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Pharmacokinetics, Oral Bioavailability, and Metabolic Disposition in Rats of (−)-*cis*-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl] Cytosine, a Nucleoside Analog Active against Human Immunodeficiency Virus and Hepatitis B Virus

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The pharmacokinetics and metabolism of the potent anti-human immunodeficiency virus and anti-hepatitis B virus compound, (−)-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC), were investigated in male CD rats. Plasma clearance of 10 mg of FTC per kg of body weight was biexponential in rats, with a half-life at α phase of 4.7 ± 1.1 min (mean ± standard deviation) and a half-life at β phase of 44 ± 8.8 min ($n = 5$). The total body clearance of FTC was 1.8 ± 0.1 liters/h/kg, and the oral bioavailability was 90% ± 8%. The volume of distribution at steady state (V_{SS}) was 1.5 ± 0.1 liters/kg. Increasing the dose to 100 mg/kg slowed clearance to 1.5 ± 0.2 liters/kg/h, lowered the V_{SS} to 1.2 ± 0.2 liters/kg, and reduced the oral bioavailability to 65% ± 15%. FTC in the brains of rats was initially less than 2% of the plasma concentration but increased to 6% by 2 h postdose. Probenecid elevated levels of FTC in plasma as well as in brains but did not alter the brain-to-plasma ratio. The urinary and fecal recoveries of unchanged FTC after a 10-mg/kg intravenous dose were 87% ± 3% and 5% ± 1.6%, respectively. After a 10-mg/kg oral dose, respective urinary and fecal recoveries were 70% ± 2.5% and 25% ± 1.6%. Two sulfoxides of FTC were observed in the urine, accounting for 0.4% ± 0.03% and 2.7% ± 0.2% of the intravenous dose and 0.4% ± 0.06% and 2.5% ± 0.3% of the oral dose. Also observed were 5-fluorocytosine, representing 0.4% ± 0.06% of the intravenous dose and 0.4% ± 0.07% of the oral dose, and FTC glucuronide, representing 0.7% ± 0.2% of the oral dose and 0.4% ± 0.2% of the intravenous dose. Neither deaminated FTC nor 5-fluorouracil was observed in the urine (less than 0.2% of dose). The high oral availability and minimal metabolism of FTC encourage its further preclinical development.

2′,3′-Dideoxynucleoside analogs, such as zidovudine, didanosine, and zalcitabine (ddC), are clinically effective in therapy against human immunodeficiency virus (HIV) infection. Both didanosine and ddC act synergistically with zidovudine in vitro (13, 23). However, toxicity and viral resistance are associated to various degrees with each of these drugs. Toxicity is most pronounced for ddC, which has excellent in vitro activity against HIV (25) but induces peripheral neuropathy in humans at relatively low doses (41). ddC is also highly active against hepatitis B virus (HBV) (36). HBV is a widespread health problem that frequently results in hepatocellular carcinoma in chronic carriers.

(−)-3′-Thia-2′-deoxycytidine (3TC) and its 5-fluoro congener (−-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC) (Fig. 1) are ddC analogs that may have improved therapeutic indices for HIV and HBV relative to that of ddC (3, 14, 33–35, 39). Racemic 5-fluoro-3′-thia-2′-deoxycytidine is highly active against HIV as well as HBV (10, 34). For both nucleoside analogs, the antivirally active (−) enantiomer, corresponding to the unnatural L form, is less toxic to bone marrow stem cells and other cultured cells than is the (+) enantiomer (6, 14, 33, 34). Inside the cell, FTC is initially phosphorylated by deoxycytidine kinase and further anabolized to the triphosphate (14, 33). Clinical trials of 3TC as a therapy for HIV infection are in progress (30).

The potent in vitro HIV and HBV activities of FTC, coupled with its low toxicity in cell culture, justify its preclinical development. This report describes the plasma pharmacokinetics and metabolic disposition of FTC in male CD rats.

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC) grade acetonitrile, 88% formic acid, and analytical reagent grade ammonium hydroxide were obtained from Mallinckrodt (Paris, Ky.). Trifluoroacetic acid, triethylamine, phosphoric acid, and isopropanol (all HPLC grade) were from Fisher Scientific (Pittsburgh, Pa.). Disodium-EDTA was from EM Science (Gibbstown, N.J.). Probenecid, *Escherichia coli* β-glucuronidase, and 5-fluorouracil were purchased from Sigma Chemical Co. (St. Louis, Mo.). Absolute ethanol was bought from Aaper Alcohol and Chemical Co. (Shelbyville, Ky.). 5-Fluorocytosine was from Aldrich Chemical Co., Inc. (Milwaukee, Wis.).

FTC was synthesized as described elsewhere (5) and was resolved with hog liver esterase (21). Chiral chromatography on a Chiral Pak column (4.6 by 250 mm; J. T. Baker, Phillipsburg, N.J.) was used to characterize the enantiomeric purity of the FTC preparation. Elution with 1:1 isopropanol-absolute ethanol gave a peak of FTC at 316 s,

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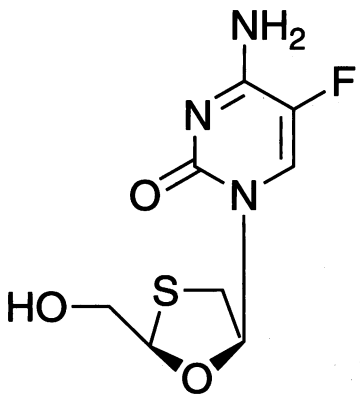

FIG. 1. The structure of FTC.

while the (+) enantiomer eluted at 450 s. This analysis indicated that FTC contained 1% of the (+) enantiomer in the resolved material.

Sulfoxides of FTC were synthesized with sodium periodate and resolved by reverse-phase HPLC. Briefly, 8 ml of 10 mM FTC was incubated for 2.5 h with 4 ml of 100 mM sodium periodate. The reaction was quenched with 100 μl of 50% glycerol. The two diastereomeric sulfoxides, corresponding to oxygen on the α and β faces of the oxathiolane ring, were separated by HPLC on a LiChroCART (E. Merck) C_{18} 5 μm column (4 by 250 mm) in 0.1% trifluoroacetic acid, with a gradient of 0 to 4.5% acetonitrile over 1,200 s. One isomer (isomer a) eluted at 13.9 min, and the other (isomer b) eluted at 14.9 min. The overall yield was about 20% for each isomer. Fast-atom bombardment mass spectra gave an *m/e* of 264, corresponding to the molecular ion $(M + H)^+$, for both compounds.

Radiolabeled [6-^{3}H]FTC was synthesized by Moravek Biochemicals (Brea, Calif.) with a specific activity of 9 Ci/mmol. To remove contaminating (+) enantiomer, tritiated FTC was further purified by digestion with *E. coli* cytidine deaminase. After alkalinization with ammonia and chromatography on QAE A25 Sephadex, chiral chromatography indicated that less than 0.1% of this material was the (+) enantiomer of FTC. Tritiated water was present (4% of total).

Cannulation, dosing, and sample collection. Male Sprague-Dawley (CD) rats (220 to 320 g; Charles Rivers Laboratories, Raleigh, N.C.) were anesthetized with acepromazine maleate (1 mg/kg of body weight; Fermenta Animal Health Co., Saint Louis, Mo.) and Ketalar (100 mg/kg; Parke-Davis, Morris Plains, N.J.), and the right jugular veins were cannulated by the procedure described by Upton (38). Six rats were used for each dose level.

A crossover experimental design was employed. The day after surgery, animals that had been made to fast were dosed with drug dissolved (10 mg/ml) in 0.9% saline. Oral doses were administered with a feeding needle; 2 days later, intravenous doses were injected via the jugular cannula as a 30-s bolus, and the cannula was rinsed with saline (0.5 ml). Compound was effectively removed from the cannula by this washing procedure. After all blood samples were obtained for the first branch of the study, the rats were transfused with 4.5 ml of heparinized whole blood.

Blood (0.40 to 0.45 ml) was drawn into syringes containing 30 μl of 5% disodium-EDTA and was centrifuged (2,000 × *g* for 15 min at 4°C). An equal volume of heparinized normal saline was infused after each sample was taken. To remove proteins, plasmas were ultrafiltered with Centrifree tubes having a 30,000-molecular-weight cutoff (Amicon, Beverly, Mass.), and an 8R centrifuge (International Equipment Co., Needham Heights, Mass.) operated at 3,000 × *g* for 45 min at 4°C. Ultrafiltrates were generally analyzed immediately.

Pharmacokinetic analysis. The equations for the oral experiment and for the intravenous experiment were fitted to the respective plasma concentration-time data with Solver, the nonlinear least-squares minimization function of Excel (Microsoft Corporation, Redmond, Wash.). These equations are as follows: plasma concentration = $Ie^{-k_{el} \cdot (t-lag)} - Ie^{-k_{abs} \cdot (t-lag)}$, where I = intercept, k_{el} is the elimination rate, k_{abs} is the absorption rate, t is time, and lag is the lag time after dosing before absorption started; plasma concentration $= Ae^{-a \cdot t} + Be^{-b \cdot t}$, where A and B are intercepts and a and b are rate constants. The discrimination criterion for choosing among monophasic, biphasic, and triphasic models was the F statistic calculated by the difference in the sums of squares weighted by the change in degrees of freedom (26). Curves were fitted to the data by minimizing the sum of the squared deviations of observed from predicted values, with $1/C^2$ weighting. The default settings of Solver were used to control the minimization algorithm. These settings result in the use of the Newton modification of the Marquardt algorithm. Pharmacokinetic parameters were derived from the intercepts and rate constants of the exponential terms of the fitted curves, with the following standard pharmacokinetic equations (18): area under the plasma concentration-time curve (AUC), $AUC_{IV} = (A/a) + (B/b)$ (where IV is intravenous dose) and $AUC_{PO} = (I/k_{el}) + (I/k_{abs})$ (where PO is oral dose); area under the first moment of the concentration-time curve (AUMC), $AUMC = (A/a^2) + (B/b^2)$; mean residence time (MRT), MRT = AUMC/AUC; clearance (CL), CL = dose/AUC (where dose was expressed in micromoles per kilogram); volume of distribution at steady state (V_{SS}), V_{SS} = CL · MRT. The AUC of the 10-mg/kg oral dose was calculated by the AUC equation given above. The AUC of the 100-mg/kg oral dose was calculated by the linear trapezoid method, with extrapolation to infinity. The rate constant of the terminal elimination phase of the 100-mg/kg oral dose was estimated from the curve generated by nonlinear least-squares minimization. Renal CL (CL_R) was calculated by multiplying the CL by the fraction of dose excreted in the urine. Oral bioavailability (F) was calculated by dividing the AUC after oral dosing by the AUC after intravenous dosing.

Brain penetration. The concentrations of FTC in the brains of six male CD rats (Charles Rivers) were determined at 15, 30, 60, 90, 120, and 180 min after an intraperitoneal dose of 10 mg of FTC per kg. An equal number of animals were pretreated intraperitoneally with 60 mg of probenecid per kg 15 min before receiving the FTC dose. In a separate experiment, groups of five male CD rats were dosed with either 10 or 50 mg of FTC per kg by intraperitoneal injection, and the concentrations of FTC in the brains and plasma were determined at 30 min and at 2 h postdose. At sacrifice, the rats were lightly anesthetized with CO_2 and decapitated, and blood was collected into a beaker containing 50 μl of 5% disodium-EDTA. The brains were rinsed in saline, the frontal lobes were dissected out, and the meninges and major blood vessels were removed. After homogenization of the frontal lobes in 2 volumes (wt/vol) of ice-cold deionized water, the homogenate was clarified by centrifugation. Brain homogenate supernatants and blood plasma samples were ultrafiltered with Centrifree tubes and analyzed by HPLC. Some brain extracts were prepared by precipitation with a

50% volume of ice-cold 10% trichloroacetic acid. Recovery of FTC spiked into a control brain before homogenization was 100% by both methods.

Plasma binding. Binding of FTC to high-molecular-weight plasma components at 37°C was determined. Rat plasma samples were spiked with authentic compound, placed into Centrifree tubes, loaded into a centrifuge maintained at 37°C, and allowed to equilibrate for 10 min. Ultrafiltrates were prepared by centrifugation and assayed by HPLC as usual.

Metabolic disposition of FTC. Fed male CD rats were intravenously or orally given 10 mg of [6-^{3}H]FTC per kg (300 μCi/kg). An additional four rats were injected 15 min before an intravenous dose of FTC with 1 mg of the potent uracil reductase inhibitor, 5-ethynyluracil (31), per kg. This dose of 5-ethynyluracil was sufficient to completely inhibit the uracil reductase in rats for 6 to 8 h (data not shown). The rats were housed individually in Nalgene metabolism cages. The animals had access to food and water throughout the study. Urine samples were collected into 100 μl of 5% sodium azide. Urine collection periods were 0 to 24, 24 to 48, and 48 to 96 h. Feces were collected for 48 h. Urine samples were centrifuged for 5 min in a Fisher 235C Microfuge (Fisher Scientific) and assayed by HPLC. The radioactivity in 200-μl aliquots of the fecal homogenates was determined with a Packard tissue oxidizer after homogenization in 200 ml (final volume) of sodium phosphate (0.2 M; pH 6.7) with a Polytron (Brinkmann Instruments, Westbury, N.Y.) at maximum power for 45 s. Urine samples and feces were stored frozen at −20°C until analysis. Recovery of FTC spiked into control feces was 98%.

HPLC analysis. Control of LKB 2150 pumps and data acquisition was performed by a Digital Specialties (Chapel Hill, N.C.) computer with CHROM software (Burroughs Wellcome Co.). Samples (50 to 100 μl) were injected with a 712 WISP injector (Waters, Milford, Mass.). Ultrafiltered plasma samples were analyzed by reverse-phase HPLC on C_{18} Microsorb columns (4.5 by 250 mm, 5-μm particle size; Rainin, Woburn, Mass.). The mobile phase consisted of 9% acetonitrile (buffer A) in ammonium phosphate (25 mM [pH 3.0]). Isocratic elution at 1 ml/min was used to measure FTC in plasma samples. The retention time of FTC was 420 s. The UV A_{280} of the column effluent was monitored with an LDC (Laboratory Data Control) (Riviera Beach, Fla.) SM5000 diode array detector. The concentration of FTC was determined by reference to a standard curve of authentic compound.

Feces were analyzed with a C_{18} column with a gradient of acetonitrile in 0.1% trifluoroacetic acid (1.2% acetonitrile isocratic for 300 s, followed by a linear gradient to 15% acetonitrile over 1,500 s). Urinary metabolites were analyzed on a Microsorb C_{18} column with a three-part gradient in triethylamine formate buffer (0.1% triethylamine, formic acid to pH 3.5). Buffer A had no acetonitrile, while buffer B had 60% acetonitrile. Part one was 0 to 4% buffer B over 1,200 s, part two was 4 to 20% buffer B over 1,200 s, and part three was 20 to 100% buffer B over 600 s. The flow rate was 1 ml/min. Metabolites eluted with the following retention times: 5-fluorocytosine, 320 s; 5-fluorouracil, 460 s; sulfoxide a, 1,270 s; sulfoxide b, 1,390 s; FTC β-glucuronide, 2,110 s; FTC, 2,170 s; and deaminated FTC, 2,350 s. The β-glucuronide of FTC was identified by enzymatic peak shifting with *E. coli* β-glucuronidase. The concentrations of radiolabeled urinary metabolites were determined by monitoring the column effluent with a Packard Flow-one/beta (Packard Instruments, Downers Grove, Ill.) equipped with a 1,000-μl flow cell. Ecolite scintillant (ICN Biomedicals, Inc., Irvine, Calif.) was used at 3 ml/min. A standard curve was constructed with the radioactive dosing solution. In some cases, 1-min fractions were collected, 5 ml of Ready Safe scintillant was added (Beckman Instruments, Inc., Fullerton, Calif.), and the radioactivity was determined in a Packard CA1900 scintillation counter.

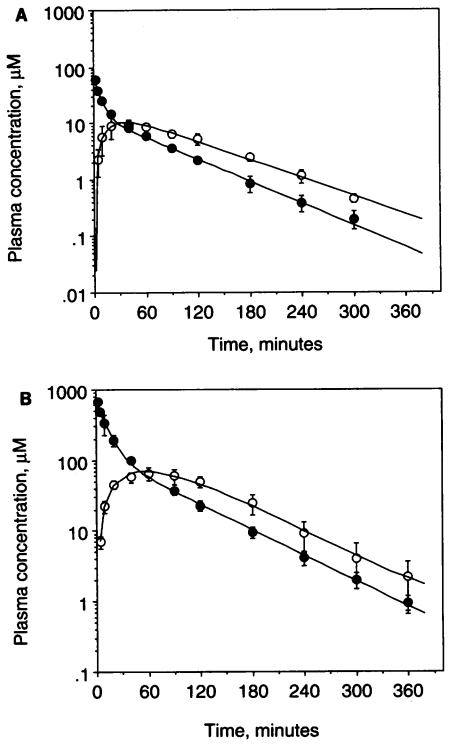

FIG. 2. (A) Plasma concentration-time curves in rats dosed orally and intravenously with 10 mg of FTC per kg. Error bars are the SD (n = 5 for the intravenous dosage and n = 6 for the oral dosage). (B) Plasma concentration-time curves in rats dosed orally and intravenously with 100 mg of FTC per kg. Error bars are the SD (n = 6).

Metabolite identification. Metabolites were identified by comparison with authentic standards and by UV spectral analysis with the diode array detector. HPLC-mass spectroscopy was used to identify sulfoxide b. HPLC-mass spectroscopy employed a Waters model 625LC system connected to a Waters 486 UV detector (Waters Associates, Danvers, Mass.). A Rainin Microsorb 5-μm C_{18} column (4.6 by 250 mm) was eluted at 1 ml/ml with a gradient of acetonitrile from 0 to 30% over 30 min. The mobile phase contained 0.1% formic acid. The eluent was analyzed directly with a Sciex API III mass spectrograph (Perkin Elmer Sciex, Toronto, Ontario, Canada) in the positive-ion heated nebulizer mode. The glucuronide metabolite was identified by digestion with *E. coli* β-glucuronidase (Sigma Chemical Co.) in the presence and absence of the β-glucuronidase inhibitor 1,4-disaccharolactone (Sigma Chemical Co.).

RESULTS

HPLC assay. The lower limit of quantitation of the HPLC assay was 0.25 μM with a 100-μl sample. The respective intra- and interassay coefficients of variation determined at 200 and 0.5 μm were 3.9 and 5.6%. Recovery of spiked FTC

TABLE 1. Pharmacokinetic parameters of FTC in rats at 10 mg/kg

Rat	After intravenous administration							After oral administration						
	AUC (μM · h)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	V_{SS} (liter/kg)	CL (liter/h/kg)[a]	CL_R (liter/h/kg)	f_e[b]	AUC (μM · h)	Lag time (min)[c]	$t_{1/2abs}$ (min)[d]	$t_{1/2el}$ (min)	C_{max}	T_{max}	F
A	19.8	5.4	40.5	1.4	2.0	1.7	0.87	19.2	3.4	28	52	10	60	97
B	ND[e]	ND	ND	ND	ND	ND	ND	23.5	1.6	33	39	11	40	ND
C	22.5	5.5	53.4	1.6	1.8	1.6	0.88	18.8	1.2	19	49	8	40	83
D[f]	22.9	3.3	31.6	1.5	1.8	1.5	0.84	22.0	3.7	4	59	14	20	96
E	23.9	5.5	51.5	1.6	1.7	1.4	0.84	22.6	2.8	5	60	13	20	95
F	21.4	3.6	42.5	1.4	1.9	1.5	0.77	17.2	1.3	13	53	9	40	80
Mean	22.1	4.7	43.9	1.5	1.8	1.5	0.84	20.6	2.3	17	52	11	43	90
SD	1.6	1.1	8.8	0.1	0.1	0.1	0.04	2.5	1.1	12	8	2	10	8

[a] CL, total body clearance.
[b] f_e, fraction excreted in the urine.
[c] The lag time calculated between dosing and onset of absorption.
[d] Also influenced by distribution phase kinetics.
[e] ND, not done.
[f] This animal had a third phase to its elimination of FTC with a $t_{1/2}$ of 80 min.

from whole rat blood after centrifugation and ultrafiltration was essentially 100%.

Pharmacokinetics. The average plasma concentration versus time curves for oral and intravenous FTC in rats at 10 and 100 mg/kg are shown in Fig. 2A and B. The pharmacokinetic parameters derived from individual rats dosed with 10 mg of FTC per kg are shown in Table 1. Similar data from rats dosed with 100 mg of FTC per kg are shown in Table 2.

Elimination of 10 mg of intravenous FTC per kg from the plasma was best described by a biphasic exponential equation. The half-life at α phase ($t_{1/2\alpha}$) was 4.7 ± 1.1 min, and the half-life at β phase ($t_{1/2\beta}$) was 44 ± 8.8 min (values are means ± standard deviations [SD]; $n = 6$). The AUC was 22 ± 1.6 μM · h, corresponding to a total body CL of 1.8 ± 0.1 liters/kg/h. This value is comparable to that for the renal plasma flow of the rat (1.6 liters/h/kg for a 300-g rat [17, 20]). The V_{SS} was 1.5 ± 0.1 liters/kg, indicating good penetration of the tissues by FTC.

After the 10-mg/kg oral dose, the AUC was 21 ± 2.5 μM · h, indicating an F of 90% ± 8%. The terminal elimination half-life ($t_{1/2el}$) after oral dosing was 52 ± 8 min, which is slower than that after intravenous dosing. The C_{max} was 11 ± 2 μM at a T_{max} of 43 ± 10 min. The apparent absorption half-life ($t_{1/2abs}$) was 17 ± 12 min at this dose, although the biphasic nature of the elimination process observed after the intravenous dose makes interpretation of this value difficult, since absorption and distribution are occurring simultaneously.

The pharmacokinetics of FTC appeared to be dependent on dosing over the range of 10 to 100 mg/kg in rats. At the high dose, CL was slower ($P < 0.02$), the V_{SS} was less ($P < 0.02$), and the F was less ($P < 0.01$) than that at the low dose. After the 100-mg/kg oral dose, absorption was slowed until it was close to the elimination rate. For this reason, nonlinear least-squares minimization was not used to determine the AUC. Instead, the linear trapezoid rule with extrapolation to infinity was employed.

Binding of FTC to rat plasma proteins was less than 10% over a range of 0.1 to 100 μM.

Brain penetration. A preliminary experiment showed that the concentration of FTC in the brain was low relative to that in the plasma (Fig. 3). At 15 min after a 10-mg/kg intraperi-

TABLE 2. Pharmacokinetic parameters of FTC in rats at 100 mg/kg

Rat	After intravenous administration							After oral administration						
	AUC (μM · h)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	V_{SS} (liter/kg)	CL (liter/h/kg)[a]	CL_R (liter/h/kg)	f_e[b]	AUC (μM · h)[c]	Lag time (min)	$t_{1/2abs}$ (min)[d]	$t_{1/2el}$ (min)[e]	C_{max}	T_{max}	F
1	250	5.9	47.8	1.4	1.6		ND[f]	219	3.1	36	41	88	60	88
2	265	6.8	50.9	1.3	1.5		ND	178	2.5	44	49	59	60	67
3	304	11.3	58.4	0.8	1.3	1.1	0.80	130	1.8	35	38	53	60	43
4	267	7.5	44.0	1.1	1.5	1.2	0.82	166	3.3	31	35	74	40	62
5	229	5.7	46.2	1.3	1.8	1.6	0.86	167	2.1	35	39	61	90	73
6	274	7.9	53.5	1.3	1.5	1.4	0.90	150	3.4	31	36	62	90	55
Mean	265	7.5[g]	50.1	1.2[h]	1.5[h]	1.3	0.84	168	2.7	35[h]	40[h]	66	57	65[h]
SD	25	2.0	5.3	0.2	0.2	0.2	0.04	30	0.7	5	5	13	21	15

[a] CL, total body clearance.
[b] f_e, fraction excreted in the urine.
[c] The AUC was calculated by the linear trapezoid rule, with extrapolation to infinity using the terminal elimination rate calculated from the nonlinear least-squares minimized curve.
[d] Because the rate of absorption is close to the rate of elimination, these values are for comparison only.
[e] These $t_{1/2}$s were calculated from the nonlinear least-squares minimized curve.
[f] ND, not done.
[g] Significantly different than at 10 mg/kg ($P < 0.05$).
[h] Significantly different than at 10 mg/kg ($P < 0.02$).

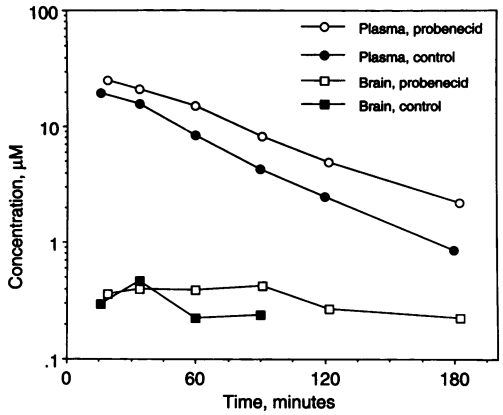

FIG. 3. Concentrations of FTC in the brains and plasma of control and probenecid-treated rats. Data points represent individual rats. The dose of FTC was 10 mg/kg by the intraperitoneal route. The FTC concentrations in the brains of control rats were too low to measure by 2 h postdose.

toneal dose, the concentration of drug in the brain was <2% of that in the plasma. However, although the plasma concentration declined rapidly, the brain concentration was relatively stable, so that the brain/blood ratio steadily increased. The concentration of FTC was higher in the brains of rats treated with probenecid, but this was offset by the higher levels of FTC in the plasma of these animals, so that the brain/blood ratio of FTC was unaffected.

Probenecid appeared to slow the elimination of FTC in rats. Linear regression of the natural log of the concentration of FTC in plasma on time indicated that the half-lives were from 32 to 39 min in control animals and from 41 to 50 min in probenecid-treated animals (± 95% confidence limits [$P < 0.05$]).

TABLE 3. Penetration of FTC into the brains of rats

Dose (mg/kg)	Time postdose (min)	Concn in plasma (μM)[a]	Concn in brain (μM)[a]	Brain/blood ratio (%)[a]
10	30	24.1 ± 2.1	0.80 ± 0.40	3.4 ± 1.8
50	30	102 ± 12.5	2.50 ± 0.67	2.5 ± 0.58
50	120	25.6 ± 3.8	1.91 ± 0.17	6.9 ± 0.56

[a] Data shown are the means ± SD ($n = 5$).

In a separate experiment, the concentration of FTC in the brain 30 min after an intraperitoneal dose of 10 mg/kg was 3.4% ± 2% of the concentration in plasma ($n = 5$; Table 3). At 50 mg/kg and 30 min postdose, the concentration of FTC in the brain was 2.5% ± 1% of the concentration in plasma ($n = 6$), indicating no effect of dose on brain penetration. At 2 h after the 50-mg/kg dose, the concentration of FTC in the brain was 6.9% ± 0.6% of the concentration in plasma ($n = 3$), consistent with the data shown in Fig. 3. The levels of FTC in the brains of rats 2 h after a dose of 10 mg of FTC per kg were too low to measure accurately (data not shown).

Metabolic disposition. An HPLC chromatogram of radioactivity in rat urine after an intravenous dose of 10 mg of (−)-[6-^{3}H]FTC per kg is shown in Fig. 4. In addition to unchanged FTC and a small amount of tritiated water, minor amounts of 5-fluorocytosine, the β-glucuronide of FTC, and the two diastereomeric sulfoxides of FTC are apparent in the chromatogram. Deaminated FTC was not observed in urine

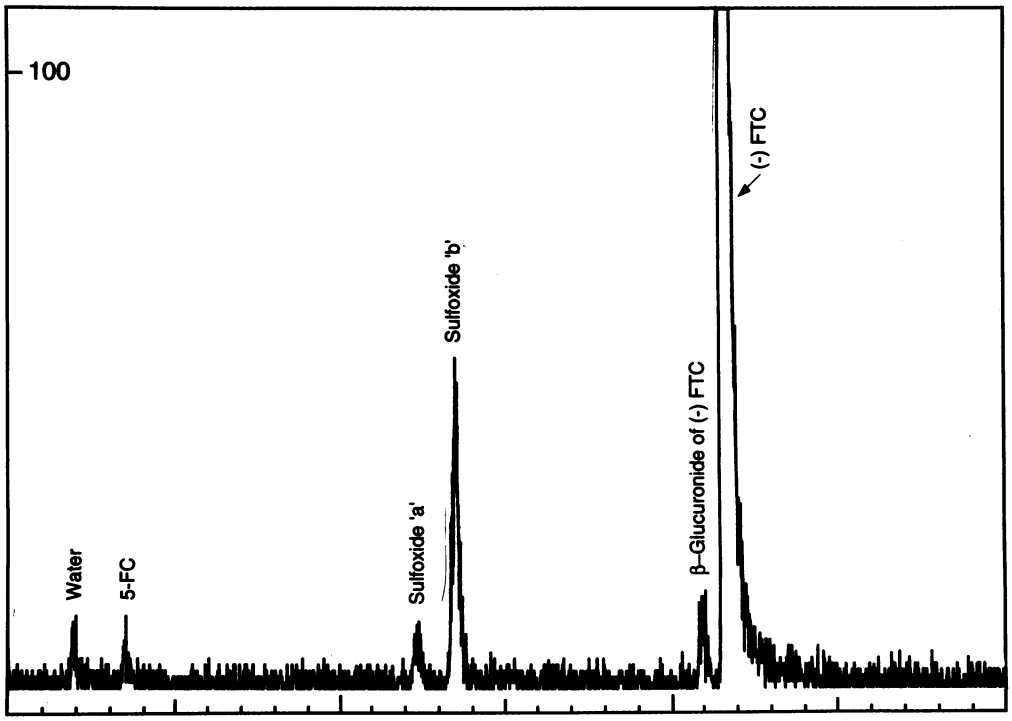

FIG. 4. Reverse-phase C_{18} HPLC of 0- to 24-h rat urine from an animal dosed intravenously with 10 mg of FTC per kg. The effluent was monitored for radioactivity with a flow monitor. 5-Fluorouracil (5-FU) eluted at 460 s, while deaminated FTC eluted at 2,350 s; neither compound was a detectable metabolite of FTC.

TABLE 4. Metabolic disposition of radiolabeled FTC in rats at 10 mg/kg[a]

Dose method	Recovery of radioactivity (% of dose)			Recovery of metabolites in urine (% of dose)							
	Urine	Feces	Total[b]	Water	5-FC[c]	5-FU[d]	Sulfoxide a	Sulfoxide b	β-Glucuronide	FTC	FTU[e]
Oral	74 ± 2.8	25 ± 1.6	99 ± 3.2	0.4 ± 0.06	0.4 ± 0.07	<0.1	0.4 ± 0.06	2.5 ± 0.26	0.7 ± 0.17	69 ± 2.5	<0.1
Intravenous	91 ± 3.4	5 ± 1.6	96 ± 3.7	0.4 ± 0.1	0.3 ± 0.02	<0.1	0.5 ± 0.05	2.7 ± 0.24	0.4 ± 0.18	86 ± 3.2	<0.1

[a] Data are the means ± SD for four animals.
[b] The total recovery is the sum of the 0- to 96-h urinary recovery and the 0- to 48-h fecal recovery.
[c] 5-FC, 5-fluorocytosine.
[d] 5-FU, 5-fluorouracil.
[e] FTU, the uridine analog of FTC.

or feces, consistent with the low activity of cytidine deaminase against this enantiomer (14). 5-Fluorouracil was also undetectable. This observation was true even for animals treated with the potent uracil reductase inhibitor 5-ethynyluracil (data not shown). The small amount of tritiated water recovered in the urine is consistent with the presence of tritiated water in the dose.

The metabolic disposition of FTC in rats is summarized in Table 4. Metabolism was minimal, and recovery of intact FTC in urine and feces was high, with 96% of the intravenous dose recovered unchanged. Of the dose recovered in the urine, 98% was excreted in the first 24 h postdose. Although only 5% of the dose was recovered in the feces after the intravenous dose, 25% of the oral dose was recovered in the feces.

DISCUSSION

Comparison of the AUCs of FTC after oral and intravenous doses indicates high F of FTC in rats (90% at 10 mg/kg). However, 25% of the oral dose was recovered in the feces. Since only 5% of intravenous FTC was recovered in the feces, it seems that a significant proportion of the oral dose is unabsorbed. These apparently conflicting results suggest that CL of FTC is slower after the oral dose, inflating the oral AUC. Even at 100 mg/kg, at which concentration oral absorption was slowed and reduced, the F of FTC was excellent (65% ± 15%).

The decrease in F and the increase in the $t_{1/2abs}$ at higher doses of FTC suggest that absorption from the gut is saturable. The observations that CL and V_{SS} are lower and that the $t_{1/2\alpha}$ is longer at the 100-mg/kg FTC dose may also be explained by saturation of transport processes at the relatively high level of FTC present in the rats at this dose.

Metabolic transformation of FTC was minimal, so that essentially all of the CL was due to renal processes (that is, $CL \simeq CL_R$). The CL of FTC was greater than the glomerular filtration rate and was similar to the renal plasma flow (17, 20). This rapid CL suggests that FTC is actively secreted by the kidney, as are other natural nucleosides such as adenosine (27). Probenecid, an inhibitor of the organic anion transporter, has been shown to inhibit the secretion of zidovudine (4, 7, 8, 19, 40), presumably by interfering with transport systems in the kidney. The ability of probenecid to slow the CL of FTC is thus consistent with the idea that an active secretory mechanism of some sort exists for FTC in the kidney, although it does not seem likely that the organic anion transporter is directly involved in the renal secretion of the weakly cationic FTC.

Table 5 compares the CLs and CL_Rs of FTC and other HIV-active pyrimidine nucleosides reported in the literature. Remarkably little variation of either CL or CL_R in rodents among the variety of pyrimidine nucleosides that have been investigated to date has been observed. The CL_R of most of these compounds is above the glomerular filtration rate of the corresponding species and is, in fact, often close to the renal plasma flow, suggesting that a highly active renal transport may be a common, rate-determining feature of their elimination in rodents.

Penetration of FTC into the brain would be a desirable feature for compounds used to treat AIDS. However, the brain/blood ratio of FTC in rats was low and could be accounted for by contamination of the brain preparation with material outside the blood-brain barrier, such as endothelial cells. The observed brain/blood ratio of FTC in rats is similar to the cerebrospinal fluid/plasma ratio of ddC in monkeys (24). For comparison, in rats orally given 6.7 mg of zidovudine per ml, the brain/blood ratio is slightly higher (0.08 [16]). The concentration of FTC in plasma declined faster than that in the brain because of a system hysteresis effect, causing the brain/blood ratio to rise over time. It was hoped that probenecid would inhibit efflux of FTC from the brain, as has been reported for zidovudine and didanosine (15, 19), but the present study showed that 60 mg of probenecid per kg did not affect the brain/blood ratio of FTC in rats.

The enzymes responsible for production of the 3′-sulfoxides of FTC have not been identified. The amount of FTC oxidized was independent of the dose route, indicating that there is no first-pass effect and suggesting that sites of oxidation other than the intestine or liver may be important.

TABLE 5. Total CL and CL_R of anti-HIV pyrimidine nucleosides in rodents

Species	Compound	Dose (mg/kg)	Total CL (liter/h/kg)	CL_R (liter/h/kg)	Reference
Rat	FTC	10	1.8	1.5	The present paper
	AzddMeC[a]	10	1.6	0.8	1
	Zidovudine	10	2.8	1.9	29
	Zidovudine	20	1.7	1.2	37
	ddC	10	1.7	0.7	22
	D_4T[b]	25	1.8	1.4	2
	FLT[c]	25	1.2	1.1	2
Mouse	D_4T[b]	25	2.0		32
	AZddU[d]	50	1.3		11
	ddC	100	1.8		24
	FTC	10	2.3	2.2	13a
	3TC	18	2.4	2.2	12

[a] AzddMeC, 3′-azido-2′,3′-dideoxy-5-methylcytidine.
[b] D_4T, 3′-deoxy-2′,3′-dideoxyhydrothymidine.
[c] FLT, 3′-fluoro-3′-deoxythymidine.
[d] AZddU, 3′-azido-2′,3′-dideoxyuridine.

The flavin-containing monooxygenase enzymes characterized by Ziegler (42) are a likely source of these 3'-sulfoxides, although it is possible that other oxidative enzymes play a role. The possibility that low urinary recovery of sulfoxides was due to rereduction of sulfoxide was tested by injecting a rat with radiolabeled sulfoxides. All of the recovered radioactivity was present as unchanged sulfoxides, with no FTC detectable (data not shown).

The production of the small amounts of 5-fluorocytosine observed in the urine samples was unexpected, since uridine phosphorylase is known to be inactive against cytidine analogs (28). The source of this metabolite is unknown. 5-Fluorocytosine is not toxic to humans except at very high doses and in individuals that convert it to 5-fluorouracil (9).

In conclusion, the high *F* of FTC and its resistance to metabolism encourage its further preclinical development. It remains to be seen what levels of FTC in the brain are achievable in humans and the effect that such levels of FTC might have on the neurological manifestations of AIDS.

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