, and K. A. Pittman. Analytical method for the quantification of 2′,3′-didehydro-3′-deoxythymidine, a new anti-human immunodeficiency virus (HIV) agent, by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection in rat and monkey plasma. *Pharm. Res.* 6:895–899 (1989).
8. L. Oppenheimer, T. P. Capizzi, R. M. Weppelman, and H. Mehta. Determining the lowest limit of reliable assay measurement. *Anal. Chem.* 55:638–643 (1983).
9. D. K. Yu and R. J. Sawchuk. Pharmacokinetics of propylene glycol in the rabbit. *J. Pharmacokinet. Biopharm.* 15:453–469 (1987).
10. C. M. Metzler and D. L. Weiner. *PCNONLIN,* Statistical Consultants Inc., Edgewood, KY, 1989.
11. K. K. H. Chan and K. Wnuck. Microcomputer based pharmacokinetic program for calculating absorption rate. *Comput. Prog. Biomed.* 16:27–34 (1983).
12. H. W. Smith. *The Kidney Structure and Function in Health and Disease,* Oxford University Press, New York, 1956.
13. M. A. Hedaya and R. J. Sawchuk. Effect of probenecid on the renal and nonrenal clearances of zidovudine and its distribution into cerebrospinal fluid in the rabbit. *J. Pharm. Sci.* 78:716–722 (1989).
14. M. A. Hedaya and R. J. Sawchuk. A sensitive and specific liquid-chromatographic assay for determination of ganciclovir in plasma and urine and its application to pharmacokinetic studies in the rabbit. *Pharm. Res.* 7:1113–1118 (1990).