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Pharmacokinetics, Oral Bioavailability, and Metabolism in Mice and Cynomolgus Monkeys of (2′*R*,5′*S*-)-*cis*-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl] Cytosine, an Agent Active against Human Immunodeficiency Virus and Human Hepatitis B Virus

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(2′*R*, 5′*S*-)-*cis*-5-Fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (524W91) is a nucleoside analog with potent anti-human immunodeficiency virus and anti-human hepatitis B virus activities in vitro. The pharmacokinetics and bioavailability of 524W91 after oral dosing were studied in mice dosed with 10, 100, and 600 mg of 524W91 per kg of body weight by the oral and intravenous routes. Cynomolgus monkeys were dosed with 10 and 80 mg of 524W91 per kg. In both species, the clearance of 524W91 was rapid, via the kidney, and was independent of dose. In monkeys, the total body clearance of 10 mg of 524W91 per kg was 0.7 ± 0.1 liter/h/kg, and the volume of distribution at steady state was 0.8 ± 0.02 liter/kg. The terminal elimination half-life was 1.0 ± 0.2 h. The absolute bioavailability after oral dosing was 63% ± 4% at 10 mg/kg. Concentrations of 524W91 in the cerebrospinal fluid were 4% ± 0.7% of the corresponding levels in plasma. In mice, the total clearance of 10 mg of 524W91 per kg was 2.3 liters/kg/h, and the volume of distribution at steady state was 0.9 liter/kg. Absolute bioavailability in mice after oral dosing was 96% at a dose of 10 mg/kg. The metabolism of orally administered [6-^{3}H]524W91 was studied in cynomolgus monkeys at a dose of 80 mg/kg and in mice at a dose of 120 mg/kg. Monkeys excreted 41% ± 6% of the radioactive dose in the 0- to 72-h urine, 33% ± 10% in the feces, and 10% ± 7% in the cage wash. Unchanged 524W91 was 64% of the total radiolabeled drug recovered in the urine. The major urinary metabolite was a 3′-sulfoxide, constituting 27% of the radiolabeled material in the urine. The glucuronide was a minor urinary metabolite. 5-Fluorouracil was not detected (less than 0.02% of the dose). Mice dosed orally with 120 mg of [6-^{3}H]524W91 per kg excreted 67% ± 7% of the radiolabel in the 0- to 48-h urine. Small amounts of the 3′-sulfoxide and glucuronide metabolites were observed in the urine, but 5-fluorouracil was not detected. Good bioavailability after oral dosing and resistance to metabolism recommend 524W91 for further preclinical evaluation.

Several 2′,3′-dideoxynucleoside analogs are used for the therapy of human immunodeficiency virus (HIV) infection, but toxicity and the rapid evolution of viral resistance to these compounds demand that additional anti-HIV drugs be developed. Antiviral agents are also being developed for use against human hepatitis B virus (HBV) infection, a worldwide health problem which results in a greater risk of hepatocellular carcinoma in chronically infected individuals. Immunomodulation with interferons is an effective therapy for HBV for some patients, but additional drugs are needed.

(2′*R*, 5′*S*-)-*cis*-5-Fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (524W91; Fig. 1) has been shown to be a potent inhibitor of HIV and HBV replication in vitro (9, 19). 524W91 resembles another oxathiolane nucleoside analog, (2*R*, 5*S*-)-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (3TC; Lamivudine). 3TC is currently undergoing clinical trials as a therapy for infection with HIV (1, 23) and HBV (22). Like 3TC, 524W91 is synthesized as part of a racemic mixture of (+) and (−) enantiomers that are analogous to D- and L-nucleosides, respectively (4). These enantiomers can be resolved with enzymes or by chiral chromatography (5, 9, 13, 18). Although the racemic mixtures of 3TC and 524W91 are active against both HIV (21) and HBV (6), most or all of the activity is due to the (−) enantiomer, which has a configuration similar to that of a sugar with the L configuration (5, 9, 14, 18). Significant cytotoxicity is associated with the (+) enantiomer of 3TC (5, 20). Neither 524W91 nor its (+) enantiomer are toxic to cultured cell lines (50% inhibitory concentrations [IC_{50}s], >200 μM) (9). The (+) enantiomer of 524W91 is somewhat more toxic to cultured bone marrow stem cells (IC_{50}, 7.5 μM) than is 524W91 (IC_{50}, 50 μM) (19). In Hep-G2 cells, 524W91 is more efficiently anabolized to the 5′-triphosphate derivative than is the (+) enantiomer (16). The (+) enantiomer is a much better substrate than the (−) enantiomer for cytidine deaminase (3, 9). For these reasons, only the pure (−) enantiomer, 524W91, is being developed as an antiviral agent at this time.

In mice, 3TC is rapidly cleared but has good availability after oral dosing (7). In rats, 524W91 is rapidly cleared by renal mechanisms that are at least partially sensitive to inhibition by probenecid, suggesting an active secretory process (8). The metabolism of racemic 524W91 has been studied in rhesus monkeys (17).

The potent anti-HIV and anti-HBV activity of 524W91 in vitro and its low level of cytotoxicity in cell culture encourage its further development. This report describes the pharmaco-

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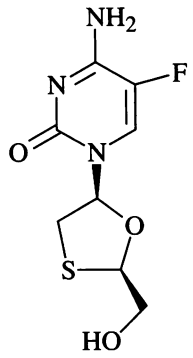

524W91

FIG. 1. Structure of 524W91, (2'*R*, 5'*S*)-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

kinetics and metabolism of 524W91 in the cynomolgus monkey (*Macaca fasicularis*) and in the male CD-1 mouse.

MATERIALS AND METHODS

Chemicals. High-pressure liquid chromatography (HPLC)-grade trifluoroacetic acid, HPLC-grade triethylamine, and analytical reagent-grade trichloroacetic acid (TCA) were purchased from Fisher Scientific Co., (Fairlawn, N.J.). HPLC-grade acetonitrile, AR-grade 88% formic acid, and AR-grade ammonium hydroxide were purchased from Mallinckrodt (Paris, Ky.). Flucytosine, 5-fluorouracil, *Escherichia coli* β-glucuronidase, and 1,4-saccharolactone were purchased from Sigma Chemical Co. (St. Louis, Mo.). Sulfoxides of 524W91 were synthesized with sodium periodate (8).

524W91 was synthesized at Burroughs Wellcome Co. (Research Triangle Park, N.C.) by the procedure of Choi et al. (4). Resolution of the enantiomers was achieved with hog liver esterase. Chiral chromatography (18) on a 4.6-by-250-mm Chiral Pak AS column (Daicel Chemical Industries Ltd., obtained from J. T. Baker, Phillipsburg, N.J.) indicated that the resolved 524W91 contained 2.6% of the (+) enantiomer.

Radiolabeled [6-^{3}H]524W91 with a specific activity of 3 Ci/mmol was synthesized by Moravek Biochemicals (Brea, Calif.). Radiochemical-HPLC analysis of the dosing solution indicated that radiochemical purity was >96%. Small amounts of water (0.8%), flucytosine (0.7%), and sulfoxides a and b (0.8 and 1.1% of the total, respectively) were present. Chiral chromatography on the Chiral Pak AS column as described above indicated that the (+) enantiomer of 524W91 was approximately 2% of the total radiolabeled material, indicating that the overall radiochemical purity was >94%. This level of the (+) enantiomer of 524W91 is similar to that present in material being used in a clinical trial of 524W91. In some cases, [6-^{3}H]524W91 was further resolved by enantioselective deamination with *E. coli* cytidine deaminase (CDA) (8).

Monkey dosing and sample collection. Three separate experiments were conducted in monkeys. In one, male cynomolgus monkeys were used for a pharmacokinetics study. In another, the concentration of 524W91 in the cerebrospinal fluid (CSF) was determined as part of a 30-day toxicology study of 524W91. Both groups of animals in these two experiments were maintained, dosed, and bled at Hazelton Washington, Inc. (Vienna, Va.). For the third study, the metabolism of 524W91 was investigated in female cynomolgus monkeys. These animals were housed and dosed at T.P.S., Inc. (Mt. Vernon, Ind.). A preliminary metabolism study with the CDA-treated [6-^{3}H]524W91 was conducted at Hazelton.

An oral and intravenous crossover experimental design was used for the pharmacokinetic experiments. Two separate groups of male monkeys (n = 4) were dosed at either 10 or 80 mg of 524W91 per kg of body weight. A 2-week interval was allowed between the oral and intravenous doses. The dosing solutions of 524W91 were prepared in 0.9% saline. The intravenous dose was injected over a 2-min period, and venous blood samples were drawn at the end of the injection and at 0.167, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, and 24 h postdose. Plasma was isolated by centrifugation (2,000 × g for 15 min at 4°C) and stored frozen at −85°C until analysis. For the oral dosing branch of the study, monkeys were dosed by nasogastric intubation, and blood samples were drawn at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 24 h postdose. EDTA was used as an anticoagulant.

CSF was drawn on day 28 from monkeys enrolled in a 30-day toxicology study. The animals (five males and five females in each of three dose groups) had been dosed orally 1 h previously with either 40, 200, or 1,000 mg of 524W91 per kg of body weight. Blood samples were drawn at the same time as the CSF samples.

The metabolism of [6-^{3}H]524W91 administered orally at 80 mg/kg of body weight was studied in four female monkeys. Urine samples were collected into receptacles containing 100 μl of 5% sodium azide. Urine and feces were collected at 0 to 8, 8 to 24, 24 to 48, and 48 to 72 h. Cages were washed with a small amount of water after each collection, and the cage wash was collected separately. A preliminary study of the metabolism of orally and intravenously administered CDA-treated [6-^{3}H]524W91 at 10 mg/kg was conducted in three female and three male monkeys. This study examined urinary metabolites and the urinary recovery of the dose for the 0- to 24-h postdose interval.

Mouse dosing and sample collection. Male mice [CD-1; strain Crl:CD-1 (ICR)br] were obtained from Charles River Laboratories (Raleigh, N.C.). Generally, samples were obtained from five mice (weight, approximately 35 g) at each time. Mice were dosed at either 10, 100, or 600 mg of 524W91 per kg of body weight. Dosing solutions were prepared in 0.9% saline or, for the 600-mg/kg dosing solution, in saline containing 10% propylene glycol (Eastman Kodak Co., Rochester, N.Y.). The dose volume was 10 ml/kg of body weight. Oral doses were administered to fed, male CD-1 mice with a feeding needle. Intravenous doses were injected into the lateral tail vein as a bolus over 5 to 10 s. Blood samples were obtained at 2, 5, 10, 20, and 40 min and 1, 2, 3, 4, 6, and 8 h postdose. In some cases, samples were taken up to 24 h postdose. Blood was obtained by cardiac puncture from lightly CO_2-anesthetized mice. Blood was drawn into 1-ml syringes containing disodium EDTA and was centrifuged (2,000 × g for 15 min at 4°C) to isolate the plasma.

During the metabolism experiments, urine and feces were collected from fed mice by using Nalge metabolism cages. Mice were dosed orally with 120 mg of [6-^{3}H]524W91 (specific activity, 0.65 mCi/mmol) per kg of body weight. Mice were housed at three to a cage in three cages. Urine samples were collected into 100 μl of 5% sodium azide. Collection periods were 0 to 24 and 24 to 48 h postdose. Male CD-1 mice were also used in a similar, preliminary study of the metabolism of orally and intravenously administered CDA-treated [6-^{3}H]524W91 at 10 mg/kg.

HPLC assays of 524W91 in plasma. Data acquisition and control of LKB 2150 pumps were performed by a Digital Specialties (Chapel Hill, N.C.) computer with CHROM soft-

ware (Burroughs Wellcome Co.). Samples were injected by using a 712 WISP autoinjector (Waters, Milford, Mass.). Plasma samples were deproteinized by mixing 200 μl of plasma with 100 μl of 10% TCA. After vigorous mixing, the TCA-plasma mixture was placed on ice for at least 5 min. TCA-precipitated plasma samples were centrifuged for at least 2 min in a Fisher microcentrifuge (model 235C). The supernatants were removed and placed into autoinjector vials containing microvial inserts, and 524W91 was assayed by reverse-phase HPLC on C_{18} Microsorb columns (4.6 by 250 mm, 5-μm particle size; Rainin, Woburn, Mass.) equipped with a precolumn of the same medium. When mouse plasma was being assayed, the mobile phase consisted of 6% acetonitrile in 0.1% triethylamine adjusted to pH 3.5 with formic acid. Under these conditions, 524W91 eluted at 600 to 700 s. When monkey plasma samples were being assayed, the mobile phase consisted of 4.8% acetonitrile in the same triethylammonium formate buffer. By the latter method, 524W91 eluted at 800 to 1,000 s. The UV absorbance of the HPLC effluent was monitored at 280 nm with either an SM4000 or an SM5000 UV detector (both from Thermo Separation Products, Riviera Beach, Fla.). The linear regression of the calibration standards was weighted by natural logarithmic transformations of the peak areas and concentrations. Calibration standards spanned the range from 0.5 to 200 μM 524W91 for the monkey samples and from 0.25 to 200 μM 524W91 for the mouse samples. Quality control standards of 1, 10, and 100 μM 524W91 were analyzed with each batch of samples.

The assay of 524W91 in monkey plasma was linear over the range of 0.5 to 200 μM. The lower limit of quantitation of 524W91 in monkey plasma was 0.5 μM. The average intra- and interassay coefficients of variation of quality control standards containing 1, 10, and 100 μM 524W91 were 5.5 and 4.3%, respectively. The assay of 524W91 in mouse plasma was linear over the range of 0.25 to 200 μM. In mouse plasma, the lower limit of quantification of 524W91 ranged from 0.25 to 0.5 μM. The average intra- and interassay coefficients of variation of quality control standards containing 1, 10, and 100 μM 524W91 were 2.5 and 2.8%, respectively.

Measurement of 524W91 in Monkey CSF. CSF samples from monkeys dosed with 40, 200, and 1,000 mg of 524W91 per kg were obtained together with plasma samples drawn at the same time as part of a 30-day toxicology study of 524W91 in cynomolgus monkeys. CSF samples were processed and analyzed by the same techniques used to analyze the plasma samples.

Urine and feces preparation and analysis. Urine samples were prepared for HPLC by microcentrifugation for 5 min. Samples of thawed monkey feces were prepared for analysis by homogenization in 0.9% saline containing 0.05% sodium azide with a Polytron P-10 instrument (Brinkmann Instruments, Westbury, N.Y.). Thawed mouse feces were similarly homogenized in 200 ml of sodium phosphate (0.2 M, pH 6.7). Fecal homogenates were microcentrifuged for 5 min before HPLC analysis. The radiometric HPLC-based assay of urine and fecal homogenate supernatants from both species was conducted with a mobile phase of 0.1% triethylamine adjusted to pH 3.8 with 88% formic acid. Buffer A contained no acetonitrile; buffer B contained 60% acetonitrile. A two-part linear gradient was used to elute 524W91 and its metabolites. In the first part, the buffer B concentration increased from 0 to 15% over 2,400 s. In the second part, the buffer B concentration increased from 15 to 100% over 300 s. The mobile-phase flow rate was 1 ml/min. The concentrations of radiolabeled urinary metabolites were determined by monitoring the radioactivity in the column effluent with a Packard Flow-one/beta instrument (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 from dilutions of the [6-^{3}H]524W91 dosing solution ranging from 3- to 3,000-fold. The linear regression of the calibration standards was weighted by natural logarithmic transformations of the peak areas and concentrations.

The radiochemical HPLC assay of 524W91 and its metabolites in urine samples and fecal homogenate supernatants was linear over a range of dose solution dilutions from 3- to 3,000-fold. Because the volume of a given sample affected the sensitivity of the assay, the limit of quantification was variable. In most urine and fecal samples, the limit of quantification was approximately equal to 0.1% of the dose.

Total radioactivity in the urine samples was measured by counting 100 μl of urine in 5 ml of Beckman Readysafe (Beckman Instruments, Inc., Fullerton, Calif.) in a Packard CA1900 scintillation counter (Packard Instruments Co.). Radioactivity in the feces was quantitated by combustion of 200-μl aliquots of the crude fecal homogenate in a Packard model 306 tissue oxidizer. Controls were run to establish that the recovery of radioactivity was greater than 95% and that carryover was less than 0.5%.

Nonlinear least-squares minimization. Plasma concentration-time curves after the administration of intravenous doses of 524W91 were modeled by bi- and triexponential equations with Solver, the nonlinear least-squares minimization program of Excel version 4.0 (Microsoft Corp., Redmond, Wash.). Solver uses the Newton variant of Marquardt's algorithm. Curves were fit to the data by using $1/C^2$ weighting of the squared deviations of observed from predicted values. The goodness of fit was assessed by inspection of residuals. The discrimination criterion between mono-, bi-, and triphasic exponential models was based on the F statistic (15).

Plasma concentration-time curves of 524W91 from mice dosed orally were modeled by Equation 1:

$$\text{micromolar 524W91 at time (t)} = A \cdot e^{[-a \cdot (t - \text{lag})]} + B \cdot e^{(-b \cdot [t - \text{lag})]} - (A + B) \cdot e^{[-k_{abs} \cdot (t - \text{lag})]} \quad (1)$$

where A and B are intercepts, a and b are the respective elimination rate constants, k_{abs} is the absorption rate constant, t is the time postdose, and lag is the lag time. Data from mice dosed orally with 600 mg of 524W91 per kg were not adequately modeled by $1/C^2$ weighting. In this instance, curves were fit to the data by minimizing the sum of the squared natural logarithms of the ratio of the observed and the expected concentrations.

Pharmacokinetic analysis. Pharmacokinetic parameters were derived from the plasma concentration-time data and from the intercepts and exponents of the exponential terms of the fitted curves by the following equations (10). The intravenous and oral areas under the plasma concentration-time curve [AUC(i.v.) and AUC(p.o.)] were calculated as follows:

$$\text{AUC(i.v.)} = A/a + B/b + C/c \quad (2)$$

$$\text{AUC(p.o.)} = A/a + B/b - (A + B)/k_{abs}$$

where A, B, and C are intercepts, and a, b, and c are the respective rate constants of the exponential equations derived from the nonlinear least-squares minimization process.

The AUCs of 524W91 in plasma after oral dosing in monkeys were calculated by the linear trapezoid rule, with

extrapolation to infinity on the basis of a rate constant determined from the last two concentrations.

The area under the first moment of the plasma concentration-time curve after intravenous dosing (AUMC) was calculated as follows:

$$\text{AUMC} = A/a^2 + B/b^2 + C/c^2 \quad (3)$$

The mean residence time after intravenous dosing (MRT) was calculated as:

$$\text{MRT} = \text{AUMC}/\text{AUC} \quad (4)$$

Total body clearance after intravenous dosing (CL) was calculated as:

$$\text{CL} = \text{dose}/\text{AUC} \quad (5)$$

where dose is expressed in micromoles per kilogram. The volume of distribution at steady state (V_{ss}) was calculated as:

$$V_{ss} = \text{CL} \times \text{MRT} \quad (6)$$

Absolute bioavailability after oral dosing (F) was calculated as:

$$F = 100 \times \text{AUC(p.o.)}/\text{AUC(i.v.)} \quad (7)$$

Identification of metabolites. The radiolabeled metabolites of 524W91 were characterized by comparison of their retention times with the retention times of authentic standards by UV spectral analysis with the SM5000 diode array detector and by liquid chromatography-mass spectrometry (LC-MS).

Prior to LC-MS analysis, the glucuronide of 524W91 was partially purified and concentrated. Monkey urine was adjusted to pH 3 with 1 N HCl. After centrifugation, 6 ml of urine was applied to a column (1.5 by 7 cm) of QAE-Sephadex A-25 (Sigma Chemical Co.). The column was washed with 2 column volumes of water, and the glucuronide was eluted with 10% acetic acid in water. Fractions containing the glucuronide were pooled and lyophilized. The dried fractions were dissolved in 200 μl of water and were applied to a C_{18} Rainin Microsorb HPLC column (4.6 by 250 mm). The 524W91 glucuronide was eluted in a gradient of acetonitrile from 0 to 4.8% over 50 min in triethylamine-trifluoroacetic acid buffer (0.1% each; pH 2.2). Fractions (1 min) were collected and dried, and those containing the 524W91 glucuronide were subjected to LC-MS analysis. The glucuronide of 524W91 was also characterized by its sensitivity to *E. coli* β-glucuronidase in the presence and absence of the glucuronidase inhibitor 1,4-saccharolactone (Sigma Chemical Co.).

MS. A Rainin Microsorb column (C_{18}; 4.6 by 250 mm; 5-μm particle size) eluted at 1 ml/min with a 30-min gradient of 0 to 30% acetonitrile in 0.1% formic acid was used for LC-MS of the sulfoxide metabolites. The 524W91 glucuronide metabolite was analyzed with a 40-min gradient of 0 to 6% acetonitrile in a buffer of 10 mM ammonium acetate.

A Waters 486 UV detector and a Waters 625LC system were interfaced with a Sciex API III mass spectrometer (Perkin-Elmer Sciex, Thornhill, Ontario, Canada) operated in the positive atmospheric pressure chemical ionization mode.

RESULTS

Mouse urine did not have any 524W91 metabolites that were not also observed in monkeys. An HPLC radiochromatogram of the 0- to 24-h urine from a monkey dosed intravenously with 80 mg of [6-^{3}H]524W91 per kg is shown in Fig. 2. 524W91 eluted at 1700 s. Flucytosine eluted at 340 s, 5-fluorouracil eluted at 410 s, sulfoxide a eluted at 950 s, sulfoxide b eluted at 1,030 s, deaminated 524W91 eluted at 1,980 s, and the β-glucuronide of 524W91 eluted at 1,500 s. Unidentified metabolites eluted at 1,080 s (M1100) and 1,940 s (M1940).

Identification of metabolites. β-Glucuronide digestion of the isolated glucuronide metabolite of 524W91 gave a radioactive compound that coeluted with authentic 524W91. This digestion was prevented by 1,4-saccharolactone. LC-MS of the partially purified glucuronide metabolite confirmed that it was 524W91 glucuronide, since the protonated molecular ion was observed at m/z 424. The mass spectrum also showed the fragment ion corresponding to intact 524W91 at m/z 248.

LC-MS of crude monkey urine confirmed the presence of 524W91 sulfoxide, with a protonated molecular weight at m/z 264.

Pharmacokinetics of 524W91 in monkeys. The mean plasma concentration-time curves after the administration of intravenous doses of 10 and 80 mg of 524W91 per kg in monkeys are shown in Fig. 3A. Figure 3B shows the corresponding plasma concentration-time curves after the administration of oral doses of 10 and 80 mg of 524W91 per kg. Data from monkeys dosed orally with 524W91 could not be successfully modeled by Equation 1, possibly because the rate of absorption was too similar to the rate of elimination. The AUCs of the plasma concentration-time curves from orally dosed monkeys were therefore determined by the linear trapezoid method, with extrapolation to infinity based on a rate constant derived from the last two samples in which drug was measurable. The elimination of 524W91 from plasma after administration of the intravenous doses was best described by biphasic exponential equations. The pharmacokinetic parameters derived from individual monkeys dosed intravenously and orally with 10 and 80 mg of 524W91 per kg are given in Table 1. The pharmacokinetics of 524W91 were dose independent over the 10- to 80-mg/kg dose range. The CL averaged 0.7 liter/kg/h and the V_{ss} 0.8 averaged liter/kg at both dose levels. The absolute bioavailability of 524W91 after oral dosing was 63% ± 4% at 10 mg/kg and 58% ± 12% at 80 mg/kg. These differences in availability after oral dosing were not statistically significant (P = 0.47 by the two-tailed Student t test).

524W91 in CSF of monkeys. The concentration of 524W91 in the CSF relative to that in plasma taken at the same time was not influenced by the dose or the concentration of 524W91 in the plasma. Two outliers, having concentrations of 524W91 in CSF that were 27 and 19% of the plasma 524W91 concentrations in plasma, were omitted from the analysis. In the other monkeys, the 524W91 concentration in CSF averaged 3.9 ± 0.7% (n = 28) of the concentration in plasma.

Metabolism of 524W91 in monkeys. After 72 h, monkeys dosed orally with 80 mg of [6-^{3}H]524W91 per kg excreted 41% ± 6% of the radiolabel into the urine, 33% ± 10% in the feces, and 10% ± 7% into the cage wash. More than 90% of the radioactivity ultimately recovered in the urine and cage washes was excreted within the first 24 h postdose, and 70% was excreted within the first 8 h postdose. Unchanged 524W91 was 64% of the radioactive material in the urine and 98% of that in the feces. The most abundant urinary metabolite was a 3′-sulfoxide of 524W91 (27% of radioactive material in urine, 11% of the dose). Traces of 5-fluorocytosine (0.3% of the dose), deaminated 524W91 (1.1% of the dose), and a glucuronide of 524W91 (2% of the dose) were also observed in the urine, as were two minor (<0.4% of dose), unidentified metabolites. Fluorouracil was undetectable in either urine or feces. Deaminated 524W91 was not observed in urine samples from monkeys dosed with 10 mg of CDA-treated [6-^{3}H]524W91 per kg of body weight by either the oral or the intravenous route (data not shown).

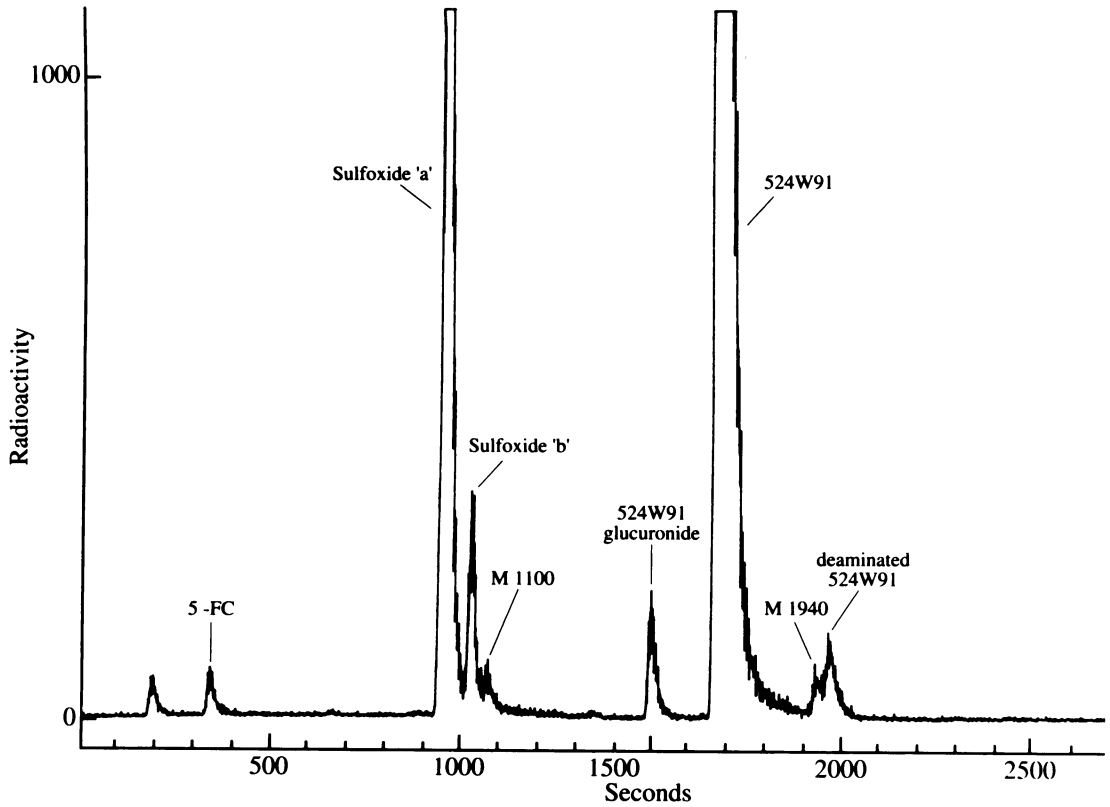

FIG. 2. HPLC radiochromatogram of a sample of the 0- to 24-h urine from a monkey dosed with 80 mg of [6-^{3}H]524W91 per kg of body weight. 524W91 eluted at 1,700 s. Tritiated water eluted at 190 s, 5-fluorocytosine eluted at 340 s, 5-fluorouracil eluted at 410 s, sulfoxide a eluted at 950 s, sulfoxide b eluted at 1,030 s, deaminated 524W91 eluted at 1,980 s, and the β-glucuronide of 524W91 eluted at 1,500 s. Unidentified metabolites eluted at 1,080 s (M1100) and 1,940 s (M1940).

Pharmacokinetics of 524W91 in mice. Figure 4A shows the plasma concentration-time curves of 524W91 in male CD-1 mice dosed intravenously with 10, 100, and 600 mg of 524W91 per kg of body weight. The elimination of 524W91 after the administration of a 10-mg/kg dose was biphasic, whereas after the administration of the higher doses the elimination of 524W91 was triphasic. Table 2 summarizes the pharmacokinetics of 524W91 in mice. CL values were 2.3, 2.2, and 2.1 liters/kg/h after the administration of doses of 10, 100, and 600 mg of 524W91 per kg of body weight, respectively. The respective V_{ss} values were 0.89, 0.94, and 1.0 liter/kg.

Figure 4B shows the plasma concentration-time curves of 524W91 in fed, male CD-1 mice dosed orally with 10, 100, and 600 mg of 524W91 per kg of body weight. 524W91 was rapidly absorbed after oral dosing. After oral dosing, the elimination of 524W91 from the plasma was biphasic. The bioavailabilities of 524W91 after oral dosing at doses of 10, 100, and 600 mg/kg of body weight were 96, 79, and 82%, respectively.

Metabolism of 524W91 in mice. After 72 h, 67% ± 7% of the radioactive material in a 120-mg/kg oral dose of [6-^{3}H]524W91 was recovered in the urine and 18% ± 3% was recovered in the feces. The overall recovery of the dose was 85% ± 4%. The metabolism of 524W91 by the mouse was negligible, with 96% of the radiolabel in the urine being due to 524W91 (data not shown). Fluorouracil was undetectable in either urine or feces.

DISCUSSION

The pharmacokinetics and metabolism of racemic 524W91 in rhesus monkeys have been reported previously (17). However, the (+) enantiomer of 524W91 is very much less active than 524W91 against both HIV (14, 18) and HBV (9). Interpretation of both the pharmacokinetics and the metabolism of racemic 524W91 is confounded by the rapid deamination of the (+) enantiomer of 524W91 by CDA, whereas the (−) enantiomer is not detectably deaminated by the mammalian form of this enzyme (9). For these reasons, the preclinical evaluation of 524W91 has largely been restricted to the pure (−) enantiomer. The radiolabeled 524W91 used in the studies described here contained 2% of the (+) enantiomer of 524W91. This contamination may account for the presence of deaminated 524W91 in the urine recovered from monkeys. Neither deaminated 524W91 nor two of the unidentified metabolites of 524W91 (eluting at 1,080 and 1,940 s in Fig. 2) were observed in the urine when monkeys were dosed with [6-^{3}H]524W91 that had been treated with *E. coli* CDA to remove traces of the (+) enantiomer of 524W91 (data not shown), suggesting that they are in fact metabolites of the (+) enantiomer of 524W91.

Chemical oxidation of the oxathiolane sulfur of 524W91 yields a pair of sulfoxide stereoisomers which we termed a and b, reflecting their order of elution from C_{18} reverse-phase columns. Metabolic oxidation of 524W91 is a minor pathway in the mouse, but it is somewhat more important in the monkey, in which approximately 27% of the radioactivity in the urine is sulfoxide a. The stereospecificity of the oxidation in the mouse is different from that in the monkey, such that roughly equal amounts of sulfoxides a and b are produced (both about 1% of dose; data not shown). The rat also produces relatively more

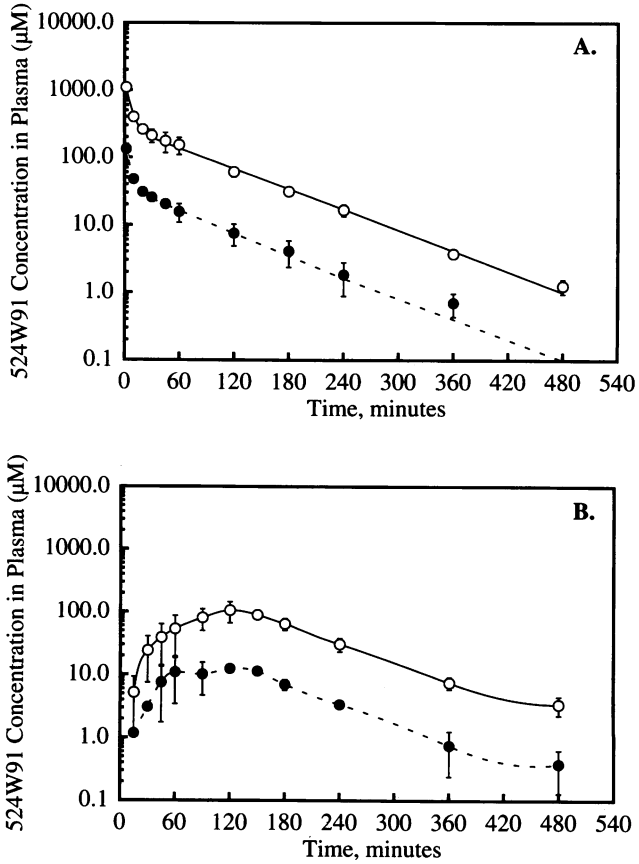

FIG. 3. Plasma concentration-time curves of 524W91 in monkeys after administration of 10 (closed circles) and 80 (open circles) mg of 524W91 per kg of body weight by the intravenous (A) and oral (B) routes. Error bars indicate the standard deviations (n = 4).

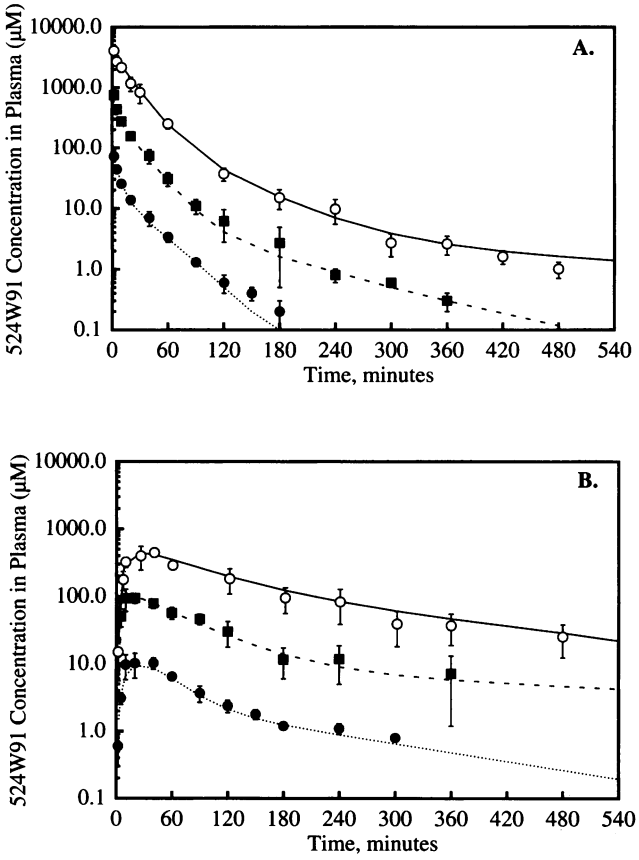

FIG. 4. Plasma-concentration time curves of 524W91 in mice after administration of doses of 10 (closed circles), 100 (closed squares), and 600 (open circles) mg of 524W91 per kg of body weight by the intravenous (A) and oral (B) routes.

sulfoxide b (8). The enzyme responsible for this oxidation has not been determined. The microsomal flavin-containing oxidase system is one possibility (for a review, see reference 24). Flavin-containing oxidases have been implicated in the S-oxidation of 4-bromophenyl-1,3-oxathiolane by human liver microsomes (2).

The pharmacokinetics of 524W91 in mice are similar to those reported earlier in rats (8). CL, which is due almost entirely to renal processes, is close to the renal plasma flow (11), suggesting that a high-capacity secretory system for 524W91 exists in the rodent kidney. In the mouse, this secretory system must have a low affinity for 524W91, since the CL did not change appreciably even after the administration of intravenous doses of 600 mg/kg, when the levels of 524W91 in plasma exceeded 3,000 μM. A transport system having a K_m for 524W91 of 4 mM has been described in Hep-G2 cells (16).

The absolute bioavailability of 524W91 after oral dosing is excellent in the mouse, even at 600 mg/kg. However, the urinary recovery of radioactive 524W91 after oral administration of 120 mg/kg was only 67% of the dose, suggesting that absorption of the dose was good but less than complete. After intravenous dosing with radiolabeled 524W91, urinary recovery was greater than 95% of the dose (data not shown). These data suggest that the availability in the mouse after oral dosing may be somewhat less than that indicated by a comparison of the plasma AUCs after oral and intravenous dosing. The reason for the discrepancy between these estimations of absorption after oral dosing is not clear.

The good availability of 524W91 in the mouse after oral

TABLE 1. Pharmacokinetics of 524W91 in cynomolgus monkeys dosed with 10 or 80 mg of 524W91 per kg of body weight[a]

Dose of 524W91 (mg/kg)	Intravenous				Oral		
	CL (liter/kg/h)	V_{ss} (liter/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C_{max} (μM)	T_{max} (h)	F (%)
10	0.70 ± 0.14	0.79 ± 0.02	0.06 ± 0.01	1.00 ± 0.19	14.1 ± 2.0	1.4 ± 0.5	63 ± 4
80	0.67 ± 0.08	0.76 ± 0.09	0.06 ± 0.01	1.02 ± 0.13	111 ± 34	2.3 ± 0.3	58 ± 12

[a] Values are means ± standard deviations (n = 4). CT, total body clearance; V_{ss}, volume of distribution at steady state; $t_{1/2\alpha}$ and $t_{1/2\beta}$, half-lives at the distribution and elimination phases, respectively; C_{max}, maximum concentration of drug in serum; T_{max}, time at which maximum concentration of drug in plasma is achieved; F, absolute oral bioavailability.

TABLE 2. Pharmacokinetics of 524W91 in male CD-1 mice dosed with 10, 100, and 600 mg of 524W91 per kg of body weight[a]

Dose of 524W91 (mg/kg)	Intravenous					Oral					
	CL (liter/kg/h)	V_{ss} (liter/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	F (%)	C_{max} (μM)	T_{max} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2abs}$ (h)
10	2.3	0.89	0.07	0.4	NA	96	9.8	0.4	0.3	2.3	0.25
100	2.2	0.94	0.03	0.3	1.4	79	89	0.4	0.4	2.0	0.07
600	2.1	1.00	0.06	0.4	3.3	82	440	0.7	0.8	4.5	0.13

[a] Abbreviations: CL, total body clearance; V_{ss}, volume of distribution at steady state; $t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$, half-lives at distribution, elimination, and terminal elimination phases, respectively; F, absolute oral bioavailability; C_{max}, maximum concentration of drug in plasma; T_{max}, time at which the maximum concentration of drug in plasma achieved; NA, not available.

dosing can result in very high concentrations of 524W91 in plasma by this route of administration. In an investigation of the toxicology of 524W91 in the mouse, doses of 3 g/kg/day have been used, resulting in 524W91 concentrations in plasma of greater than 3,000 μM at 30 min postdose (data not shown).

524W91 was present in the CSF of monkeys dosed with 524W91. The concentration of 524W91 in the CSF relative to that in the plasma did not change, remaining near 4% after the administration of a wide range of concentrations. The ratio of 524W91 in CSF to that in plasma is similar to that reported for ddC (12). However, the low level of toxicity and potent in vitro activity of 524W91 against HIV offer hope that achievable levels of 524W91 in the brain will provide therapeutic benefit for the encephalopathy associated with AIDS.

The clearance of 524W91 from the plasma of the monkey depended at least in part on the metabolism of 524W91. The extent of metabolism can be estimated from the composition of the radiolabel in the urine: approximately 64% of the radioactive material excreted in the urine was 524W91, suggesting that 36% of the absorbed dose was metabolized. These data are consistent with those from a study in monkeys of the metabolism of a 10-mg/kg intravenous dose of CDA-treated [6-^{3}H]524W91 in which 61% ± 8% (n = 6) of the radiolabeled material recovered in the urine was 524W91 (data not shown). When the CL is corrected for this nonrenal clearance, the renal clearance of 0.45 liter/kg/h is still greater than the estimated glomerular filtration rate of 0.2 liter/kg/h for a 4-kg animal but less than the estimated renal plasma flow of 1 liter/kg/h (11). These rate comparisons suggest that renal secretion of 524W91 occurs in the monkey, although the capacity of this system is less in the monkey than it is in the mouse and rat.

The pharmacokinetics of 524W91 in humans are under investigation as part of a phase I clinical trial. The major route of elimination of 524W91 in rodents and monkeys is renal, but the rate of elimination appears to be dependent on the magnitude of active transport processes in the kidney as well as on rates that vary with body size. Clearance rates in humans are therefore likely to depend in large part on kidney function, but are otherwise difficult to predict, since the ability of the human kidney to secrete 524W91 is not known.

The good bioavailability of 524W91 after oral dosing coupled with its simple metabolic profile, in vitro antiviral potency, and low toxicity in model species makes it a promising candidate for further evaluation as a therapy for infections with HIV and HBV.

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