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Preclinical Pharmacology and Pharmacokinetics of the Anti-Hepatitis Virus Agent 2′-Fluoro-5-Ethyl-1-β-D-Arabinofuranosyluracil in Mice and Rats

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The preclinical pharmacology and pharmacokinetics of 2′-fluoro-5-ethyl-1-β-D-arabinofuranosyluracil (FEAU), a selective inhibitor of herpesvirus and hepatitis virus replication, were investigated in the mouse and rat. Following intravenous (i.v.) or oral (p.o.) administration, FEAU was cleared from the plasma primarily unchanged, with a terminal half-life of 58 to 80 min in the mouse and 63 to 78 min in the rat. The steady-state volumes of distribution times bioavailabilities of FEAU were approximately 2.1 and 3.4 times the total body water volumes after p.o. administration of 10 mg of drug per kg of body weight in mice and rats, respectively. A comparison of the area under the concentration-time curve after i.v. and p.o. FEAU administration indicated that the p.o. dose was completely absorbed in both species. When tritiated FEAU was used in mice, 35.0% of the i.v. dose and 33.5% of the p.o. dose were excreted in urine as unchanged FEAU, 8.1% (i.v. dose) and 9.2% (p.o. dose) were excreted as tritiated water, and 15.6% (i.v. dose) and 18.1% (p.o. dose) were excreted as unknown metabolite(s) in urine within 24 h of dosing. Only 1.24% (i.v. dose) and 2.6% (p.o. dose) of the total doses were found in urine as ^3H_2O when the FEAU dose was increased to 50 mg/kg. However, a higher percentage of the total dose (59.6% for the i.v. dose and 61.3% for the p.o. dose) was recovered within 24 h as intact FEAU in rat urine, less than 1.4% (i.v. dose) and 2.7% (p.o. dose) of the total dose were found to be ^3H_2O, and 5.6% (i.v. dose) and 6.7% (p.o. dose) of the total dose were excreted as unknown metabolite(s). The distribution ratios for total radioactivity in tissue relative to those in plasma were 0.5 to 1.3 in spleen, testes, muscle, and liver during the first hour after a 10-mg/kg dose in rats. Of the total FEAU radioactivity administered, only 1.38% was excreted in bile as unchanged FEAU. No FEAU glucuronide metabolite was detected. Tissue concentrations of 0.15 to 0.6 μM at 6 h after dosing are in the range of the effective antiviral concentration for FEAU. In conclusion, FEAU administered p.o. to mice and rats was well absorbed; FEAU was rapidly distributed into tissues and remained above in vitro antiviral concentrations for more than 6 h; in mice, [^{3}H]FEAU showed metabolism-mediated tritium exchange with water; and in rats, FEAU was less extensively metabolized than in mice and clearance was primarily via renal processes, mainly in the form of unchanged FEAU.

Our earlier studies of structure-activity relationships for a series of 2′-fluoro-5-substituted 1-β-D-arabinofuranosyl pyrimidine analogs showed that 2′-fluoro-5-ethyl-1-β-D-arabinofuranosyluracil (FEAU) is a potent agent against herpes simplex virus with high selectivity in vitro (1, 3, 5, 8, 12). FEAU has a higher affinity for HSV type 1 (HSV-1) and HSV-2 thymidine kinase than for host cell thymidine kinase (1). In vitro metabolic studies using HSV-1-infected and mock-infected Vero cells showed that 84% of FEAU is incorporated into the DNA of HSV-1-infected cells (8). FEAU is also highly effective in the treatment of simian varicella virus infection in vivo (10). In addition, recent studies have demonstrated that FEAU given either intraperitoneally or orally (p.o.) selectively inhibits the virus-encoded DNA polymerase of woodchuck hepatitis virus found in the serum of chronically infected woodchucks. This inhibitory effect was immediate, nontoxic, and long lasting compared with the effects of 2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU), 6-deoxyacyclovir(9-(2-hydroxethoxymethyl)-2-aminopurine), 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG), and 9-β-D-arabinofuranosyladenine monophosphate (Ara-AMP) (4). Preclinical pharmacology and toxicology studies have indicated that FEAU is much safer than FMAU and has a very low toxicity in vivo (1). This communication reports our evaluation of the tissue distribution, elimination, metabolism, and absorption of FEAU in rodents.

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MATERIALS AND METHODS

Preparation of tritiated FEAU. [^{3}H-ethyl]FEAU with 92% radiochemical purity was prepared by Amersham Corp. (Arlington Heights, Ill.). An aliquot containing approximately 1 mCi FEAU in 50% ethanol was evaporated to dryness, dissolved in distilled water, passed through a C_{18} (6-ml) Bond Elut column, washed with water and 2% methanol to remove impurities, and then eluted with methanol. This preparation was found by high-pressure liquid chromatography (HPLC) to be 96% pure FEAU. Specific activity was 9.262 Ci/mmol. Solutions for injection were prepared by combining an aliquot of [^{3}H-ethyl]FEAU with nonradiolabeled FEAU in 0.9% saline provided by K. A. Watanabe,

Laboratory of Organic Chemistry, Memorial Sloan-Kettering Cancer Center.

Animals and sampling of tissues and plasma. Male CD rats, 130 to 166 g each, and male CD-1 mice, 25 to 32 g each, (Charles River Breeding Laboratories, Wilmington, Mass.) were received when 3 weeks old and acclimated to laboratory conditions for 2 weeks before use. Doses were given intravenously via the tail vein for mice. For rats, intravenous (i.v.) doses were given via a femoral vein. The p.o. doses were given by intragastric intubation of mice and rats. All doses were given at a constant volume of 1.0 ml/100 g of body weight.

To obtain blood and tissue samples, rats were anesthetized with Metofane. Blood (5 ml) was drawn into a heparinized syringe from the rat abdominal aorta after an abdominal incision. The animals were sacrificed by exsanguination. Liver, spleen, muscle, testes, and brain were quickly removed, rinsed in ice-cold 0.9% NaCl solution, blotted dry, weighed, frozen between blocks of dry ice, wrapped in aluminum foil, and stored at −70°C. From anesthetized mice, blood from brachial vessels (0.5 to 1.0 ml for each mouse) was drawn into heparinized syringes. Plasma from the blood was collected by centrifugation at 4°C and then stored at −70°C. Each datum point (15 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h) represents the average amount of FEAU found in tissue or plasma samples taken from two animals.

Urine and bile samples. [^{3}H-ethyl]FEAU was administered to rats (two rats per dose group, with one group receiving 0.9% NaCl solution as control) and mice (two animals per dose group and one control group) as described above. Immediately after being dosed, rodents were placed into metabolism cages for the collection of urine. Rats were housed one per cage, and mice were housed in groups of four per cage. Drinking solution consisting of 5% glucose and 0.3% NaCl and food were given ad libitum during the course of the experiment. Urine was collected into iced receivers over 24-h periods up to 48 h postdose.

Two rats with body weights of 261 and 330 g were anesthetized with sodium pentobarbital, 24 mg/kg given intraperitoneally, and urine and bile were collected as described previously (9). Bile was collected into iced receivers for 30-min periods up to 4 h postdose.

Measurement of radioactivity. Radioactivity was measured with a Packard Tri-Carb model 1900 CA liquid scintillation analyzer using Liquiscint scintillation solution (National Diagnostic, Somerville, N.J.). Counting efficiencies were determined in a representative sample for each of the biological fluids and tissue supernatants by adding ^3H_2O as an internal counting standard.

Chromatographic analysis for FEAU. Before HPLC assay, samples were extracted by using the Centrifree micropartition system (Amicon Div., W. R. Grace and Co., Danvers, Mass.) or the Seppack filtration method (Waters Associates, Milford, Mass.). Recoveries for Centrifree and Seppack methods were 85 and 94%, respectively. Plasma samples were assayed by both the Centrifree and the Seppack HPLC methods. Urine samples were analyzed by the Seppack HPLC method. Bile samples were assayed directly by HPLC. HPLC was carried out with a Waters HPLC system (model 510) with two dual pumps WISP 710B, data module, automatic gradient controller, and model SP 8450 variable-wavelength detector.

The concentration of FEAU in plasma was determined by HPLC with detection of A_{260}. The sensitivity of detection was 0.02 absorbance units full scale. A BrownLee reverse-phase column (RP-18, 4.6 mm by 10 cm) with a guard column (4.6 mm by 3.0 cm) was used for the assay. The isocratic chromatographic procedure used a mobile phase of 92% 20 mM KH_2PO_4–8% acetonitrile buffer (pH 3.8) for 9 min followed by a wash with 80% 20 mM KH_2PO_4–20% acetonitrile for 8 min. Flow rate was 1.2 ml/min at ambient column temperature.

The radioactivity levels of aliquots of all samples were measured before and after evaporation to determine the presence of tritiated water. HPLC was run on samples free of tritiated water. The retention time of the FEAU standard was 8.7 min. Other radioactive peaks had retention times of 2.5, 2.6, and 14 min. Fractions (0.60 ml) of eluent were collected, and the amount of radioactivity which coeluted with the FEAU standard was measured in each fraction. The limit of sensitivity (twice the background radioactivity) was 0.04 μM with a plasma sample size of 25 μl or with a bile sample size of 10 μl. Concentrations of total radioactivity, loss of radioactivity as tritiated water, and FEAU recovery from plasma were considered in the calculation of the final concentration of FEAU in the plasma.

Alternatively, FEAU concentrations in plasma and urine were determined by HPLC-UV detection at 260 nm under the conditions described above for radioactivity measurements. The linear correlation coefficients (r^2) of the standard curves were always at least 0.998, and the variations of assays at high and low concentrations for repeated assays were 0.3 and 3.6% from the means, respectively. The limit of sensitivity was 0.78 μM in 50-μl samples, and the recovery of FEAU was 93%.

Determination of glucuronide conjugates. Glucuronide conjugates of FEAU were assayed as previously described (2). Each purified component from the unknown metabolite peaks in urine samples was dissolved in 100 μl of water and incubated with 2 mg of β-glucuronidase (10^6 U/mg) (Sigma Chemical Co., St. Louis, Mo.) for 18 h at 37°C in 50 μl of 0.05 M acetate buffer (pH 4.5). The [^{3}H]FEAU was stable under these experimental conditions. Aliquots of 50 μl were then injected onto the HPLC column as described above. The difference in FEAU concentrations before and after hydrolysis with β-glucuronidase was considered to be the amount of FEAU glucuronide that had been formed by the animal.

Calculation of pharmacokinetic parameters. The pharmacokinetic parameters were estimated by noncompartmental methods with the computer software Automod (automatic multiexponential regression; IBM version by G. A. McPherson) (7). The areas under the concentration-time curve (AUC) from time zero to t^* (time of the last measurable FEAU concentration) were calculated by trapezoidal approximation. The AUC from t^* to ∞ was estimated by the formula $AUC_{t^*-\infty} = C^*/\beta$, where C^* is the last measurable concentration in plasma and β is the slope of the linear regression line through the log-linear portion of the concentration-time plot. The terminal half-life, $t_{1/2}$, the apparent volume of distribution at steady state (V_{ss}), and the total body clearance were determined (7).

RESULTS

Pharmacokinetics of [^{3}H]FEAU in plasma of CD mice and CD rats. The concentrations of FEAU in plasma obtained from mice within 6 h after an i.v. bolus dose of 10 mg/kg are summarized in Fig. 1. The concentration of FEAU in plasma declined rapidly, with relatively little radioactivity detected by 6 h postdose. As shown in Table 1, FEAU was eliminated from the plasma in mice after i.v. dosing with a $t_{1/2}$ of 58 min.

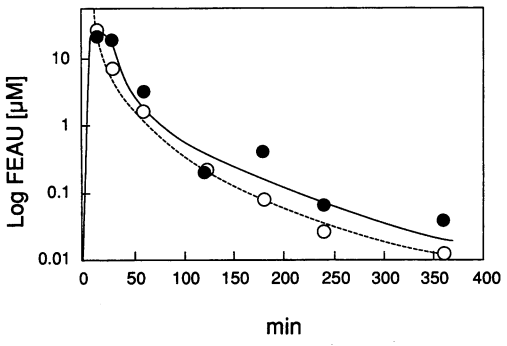

FIG. 1. Mean concentrations of FEAU in plasma in mice up to 6 h after i.v. (○) or p.o. (●) administration of [^{3}H]FEAU (10 mg/kg). Each point is the average of results for two animals. Variations are given in footnote *a*, Table 1.

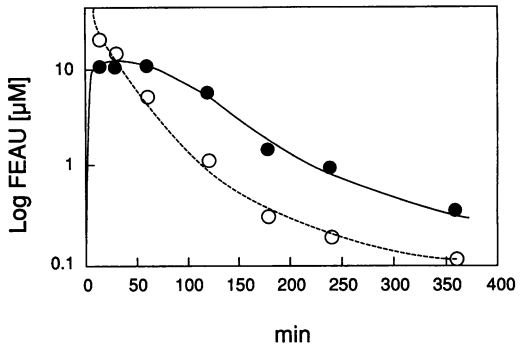

FIG. 2. Mean concentrations of FEAU in plasma in rats up to 6 h after i.v. (○) or p.o. (●) administration of [^{3}H]FEAU (10 mg/kg) as determined by the measurement of FEAU radioactivity. Each point is the average of results for two animals. Variations are given in footnote *a*, Table 1.

The V_{ss} for FEAU was approximately 2.7 times the volume of total body water (0.75 liter/kg for the mouse). Concentrations of FEAU in plasma after p.o. administration of 10 mg/kg peaked at 21 μM in about 30 min (Fig. 1). With p.o. dosing, FEAU was eliminated from the plasma with a $t_{1/2}$ of 80 min (Table 1 and Fig. 1), and V_{ss} times bioavailability of FEAU was calculated to be approximately 2.1 times total body water volume. The AUC for p.o. administration was greater than that for i.v. administration of the same dose, resulting in a calculated bioavailability of a p.o. dose of over 100%, which indicates that FEAU was very well absorbed by the gastrointestinal tract.

The concentrations of FEAU in plasma obtained from rats within 6 h after i.v. bolus or p.o. doses of 10 mg/kg are shown in Fig. 2, and the pharmacokinetic parameters for FEAU in rat plasma are presented in Table 1. The pattern of FEAU elimination from rat plasma was similar to that observed in mice. The V_{ss} for FEAU was about 3.4 times the volume of total body water (0.64 liter/kg for the rat) after either i.v. or p.o. dosing. This is similar to that seen in mice. The total body clearance may be somewhat more rapid in rats than in mice, although any difference here was not clearly reflected in the $t_{1/2}$. Concentrations of FEAU in plasma following a 50-mg/kg i.v. dose in rats were also measured (data not shown), and the overall pharmacokinetics were similar to those for a 10-mg/kg i.v. dose. Doses of 50 mg/kg of FEAU given p.o. were not administered to rats.

Comparison of rat and mouse urinary excretion. The recovery of FEAU from the urine of mice receiving 10 mg of [^{3}H]FEAU per kg either i.v. or p.o. was assayed by HPLC as measured by radioactivity (Table 2) and UV absorbance (data not shown). Regarding total radioactivity in urine, 58% (i.v. dose) and 60.8% (p.o. dose) of the administered radioactivity was recovered in the urine over 24 h, whereas 2.6% (i.v. dose) and 2.64% (p.o. dose) of administered [^{3}H]FEAU radioactivity was recovered from 24- to 48-h postdose. Of the total dose, 35% (i.v. dose) and 33.5% (p.o. dose) were excreted as FEAU in urine within 24 h after dosing, while less than 1% of the total dose was excreted as FEAU during the 24 to 48 h following dosing by either of these routes. The remainder of the radioactivity in urine was identified as tritiated water (8.1% of the total i.v. dose and 9.2% of the total p.o. dose) and unknown metabolite(s) (15.6% of the total i.v. dose and 18.1 of the total p.o. dose). A similar range of percentages of FEAU recovery from urine over the same period was observed when the dose of FEAU was increased to 50 mg/kg i.v. or p.o., but less ^3H_2O (1.2% of the i.v. dose and 2.6% of the p.o. dose) was detected than was observed at the lower dose level (10 mg/kg) (Table 2). In rats, the 0- to 24-h urinary recovery of total administered [^{3}H]FEAU radioactivity for an i.v. dose of 10 mg/kg was 66.6%; 59.6% of the total dose was FEAU. When 10 mg/kg was given p.o.,

TABLE 1. Pharmacokinetic parameters of FEAU in CD mouse and CD rat plasma after a single 10-mg/kg dose[a]

Animal and route of administration	Total dose (μmol)	Kinetic parameters[b]			
		$t_{1/2}$[c] (min)	V_{ss}[d] (liter)	CL_t[e] (L/min)	AUC[f] (μM-min)
Mouse					
i.v.	1.086	58	0.054 (2.0)	0.0018	595
p.o.	0.948	80	0.043 (1.6)	0.0011	860
Rat					
i.v.	5.784	78	0.314 (2.1)	0.006	992
p.o.	5.280	63	0.33 (2.2)	0.004	1,506

[a] [^{3}H]FEAU in plasma was measured by Centrifree HPLC analysis followed by radioactivity counting. Values are the means from two animals per time point. Variations of the concentrations for each time point were calculated, and the average variations were 9.2% for mouse i.v., 24% for mouse p.o., 9.4% for rat i.v., and 22% for rat p.o.

[b] Kinetic parameters were determined by noncompartmental methods.

[c] Determined from the last four datum points of the terminal phase.

[d] Liter per animal. Values in parentheses are V_{ss} in liter per kilogram. Values for V_{ss} p.o. were extrapolated by assuming that oral bioavailability was 100%.

[e] CL_t, total body clearance. Values for p.o. dosing were determined by assuming that oral bioavailability was 100%.

[f] The AUC from 6 h to infinity was estimated by extrapolation of the concentration-time plot. The extrapolated proportion of total AUC was 0.2% for mice i.v., 0.5% for mice p.o., 1.1% for rat i.v., and 1.8% for rat p.o.

TABLE 2. Recovery of [^{3}H]FEAU from CD mouse and CD rat urine as measured by radioactivity in HPLC fractions[a]

Animal	Dose (mg/kg)	Route of administration	Time (h)	Percentage of total dose as:			
				Total radioactivity	^3H_2O	FEAU	Unknown metabolite(s)
Mouse	10	i.v.	0–24	58.3	8.1	35.0	15.7
			24–48	2.6	1.1	0.63	0.8
			Total	60.9	9.2	35.6	16.5
	10	p.o.	0–24	60.8	9.2	33.5	18.1
			24–48	2.64	1.5	0.46	0.69
			Total	63.5	10.7	34.0	18.8
	50	i.v.	0–24	56.4	1.2	37.6	15.6
			24–48	0.58	0.18	0.18	0.22
			Total	59.5	1.42	39.8	15.8
	50	p.o.	0–24	60.9	2.60	37.3	21.0
			24–48	2.6	1.21	0.3	0.74
			Total	63.5	3.85	37.6	22.0
Rat	10	i.v.	0–24	66.6	1.42	59.6	5.6
			24–48	0.3	0.07	0.25	0.09
			Total	66.9	1.49	59.8	5.7
	10	p.o.	0–24	70.8	2.7	61.3	6.7
			24–48	1.8	0.2	1.0	0.68
			Total	72.6	2.9	62.3	7.4
	50	i.v.	0–24	77.6	0.79	70.1	6.7
			24–48	0.74	0.03	0.63	0.08
			Total	78.3	0.83	70.7	6.8

[a] For mice, values are the means of two separate experiments, each with four mice kept in one cage. Urine samples for four mice were therefore pooled. Average variations from the mean were 16.6% for mouse i.v. and 13% for mouse p.o. dosing. For rats, values are the means for two or three rats. Average variations from the mean were 18% for rat i.v. and 17% for rat p.o. dosing.

70.8% of the total radioactivity was recovered within 24 h of dosing and 61.3% of the total dose was excreted as unchanged FEAU. For the 0- to 48-h interval after i.v. or p.o. dosing, 1.49% (i.v. dose) and 2.9% (p.o. dose) of the total dose were found in urine as ^3H_2O, and 5.7% (i.v. dose) and 7.4% (p.o. dose) of the total dose were excreted in urine as unknown metabolite(s). Similar percentages of recovery in rat urine over the same periods were observed for rats given 50 mg/kg i.v.

In a separate experiment, the stability of [^{3}H]FEAU was determined before it was administered to animals. [^{3}H]FEAU in 0.9% saline was kept at either −20 or 37°C for up to 72 h and then evaporated and reconstituted to the initial volume. It was found that [^{3}H]FEAU is very stable (99% [^{3}H]FEAU was recovered by HPLC analysis) under these conditions, indicating that the ^3H_2O is not being formed as a free exchange of tritium from FEAU in solution.

A comparison of the HPLC profile of FEAU in mouse and rat urine after 10-mg/kg i.v. doses is shown in Fig. 3. Rat urine contained mainly unchanged FEAU with little metabolite. However, after the same dose, mouse urine contained two metabolites with retention times of 4 and 5.6 min that constituted 26.4% of the peak areas measured. The retention time for FEAU was 9.8 min.

Distribution of [^{3}H]FEAU in rat tissues. After p.o. administration of 10 mg of drug per kg to rats (Fig. 4), the total radioactivity of [^{3}H]FEAU in liver and plasma reached a maximum at 15 min. For spleen, testes, muscle, and brain, the peak total radioactivity was attained at 1 h postdose. Ratios of distribution relative to that in plasma were approximately 0.5 to 1.3 for spleen, testes, muscle, and liver during the first hour after administration. Total radioactivity in rat brain was among the lowest values (1 log lower than in plasma at the peak), whereas total radioactivity in rat liver was among the highest values (nearly twofold greater than in plasma at 15 min and remaining higher than concentrations in plasma for up to 1.5 h postdose. In the case of i.v. administration (10 mg/kg), the FEAU ratios of distribution relative to that in plasma were about 1 for liver, spleen, testes, and muscle at 0.5 h, and the total radioactivity in liver, spleen, testes, and muscle declined rapidly, with a rate similar to that in plasma (Fig. 5). Again, among the organs measured, the brain showed the lowest total radioactivity. For both i.v. and p.o. routes, 0.15 to 0.6 μM radioactivity was detected in the organs evaluated at 6 h after administration. These concentrations are in the range of the effective in vitro antiviral concentration for FEAU.

Elimination of [^{3}H]FEAU in bile. The recovery of radioac-

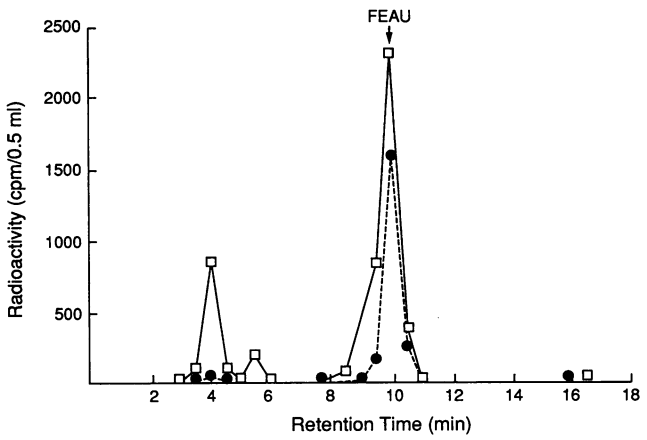

FIG. 3. HPLC profiles of FEAU and its metabolites in mouse (□) and rat (●) urine after i.v. administration of [^{3}H]FEAU (10 mg/kg). Each point represents the average of results for two or three rats or for two groups of four mice each. Average variations are 12% for mice and 9% for rats.

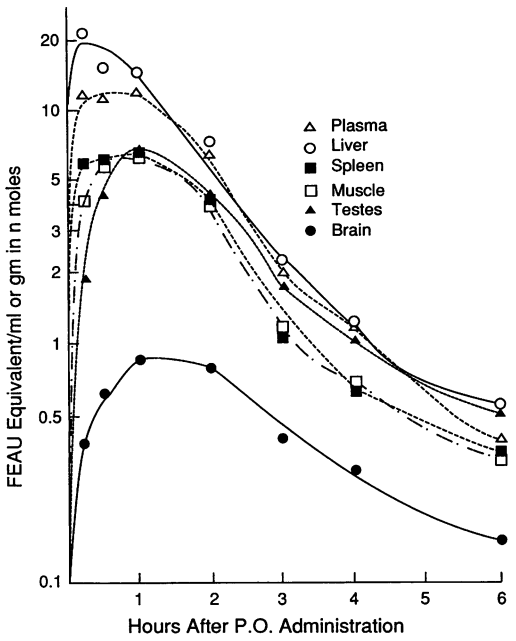

FIG. 4. Tissue distribution of total radioactivity in CD rats after p.o. administration of [^{3}H]FEAU (10 mg/kg). No HPLC analysis for FEAU radioactivity was performed. Data shown are averages for two animals. Average variations are 20% for plasma, 20% for liver, 21% for spleen, 19% for muscle, 21% for testes, and 22% for brain.

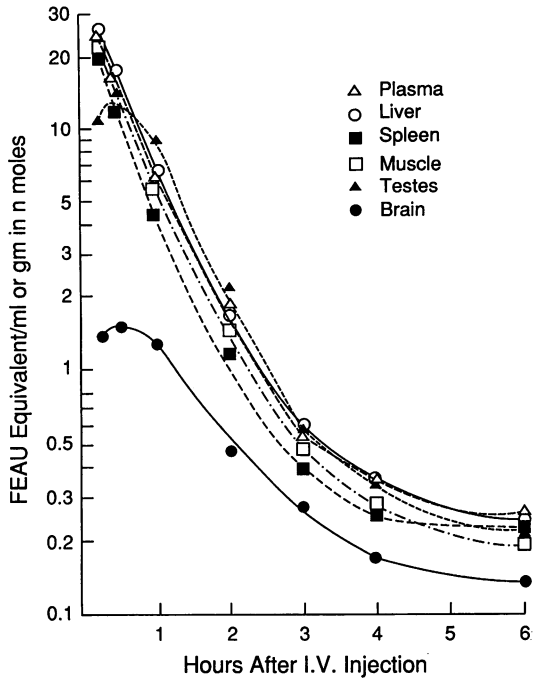

FIG. 5. Tissue distribution of total radioactivity in CD rats after i.v. administration of [^{3}H]FEAU (10 mg/kg). No HPLC analysis for FEAU radioactivity was performed. Data shown are averages for two animals. Average variations are 8% for plasma, 6% for liver, 7% for spleen, 5% for muscle, 10% for testes, and 9% for brain.

tivity from cannulated common bile ducts of two rats given 10 mg of [^{3}H]FEAU per kg i.v. accounted for only 3.45% of the administered dose in cumulative collections of 4 h duration (data not shown), indicating that the elimination of [^{3}H]FEAU in the bile accounted for only a small portion of the total dose. Of the total radioactive FEAU given, only 1.38% was excreted in bile as unchanged FEAU. No significant amount of ^3H_2O was recovered but two apparent metabolites appearing in the HPLC profile accounted for approximately 60% of the total radioactivity detected in the bile.

DISCUSSION

This study demonstrated that [^{3}H]FEAU is cleared from the plasma of mice and rats primarily as unchanged FEAU after either i.v. or p.o. administration. This result confirms our in vitro results that FEAU, like FMAU (9), is metabolically stable (8). FEAU is eliminated rapidly in the mouse, with total clearance values approximating hepatic (0.05 ml/min/g) and renal (0.04 ml/min/g) blood flow rates (6). Similar clearance values were observed in rats receiving FEAU by the i.v. and p.o. routes. FEAU appears to be distributed intracellularly, at least in some tissues of the body, since its V_{ss} is greater than that of total body water. This finding is at least partially consistent with the distribution results for [^{3}H]FEAU in rats, where the ratios of organ to plasma total radioactivity ranged from 0.5 to 1.3 in spleen, testes, muscle, and liver. However, as a nucleoside analog, FEAU is able to enter the intracellular compartment and is incorporated into nucleotide pools and DNA of normal proliferating cells. The presence of this metabolic pathway may give rise to substantial error in the calculation of V_{ss}, since this pathway represents drug elimination from an unmeasured compartment and may explain why the V_{ss} is greater than total body water volume. In addition, protein binding of FEAU may also be a factor affecting the calculated V_{ss}.

In mice, the $t_{1/2}$ of radioactive FEAU was greater after p.o. administration than after i.v. dosing. This may be due to the kinetics of absorption from the gut or to a drug-induced reduction in metabolism and clearance. However, in both mice and rats, a comparison of AUC for FEAU radioactivity following i.v. and p.o. administration showed the bioavailability of FEAU after a p.o. dose of 10 mg/kg to be virtually complete (over 100%). This suggests that theoretically, all of the dose of FEAU administered p.o. was absorbed and that the first-pass metabolism of FEAU in mice and rats is thus negligible. The $t_{1/2}$ in plasma measured after a p.o. dose is longer than that after an i.v. dose (Table 1). This may in part explain why the bioavailability is calculated to be greater than 100%.

The study of tissue distribution in the rat showed that 6 h after a 10-mg/kg dose by the i.v. or p.o. route, 0.15 to 0.6 μM radioactivity was still detected in organs assayed. These concentrations are in the range of the in vitro effective antiviral concentration for FEAU, since 90% concentrations of FEAU that inhibit the replication of different strains of HSV-1 and HSV-2 range between 0.24 and 0.9 μM (4). In preliminary in vivo efficacy evaluation, FEAU given p.o. at a dose of 0.2 mg/kg/day for 10 days showed significant suppression of woodchuck hepatitis virus replication without any observed toxicity (4). Furthermore, the toxic dose of FEAU in mice was 800 mg/kg/day for 4 days when given intraperitoneally, and the toxic and lethal doses in dogs were 50 mg/kg/day for 10 days and 100 mg/kg/day for 10 days, respectively. Therefore, FEAU may be a safe antiviral agent with a substantial therapeutic index.

In this study, it was found that only 58 or 61% of administered radioactivity was recovered in mouse urine within 24 h of a 10-mg/kg dose of [^{3}H]FEAU given i.v. or p.o., respectively. As shown by the pharmacokinetics in plasma, very little if any radioactivity was detected 6 h after i.v. injection. Tissue distribution of FEAU, metabolite(s), and ^3H_2O may account for a substantial proportion of the radioactivity not yet excreted. Fecal and respiratory routes may also contribute to the elimination of FEAU, but previous studies with mice reported that less than 5% of FMAU or 2′-fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAC) (compounds related to FEAU) ^{14}C radioactivity was excreted in the feces or as $^{14}CO_2$ (9).

When the dose of FEAU was increased to 50 mg/kg given i.v. or p.o. to mice, a similar percentage of recovery of FEAU in the urine over the same period was observed, but less ^3H_2O was detected than was observed with the 10-mg/kg dose. Furthermore, in rats, only small proportions of the total FEAU dose were identified as ^3H_2O and unknown metabolite(s) after a 10-mg/kg dose of FEAU given i.v. This observation is in agreement with the results of the FEAU stability experiment, which show that FEAU is very stable before being injected into the animal. Taken together, it appears that ^3H_2O detected in mouse urine may result from the metabolism-mediated tritium exchange of [^{3}H]FEAU after the drug is administered to the mouse but that this metabolic pathway may not be significant in the rat. These data suggest that FEAU is partially metabolized in the mouse and that excretion of unchanged FEAU in the urine is the primary route of elimination in both the mouse and the rat.

Glucuronidation of 2′-fluoro-5-iodo-1-β-D-arabinofuranosyluracil (FIAU) was the primary clearance pathway for this nucleoside analog in mice, rats, and humans (2). Attempts have been made in this study to measure levels of glucuronide metabolites of FEAU. To our surprise, no FEAU glucuronide metabolite was detected. These results suggest that glucuronidation is not a significant clearance pathway for FEAU in the mouse and the rat and that another route(s) of elimination, presumably renal elimination of unchanged FEAU, predominates for FEAU, even though FEAU is structurally similar to both FMAU and FIAU.

In summary, we conclude that FEAU administered to rats and mice is well absorbed from the gastrointestinal tract, rapidly distributed into the tissues, and mostly cleared from the plasma within 6 h; in mice, [^{3}H]FEAU showed metabolism-mediated tritium exchange with water. In rats, FEAU was less extensively metabolized than in mice and was removed primarily via the renal processes mainly as unchanged FEAU; the excretion of [^{3}H]FEAU in unchanged form in the bile is very limited, and the unidentified metabolite(s) of FEAU in the urine of mice and rats is not FEAU glucuronide. The present studies with rodent models should provide some guidance for future clinical trials in humans, although the data obtained from animal studies cannot always be directly extrapolated to the clinical setting.

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