Pharmacokinetics of β -L-2',3'-Dideoxy-5-Fluorocytidine in Rhesus Monkeys

LEE T. MARTIN,¹ ERIKA CRETTON-SCOTT,¹ RAYMOND F. SCHINAZI,² XIAO-JIAN ZHOU,¹
HAROLD M. McCLURE,³ CHRISTOPHE MATHE,⁴ GILLES GOSSELIN,⁴ JEAN-LOUIS IMBACH,⁴
AND JEAN-PIERRE SOMMADOSSI^{1*}

Department of Clinical Pharmacology, Center for AIDS Research, The Liver Center, University of Alabama at Birmingham, Birmingham, Alabama¹; Georgia Veterans Affairs Research Center for AIDS and HIV Infections, Veterans Affairs Medical Center, Decatur, Georgia²; Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia³; and Laboratory of Bioorganic Chemistry, University of Montpellier II, Unité Mixte De Recherche 5625, CNRS, 34095, Montpellier, France⁴

Received 12 December 1997/Returned for modification 26 April 1998/Accepted 2 January 1999

β -L-2',3'-Dideoxy-5-fluorocytidine (β -L-FddC), a novel cytidine analog with an unnatural β -L sugar configuration, has been demonstrated by our group and others to exhibit highly selective in vitro activity against human immunodeficiency virus types 1 and 2 and hepatitis B virus. This encouraging in vitro antiviral activity prompted us to assess its pharmacokinetics in rhesus monkeys. Three monkeys were administered an intravenous dose of [³H] β -L-FddC at 5 mg/kg of body weight. Following a 3-month washout period, an equivalent oral dose was administered. Plasma and urine samples were collected at various times for up to 24 h after dosing, and drug levels were quantitated by high-pressure liquid chromatography. Pharmacokinetic parameters were obtained on the basis of a two-compartment open model with a first-order elimination from the central compartment. After intravenous administration, the mean peak concentration in plasma ($C_{\max}$) was 29.8 ± 10.5 μ M. Total clearance, steady-state volume of distribution, terminal-phase plasma half-life ($t_{1/2\beta}$), and mean residence time were 0.7 ± 0.1 liters/h/kg, 1.3 ± 0.1 liters/kg, 1.8 ± 0.2 h, and 1.9 ± 0.2 h, respectively. Approximately $47\% \pm 16\%$ of the intravenously administered radioactivity was recovered in the urine as the unchanged drug with no apparent metabolites. β -L-FddC exhibited a $C_{\max}$ of 3.2 μ M after oral administration, with a time to peak drug concentration of approximately 1.5 h and a $t_{1/2}$ of 2.2 h. One monkey in the oral administration arm of the study had a significant delay in the absorption of the aqueous administered dose. The absolute bioavailability of orally administered β -L-FddC ranged from 56 to 66%.

Nucleoside analogs have been demonstrated to be potent drugs in the treatment of human immunodeficiency virus (HIV) infections. These antiretroviral agents target the HIV-encoded reverse transcriptase (HIV-RT) enzyme and share the same mechanism of action, including competitive inhibition of viral RNA-directed DNA polymerase and, in some cases, incorporation into viral DNA, which potentiates termination of viral chain elongation. Clinically approved 2',3'-dideoxynucleoside analogs include β -D-3'-deoxy-3'-azidothymidine (AZT), β -D-2',3'-dideoxycytidine (β -D-ddC), β -D-2',3'-dideoxyinosine (ddI), β -D-3'-deoxy-2',3'-didehydrothymidine (D4T), and β -L-2',3'-dideoxy-3'-thiacytidine (3TC). Recently, an extremely promising three-drug anti-HIV regimen which includes AZT, 3TC, and an HIV protease inhibitor has provided clinical benefits to HIV-infected patients (9). Interestingly, 3TC is the first of a new generation of nucleoside analogs with an unnatural β -L configuration, and it is more selective than its corresponding β -D enantiomer, β -D-2',3'-dideoxy-3'-thiacytidine [(+)-BCH-189] (5, 29, 30). Mutated HIV-RT (M184V) that confers resistance to 3TC has been identified both in vitro and in vivo (31). In vitro chain termination assays have indicated that the 5'-triphosphates of 3TC, β -L-2',3'-dideoxy-5-fluoro-3'-thiacytidine [(-)-FTC], β -L-ddC, and β -L-2',3'-dideoxy-5-fluorocytidine (β -L-FddC) were all recognized by wild-type HIV-RT, but the mutated HIV-RT, M184V,

failed to recognize this group of β -L-cytidine analogs (12). The HIV-RT mutation associated with AZT monotherapy combined with the M184V mutation induced by 3TC facilitates the emergence of a viral population that is more susceptible to inhibition by AZT in vitro (20). This unexpected mechanism of action has been proposed to be responsible for the observed synergistic anti-HIV activity of 3TC and AZT in vivo (20). Furthermore, Bridges et al. (4) reported on the in vitro synergistic inhibition of HIV replication with AZT in combination with 3TC, β -L-FddC, or (-)-FTC. In addition to their impressive anti-HIV selectivities, these unnatural β -L-configured analogs including 3TC and its 5-fluoro derivative, FTC, and β -L-FddC are highly selective agents against hepatitis B virus in vitro and in vivo (1, 6, 10, 11, 21, 22, 30, 35). The development of β -L-cytidine analogs remains of major interest.

Studies exploring the cellular pharmacology of β -L-ddC, β -L-FddC, and its corresponding β -D-enantiomer β -D-2',3'-dideoxy-5-fluorocytidine (β -D-FddC) were performed by our laboratories to determine the mechanism(s) responsible for the increased anti-HIV and anti-hepatitis B virus selectivities of β -L-FddC (23). Activation of 10 μ M β -L-FddC in Hep-G2 cells resulted in intracellular levels of 26.6 ± 10.9 pmol of β -L-FddC triphosphate (β -L-FddCTP) per 10^6 cells, a value that is approximately 10-fold higher than the intracellular β -D-FddC triphosphate (β -D-FddCTP) levels achieved under the same conditions (23). Furthermore, deamination of β -L-FddC to β -L-2',3'-dideoxy-5-fluorouridine was not detected in these experiments (23). Similar patterns were also observed by our group in phytohemagglutinin-stimulated peripheral blood mononuclear cells (unpublished data). Additionally, β -L-FddCTP

* Corresponding author. Mailing address: University of Alabama at Birmingham, Box 600, Volker Hall G019, University Station, Birmingham, AL 35294. Phone: (205) 934-8226. Fax: (205) 975-4871. E-mail: jean-pierre.sommadosi@ccc.uab.edu.

underwent a single-phase elimination process and had an extended intracellular half-life ($t_{1/2}$) of 14.8 h, with 6.7 ± 2.3 pmol/ 10^6 cells remaining intracellularly after 24 h of incubation in drug-free medium (23). Faraj et al. (12) reported the competitive inhibition of wild-type HIV-RT by β -L-FddCTP with a K_i of 1.60 ± 0.10 μ M with a poly(rI) n · oligo(dC) $_{10-15}$ template primer (12). β -L-FddCTP also has been demonstrated to inhibit woodchuck hepatitis virus DNA polymerase (50% inhibitory concentration, 2.0 μ M) in an endogenous assay conducted with disrupted woodchuck hepatitis virus particles (30). Cui et al. (8) also reported that 10 μ M β -L-FddC had no effect on mitochondrial DNA content, mitochondrial morphology, or induction of lipid droplet formation in Hep-G2 cells, but an increase in lactate production was noted. Because of the selective in vitro antiviral characteristics of β -L-FddC, our group assessed the pharmacokinetics of β -L-FddC in rhesus monkeys (*Macaca mulatta*) to determine its in vivo metabolism and pharmacokinetic parameters.

MATERIALS AND METHODS

Chemicals. The stereoselective synthesis of β -L-FddC from L-xylose has been reported elsewhere (17). β -L-FddC was fully characterized by 1 H and 19 F nuclear magnetic resonance spectroscopy, fast-atom bombardment mass spectroscopy, and UV spectroscopy, and its purity was confirmed by reverse-phase high-pressure liquid chromatography (HPLC) analysis as being greater than 98%. [6 - 3 H] β -L-FddC (2.5 Ci/mmol) was custom synthesized by Moravsek Biochemical (Brea, Calif.), and its purity was assessed to be greater than 97% (23). All other chemicals and reagents were of the highest analytical grade available.

Study design. Rhesus monkeys (*M. mulatta*) were used for the in vivo metabolism and pharmacokinetic studies. These animals were maintained at the Yerkes Regional Primate Research Center at Emory University in accordance with guidelines established by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals of the National Institutes of Health* (24a). The Yerkes Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Three young adult female rhesus monkeys weighing between 4.7 and 5.6 kg were used for the intravenous and oral administration of β -L-FddC in this study. The monkeys were fasted for 12 h prior to dose administration, and water was made available throughout the fasting and postanesthesia period. For intravenous dosing, three monkeys (monkeys RJv2, RPD3, and RRM3) received a bolus dose of β -L-FddC at 5 mg/kg of body weight with 250 μ Ci of a [3 H] β -L-FddC tracer dissolved in sterile phosphate-buffered saline (pH 7.4). Following a 3-month washout period, the same animals were administered an equivalent oral dose by nasogastric intubation with thorough flushing of the administration tube. There was no substantial weight fluctuation of the animals between the times of the intravenous and oral dose administrations.

Two milliliters of blood was collected in a clot tube prior to and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after dose administration. A catheter was inserted into the bladder for urine collection at the times noted above. The monkeys were initially anesthetized with a combination of tiletamine hydrochloride-zolazepam hydrochloride (Telazol) and ketamine, with supplemental doses administered periodically as needed. Anesthesia was halted after 8 h, and the bladder catheter was removed. The animals were briefly anesthetized at 24 h for blood and urine collection. All urine excreted between 8 and 24 h was collected from the pan beneath the cage. No fecal samples were collected. The urine and feces excreted after 24 h were monitored for radioactivity by the wipe test until the levels were below the baseline. Plasma and urine samples were then frozen at -20°C until analysis.

Analytical methodology. Plasma sample aliquots were extracted with an equal volume of 100% acetonitrile overnight at -20°C . The precipitated protein was separated by centrifugation, and plasma extracts were dried under nitrogen and reconstituted in distilled water. The recovery from the extraction process was 92%. Urine samples were filtered through a 0.2- μ m-pore-size Acrodisc LC 13 polyvinylidene difluoride syringe filter (Gelman Sciences, Ann Arbor, Mich.). Plasma and urine samples were analyzed by reverse-phase HPLC with a Hewlett-Packard model 1050 liquid chromatograph equipped with a manual injector and a variable-wavelength UV detector. Reverse-phase chromatography was conducted with a Hypersil ODS 5- μ m column (Jones Chromatography, Littleton, Colo.). A gradient elution was performed at 1 ml/min with 50 mM phosphoric acid (pH 3.0) and a 35-min linear gradient of acetonitrile from 0 to 30% starting at the time of injection. Column eluent was monitored by UV at 254 nm, and fractions were collected every 1 min in a Redifrac fraction collector (Pharmacia LKB, Piscataway, N.J.) and combined with 5 ml of Econosafe scintillant (Research Products International Corp., Mount Prospect, Ill.). Under these conditions, β -L-FddC eluted at 16 min. Radioactivity was quantitated in a Beckman LS5000 TA counter. Plasma and urine β -L-FddC concentrations were based on

FIG. 1. Mean plasma concentration-versus-time curves for β -L-FddC following intravenous (■) and oral (□) administration of 5 mg of β -L-FddC per kg.

the detected radioactivity and the specific activity of the administered dose (specific activity, 5 dpm/pmol). The limit of quantitation was 0.1 μ M.

Pharmacokinetic analysis. The pharmacokinetic parameters of β -L-FddC for the intravenous administration were estimated by a two-compartment open model with first-order elimination from the central plasma compartment, and those for oral administration were characterized by a one-compartment open model with first-order absorption with SIPHAR/Base software (14–16). Biexponential curves were generated by a least-squares algorithm with extrapolation to infinity based on the terminal slope of the elimination phase. The plasma concentration-versus-time curve for oral administration was not forced through the origin. The area under the plasma concentration-versus-time curve (AUC) was calculated by the trapezoidal rule. Transfer constants (K , K_{12} , and K_{21}) were derived from the hybrid first-order rate constants (a and b) and their respective coefficients (A and B) that were generated by the method of residuals (14, 32). The volume of the central plasma compartment (V_p) and the peripheral tissue compartment (V_t) were calculated as the quotient of the administered dose (D_0)/ $A + B$ and $V_p \cdot K_{12}/K_{21}$, respectively. The volume of distribution at steady state (V_{ss}) was taken as the sum of V_p and V_t and was normalized to the monkey's weight. Total clearance (CL) was calculated as the quotient D_0/AUC . Renal clearance (CL_R) was calculated as the amount of drug excreted in urine in 8 h divided by the AUC from time zero to 8 h (AUC_{0-8}). Nonrenal clearance (CL_{NR}) was calculated as $\text{CL} - \text{CL}_R$. All clearance parameters were normalized to the monkey's weight. The $t_{1/2}$ of the distribution phase ($t_{1/2\alpha}$) and the elimination phase ($t_{1/2\beta}$) were determined as $0.693/a$ and $0.693/b$, respectively. The mean residence time (MRT) was the quotient V_{ss}/CL . The maximum concentration of drug in plasma (C_{max}) was observed from the experimental data, and T_{max} was the time to C_{max} after oral dosing. The lag time was based on the intersection of the two residual lines based upon an initial peeling algorithm. Oral bioavailability was estimated by the product of the ratios $\text{AUC}_{\text{p.o.}}/\text{AUC}_{\text{i.v.}}$ and $\text{dose}_{\text{i.v.}}/\text{dose}_{\text{p.o.}}$, where i.v. represents intravenous administration, and p.o. represents oral administration.

RESULTS

In vivo metabolism and toxicity of β -L-FddC. Following administration of 5 mg of [3 H] β -L-FddC per kg, no metabolites were detected by HPLC analysis in plasma or urine samples up to 24 h. Intravenously and orally administered β -L-FddC was well tolerated by all animals. One animal regurgitated a small amount of bile-colored material after intravenous administration of β -L-FddC.

Kinetics of β -L-FddC in plasma. The mean plasma concentration-versus-time curve following intravenous administration of 5 mg of [3 H] β -L-FddC per kg is presented in Fig. 1. The C_{max} after intravenous administration was 29.8 ± 10.5 μ M/liter, and plasma β -L-FddC concentrations rapidly declined with an MRT of 1.9 ± 0.2 h. β -L-FddC exhibited a biphasic elimination from plasma with a $t_{1/2\alpha}$ of 0.2 ± 0.1 h followed by a $t_{1/2\beta}$ of 1.8 ± 0.2 h. By 8 h, plasma β -L-FddC levels were below 1 μ M and were undetectable by 24 h. The V_{ss} was $1.3 \pm$

TABLE 1. Coefficients, hybrid first-order rate constants, transfer constants, and absorption constants following intravenous or oral administration of 5 mg of β -L-FddC per kg

Monkey	A (μ M)	B (μ M)	a (h^{-1})	b (h^{-1})	K_{12} (h^{-1})	K_{21} (h^{-1})	K_{10} (h^{-1})	K_a (h^{-1})	$K_{p.o.}$ (h^{-1})
RRm3	19.0	10.3	3.4	0.4	1.4	1.5	1.0	0.9	0.3
RJv2	40.0	7.1	3.1	0.4	1.2	0.8	1.4	0.7	0.4
RPd3	30.5	8.1	2.6	0.3	1.0	0.8	1.1	NA ^a	NA
Mean $\pm$ SD	29.8 $\pm$ 10.5	8.5 $\pm$ 1.6	3.0 $\pm$ 0.4	0.4 $\pm$ 0.1	1.2 $\pm$ 0.2	1.0 $\pm$ 0.4	1.2 $\pm$ 0.2	0.8	0.4

^a NA, not available due to a delay in gastric absorption.

0.1 liters/kg, with a V_p and a V_t of 0.6 ± 0.1 and 0.7 ± 0.1 liters/kg, respectively. The coefficients, hybrid first-order rate constants, and transfer constants for the two-compartment open model are presented in Table 1, and the derived pharmacokinetic parameters of β -L-FddC following intravenous and oral administration are summarized in Table 2.

After a 3-month washout period, the same monkeys were administered an equivalent oral dose of [3 H] β -L-FddC by gastric gavage. One monkey (monkey RPd3) in the oral administration arm of the study had a significant delay in absorption; therefore, the mean plasma concentration-versus-time curves for monkeys RRm3 and RJv2 following oral administration of 5 mg/kg is presented in Fig. 1. With the mean K_a ($0.8 h^{-1}$) approaching K_{21} , no distribution phase was observed, and the data were fit with a one-compartment open model with first-order absorption (14, 15). The mean C_{max} was 3.2 μ M, with lag time of 0.2 h and a mean T_{max} of 1.5 h. In monkey RPd3 a higher C_{max} of 5.1 μ M with an extended T_{max} of 8 h occurred. After the maximum concentration in plasma was achieved, plasma β -L-FddC levels declined to below detection levels by 24 h in all monkeys except monkey RPd3. The absolute oral bioavailability of β -L-FddC ranged from 56 to 66%.

Urinary excretion of β -L-FddC. β -L-FddC accounted for all of the radioactivity recovered in the urine. However, the percentage of the dose recovered in the urine was incomplete for both routes of administration. In the intravenous administration arm, only $47\% \pm 16\%$ of the administered dose was recovered in the urine after 24 h, and in the oral administration arm, only 12% of the administered dose was recovered in the urine after 24 h. Urinary excretion of β -L-FddC was rapid, with essentially all (98%) of the recovered dose being excreted within 8 h following intravenous dose administration. Urinary output was variable at 77.2 ± 32.9 ml following intravenous

administration and 93.8 ± 48.7 ml following oral administration. Interestingly, monkey RRm3 had a β -L-FddC concentration in urine of 63 μ M at 24 h following administration of the dose, and this monkey had a K_{21} transfer constant that was 100% higher than those for the other two monkeys.

The AUC of β -L-FddC was 32.5 ± 3.5 h $\cdot$ μ mol/liter, corresponding to a CL of 0.7 ± 0.1 liters/h/kg. However, CL_R was 0.3 ± 0.1 liters/h/kg, which was approximately 43% of the CL, suggesting 57% CL_{NR} . Wipe tests indicated that a significant amount of radioactivity (twice the background level) was present in the feces for up to 10 days after intravenous administration of the drug. However, quantitation of total fecal radioactivity was not possible because the fecal samples were not weighed. CL_R was reduced by 50% in monkey RRm3 and by 66% in monkey RJv2 following oral administration of β -L-FddC.

DISCUSSION

β -L-FddC exhibited a biphasic elimination from the plasma; therefore, a two-compartment open model was used to describe the pharmacokinetics. Rapid disposition to the peripheral tissue compartment and a subsequent slow redistribution back into the central plasma compartment from the peripheral tissues are indicated by the derived transfer constants for two of the monkeys. This pattern of biphasic elimination from the plasma is also characteristic of 3TC, D4T, ddI, and ($\pm$)-FTC (2, 7, 25, 26, 28). β -L-FddC had a $t_{1/2\alpha}$ of 0.2 ± 0.1 and a $t_{1/2\beta}$ of 1.8 ± 0.2 h following intravenous administration, and these are similar to those for the cytidine analogs mentioned above. However, after oral administration, β -L-FddC elimination from the plasma was characterized as monoexponential due to the moderate K_a that approached the K_{21} (14). Several nucle-

TABLE 2. Pharmacokinetic parameters after intravenous and oral administration of 5 mg of β -L-FddC per kg to rhesus monkeys

Arm of study and monkey	V_{ss} (liters/kg)	AUC (h $\cdot$ μ mol/liter)	CL (liters/h/kg)	CL_R (liters/h/kg)	CL_{NR} (liters/h/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	Lag (h)	C_{max} (μ M)	T_{max} (h)	Amt excreted (%)	Bioavailability (%)
Intravenous administration													
RRm3	1.4	28.8	0.8	0.4	0.4	0.2	1.6	1.9				59	
RJv2	1.2	32.9	0.7	0.3	0.4	0.2	1.9	1.8				53	
RPd3	1.3	35.8	0.6	0.2	0.4	0.3	2.0	2.1				28	
Mean $\pm$ SD	1.3 0.1	32.5 3.5	0.7 0.1	0.3 0.1	0.4 0.1	0.2 0.1	1.8 0.2	1.9 0.2				47 16	
Oral administration													
RRm3		18.9		0.2		2.5			0.4	3.1	2	14	66
RJv2		18.6		0.1		1.8			0.0	3.2	1	10	56
RPd3		NA ^a		NA		NA			NA	5.1 ^b	8 ^b	7 ^b	NA
Mean		18.8		0.2		2.2			0.2	3.2	1.5	12	61

^a NA, not available due to the delay gastric absorption.

^b Value not used in calculation of the mean.

oside analogs are characterized by short plasma $t_{1/2\alpha}$ and $t_{1/2\beta}$ or a short single-phase $t_{1/2}$ (2, 3, 7, 19, 24–28).

The CL of β -L-FddC, 0.7 ± 0.1 liters/h/kg, was similar to those of ddC and 3TC, which were 0.87 ± 0.1 and 0.8 ± 0.1 liters/h/kg, respectively (2, 26). In contrast, the cytidine analog ($\pm$)-FTC displayed a dramatically higher CL of 1.49 ± 0.18 liters/h/kg (28). The CL of β -L-FddC was also comparable to those of other noncytidine analogs. ddI had a similar CL of 0.74 ± 0.08 liter/h/kg in cynomolgus monkeys (*Macaca fascicularis*) (25). However, CL variations are more prevalent with thymidine analogs than with cytidine analogs (3, 7, 27). In rhesus monkeys, after administration of a dose of 60 mg/kg, D4T was cleared at a rate of 0.69 ± 0.15 liters/h/kg (27), whereas the CL of 60 mg of AZT per kg was approximately twofold greater (1.57 liters/h/kg) in the same species (3). However, Cretton et al. (7) noted a CL of 0.261 ± 0.039 liters/h/kg for D4T following intravenous administration of a lower dose of 5 mg/kg, with a V_{ss} of 0.74 ± 0.14 liters/kg in primates (7). The V_{ss} of β -L-FddC is greater than the volume of total body water, which indicates a disposition in tissue outside the circulatory system (13). Interestingly, Cretton et al. (7) noted that the CL_R for D4T was only 51% of the CL and that the CL_R of β -L-FddC, ddC, and 3TC in monkeys were all approximately half of the observed CL (2, 26). Pharmacokinetic studies of 3TC with humans have also observed this relationship between CL and CL_R (34). Therefore, this phenomenon is not just characteristic of the cytidine analogs. Consequently, the biliary clearance of β -L-FddC in mice was investigated by our group. Female CD-1 mice received a 30-mg/kg bolus intravenous dose of [3 H] β -L-FddC via the tail vein and were housed in metabolic cages for 96 h. Urine and fecal samples were collected at 24, 72, and 96 h and their volumes and weights, respectively, were noted. The total radioactivity in the urine was determined by scintillation counting, and the radioactivity in the feces was determined after dissolution of a known weight in 0.1 N NaOH at 50°C for 24 h, neutralization with 0.1 N HCl, and scintillation counting. Low levels of radioactivity representing 2.4% of the total administered dose appeared in the feces within the first 24 h, while a majority of the dose (84%) was excreted in the urine within 24 h. One hundred percent of the administered dose was recovered within 96 h, with no radioactivity detected in feces at 72 and 96 h. These experiments indicate that fecal excretion of β -L-FddC does occur in mice, but only to a small extent compared with urinary excretion.

In contrast to mice, the wipe tests performed with urine and fecal samples from rhesus monkeys indicate that significant amounts of radioactivity are excreted in the feces after administration of the intravenous dose. In one monkey (monkey RPD3), the relative amount of radioactivity detected in feces 24 to 48 h after intravenous drug administration was 25% of that detected in urine, as determined by the wipe test. For all monkeys an increase in the radioactivity in the feces was detected after administration of the oral dose compared with that observed after administration of the intravenous dose. This is indicative of the incomplete oral bioavailability of β -L-FddC. Prolonged excretion of low β -L-FddC levels below our limit of quantitation ($0.1 \mu\text{M}$) in the urine and feces may be a plausible explanation on the basis of an analysis of the intercompartment transfer constants. Two monkeys (monkeys RJv2 and RPD3) presented with slow elimination from the peripheral tissue compartment, with K_{12} being greater than K_{21} . However, monkey RRM3 presented with a K_{12} that was less than the K_{21} . Low levels of radioactivity were detected by the wipe test in the urine and feces 9 days after intravenous administration of [3 H] β -L-FddC. Phase II conjugation metabolism would facilitate rapid excretion; however, no glucuronide, glutathione,

sulfate, or amino acid conjugates were detected in the urine or plasma of monkeys.

One issue that must be addressed with β -L-FddC is the low recovery, $47\% \pm 16\%$, of the total administered intravenous dose in the urine. The nonvolatile nature of 2',3'-dideoxynucleosides, in general, precludes the possibility of respiratory elimination, and elimination in the sweat is highly unlikely. Three possible explanations may account for the remaining 53% of the administered radioactivity. First, β -L-FddC elimination in the feces resulting from hepatic extraction with subsequent secretion into the bile may provide evidence for the radioactivity detected in the feces. Positive wipe tests with feces confirm that fecal excretion of β -L-FddC does occur, but the extent of fecal clearance has yet to be established. Second, rapid tissue distribution in conjunction with slow redistribution back into the central plasma compartment may permit the accumulation of β -L-FddC outside the vasculature. Furthermore, β -L-FddCTP has been demonstrated to have an extended intracellular $t_{1/2}$ (23). These two characteristics provide the rationale for a long-term disposition in tissue. Finally, tritium exchange with body water and the subsequent formation of deuterium may occur. This phenomenon was observed by Cretton et al. (7) when D4T was administered to primates.

In summary, this study has detailed the pharmacokinetic parameters and the in vivo metabolism of β -L-FddC in rhesus monkeys. No metabolites of β -L-FddC were detected in either the plasma or the urine of rhesus monkeys. The V_{ss} indicated the distribution of β -L-FddC in a volume of body water which is greater than that of total body water. Our data suggest that CL_{NR} in feces may also be a significant route of elimination. This, in conjunction with possible prolonged excretion of low levels in the urine, may account for approximately 50% of the administered dose. The extent of the long-term disposition in tissue warrants further investigation to facilitate estimation of the correct dosing regimens to prevent the accumulation of β -L-FddC in the body.

ACKNOWLEDGMENTS

We thank Ellen Lockwood and the staff of the Yerkes Regional Research Center for valuable assistance with the experiments with monkeys.

This work was supported in part by Public Health Service grant AI-33239 (to J.-P.S.), grant RR-00165 from the National Institutes of Health, and grant AI-25899 (to R.F.S.) and by grants from the Centre National de la Recherche Scientifique and Agence National de la Recherche sur le Sida.

REFERENCES

- Benhamou, Y., E. Dohin, F. Lunel-Fabiani, T. Poynard, J. M. Huraux, C. Katlama, P. Opolon, and M. Gentilini. 1995. Efficacy of lamivudine on replication of hepatitis B virus in HIV-infected patients. *Lancet* **345**:396–397.
- Blaney, S. M., M. J. Daniel, A. J. Harker, K. Godwin, and F. M. Balis. 1995. Pharmacokinetics of lamivudine and BCH-189 in plasma and cerebrospinal fluid of nonhuman primates. *Antimicrob. Agents Chemother.* **39**:2779–2782.
- Boudinot, F. D., R. F. Schinazi, J. M. Gallo, H. M. McClure, D. C. Anderson, K. J. Doshi, P. C. Kambhampathi, and C. K. Chu. 1990. 3'-Azido-2',3'-dideoxyuridine (AZddU): comparative pharmacokinetics with 3'-azido-2',3'-deoxythymidine (AZT) in monkeys. *AIDS Res. Hum. Retroviruses* **6**:219–228.
- Bridges, E. G., G. E. Dutschman, E. A. Gullen, and Y.-C. Cheng. 1996. Favorable interaction of β -L-(-) nucleoside analogues with clinically approved anti-HIV nucleoside analogues for the treatment of human immunodeficiency virus. *Biochem. Pharmacol.* **51**:731–736.
- Coates, J. A. V., N. Cammack, H. J. Jenkinson, I. M. Mutton, B. A. Pearson, R. Storer, J. M. Cameron, and C. R. Penn. 1992. The separated enantiomers of 2'-deoxy-3'-thiacytidine (BCH-189) both inhibit human immunodeficiency virus replication in vitro. *Antimicrob. Agents Chemother.* **36**:202–205.
- Condreay, L. D., J. P. Condreay, R. W. Jansen, M. T. Paff, and D. R. Averett. 1996. (-)-cis-5-Fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (524W91) inhibits hepatitis B virus replication in primary human hepatocytes. *Antimicrob. Agents Chemother.* **40**:520–523.

7. Cretton, E. M., Z. Zhou, L. B. Kidd, H. M. McClure, S. Kaul, M. J. M. Hitchcock, and J.-P. Sommadossi. 1993. In vitro and in vivo disposition and metabolism of 3'-deoxy-2',3'-didehydrothymidine. *Antimicrob. Agents Chemother.* **37**:1816-1825.
8. Cui, L., R. F. Schinazi, G. Gosselin, J.-L. Imbach, C. K. Chu, R. F. Rando, G. R. Revankar, and J.-P. Sommadossi. 1996. Effect of β -enantiomeric and racemic nucleoside analogues on mitochondrial functions in HepG2 cells. *Biochem. Pharmacol.* **52**:1577-1584.
9. Deeks, S. G., M. Smith, M. Holodniy, and J. O. Kahn. 1997. HIV-1 protease inhibitors: a review for clinicians. *JAMA* **277**:145-153.
10. Dienstag, J. L., R. P. Perrillo, E. R. Schiff, M. Bartholomew, C. Vicary, and M. Rubin. 1995. A preliminary trial of lamivudine for chronic hepatitis B infection. *N. Engl. J. Med.* **333**:1657-1661.
11. Doong, S.-L., C.-H. Tsai, R. F. Schinazi, D. C. Liotta, and Y.-C. Cheng. 1991. Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues. *Proc. Natl. Acad. Sci. USA* **88**:8495-8499.
12. Faraj, A., L. A. Agrofoglio, J. K. Wakefield, S. McPherson, C. D. Morrow, G. Gosselin, C. Mathe, J.-L. Imbach, R. F. Schinazi, and J.-P. Sommadossi. 1994. Inhibition of human immunodeficiency virus by the 5'-triphosphate β enantiomers of cytidine analogs. *Antimicrob. Agents Chemother.* **38**:2300-2305.
13. Gerlowski, L. E., and R. K. Jain. 1983. Physiological based pharmacokinetic modeling: principles and applications. *J. Pharm. Sci.* **72**:1103-1126.
14. Gibaldi, M., and D. Perrier. 1982. Multicompartmental models, p. 45-112. *In* Pharmacokinetics. Marcel Dekker, Inc., New York, N.Y.
15. Gibaldi, M., and D. Perrier. 1982. One-compartmental Model, p. 1-44. *In* Pharmacokinetics. Marcel Dekker, Inc., New York, N.Y.
16. Gomeni, R., and C. Gomeni. 1979. Interactive graphic package for pharmacokinetic analysis. *Comput. Biol. Med.* **9**:38-48.
17. Gosselin, G., C. Mathé, M.-C. Bergogne, A.-M. Aubertin, A. Kirn, R. F. Schinazi, J.-P. Sommadossi, and J.-L. Imbach. 1994. Enantiomeric 2',3'-dideoxycytidine derivatives are potent human immunodeficiency virus inhibitors in cell culture. *C. R. Acad. Sci. [III]* **317**:85-89.
18. Gosselin, G., R. F. Schinazi, J.-P. Sommadossi, C. Mathé, M.-C. Bergogne, A.-M. Aubertin, A. Kirn, and J.-L. Imbach. 1994. Anti-human immunodeficiency virus activities of the β -L enantiomer of 2',3'-dideoxycytidine and its 5-fluoro derivative in vitro. *Antimicrob. Agents Chemother.* **38**:1292-1297.
19. Kelly, J. A., C. L. Litterst, J. S. Roth, D. T. Vistika, D. G. Poplack, D. A. Cooney, M. Nadkarni, F. M. Balis, S. Broder, and D. G. Johns. 1987. The disposition and metabolism of 2',3'-dideoxycytidine, an in vitro inhibitor of human T-lymphotropic virus type III infectivity, in mice and monkeys. *Drug Metab. Dispos.* **15**:595-601.
20. Larder, B. A., S. D. Kemp, and P. R. Harrigan. 1995. Potential mechanism for the sustained antiretroviral efficacy of AZT-3TC combination therapy. *Science* **269**:696-698.
21. Lin, T.-S., M.-Z. Luo, M.-C. Liu, S. B. Pai, G. E. Dutschman, and Y.-C. Cheng. 1994. Synthesis and biological evaluation of 2',3'-dideoxy-L-pyrimidine nucleosides as potential antiviral agents against human immunodeficiency virus (HIV) and hepatitis B virus (HBV). *J. Med. Chem.* **37**:798-803.
22. Lin, T.-S., M.-Z. Luo, M.-C. Liu, S. B. Pai, G. E. Dutschman, and Y.-C. Cheng. 1994. Antiviral activity of 2',3'-dideoxy- β -L-5-fluorocytidine (β -L-FddC) and 2',3'-dideoxycytidine (β -L-ddC) against hepatitis B virus and human immunodeficiency virus type 1 in vitro. *Biochem. Pharmacol.* **47**:171-174.
23. Martin, L. T., A. Faraj, R. F. Schinazi, G. Gosselin, C. Mathé, J.-L. Imbach, and J.-P. Sommadossi. 1997. Effect of stereoisomerism on the cellular pharmacology of β -enantiomers of cytidine analogs in Hep-G2 cells. *Biochem. Pharmacol.* **53**:75-87.
24. Moore, L. E., L. Ni, F. D. Boudinot, H. M. McClure, and R. F. Schinazi. 1997. Pharmacokinetics of (-)-2',3'-dideoxy-5-fluoro-3'-thiacytidine [(-)-FTC] and its metabolites in rhesus monkeys, abstr. 93. *In* Abstracts of the Tenth International Conference on Antiviral Research.
- 24a. National Institutes of Health. 1996. Guide for the care and use of laboratory animals. National Institutes of Health, Bethesda, Md.
25. Qian, M., T. S. Finco, A. R. Swagler, and J. M. Gallo. 1991. Pharmacokinetics of 2',3'-dideoxyinosine in monkeys. *Antimicrob. Agents Chemother.* **35**:1247-1249.
26. Qian, M., A. R. Swagler, K.-L. Fong, C. S. Cryslar, M. Mehta, and J. M. Gallo. 1992. Pharmacokinetic evaluation of drug interactions with anti-human immunodeficiency virus drugs: effect of soluble CD4 on 2',3'-dideoxycytidine kinetics in monkeys. *Drug Metab. Dispos.* **20**:396-401.
27. Schinazi, R. F., F. D. Boudinot, K. J. Doshi, and H. M. McClure. 1990. Pharmacokinetics of 3'-fluoro-3'-deoxythymidine and 3'-deoxy-2',3'-didehydrothymidine in rhesus monkeys. *Antimicrob. Agents Chemother.* **34**:1214-1219.
28. Schinazi, R. F., F. D. Boudinot, S. S. Ibrahim, C. Manning, H. M. McClure, and D. C. Liotta. 1992. Pharmacokinetics and metabolism of 2',3'-dideoxy-5-fluoro-3'-thiacytidine in rhesus monkeys. *Antimicrob. Agents Chemother.* **36**:2432-2438.
29. Schinazi, R. F., C. K. Chu, A. Peck, A. McMillan, R. Mathis, D. Cannon, L.-S. Jeong, J. W. Beach, W.-B. Choi, S. Yeola, and D. C. Liotta. 1992. Activity of the four optical isomers of 2',3'-dideoxy-3'-thiacytidine (BCH-189) against HIV-1 in human lymphocytes. *Antimicrob. Agents Chemother.* **36**:672-676.
30. Schinazi, R. F., G. Gosselin, A. Faraj, B. E. Korba, D. C. Liotta, C. K. Chu, C. Mathé, J.-L. Imbach, and J.-P. Sommadossi. 1994. Pure nucleoside enantiomers of β -2',3'-dideoxycytidine analogs are selective inhibitors of hepatitis B virus in vitro. *Antimicrob. Agents Chemother.* **38**:2172-2174.
31. Schinazi, R. F., R. M. Lloyd, Jr., M.-H. Nguyen, D. L. Cannon, A. McMillan, N. Ilksoy, C. K. Chu, D. C. Liotta, H. Z. Bazmi, and J. W. Mellors. 1993. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrob. Agents Chemother.* **37**:875-881.
32. Shargel, L., and A. B. C. Yu. 1993. Multicompartment models, p. 61-76. *In* Applied biopharmaceutics and pharmacokinetics. Appleton and Lange, Norwalk, Conn.
33. Sommadossi, J.-P., R. F. Schinazi, C. K. Chu, and M.-Y. Xie. 1992. Comparison of cytotoxicity of the (-) and (+) enantiomer of 2',3'-dideoxy-3'-thiacytidine in normal human bone marrow progenitor cells. *Biochem. Pharmacol.* **44**:1921-1925.
34. Van Leeuwen, R., J. O. A. Lange, E. K. Hussey, K. H. Donn, S. T. Hall, A. J. Harker, and S. A. Danner. 1992. The safety and pharmacokinetics of a reverse transcriptase inhibitor, 3TC, in patients with HIV infection: a phase I study. *AIDS* **6**:1471-1475.
35. Zoulim, F., E. Dannaoui, C. Borel, O. Hantz, T.-S. Lin, S.-H. Liu, C. Trépo, and Y.-C. Cheng. 1996. 2',3'-Dideoxy- β -L-5-fluorocytidine inhibits duck hepatitis B virus reverse transcription and suppresses viral DNA synthesis in hepatocytes, both in vitro and in vivo. *Antimicrob. Agents Chemother.* **40**:448-453.