ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1996, p. 642–645 Vol. 40, No. 3
0066-4804/96/$04.00+0

Pharmacokinetics of (−)-2′-3′-Dideoxy-3′-Thiacytidine in Woodchucks

PRABHU RAJAGOPALAN,[1] F. DOUGLAS BOUDINOT,[1]* CHUNG K. CHU,[2] BUD C. TENNANT,[3] BETTY H. BALDWIN,[3] AND RAYMOND F. SCHINAZI[4]

Department of Pharmaceutics[1] and Department of Medicinal Chemistry,[2] College of Pharmacy, University of Georgia, Athens, Georgia 30602; Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853[3]; and Veterans Affairs Medical Center, Decatur, Georgia 30033, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322[4]

Received 17 July 1995/Returned for modification 23 November 1995/Accepted 22 December 1995

The woodchuck (*Marmota monax*) has proven to be a suitable animal model for studying hepatitis B virus (HBV) infection owing to similarities in the course of infection between woodchuck hepatitis virus (WHV) in woodchucks and HBV in humans. (−)-β-L-2′,3′-Dideoxy-3′-thiacytidine (3TC; lamivudine) is a nucleoside analog which has demonstrated antiviral activity against HBV as well as human immunodeficiency virus (HIV). The purpose of the present investigation was to characterize the pharmacokinetics of 3TC following intravenous and oral administration of 20 mg of 3TC per kg of body weight to woodchucks. Following intravenous administration, the concentrations of 3TC in plasma declined, with a terminal half-life of 2.84 ± 0.85 h (mean ± standard deviation). The systemic clearance and steady-state volume of distribution of 3TC were 0.22 ± 0.078 liters/h/kg and 0.75 ± 0.13 liters/kg, respectively. The renal clearance of the nucleoside analog was 0.063 ± 0.016 liters/h/kg. The oral bioavailability of 3TC ranged from 18 to 54%. Allometric relationships between pharmacokinetic parameters and body weight developed by Hussey et al. (E. K. Hussey, K. H. Donn, M. J. Daniel, S. T. Hall, A. J. Harker, and G. L. Evans, J. Clin. Pharmacol. 34:975–977, 1994) were augmented by including data from woodchucks, monkeys (S. M. Blaney, M. J. Daniel, A. J. Harker, K. Godwin, and F. M. Balis, Antimicrob. Agents Chemother. 39:2779–2782, 1995), and additional data from rats (P. Rajagopalan, L. Moore, C. K. Chu, R. F. Schinazi, and F. D. Boudinot, submitted for publication). Interspecies scaling of the pharmacokinetic parameters of 3TC demonstrated a good correlation between clearance ($0.74 \cdot W^{0.76}$ [where W is body weight]; $r = 0.93$; $P < 0.025$), apparent volume of distribution ($1.62 \cdot W^{0.81}$; $r = 0.98$; $P < 0.005$), and steady-state volume of distribution ($1.09 \cdot W^{0.94}$; $r = 0.99$; $P < 0.05$) and species body weight. The allometric relationships for clearance and volume of distribution at steady state predicted the observed pharmacokinetic parameters in humans quite well; however, the apparent volume of distribution was underestimated in humans. Thus, the pharmacokinetic data obtained with the woodchuck HBV animal model should be useful for designing clinical trials.

Hepatitis B virus (HBV) infection usually results in acute and chronic hepatitis, which is closely associated with the development of hepatocellular carcinoma. Although a vaccine is available for the prevention of HBV, only a few antiviral agents are effective for the treatment of chronic HBV infection. (−)-β-L-2′,3′-Dideoxy-3′-thiacytidine (3TC; lamivudine) and its 5-fluoro analog (FTC) have been found to exhibit antiviral activity against HBV, as well as human immunodeficiency virus (HIV), in vitro (6, 9) and in vivo (1, 29). Cellular metabolism studies have shown that 3TC is converted to its mono-, di-, and triphosphates in HIV type 1-infected (5) and HBV-transfected 2215 cell lines (6). 3TC-triphosphate has been shown to be the active form of 3TC against viral DNA. 3TC is at least 50-fold more potent in inhibiting HBV (6) and HIV (5) in vitro compared with the (+)-β-D-enantiomer, which is probably due, in part, to its resistance to deamination by deoxycytidine deaminase (6). The pharmacokinetics of 3TC in several laboratory animal models (2, 15, 21), as well as in humans (28, 30), have been reported previously.

Preclinical pharmacokinetic studies conducted in suitable animal models provide the important information needed to conduct efficacy studies in these animal models and clinical investigations in humans. Woodchucks (*Marmota monax*) have a high incidence of hepatocellular carcinoma (26). The virus causing this condition in woodchucks has been identified as woodchuck hepatitis virus (WHV) (27), and a close correlation between WHV infection and hepatocellular carcinoma has been observed (25). HBV and WHV have a closer phylogenetic relationship, as illustrated by nucleic acid homology (11) and immunological cross-reactivity (19, 31), than that between HBV and the duck hepatitis virus, which is also used as an animal model for HBV (7). A comparison of the endogenous DNA polymerase properties of HBV and WHV shows striking similarities in terms of the conditions required for optimal activity, and these enzymes are inhibited by certain nucleotide triphosphates to the same extent in vitro (12). The woodchuck model has also been shown to be a suitable animal model for studying the efficacy of antiviral agents against HBV in vivo (10, 17). However, there are no reports concerning drug disposition in this animal model. Thus, results from pharmacokinetic studies in woodchucks should provide useful information for testing antiviral agents against HBV.

The purpose of the present study was to characterize the pharmacokinetics of 3TC in woodchucks following intravenous (i.v.) and oral (p.o.) administration of the compound. Furthermore, allometric equations relating the pharmacokinetic parameters total clearance (CL) and the apparent volume of

* Corresponding author. Mailing address: Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA 30602-2353.

distribution (V) of 3TC to body weight were augmented by using data obtained from the present study and interspecies scale-up data available in the literature (2, 15, 21).

MATERIALS AND METHODS

Chemicals. 3TC was synthesized as described previously (8, 14). 3TC was greater than 98.5% enantionmerically enriched, as determined by chiral chromatography. 2′,3′-Didehydro-2′-deoxythymidine (D4T), which was used as an internal standard, was provided by the Developmental Therapeutic Branch, AIDS Program, National Institutes of Health (Rockville, Md.). Acetonitrile (high-performance liquid chromatography [HPLC] grade) and other chemicals (analytical grade) were purchased from J. T. Baker (Phillipsburg, N.J.).

Experimental design. Adult male woodchucks (*M. monax*) weighing 3.73 ± 0.58 kg (mean ± standard deviation) were used for the pharmacokinetic study. The study was conducted between 27 October and 10 November 1994. The animals were housed in standard stainless steel cages in a cycle of 12 h of light and 12 h of dark. The animal studies were approved by the Cornell University Animal Care and Use Committee and were conducted in accordance with the guidelines established by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (19a).

Three woodchucks (animals 3259, 4257, and 4279) were used for characterizing the pharmacokinetics of 3TC following p.o. and i.v. administration of 20 mg of the nucleoside analog per kg of body weight. An additional woodchuck administered saline served as a control. Woodchucks were administered 20 mg of 3TC per kg of body weight p.o., and after a washout period of 2 weeks, the same dose was administered i.v. in a femoral vein. Blood samples were collected prior to drug administration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after dosing through an i.v. catheter placed in the opposite femoral vein. The blood samples were centrifuged immediately, and serum was stored at −80°C until analysis. Complete urine samples were collected either by housing the woodchuck in metabolic cages or via a tomcat urinary catheter (woodchuck 4257). The urine samples were also stored at −80°C until analysis.

Analytical methodology. Serum and urine samples obtained from woodchucks after administration of the nucleoside were analyzed by HPLC (21). Briefly, a 50-μl serum sample was placed in a 1.5-ml polypropylene microcentrifuge tube. To this, 100 μl of the internal standard (2 μg/ml of D4T) and 50 μl of 2 M perchloric acid were added. The tubes were vortexed and centrifuged at 9,500 rpm (Fisher Microcentrifuge model 59A) for 10 min. The supernatant (40 to 125 μl) was injected onto the HPLC column. The urine samples were diluted 100-fold with water. An aliquot (100 μl) was mixed with an equal volume of internal standard (10 μg/ml), and 40 μl was injected onto the column.

Chromatographic separation was achieved with an octyldecyl silane (ODS) column (4.6 mm [inner diameter] by 25 cm; 5-μm particle size; Jones Chromatography, Lakewood, Colo.). The mobile phase consisted of 4% acetonitrile in 5 mM potassium phosphate and 5 or 2 mM triethylamine for serum or urine analysis, respectively. The flow rate of the mobile phase was 1.5 ml/min, the UV detection wavelength was 270 nm, and the detector was set at 0.005 absorbance units, full scale. The retention times of 3TC and D4T were 7.2 and 12.1 min, respectively.

The concentration range for the standard curves for 3TC in serum and urine ranged from 0.25 to 25 μg/ml (1.1 to 109 μM) and 0.50 to 125 μg/ml (2.2 to 545 μM), respectively. Standard curve slopes were generated by weighted ($1/x^2$) linear least-squares regression (17). The standard curves were linear over the ranges of 0.25 to 25 μg/ml in plasma and 0.5 to 125 μg/ml in urine. Intraday relative standard deviations were assessed at 0.25, 5.0, and 25.0 μg/ml, and the interday relative standard deviation was studied over the entire concentration range of the standard curve on 3 different days. The inter- and intraday relative standard deviations were less than 6.7% at all concentrations except 0.25 μg/ml in serum, in which they were 14%.

Pharmacokinetics. Area-moment analysis was used to calculate pharmacokinetic parameters for 3TC. The area under the serum concentration-versus-time curve (AUC) and the area under the first moment curve (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time (23), with extrapolation to infinity by using the terminal slope (λ_z) generated by weighted ($1/y$) NONLIN least-squares regression (18). CL was calculated from $\text{dose}_{\text{i.v.}}/\text{AUC}_{\text{i.v.}}$. Renal clearance ($\text{CL}_\text{R}$) was calculated as $A_{u,t}/\text{AUC}_{0-t}$, where $A_{u,t}$ is the amount of 3TC excreted to time t and AUC_{0-t} is the AUC from time zero to time t. Nonrenal clearance (CL_NR) was determined as the difference between CL and CL_R. The steady-state volume of distribution (V_{SS}) was calculated from $(\text{dose}_{\text{i.v.}} \times \text{AUMC}_{\text{i.v.}})/(\text{AUC}_{\text{i.v.}})^2$, and V was calculated from CL/λ_z. The half-life ($t_{1/2}$) was calculated from $0.693/\lambda_z$. Oral bioavailability (F) was calculated from $(\text{AUC}_{\text{p.o.}} \times \text{dose}_{\text{i.v.}})/(\text{AUC}_{\text{i.v.}} \times \text{dose}_{\text{p.o.}})$.

Interspecies scaling. The mean values for CL and V in woodchucks obtained from the study along with data from rats (15, 21), dogs (15), and monkeys (2) were plotted against the mean body weight on a log-log scale. In addition, an allometric relationship between V_{SS} for rats (21), woodchucks, and monkeys (2) was developed. Neither V_{SS} values nor 3TC concentration-versus-time data were reported for dogs (15). Linear regression (22) was performed with the laboratory animal data, and a correlation between the pharmacokinetic parameters and body weight was generated (3). The resulting allometric relationships were used to predict pharmacokinetic parameters for humans, and the predicted values were compared with the observed values (30). The values for V_{SS} in humans were calculated from reported plasma 3TC concentration-versus-time data (30).

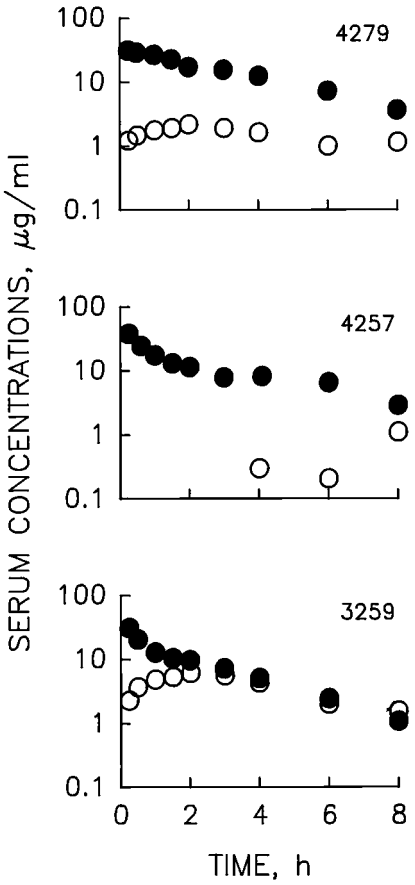

FIG. 1. Concentrations of 3TC in serum following i.v. (●) and p.o. (○) administration of 20 mg of 3TC per kg to woodchucks.

RESULTS AND DISCUSSION

The pyrimidine analog 3TC is a potent inhibitor of HBV in vitro (6). The polymerase involved in the replication cycle of hepadnaviruses shares a significant sequence homology with reverse transcriptase from HIV, including a conserved YMDD motif (24). Therefore, several compounds that inhibit HBV replication in vitro also inhibit HIV replication. However, pharmacokinetic studies of antiviral compounds possessing activity against HBV in animal models suitable for efficacy studies, such as woodchucks, have not been reported previously. Hence, the present investigation was conducted to characterize the disposition of 3TC in the woodchuck model as a prelude to therapeutic studies with this drug.

The concentrations of 3TC in serum following i.v. and p.o. administration of 20 mg of 3TC per kg to woodchucks are illustrated in Fig. 1. Following i.v. administration, 3TC concentrations declined in a mono- or biexponential manner, with an average terminal-phase $t_{1/2}$ of 2.84 ± 0.85 h (mean ± standard deviation). The concentrations of 3TC in serum following p.o. administration of the nucleoside were generally lower than those after i.v. administration. In two woodchucks (animals 4279 and 3259), peak serum 3TC concentrations were observed at 2 h after drug administration. Absorption of 3TC appeared to be much slower in woodchuck 4257, in which the

TABLE 1. Pharmacokinetic parameters of 3TC following i.v. and p.o. administration of 20 mg of 3TC per kg to woodchucks

Woodchuck (route of administration)	AUC (mg · h/liter)	CL (liters/h/kg)	CL_R (liters/h/kg)	CL_{NR} (liters/h/kg)	V_{SS} (liters/kg)	$t_{1/2}$ (h)	f_e[a]	F (%)
3259								
i.v.	63.87	0.31	0.081	0.23	0.80	2.12	0.26	
p.o.	34.47					2.36		53.9
4257								
i.v.	101.19	0.20	0.055[b]	0.14	0.85	3.78	0.20	
p.o.	ND[c]					ND		ND
4279								
i.v.	124.90	0.16	0.053	0.11	0.60	2.63	0.33	
p.o.	22.11					6.17		17.7
Mean (i.v.)	96.65	0.22	0.063	0.16	0.75	2.84	0.26	
(SD)	(30.77)	(0.078)	(0.016)	(0.063)	(0.13)	(0.85)	(0.065)	

[a] f_e, fraction excreted unchanged in urine.
[b] Urine collection interval to 5.5 h.
[c] ND, AUC, $t_{1/2}$, and F could not be calculated because serum 3TC concentrations were detected at only three time points.

first measurable 3TC concentration occurred at 4 h. Serum 3TC concentrations were below the limit of quantitation for the assay at 24 h following i.v. and p.o. administration.

The values of the pharmacokinetic parameters of 3TC following i.v. and p.o. administration of 20 mg of 3TC per kg to woodchucks are presented in Table 1. The CL, CL_R, and CL_{NR} of 3TC following i.v. administration averaged 0.22 ± 0.078, 0.063 ± 0.016, and 0.16 ± 0.063 liters/h/kg, respectively. CL_R accounted for approximately 25% of the CL of 3TC. The V_{SS} of 0.75 ± 0.13 liters/kg is slightly greater than the total body water of woodchucks, indicating an intracellular distribution of 3TC. Once it is distributed intracellularly, the compound may be trapped, in part, as the anabolite 3TC-triphosphate. The average AUC value following the i.v. administration of 3TC was 96.65 ± 30.77 mg · h/liter, and that following p.o. administration was 28.29 mg · h/liter. Integrated areas (AUC_{0-t}) accounted for approximately 85% of the total AUC values. Absorption of 3TC after oral administration was variable (Fig. 1), with F values of 18 and 54% in two woodchucks, respectively (Table 1). In another woodchuck (animal 4257), nucleoside concentrations after p.o. administration were above the limit of quantitation of the assay at only a few sample collection times (Fig. 1); thus, it was not possible to calculate AUC, $t_{1/2}$, or F for this woodchuck.

These pharmacokinetic studies were conducted between 27 October and 10 November 1994, which is approximately when indigenous woodchucks would begin winter hibernation. Although the animals used in the present study were not hibernating, the physiological functions of these woodchucks may have been altered from those in the euthermic state (4, 13). Hibernation typically results in a decrease in cardiac output (4); however, values for renal and hepatic blood flow in hibernating woodchucks are not available in the literature. A decreased urine output, an indicator of hibernation, was noted in these woodchucks. Furthermore, the percent of 3TC excreted unchanged in the urine in woodchucks (26%) was lower than that reported for rats (75%) (21), monkeys (32 to 59%) (2), and humans (60 to 80%) (30). Thus, the CL_R of 3TC may have been decreased in the woodchucks owing to seasonal variations in the physiological functions of the hibernating species. In addition, the low and variable F of 3TC compared with that in humans (82%) (30) may also be due to seasonal variations in physiological functions.

Interspecies scaling is the use of allometric equations to relate physiological parameters or, in general, any biological function to body weight. This technique has been used successfully for various classes of compounds to obtain useful information from preclinical pharmacokinetic investigations for designing dosage regimens for clinical trials, and it gives a rationale for the selection of an appropriate dose (3). Allometric relationships have been established between pharmacokinetic parameters and body weight for several other nucleoside analogs (16, 20). Indeed, a recent report compared clinical pharmacokinetic parameter values for 3TC with the parameters estimated from data obtained from rats and dogs (15). In the present study, the interspecies scaling was expanded to include additional data from rats (21), monkeys (2), and woodchucks.

The relationships between CL, V, and V_{SS} and species body weight (W) are illustrated in Fig. 2. The allometric equation that was generated for CL was $CL = 0.74 \cdot W^{0.76}$ ($r = 0.93$; $P < 0.025$), and that for V was $V = 1.62 \cdot W^{0.81}$ ($r = 0.98$; $P < 0.005$). The allometric relationship for V_{SS} was $V_{SS} = 1.09 \cdot W^{0.94}$ ($r = 0.99$; $P < 0.05$). Interspecies scaling of CL and V of 3TC in rats, woodchucks, dogs, and monkeys indicates a good correlation, with correlation coefficient values of greater than 0.93. The predicted CL, V, and V_{SS} for humans obtained by using the allometric relationships were 20 liters/h, 53 liters, and 62 liters, respectively. The estimates for CL and V_{SS} were similar to the observed values of 24 liters/h for CL and 59 liters for V_{SS} in humans (30). However, similar to the findings of Hussey et al. (15), the predicted V underestimated V in humans (96 liters). Thus, V_{SS} yielded a more germane allometric relationship than V.

In summary, the values for the pharmacokinetic parameters of 3TC in woodchucks obtained in the present study are similar to the values for the pharmacokinetic parameters obtained for the compound in other species. The pharmacokinetic data obtained with the woodchuck HBV animal model should be insightful for designing clinical trials. Therefore, the woodchuck model is a suitable animal model for further investigations on the pharmacokinetics and in vivo efficacy of 3TC. On the basis of a median effective concentration of 0.05 μM for HBV in 2.2.15-transfected cells (6), it is estimated that a dosage regimen of 2 mg/kg every 12 h should be adequate to maintain an antiviral effect (10 times the median effective concentration) in woodchucks.

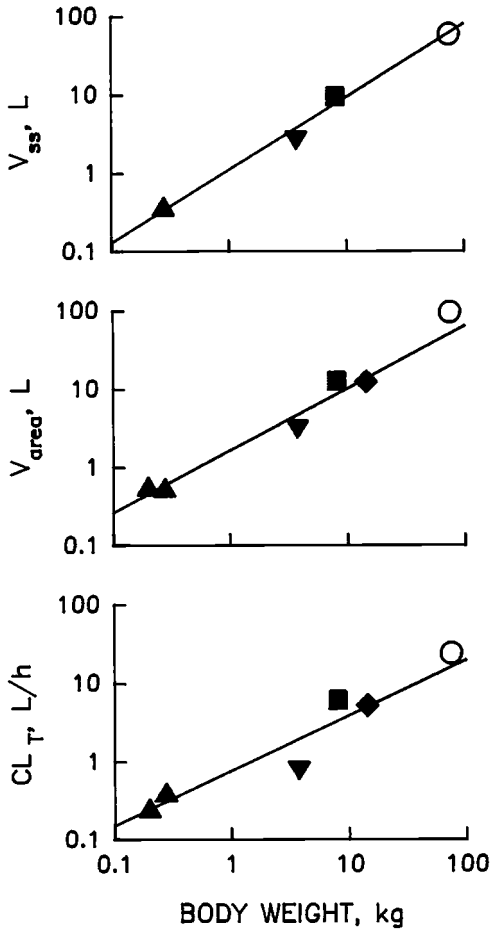

FIG. 2. Allometric relationship between CL, V, and V_{SS} of 3TC and species body weight. Symbols: ▲, rat (15, 21); ▼, woodchuck; ◆, dog (15); ■, monkeys (2); ○, humans (15, 30). Regression lines depict the relationship between the pharmacokinetic parameter and the laboratory animal's body weight.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AI-25889 and N01-AI-35164 from the National Institutes of Health and the U.S. Department of Veterans Affairs.

REFERENCES

1. **Benhamou, Y., E. Dohin, F. L. 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.
2. **Blaney, S. M., M. J. Daniel, A. J. Harker, K. Godwin, and F. M. Balis.** 1995. Pharmacokinetics of limivudien and BCH-189 in plasma and cerebrospinal fluid of nonhuman primates. Antimicrob. Agents Chemother. **39:**2779–2782.
3. **Boxenbaum, H.** 1982. Interspecies scaling, allometry, physiological time, and the ground plan for pharmacokinetics. J. Pharmacokinet. Biopharm. **10:**201–227.
4. **Bullard, R. W., and G. E. Funkhouser.** 1962. Estimated regional blood flow by rubidium 86 distribution during arousal from hibernation. Am. J. Physiol. **203:**266–270.
5. **Cammack, N., P. Rouse, C. L. P. Marr, P. J. Reid, R. E. Boehme, A. V. Coates, C. R. Penn, and J. M. Cameron.** 1992. Cellular metabolism of (−) enantiomeric 2′-deoxy-3′-thiacytidine. Biochem. Pharmacol. **43:**2059–2064.
6. **Chang, C. N., S. L. Doong, J. H. Zhou, J. W. Beach, L. S. Jeong, C. K. Chu, C. H. Tsai, and Y. C. Cheng.** 1992. Deoxycytidine deaminase-resistant stereoisomer is the active form of (±)-2′,3′-dideoxy-3′-thiacytidine in the inhibition of hepatitis B virus replication. J. Biol. Chem. **267:**13938–13942.
7. **Chen, H. S., S. Kaneko, R. Girones, R. W. Anderson, W. E. Hornbuckle, B. C. Tennant, P. J. Cote, J. L. Gerin, R. H. Purcell, and R. H. Miller.** 1993. The woodchuck hepatitis virus X gene is important in the establishment of virus infection in woodchucks. J. Virol. **67:**1218–1226.
8. **Choi, W.-B., L. J. Wilson, S. Yeola, D. C. Liotta, and R. F. Schinazi.** 1991. *In situ* complexation directs the stereochemistry of *N*-glycosylation in the synthesis of oxathiolanyl and dioxolanyl nucleoside analogues. J. Am. Chem. Soc. **113:**9377–9379.
9. **Coates, J. A., N. Cammick, H. J. Jenkinson, A. J. Jowett, M. I. Jowett, B. A. Pearson, C. R. Penn, P. L. Rouse, K. C. Viner, and J. M. Cameron.** 1992. (−)-2′-deoxy-3′-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and 2 replication in vitro. Antimicrob. Agents Chemother. **36:**733–739.
10. **Fourel, I., O. Hantz, K. A. Watanabe, C. Jacquet, B. Chomel, J. J. Fox, and C. Trepo.** 1990. Inhibitory effects of 2′-fluorinated arabinosyl-pyrimidine nucleosides on woodchuck hepatitis B virus replication in chronically infected woodchucks. Antimicrob. Agents Chemother. **34:**473–475.
11. **Galibert, F., T. V. Chen, and E. Mandart.** 1981. Localization and nucleotide sequence of the gene coding for the woodchuck hepatitis virus surface antigen: comparison with the gene coding for the human hepatitis B virus surface antigen. Proc. Natl. Acad. Sci. USA **78:**5315–5319.
12. **Hantz, O., T. Oka, L. Vitvitski, C. Pichoud, and C. Trepo.** 1984. Comparison of properties of woodchuck hepatitis virus and human virus endogenous DNA polymerases. Antimicrob. Agents Chemother. **25:**242–246.
13. **Hong, S. K.** 1957. Renal function during hypothermia and hibernation. Am. J. Physiol. **188:**137–150.
14. **Hoong, L. K., L. E. Stange, D. C. Liotta, G. W. Koszalka, C. L. Burns, and R. F. Schinazi.** 1992. Enzyme-mediated enantioselective preparation of pure enantiomers of the antiviral agent 2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC) and related compounds. J. Org. Chem. **57:**5563–5565.
15. **Hussey, E. K., K. H. Donn, M. J. Daniel, S. T. Hall, A. J. Harker, and G. L. Evans.** 1994. Interspecies scaling and pharmacokinetic parameters of 3TC in humans. J. Clin. Pharmacol. **34:**975–977.
16. **Ibrahim, S. S., and F. D. Boudinot.** 1989. Pharmacokinetics of 2′,3′-dideoxycytidine in rats: application to interspecies scale-up. J. Pharm. Pharmacol. **41:**829–834.
17. **Ikeda, N., S. Kaneko, A. Shimoda, Y. Inagaki, M. Unoura, M. Okada, Y. Yonekawa, K. Takahashi, and K. Kobayashi.** 1994. Efficiency of oxetanocin-G, a novel nucleoside against the woodchuck hepatitis virus. J. Antimicrob. Chemother. **33:**83–89.
18. **Metzler, D. M., G. L. Elfring, and A. J. McEwen.** 1974. NONLIN, a computer program for nonlinear least-squares regression. Biometrics **30:**562–563.
19. **Millman, I., T. Halbherr, and H. Simmons.** 1982. Immunological cross-reactivities of woodchuck and hepatitis B viral antigens. Infect. Immun. **35:**752–757.
19a. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals. National Institutes of Health, Rockville, Md.
20. **Patel, B. A., F. D. Boudinot, R. F. Schinazi, J. M. Gallo, and C. K. Chu.** 1990. Comparative pharmacokinetics and interspecies scaling of 3′-azido-3′-deoxythymidine (AZT) in several mammalian species. J. Pharmacobiodyn. **13:**206–211.
21. **Rajagopalan, P., L. Moore, C. K. Chu, R. F. Schinazi, and F. D. Boudinot.** Pharmacokinetics of (−)-2′-3′-dideoxy-3′-thiacytidine in rats. Submitted for publication.
22. **Riggs, D. S., J. A. Guarnieri, and S. Addelman.** 1978. Fitting straight lines when both variables are subject to error. Life Sci. **22:**1305–1360.
23. **Rocci, M. L., and W. J. Jusko.** 1983. LAGRAN program for area and moments in pharmacokinetic analysis. Comp. Prog. Biomed. **16:**203–216.
24. **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 oxathilanecytosine nucleosides. Antimicrob. Agents Chemother. **37:**875–881.
25. **Snyder, R. L.** 1985. The laboratory woodchuck. Lab. Anim. **14:**20–32.
26. **Snyder, R. L., and H. L. Ratcliffe.** 1969. *Marmota monax*: a model for studies of cardiovascular, cerebrovascular and neoplastic disease. Acta Zool. Pathol. Antverp. **48:**265–273.
27. **Summers, J., J. M. Smolec, and R. Snyder.** 1978. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc. Natl. Acad. Sci. USA **75:**4533–4537.
28. **Tyrell, D. L. J., M. C. Mitchell, R. A. Deman, S. W. Schalm, J. Main, H. C. Thomas, J. Fevery, F. Nevens, P. Beranek, and C. Vicary.** 1993. Phase II trial of lamivudine for chronic hepatitis-B. Hepatology **18:**A112.
29. **van Leeuwen, R., C. Katlama, V. Kitchen, C. A. B. Boucher, R. Tubiana, M. Mcbride, D. Ingrand, J. Weber, A. Hill, H. Mcdade, and S. A. Danner.** 1995. Evaluation of safety and efficacy of 3TC (lamivudine) in patients with asymptomatic or mildly symtomatic human-immunodeficiency-virus infection—phase I/II study. J. Infect. Dis. **171:**1166–1171.
30. **van Leeuwen, R., J. M. A. Lange, E. K. Hussey, K. H. Donn, S. T. Hall, A. J. Harker, P. Jonker, and S. A. Donner.** 1992. The safety and pharmacokinetics of a reverse transcriptase inhibitor, 3TC, in patients with HIV infection: phase I study. AIDS **6:**1471–1475.
31. **Werner, B. G., J. M. Smolec, R. Snyder, and J. Summers.** 1979. Serological relationship of woodchuck hepatitis virus to human hepatitis B virus. J. Virol. **32:**314–322.