Pharmacokinetics and Metabolism of Racemic 2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine in Rhesus Monkeys

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Received 7 May 1992/Accepted 7 September 1992

2',3'-Dideoxy-5-fluoro-3'-thiacytidine (FTC) is a nucleoside analog that selectively inhibits human immunodeficiency and hepatitis B viruses in vitro. In this study, the preclinical pharmacokinetics of racemic FTC in rhesus monkeys following intravenous and oral administration were characterized. The terminal half-life of FTC was independent of the route of administration and averaged 1.34 ± 0.18 h (mean ± standard deviation). Total clearance of FTC was moderate to high, averaging 1.49 ± 0.24 liters/h/kg. Qualitative assessment of urine samples suggests that renal excretion of unchanged FTC was the major route of elimination of the nucleoside. The compound was also eliminated by metabolism and the deaminated biotransformation product 2,3'-dideoxy-5-fluoro-3'-thiauridine (FTU) was detected in serum and urine. This metabolite has no antiviral activity in human lymphocytes and liver cells. FTC and the metabolite FTU were conjugated, to a minor extent yielding the corresponding glucuronides. No 5-fluorouracil was detected in serum or urine. This is consistent with chromatographic studies using a chiral column that indicated that when racemic FTC is treated with cellular cytidine-deoxycytidine deaminase, the α-(+)

-FTU, whereas the α-(−)-enantiomer is essentially resistant to this enzyme. The steady-state volume of distribution of FTC in serum averaged 2.23 ± 0.42 liters/kg, and the nucleoside analog was distributed into the cerebrospinal fluid, which suggests that this drug penetrated the blood-brain barrier. Absorption of FTC after oral administration was rapid, with bioavailability averaging 73 ± 6%. Taken together, the results indicate that the unusual α-(−)-enantiomer of FTC should be evaluated further in rhesus monkeys prior to determination of whether this compound is useful for treatment of human immunodeficiency and hepatitis B virus infections.

Discovery of new antiviral agents with superior virological and pharmacological profiles than currently available drugs remains an important goal for treatment of viral infections caused by human immunodeficiency virus type 1 (HIV-1), HIV-2, and hepatitis B virus (HBV) (29). 3'-Azido-3'-deoxythymidine, 2',3'-dideoxinosine, and 2',3'-dideoxythidine (DDC) are nucleoside analogs that are licensed for treatment of AIDS. These compounds have been studied extensively and found to provide significant benefit to AIDS patients despite their side effects, such as anemia and neutropenia for 3'-azido-3'-deoxythymidine, peripheral neuropathy and pancreatitis for 2',3'-dideoxynosine, and peripheral neuropathy for DDC (29). Fortunately, these toxicities can be controlled by reducing the dosage and by using these compounds in combination (22). Racemic 2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC) is a new nucleoside analog with unusually potent activity against HIV-1, HIV-2, and HBV in culture, with median effective concentrations in the submicromolar range (10, 11, 24, 27, 28). The compound is markedly less toxic in certain cells than other, related cytidine analogs. For example, unlike DDC and racemic 2',3'-dideoxy-3'-thiacytidine (BCH-189), FTC does not interfere with mitochondrial DNA synthesis, even at a concentration greater than 200 μM (10). Inhibition of this function by nucleoside analogs has been associated with peripheral neuropathy in humans (4).

The purpose of this study was to characterize the preclinical pharmacokinetics of FTC in rhesus monkeys prior to studies of this compound with humans. The disposition of FTC was assessed after intravenous (i.v.) and oral (p.o.) administration. The metabolic fate of the nucleoside was also examined.

MATERIALS AND METHODS

Chemicals. FTC and 2',3'-dideoxy-5-fluoro-3'-thiouridine (FTU) were synthesized in our laboratories as described by Choi et al. (5). The detailed synthesis and characterization of these new compounds are described elsewhere (13). 3'-Deoxy-2',3'-dehydrothymidine was synthesized as described previously (8). The chemical structure and purity of all compounds, confirmed by 1H nuclear magnetic resonance spectral and high-performance liquid chromatographic (HPLC) analyses, was greater than 99%. For i.v. administration, FTC was dissolved in 10 to 11 ml of sterile phosphate-buffered saline, pH 7.2, at 55°C. The drug solution was sterilized by using a 0.22-μm-pore-size filter. For p.o. administration, the nucleoside was dissolved in 10 to 11 ml of water warmed to 55°C. HPLC grade acetonitrile and all other chemicals, analytical grade, were obtained from J. T. Baker Chemical Co. (Phillipsburg, N.J.). β-Glucuronidase and 5-fluorouracil (5-FU) were purchased from Sigma Chemical Company (St. Louis, Mo.).

Experimental design. Four young adult male rhesus monkeys (Macaca mulatta) weighing 4.4 to 6.4 kg were used for the pharmacokinetic studies. The animals were maintained
at the Yerkes Regional Primate Research Center of Emory University, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care, in accordance with guidelines established by the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

Three monkeys were administered FTC at 33.3 mg/kg i.v., and after a 4-week washout period, the same animals received 33.3 mg/kg p.o. by gastric intubation. One control animal was administered phosphate-buffered saline i.v. and water p.o. without the drug. Blood samples were collected prior to and at 0.25, 0.5, 1.5, 2, 3, 4, 6, 8, and 24 h after drug administration. Monkeys were maintained under ketamine anesthesia for the first 4 h after dosing. A single cerebrospinal fluid (CSF) sample was taken from monkeys 1 h after drug administration, and urine was collected by catheter or cystocentesis at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after dosing. Serum, CSF, and urine samples were frozen at −20°C until analysis.

All animals in this study were monitored by frequent visual observation to evaluate any adverse effects following drug administration, and blood was collected prior to drug administration and at 1 h, 24 h, and 1 week after drug administration for complete blood count and blood chemistry determinations. Each animal's body weight was recorded at the beginning and at the termination of the study.

**Analytical methodology.** HPLC, UV spectroscopy, and mass spectroscopy were employed to identify FTU as a metabolite of FTC. Eluent fractions of the metabolite peak were collected from samples by HPLC as described below. The metabolite was then extracted from the mobile phase into ethyl acetate and evaporated to dryness at 25°C under a stream of nitrogen gas. The extraction residue was dissolved in water for UV spectroscopy and in chloroform for mass spectrometry. Mass spectra were determined with a VG 70-S Nier Johnson mass spectrometer. Samples were analyzed by electron impact and fast atom bombardment mass spectrometry.

FTC and FTU concentrations in biological fluids were determined by HPLC. To measure nucleoside concentrations in serum, a 100-μl serum sample, 50 μl of 3'-deoxy-2',3'-dideoxythymidine (20 μg/ml) as an internal standard, and 50 μl of 2 M perchloric acid as a protein precipitant were added to polypropylene microcentrifuge tubes (400 μl), thoroughly mixed, and centrifuged at 5,000 × g for 5 min. Fifty microliters of 2 M KOH was added to the supernatant, mixed well, and centrifuged at 5,000 × g for 5 min. Previous studies had indicated that the stability of the nucleosides was not affected by the extraction procedure. Supernatant (15 to 200 μl) was injected onto the HPLC column. For drug concentrations in CSF, 100 μl of CSF was added to 20 μl of the internal standard and 80 μl of water. A 100-μl sample was then injected onto the HPLC column. The presence of unchanged FTC and the metabolite FTU in urine was determined by diluting 50-μl urine samples and 100 μl of the internal standard to 1 ml with water and injecting 15 to 100 μl of the sample onto the column.

Chromatography was performed on an Alltech Hypersil octadecanoylsulfate column (4.6 by 150 mm; 5-μm particle diameter; Alltech Associates, Deerfield, Ill.) with an isocratic mobile phase of 5.5% methanol in 40 mM potassium phosphate, pH 2.5, at a flow rate of 2 ml/min. Compounds were detected at a wavelength of 279 nm with a detector range setting of 0.005 absorbance unit, full scale. The retention times for FTC, FTU, and 3'-deoxy-2',3'-dideoxythymidine were 5.5, 8.3, and 9.5 min, respectively.

FTC standards ranging from 0.1 to 100 μg/ml and FTU standards ranging from 0.05 to 100 μg/ml were prepared in control monkey serum, urine, or water (for CSF samples). Standard-curve slopes and intercepts were generated by weighted (1/y) least-squares regression. The assay for FTC was linear in the range of 0.1 to 100 μg/ml, and the lower limit of quantitation was 0.1 μg/ml (10 ng). The assay for FTU was linear in the range of 0.05 to 100 μg/ml, and the lower limit of quantitation was 0.05 μg/ml (5 ng). Extraction recoveries of FTC, FTU, and 3'-deoxy-2',3'-dideoxythymidine were 87, 93, and 96%, respectively. The intra- and interday coefficients of variation for the assay were less than 10% for both drugs at all drug concentrations.

To ascertain whether 5-FU was a metabolite of FTC, serum and urine samples were analyzed for 5-FU by a previously described HPLC method (31).

**Separation of enantiomers of FTC and FTU.** A Chiralpak AS (10 μm, 25 by 4.6 mm) column (catalog no. 7406-00; J. T. Baker Chemical Co.) was used to separate the enantiomers. The mobile phase was isocratic HPLC grade isopropyl alcohol (Fisher Scientific, Pittsburgh, Pa.). The flow rate was 0.8 ml/min. The eluent was monitored by UV detection at 262 nm. The retention times for (-)-FTC, (+)-FTC, (-)-FTU, and (+)-FTU were 5.9, 9.5, 6.4, and 8.4 min, respectively.

**Enzyme assays.** The concentrations of FTC and FTU glucuronides in urine were determined after the glucuronate was hydrolyzed with β-glucuronidase (16). To 100 μl of urine were added 15 μl of 0.12 N acetic acid, 100 μl of β-glucuronidase (500 U/ml in water), and 35 μl of phosphate buffer, pH 5.8. The solution was mixed and incubated at 37°C in a shaker water bath for 12 h. β-Glucuronidase-treated urine was assayed for FTC and FTU as described for urine samples. Glucuronide concentration was calculated as the difference between nucleoside concentrations measured before and after hydrolysis with β-glucuronidase.

The ability of FTC to be deaminated to cytidine-deaminocytidine deaminase activity was determined by using extracts obtained from Hep-2 (human epidermoid carcinoma) cells, which were found to have large amounts of this enzyme (21). The extraction and assay procedure has been described previously (21).

**Pharmacokinetics.** Pharmacokinetic parameters for FTC and its metabolite FTU were calculated by using noncompartmental analysis. Areas under the serum concentration-time curves (AUCs) and first nonnormalized moments (AUMCs) were determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time (20, 33), with extrapolation to time infinity by using the least-square terminal slope λz generated by weighted NONLIN least-squares regression (17). Reciprocal concentration values were found to be acceptable as weighting factors for generation of a normal distribution of weighted residuals in NONLIN. Mean residence time (MRT) was calculated as AUMC/AUC. For the parent compound, FTC, total clearance (CLr) was calculated as Dosei,v/AUCi,v, steady-state volume of distribution was calculated as (Dosei,v × AUMCi,v)/(AUCi,v), and half-life (t1/2) was calculated as 0.693/λz. Bioavailability (F) of FTC after p.o. administration was calculated as AUCp.o./AUCi.v. The first-order absorption rate constant was calculated as (1/MRTp.o.)−(1/MRTi,v), and half-life (t1/2) was calculated as 0.693/λz.

**Statistics.** Statistical analysis comparing the pharmacokinetic parameters of FTC after i.v. and p.o. administration
and parameters for FTC and FTU was performed by using the t test.

RESULTS

Concentrations of FTC in serum after i.v. and p.o. administrations of the nucleoside to three monkeys at 33.3 mg/kg are illustrated in Fig. 1 and 2, respectively. FTC concentrations in serum declined rapidly following i.v. administration (Fig. 1), with a terminal half-life of 1.30 ± 0.07 h (mean ± standard deviation). Following p.o. administration, peak concentrations of FTC in serum were observed within 1 h after administration (Fig. 2) and FTC concentrations subsequently declined rapidly with a terminal half-life of 1.4 ± 0.3 h. There were no statistically significant differences in half-life between i.v. and p.o. administrations of the nucleoside. Pharmacokinetic parameters of FTC after i.v. and p.o. administrations of the compound are presented in Table 1. CL(r) averaged 1.49 ± 0.24 liters/h/kg, and the steady-state volume of distribution was 2.23 ± 0.42 liters/kg. F averaged 73 ± 6%. The first-order absorption rate constant for p.o. administration of the nucleoside was 1.3 ± 0.7 h⁻¹. No drug was detected in any of the serum samples obtained at 24 h.

The metabolic fate of FTC was examined after both i.v. and p.o. administration of the compound. Unequivocal identification of FTU as a metabolite of FTC was established by comparison of chromatographic, UV, and mass spectral data on the metabolite with data on an authentic FTU reference sample. Chromatographic retention times for the metabolite and the FTU reference moved coincidentally when various ratios of acetonitrile-sodium acetate buffer were used as the mobile phases. Furthermore, the UV and mass spectra of the metabolite were identical to those of reference FTU. In agreement with the molecular weight of FTU, the fast atom bombardment mass spectrum showed a strong molecular ion at m/z 247. In the electron impact spectrum, there was no molecular ion. However, a major fragment ion at m/z 119 was apparent, which corresponds to 5-FU. The base peak is at m/z 101, which may correspond to the 2-methyl-1,3-oxathiolane ring. We also determined that racemic FTC was a substrate for cytidine-deoxyxycytidine deaminase obtained from human Hep-2 cells (data not shown). Only the d-(+)-enantiomer of FTC is a substrate for this mammalian enzyme. Thus, the results strongly suggest that FTC was metabolized by a deamination pathway in monkeys to yield d-(+)-FTU. Confirmation that the FTU detected in urine was the (+)-enantiomer was obtained by using an HPLC chiral column separation method (data not shown).

Concentrations of FTU in serum following i.v. and p.o. administration of FTC to three rhesus monkeys are also illustrated in Fig. 1 and 2, respectively. FTC was rapidly biotransformed to form FTU, with peak FTU concentrations in serum occurring 15 min after i.v. drug administration (Fig. 1) and coinciding with FTU peaks after p.o. administration (Fig. 2). FTU concentrations subsequently declined in parallel with FTC, with terminal t₁/₂ values after i.v. and p.o. administrations of FTC of 1.0 ± 0.5 and 1.7 ± 0.5 h, respectively. There were no statistically significant differences between t₁/₂ values for FTC or between values for FTC and FTU after i.v. and p.o. administration of FTC. Pharmacokinetic parameters describing FTU formed from FTC are presented in Table 2. Since the molecular weights of the parent compound and metabolite are almost equal (247.24 and 248.22, respectively), comparisons of their respective concentrations in serum can be made without converting to molar units. Interestingly, the AUC for the metabolite after i.v. administration of FTC was approximately one-half of that of the parent compound. In contrast, the AUCs for FTU and FTC were approximately equal after p.o. administration of the parent nucleoside. The ratios of AUC<sub>FTU</sub> to AUC<sub>FTC</sub> were 0.52 ± 0.08 and 0.95 ± 0.17 after i.v. and p.o. administrations of FTC, respectively.

Serum and urine samples were analyzed to determine whether the 1,3-oxathiolane ring was cleaved from FTU, yielding the toxic compound 5-FU. Fortunately, no 5-FU was detected in serum or urine samples after either single i.v. or p.o. administration of FTC. Consistent with this was the finding that neither FTC nor FTU was a substrate for thymidine phosphorylase derived from Escherichia coli (30).

Urine samples were analyzed for the presence of unchanged FTC, the unchanged metabolite FTU, and glucuronide metabolites of FTC and FTU. Relatively high concentrations of unconjugated FTC and FTU were measured in
urine samples. Concentrations of the parent compound, FTC, in urine were twofold greater than those of the metabolite. FTC glucuronide and FTU glucuronide were detected in urine. Concentrations of the glucuronides in urine were low, however, accounting for only 5 to 20% of the total FTC and FTU excreted in urine. The ratio of conjugated FTU to unconjugated FTU in urine was approximately 1.5-fold greater after p.o. administration of FTC than after i.v. administration of the nucleoside. The ratio of conjugated FTC to unconjugated FTC was independent of the route of administration. Renal excretion of unchanged FTC accounted for the greatest portion of the administered dose found in urine, while the metabolite FTU was excreted primarily as unconjugated FTU. It should be noted that urine volumes were not measured. Thus, while concentrations of the parent nucleoside and metabolite in urine were compared, renal clearance values could not be calculated. Furthermore, there is the possibility of slight inaccuracies in the glucuronide determinations in urine owing to the indirect analytical methodology employed and the low glucuronide concentrations relative to those of FTC and FTU.

Concentrations of FTC and the metabolite FTU in CSF after i.v. and p.o. administrations of the parent nucleoside are illustrated in Fig. 1 and 2, respectively. The ratios of nucleoside concentrations in CSF-serum for FTC and FTU are presented in Tables 1 and 2, respectively. The ratio of FTC concentrations in CSF-serum after i.v. administration averaged 0.10 ± 0.54, while that determined after p.o. drug administration was 0.068 ± 0.043. These values were statistically significantly different (P < 0.05). The ratios of FTU concentrations in CSF-serum after i.v. and p.o. administrations averaged 0.036 ± 0.014 and 0.039 ± 0.015, respectively. There were no significant differences in this ratio for FTU, and the average ratio of concentrations in CSF-serum for both routes of administration was 0.036 ± 0.013.

None of the animals showed any clinical signs that could be related to drug administration during the 1-week observation period. Similarly, none of the animals showed loss of body weight. Other than a slight decrease in erythrocyte counts, hematocrit, and hemoglobin and a slight increase in the reticulocyte count, there were no significant changes in the hemogram following drug administration (data not shown). The variable and transient changes seen were believed to be associated with frequent blood collections for pharmacokinetic studies during the first 24 h following drug administration. All animals, including the control, showed slight, transient increases in creatine phosphokinase, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, and lactate dehydrogenase during the first 24 h following drug administration. Since comparable elevations

### Table 1. Pharmacokinetic parameters of FTC after i.v. and p.o. administration at 33.3 mg/kg to rhesus monkeys

<table>
<thead>
<tr>
<th>Route and monkey</th>
<th>AUC&lt;sub&gt;FTC&lt;/sub&gt; (mg · h/liter)</th>
<th>CL&lt;sub&gt;FTC&lt;/sub&gt; (liters/h/kg)</th>
<th>V&lt;sub&gt;ss&lt;/sub&gt; (liters/kg)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>F</th>
<th>MRT (h)</th>
<th>k&lt;sub&gt;e&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Ratio of concn in CSF-serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUh-2</td>
<td>19.14</td>
<td>1.74</td>
<td>2.71</td>
<td>1.29</td>
<td>1.56</td>
<td>0.076</td>
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<tr>
<td>RMi-2</td>
<td>26.31</td>
<td>1.26</td>
<td>1.97</td>
<td>1.22</td>
<td>1.56</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RJd-2</td>
<td>22.51</td>
<td>1.48</td>
<td>2.00</td>
<td>1.36</td>
<td>1.36</td>
<td>0.162</td>
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<tr>
<td>Mean ± SD</td>
<td>22.65 ± 3.59</td>
<td>1.49 ± 0.24</td>
<td>2.23 ± 0.42</td>
<td>1.29 ± 0.07</td>
<td>1.49 ± 0.12</td>
<td>0.100 ± 0.054</td>
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<tr>
<td>p.o.</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>RUh-2</td>
<td>13.54</td>
<td>1.59</td>
<td>0.71</td>
<td>2.07</td>
<td>1.96</td>
<td>0.048</td>
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<td>RMi-2</td>
<td>21.11</td>
<td>1.08</td>
<td>0.80</td>
<td>2.32</td>
<td>1.32</td>
<td>0.039</td>
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<tr>
<td>RJd-2</td>
<td>15.29</td>
<td>1.47</td>
<td>0.68</td>
<td>3.23</td>
<td>0.53</td>
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<tr>
<td>Mean ± SD</td>
<td>16.65 ± 3.96</td>
<td>1.38 ± 0.27</td>
<td>0.73 ± 0.06</td>
<td>2.54 ± 0.61</td>
<td>1.27 ± 0.72</td>
<td>0.068 ± 0.043</td>
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* Abbreviations: V<sub>ss</sub>, steady-state volume of distribution; k<sub>e</sub>, first-order absorption rate constant.

### Table 2. Pharmacokinetic parameters describing the metabolite FTU after i.v. and p.o. administration of FTC at 33.3 mg/kg to rhesus monkeys

<table>
<thead>
<tr>
<th>Route and monkey</th>
<th>AUC&lt;sub&gt;FTU&lt;/sub&gt; (mg · h/liter)</th>
<th>AUC&lt;sub&gt;FTU&lt;/sub&gt;/AUC&lt;sub&gt;FTC&lt;/sub&gt;</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>Ratio of concn in CSF-serum</th>
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<tbody>
<tr>
<td>i.v.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUh-2</td>
<td>10.55</td>
<td>0.55</td>
<td>0.48</td>
<td>0.024</td>
</tr>
<tr>
<td>RMi-2</td>
<td>11.38</td>
<td>0.43</td>
<td>1.10</td>
<td>0.032</td>
</tr>
<tr>
<td>RJd-2</td>
<td>13.20</td>
<td>0.59</td>
<td>1.38</td>
<td>0.052</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.71 ± 1.36</td>
<td>0.52 ± 0.08</td>
<td>0.99 ± 0.46</td>
<td>0.036 ± 0.014</td>
</tr>
<tr>
<td>p.o.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUh-2</td>
<td>14.25</td>
<td>1.05</td>
<td>1.18</td>
<td>0.026</td>
</tr>
<tr>
<td>RMi-2</td>
<td>16.07</td>
<td>0.76</td>
<td>1.60</td>
<td>0.037</td>
</tr>
<tr>
<td>RJd-2</td>
<td>16.04</td>
<td>1.05</td>
<td>2.19</td>
<td>0.055</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.45 ± 1.04</td>
<td>0.95 ± 0.17</td>
<td>1.66 ± 0.51</td>
<td>0.039 ± 0.015</td>
</tr>
</tbody>
</table>
were seen in the control animal and all values returned to normal at 1 week following drug administration, these changes were attributed to frequent intramuscular injections of the anesthetic agent and frequent blood collections during the first 24 h following drug administration. No significant change in weight was noted for the animals treated with FTC 1 week after initiation of this study compared with weight prior to treatment.

**DISCUSSION**

Previous studies assessing the disposition of nucleoside analogs have demonstrated similar distribution and elimination characteristics, as well as glucuronide formation, in rhesus monkeys and human patients (2, 15, 19, 23). Thus, the rhesus monkey appears to be an excellent animal model for examining the pharmacokinetics of nucleoside analogs. FTC is a novel cytidine derivative with potent and selective anti-HIV and anti-HBV activity in vitro (10, 11, 13, 24). However, the deaminated form of FTC, FTU, is inactive in human peripheral blood mononuclear cells infected with HIV-1 or liver cells (2.2.15 cells) transfected with HBV (10, 13). Tissues and blood of rhesus monkeys have a very high level of deaminase activity, whereas in humans this activity is found primarily in the liver (3). This raises the possibility that FTC could be converted to FTU in monkeys and possibly in humans. We had previously demonstrated that cytosine-containing antiviral nucleosides, such as 3'-azido-2',3'-dideoxy-5-methylcytidine, are deaminated in monkeys but not in rats (25). This is expected, since rats have no measurable deaminase activity (3). Thus, it was important to determine the preclinical pharmacokinetics of FTC by using the rhesus monkey as an animal model that is closer to humans.

FTC is structurally similar to the anti-HIV agent DDC. The structural differences between FTC and DDC are (i) the presence of a sulfur atom instead of a methylene group at the 3' position of the furanose ring normally found in natural nucleosides and (ii) a fluorine atom instead of a hydrogen atom at the 5 position of the nucleoside base. FTC consists of two compounds that are mirror images of each other (enantiomers) in equal proportions, known as the (+)- and (-)-enantiomers. Another, similar nucleoside analog with anti-HIV activity is 2',3'-dideoxy-3'-thiacytidine (BCH-189) (1, 6, 26, 32); however, the pharmacokinetics of this compound in a mammal have not been reported. The (-)-enantiomers of FTC and BCH-189 (also known as 3TC) were more potent against HIV-1 than was the (+)-compound in acutely infected primary lymphocytes (24).

FTC was eliminated at a moderate to high rate with CLT values that approached hepatic blood flow and renal serum flow rates (12). The nucleoside appeared to be distributed intracellularly and to accumulate in at least some tissues in the body, since its steady-state volume of distribution (2.23 liters/kg) was three- to fourfold greater than that of the total body water of the monkey (12). FTC was absorbed rapidly after p.o. administration, with a t½ for absorption of approximately 0.5 h. The extent of absorption was incomplete, yet substantial, with an oral F of 73%.

The CLT of FTC (1.49 liters/h/kg) in monkeys was approximately twofold greater than that of DDC (0.65 liters/h/kg) in rhesus monkeys (15). However, FTC and DDC were eliminated by similar mechanisms. Renal excretion of the unchanged nucleoside was the major elimination pathway for both nucleosides. Metabolism is also known to play a role in the elimination of various other nucleosides. Kelley et al. (15) detected 2',3'-dideoxyuridine in monkey serum and urine after administration of DDC, demonstrating that the parent nucleoside was metabolized by a deamination pathway. Similarly, Schinazi et al. (25) demonstrated that 3'-azido-2',3'-dideoxy-5-methylcytidine is rapidly deaminated to 3'-azido-3'-deoxythymidine in rhesus monkeys. FTC also underwent deamination metabolism to form FTU. The t½ of the metabolite paralleled that of the parent compound, indicating that the disposition of FTU was formation rate limited. It also suggests that the elimination rate of the metabolite was greater than that of the parent compound. In contrast, Collins et al. (7) noted that the metabolite 2',3'-dideoxyuridine formed from DCC was eliminated at a slower rate than the parent compound. These investigators observed that 2',3'-dideoxyuridine concentrations increased relative to those of the parent compound, DDC, over an 8-h period.

Peak concentrations of FTU in serum occurred simultaneously with those of FTC in the present study, indicating that the formation clearance of the metabolite is relatively rapid. However, the formation clearance of FTU from FTC could not be determined since the elimination clearance of the metabolite is not known. Although the F of p.o. FTC was incomplete (73%), the AUC of the metabolite FTU was greater after p.o. administration of FTC than following i.v. administration. The ratio of the AUC of the metabolite FTU to that of the parent compound, FTC, was twofold greater after p.o. administration than after i.v. administration of FTC. This AUC ratio is equal to the ratio of the formation clearance of the metabolite from the parent compound to the elimination clearance of the metabolite (AUCFTU/AUCFTC = CLFTC/CLFTU) and thus provides data on the relative rates of metabolite formation and elimination. The greater AUCFTU:AUCFTC ratio after p.o. administration of FTC then results from increased formation of the metabolite from FTC or decreased clearance of FTU. Since peak concentrations of FTU were lower after p.o. FTC administration than after i.v. administration (Fig. 1 and 2), it is unlikely that the elimination clearance of FTU is decreased after p.o. drug administration. More likely, formation of the metabolite from FTC is greater after p.o. administration of FTC than after i.v. administration. This suggests that the incomplete F of p.o. FTC is due to first-pass metabolism or gastrointestinal tract degradation of the parent compound, yielding FTU. Thus, the lower F of FTC and greater AUCFTU after p.o. FTC administration result, most likely, from first-pass metabolism of the parent nucleoside following p.o. administration. Another complication with FTC was the finding that the (+)-enantiomer is readily susceptible to monkey serum deamination, whereas the (-)-enantiomer is not. This could explain the lower levels of FTU than FTC found in urine.

Both FTC and FTU were metabolized to a limited extent by a glucuronidation pathway, yielding FTC and FTU glucuronides. Chemical characterization of these glucuronides is under way. There was the possibility that FTC is biotransformed to yield 5-FU, an anticancer agent which has been shown to have toxic effects in humans (9). Further analysis of serum and urine samples, however, failed to demonstrate the presence of 5-FU, suggesting that FTC is not metabolized to this toxic compound either directly or through FTU. This is consistent with the finding that FTC and FTU are not substrates for thymidine phosphorylase (30).

A critical facet of anti-HIV drug therapy is the ability of the agent to inhibit viral replication within the central nervous system (14, 18). Thus, the ability of an antiviral
agent to penetrate the central nervous system is imperative for successful anti-HIV therapy. FTC penetrated into the CSF, with drug concentration ratios in CSF-serum at 0.10 after i.v. and p.o. drug administrations averaging 0.010 and 0.043, respectively. When interpreting drug concentrations in CSF at a single time point after administration of a single dose, it must be noted that the drug concentration ratio for CSF-serum may be time dependent. The differences in the FTC ratios in CSF-serum observed after i.v. and p.o. administrations are probably due to time dependency and the differing routes of drug administration. Collins et al. (7) demonstrated that for several nucleosides, peak concentrations in CSF were achieved within 1 h after i.v. drug administration. Subsequently, nucleoside concentrations in CSF declined in parallel with concentrations in serum, yielding a constant CSF-serum concentration ratio (7). Kelley et al. (15) also noted that elimination of DDC from CSF paralleled its elimination from plasma. The FTC concentration ratio in CSF-serum in the present study was determined 1 h after drug administration; thus, the FTC distribution ratio for CSF-serum of 0.10 after i.v. administration probably represents nearly pseudoequilibrium values. However, pseudoequilibrium conditions are most likely not yet achieved 1 h after p.o. FTC administration. Hence, the CSF-serum FTC concentration ratio of 0.043 after p.o. drug administration probably underestimates the equilibrium ratio. The metabolite FTU was also detected in the CSF, with a drug concentration ratio for CSF-serum of 0.038 h after FTC administration. The CSF-serum DDC concentration ratio of 0.033 at 1 h (15) was less than that determined for FTC.

There was no evidence of clinical, hematological, or blood chemistry changes that could be attributed to FTC administration in any of the experimental animals following i.v. or p.o. administration of the drug at 33.3 mg/kg. However, it should be noted that this was a short-term follow-up after a one-time drug administration, and this should not be considered as evidence for lack of toxicity that might be associated with chronic administration of FTC.

In summary, the pharmacokinetics of FTC in rhesus monkeys were, in general, similar to those of other anti-HIV-1 nucleosides (2, 15, 19, 23, 25). The presence of two possible enantiomers with the β conformation [L(-) and D(+)+enantiomer] presents a more complicated dimension, since only one enantiomer is a good substrate for cytidine-deoxyctydine deaminase. The compound was rapidly and relatively well absorbed after p.o. administration. FTC clearance was moderate to high. The compound was distributed extravascularly and penetrated the CSF. FTC concentrations exceeded the in vitro HIV-1, HIV-2, and HBV inhibitory concentration after both i.v. and p.o. administrations of 33.3 mg/kg by a factor of at least 5. We have recently reported that the L(-)-enantiomer of FTC is significantly more potent and less toxic than the D(+)+enantiomer in human lymphocytes and liver cells (11, 24, 27). The finding that the L(-)-form of FTC is a more potent and less toxic enantiomer against HIV and HBV in cell culture and is also more resistant to deamination suggests that this compound should be further evaluated instead of racemic FTC or (+)-FTC. Initial studies with radiolabeled (-)-FTC indicate that this compound is not deaminated or racemized in rhesus monkeys. In addition, three previously unrecognized minor metabolites, 5-fluorocytosine and the two sulfoxides of FTC, were detected and characterized (28). The extent to which (-)-FTC is a viable alternative to currently available anti-HIV and anti-HBV agents remains to be determined.

ACKNOWLEDGMENTS

We thank the staff at Yerkes Regional Primate Research Center for assisting in the monkey experiments.

This work was supported in part by Public Health Service grants AI-25889, AI-26055, and RR-00165 from the National Institutes of Health and by the Department of Veterans Affairs.

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