ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1165–1173
0066-4804/91/061165-09$02.00/0

Vol. 35, No. 6

Metabolic Disposition and Pharmacokinetics of the Antiviral Agent 6-Methoxypurine Arabinoside in Rats and Monkeys

THIMYSTA C. BURNETTE,* GEORGE W. KOSZALKA, THOMAS A. KRENITSKY, AND PAULO DE MIRANDA

Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709

Received 7 September 1990/Accepted 4 April 1991

The metabolism and pharmacokinetics of 6-methoxypurine arabinoside (ara-M), a potent and selective inhibitor of varicella-zoster virus, were investigated in rats and monkeys. In Long Evans rats, orally administered [8-^{14}C]ara-M (10 mg/kg) was well absorbed but extensively metabolized to hypoxanthine arabinoside (ara-H), hypoxanthine, xanthine, uric acid, and allantoin. Only 4% of an oral dose was recovered in the urine as unchanged drug, compared with 40% of an intravenous dose, indicating significant presystemic metabolism. Pretreatment of rats with 1-aminobenzotriazole, an inhibitor of cytochrome P-450, did not alter this metabolism. Pretreatment with deoxycoformycin or erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride, inhibitors of adenosine deaminase, resulted in a marked decrease in ara-M metabolism, indicating that adenosine deaminase plays a major role in the biotransformation of ara-M. In cynomolgus monkeys, [8-^{14}C]ara-M (10 mg/kg) administered intravenously or orally was extensively metabolized to ara-H. Several minor urinary metabolites were detected in both rats and monkeys. However, adenine arabinoside was not found in urine or plasma from either rats or monkeys after administration of ara-M, except for a very low level detected in the urine of rats pretreated with deoxycoformycin. The elimination half-lives of intravenously administered ara-M in rats and monkeys were 29 and 45 min, respectively. The corresponding half-lives of the primary metabolite, ara-H, were 44 min and 2.3 h. Plasma profiles of orally administered ara-M in both rats and monkeys demonstrated the poor oral bioavailability of this arabinoside. The results of these studies indicate that ara-M is not well suited for oral administration because of extensive presystemic metabolism.

Recently, a series of 6-alkoxypurine arabinosides was demonstrated to have activity against varicella-zoster virus (VZV) in vitro (2). In that series of compounds, 6-methoxypurine arabinoside (ara-M) was the most potent. In addition, ara-M was more potent than the agents used clinically for the treatment of herpes zoster, acyclovir (ZOVIRAX) and adenine arabinoside (ara-A; Vidarabine) (28). ara-M is an efficient substrate for VZV-encoded thymidine kinase (2) and undergoes highly selective anabolism in VZV-infected human foreskin and lung fibroblasts (4, 8).

This paper describes the metabolism and pharmacokinetics of ara-M in two animal models, Long Evans rats and cynomolgus monkeys. These studies were conducted to aid in evaluating the in vivo suitability of this arabinoside as an anti-VZV agent.

MATERIALS AND METHODS

Chemicals. ara-M was synthesized at Wellcome Research Laboratories as reported previously (2). [8-^{14}C]ara-M (49 Ci/mol) was prepared by combining 26.6 mg of [8-^{14}C]6-methoxypurine (56 Ci/mol; Wizard Laboratories, Davis, Calif.) in 1 ml of 95% ethanol with 0.15 g of uracil arabinoside and 8 ml of 10 mM potassium phosphate (pH 7.4). *Escherichia coli* uridine phosphorylase (153 U) and purine nucleoside phosphorylase (PNP) (277 U) were added. The reaction mixture was stirred overnight at room temperature. The progress of the reaction was monitored by thin-layer chromatography on a silica gel with chloroform-methanol (9:1) as the mobile phase. R_f values for 6-methoxypurine and ara-M were 0.64 and 0.39, respectively. [8-^{14}C]ara-M was isolated by chromatography on Dowex-1 X2 (hydroxide form) resin in a column (1.5 by 4 cm) with 90% methanol as the mobile phase. The fractions containing the product were combined. The solvent was removed under vacuum, and the residue was dissolved in 95% ethanol. The radiochemical purity of [8-^{14}C]ara-M, as determined by high-performance liquid chromatography (HPLC), was greater than 99.5%.

1-Aminobenzotriazole (ABT) and erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA) were synthesized at Wellcome Research Laboratories by published procedures (6, 22). Deoxycoformycin (DCF) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Acepromazine maleate is a product of Fermenta Animal Health Co. (Kansas City, Mo.). Ketalar (ketamine HCl) is a product of Parke-Davis (Morris Plains, N.J.). All other chemicals were reagent grade or better. All solvents and buffers used were HPLC grade.

Rat study. Jugular cannulations were performed as described by Upton (27), with some modifications. Prior to surgery, rats were anesthetized by intraperitoneal injection of ketamine HCl (42 mg/kg) in combination with acepromazine maleate (1.7 mg/kg). The cannula was a 200-mm length of Dow Corning Silastic medical-grade tubing (0.64-mm inner diameter; 1.2-mm outer diameter). A 1-ml syringe charged with heparinized saline (0.9% sodium chloride containing 20 U of heparin per ml) and attached to the end of the cannula with a blunt 22-gauge dispensing needle (Tridak Division, Indicon, Inc., Brookfield, Conn.) was used to monitor the condition of the cannula throughout the procedure. After surgery, the rats were housed individually, with access to both food and water ad libitum, and were allowed to recover overnight.

Dose solutions were prepared on the day of the experiment. ara-M was mixed with [8-^{14}C]ara-M to a final concentration of 5 mg/ml (50 μCi/ml) in normal saline. Analyzed by HPLC after dosing, the radiochemical purity of the unused ara-M dose solution was 99.2%. Aqueous solutions of ABT (25 mg/ml), DCF (5 and 0.5 mg/ml), and EHNA (50 mg/ml)

* Corresponding author.

were prepared in deionized water. The dosage schemes for ABT, DCF, and EHNA were based on the results of previous in vivo studies with these inhibitors (10, 16, 20).

Fifteen male Long Evans rats (Charles River Breeding Laboratories, Wilmington, Mass.) each weighing 268 ± 20 g were used in this crossover study. On day 1, the rats were given a single dose of [8-^{14}C]ara-M (10 mg/kg; 100 μCi/kg) either orally by gavage (12 rats) or intravenously (3 rats). Intravenous doses were administered via the jugular cannula over 30 to 45 s, and the cannula was then flushed slowly with 0.5 ml of heparinized saline. The orally dosed rats were split into four groups (three rats per group). The first group was pretreated with ABT (50 mg/kg; intraperitoneally) at 19 and 2 h predose. The second group was pretreated with DCF (3.5 mg/kg; intraperitoneally) at 19 h predose and given booster doses (0.35 mg/kg; intraperitoneally) at 2 h predose and at 2 and 5 h postdose. The third group was treated with EHNA (50 mg/kg; intraperitoneally) at 2 h predose and at 2 and 5 h postdose. The fourth group of orally dosed rats received no pretreatment. The rats were housed in individual Nalgene metabolism cages (Nalge Co., Rochester, N.Y.) for the separate collection of urine and feces over a 5-day period. The level of radioactivity in the urine from each rat was monitored for an additional 2 days to ensure complete elimination of the radiolabeled dose.

On day 8 of the study, nine rats were dosed with [8-^{14}C]ara-M (10 mg/kg; 100 μCi/kg) either orally by gavage (six rats) or intravenously via the jugular cannula (three rats). The orally dosed rats were split into two groups (three rats per group). One group of orally dosed rats was treated with EHNA (50 mg/kg; intraperitoneally) at 1 h predose and at 3 and 6 h postdose, and the other group was not pretreated. Blood samples were obtained from each animal at 2, 5, 10, 20, 40, and 60 min and 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 h postdose. Blood samples (0.4 to 0.5 ml) were withdrawn through the cannula into syringes containing EDTA. The cannula was then flushed with a volume of heparinized saline sufficient to clear the cannula and replace the volume of blood withdrawn. Each blood sample was centrifuged in the sampling syringe, and the plasma was withdrawn directly from the syringe. Immediately after collection, the plasma samples were extracted with acetonitrile at an acetonitrile/plasma ratio of 5:1. The extracts were dried, reconstituted with deionized water, and analyzed by HPLC.

Monkey study. A dose solution (5 mg/ml; 200 μCi/ml) of [8-^{14}C]ara-M in normal saline was prepared, frozen, and shipped to Hazleton Laboratories America, Inc. (Vienna, Va.), where dosing and sampling procedures were carried out under the direction of Dan W. Dalgard. Three cynomolgus monkeys (*Macaca fascicularis*) were given a single oral dose of [8-^{14}C]ara-M (10 mg/kg; 400 μCi/kg). The monkeys were placed in individual cages with urine collection pans attached. Blood samples (2 ml) were withdrawn from the femoral vein predose and at 5, 15, 30, 45, and 60 min and 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 8.0 h postdose. The samples were collected in tubes containing EDTA for anticoagulation and DCF (50 μl of a 100 μM solution) for stabilization of ara-M during storage and shipment of samples (based on rat study data; see Results). Plasma was obtained from each blood sample after separation by centrifugation. Urine was collected during the first 8-h period after dosing, and DCF (100 μl of a 100 μM solution) was added to a 10-ml aliquot saved for analysis. After a 2-week interval, the same monkeys were given a single intravenous bolus injection of [8-^{14}C]ara-M (10 mg/kg; 400 μCi/kg). Blood and urine collection following the intravenous [8-^{14}C]ara-M dose was identical to that following the oral dose, except for the blood sampling times (i.e., predose and at 5, 10, 20, 30, and 60 min and 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 8.0 h postdose). The unused ara-M dose solution and all samples were stored frozen until the study was completed and were then shipped on dry ice to Burroughs Wellcome Co. The radiochemical purity of the unused ara-M dose solution was 99.4% by HPLC.

Rat liver microsome experiment. Rat liver microsomes in 0.1 mM potassium phosphate buffer (pH 7.4), were prepared by R. L. Hawke at Wellcome Research Laboratories by a published procedure (7). The protein concentration (2.1 mg/ml) was determined by the procedure of Lowry et al. (18). The cytochrome P-450 content (0.98 nmol/mg of protein) was measured by difference spectroscopy (19). [8-^{14}C]ara-M (0.95 mM; 2.4 μCi/ml) was incubated in the absence or presence of DCF (70 μM) or ABT (10.7 mM) in the microsome preparation with 1.8 μM NADPH, 10 mM glucose 6-phosphate, 1 U of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) per ml, and 5 mM magnesium chloride. The reaction mixtures, containing all reagents except [8-^{14}C]ara-M, were preincubated for 30 min at 37°C. The reaction was initiated by the addition of the radiolabeled drug and was conducted at 37°C for 2 h. Sample aliquots (0.5 ml) were withdrawn immediately after the addition of [8-^{14}C]ara-M and at 15, 30, 60, and 120 min and were extracted with 5 ml of 60% cold methanol. The methanolic extracts were evaporated to dryness. The dried residues were reconstituted with 1 ml of water and analyzed by HPLC.

Protein binding determinations. Pooled plasma obtained from untreated animals was spiked with ara-M by serial dilution of an aqueous stock solution (≥10 mM). Rat plasma was spiked with radiolabeled drug at concentrations of 3.5, 35, and 350 μM (one sample per concentration). Monkey plasma was spiked with unlabeled drug at concentrations of 1, 10, and 100 μM (one sample per concentration). Aqueous solutions at identical concentrations were prepared for use as external standards. The spiked plasma samples and standards were incubated at 37°C for 30 min in a shaking water bath. The separation of free from protein-bound drug was achieved by ultrafiltration (24, 29). Aliquots (0.5 ml) of each sample were filtered through Centrifree micropartition units (Amicon Corp., Danvers, Mass.) by centrifugation at 2,000 × *g* for 45 min, and the ultrafiltrates (approximately 200 μl per sample), were collected and analyzed by HPLC.

Radioactivity level determinations. The radioactivity levels in plasma, urine, and feces were determined by liquid scintillation counting. The radioactivity in feces was determined with aqueous fecal extracts prepared by homogenization of feces with deionized water (4 ml/g of feces) and centrifugation at 8,000 × *g* for 60 min. Aliquots (50 to 200 μl) of the plasma samples, plasma ultrafiltrates or extracts, urine samples, and fecal homogenate supernatants were mixed with 5 to 6 ml of ScintiVerse II scintillation cocktail (Fisher Scientific, Pittsburgh, Pa.) and counted with a 1900CA Tri-Carb liquid scintillation analyzer (Packard Instrument Co., Sterling, Va.) for 10 or 20 min per sample.

HPLC analysis. Reversed-phase HPLC was used to analyze the samples for ara-M and its metabolites. Prior to analysis, all biological fluids, fecal extracts, and in vitro samples were clarified and/or deproteinated by ultrafiltration, except for rat plasma samples, which were deproteinated by acetonitrile precipitation (5).

The HPLC system used for these analyses consisted of a reversed-phase analytical column (Adsorbosphere C18; 5 μm; 4.6 mm [inner diameter] by 25 cm; Alltech Associates/

Applied Science, Avondale, Pa.) equipped with a compatible guard column, a 0.5-μm in-line filter, an automatic injector (WISP 712; Waters Associates/Millipore Corp., Milford, Mass.), a 254-nm fixed-wavelength detector (uvMonitor D; LDC/Milton Roy, Riviera, Fla.), and a variable-wavelength detector (spectroMonitor 3000; LDC/Milton Roy). Microcomputer-controlled pumps (constaMetric IIG and III; LDC/Milton Roy) were used for the delivery of the mobile phases: 25 mM ammonium formate buffer, pH 3.5 (buffer A), and 50% acetonitrile in 50 mM ammonium acetate buffer, pH 5.5 (buffer B). After the injection of a sample (100 to 200 μl), the column was eluted at a flow rate of 1 ml/min with a 20-min linear gradient from 100% buffer A to a final mobile phase of 80% buffer A–20% buffer B followed by a 15-min linear gradient from 20 to 100% buffer B with a corresponding decrease in buffer A from 80 to 0%. Between samples, the column was purged with buffer B for 2 min and equilibrated with buffer A for 10 min. For enhanced resolution of ara-M and its metabolites in urine samples, the following three-step gradient modification was used: step 1, 25-min isocratic elution with 95% buffer A–5% buffer B; step 2, 15-min linear gradient from 5 to 30% buffer B with a corresponding decrease in buffer A from 95 to 70%; and step 3, 10-min isocratic elution with 70% buffer A–30% buffer B.

The UV absorbance of the column effluent was monitored at 254 and 267 nm. Radioactivity was monitored with a Flo-One/βeta radioactive flow detector (model IC; Radiomatic Instrument and Chemical Co., Inc., Tampa, Fla.) incorporated into the HPLC system as the third postcolumn detector. The Flo-One/βeta monitored the column effluent in a continuous flow, mixing the effluent at a flow rate of 1 ml/min with Flo-Scint III scintillator (Radiomatic Instrument and Chemical Co.) at a flow rate of 3 ml/min. All results, except for protein binding in monkey plasma, were based on radioactivity measured either by liquid scintillation counting or by HPLC analysis. The equivalent micromolar concentrations were calculated by using the specific activity of the radiolabel. Calculation of protein binding of ara-M in monkey plasma was based on the drug peak area at 254 nm.

Metabolite identification. Initial identification of all radiolabeled metabolites was based on comparison of their retention times with those of authentic standards. Subsequently, selected metabolites were isolated from rat or monkey urine by HPLC and then characterized enzymatically.

(i) ADA. A rat metabolite with a retention time corresponding to that of ara-A was treated with 80 U of adenosine deaminase (ADA) from calf intestine (EC 3.5.4.4; adenosine aminohydrolase; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml in the presence of authentic ara-A and hypoxanthine arabinoside (ara-H). The mixture was incubated at room temperature for 4 h, and the ultrafiltrate was analyzed by HPLC.

(ii) PNP. Monkey metabolites corresponding to ara-H and ara-M were incubated with 120 U of *E. coli* PNP (EC 2.4.2.1) (13) per ml in 75 mM potassium phosphate buffer (pH 6.8) at room temperature for 1 h. The ultrafiltrates were analyzed by HPLC.

(iii) β-Glucuronidase. An aliquot of a monkey urine sample was incubated with 86 U of β-glucuronidase from *E. coli* (EC 3.2.1.31; β-D-glucuronide glucuronosohydrolase; Sigma) per μl at 37°C for 30 min. An ultrafiltrate of the mixture was analyzed by HPLC. The radiolabeled metabolites which had diminished or disappeared were then isolated from untreated urine and tested separately. Each metabolite was incubated at 37°C with 1 U of enzyme per μl in 75 mM potassium phosphate buffer (pH 6.8) in the absence and presence of 9 mM D-saccharic acid 1,4-lactone (Sigma). Ultrafiltrates were analyzed by HPLC after 1-h and overnight incubations.

TABLE 1. Recovery of [8-^{14}C]ara-M in Long Evans rats

Route of administration[a]	Concurrent treatment	Dose recovery (%)[b]		
		Urine	Feces	Total
Intravenous	None	99.1 ± 0.1	0.5 ± 0.1	99.6 ± 0.1
Oral	None	87.7 ± 9.0	2.1 ± 1.7	89.8 ± 7.3
Oral	ABT[c]	87.0 ± 9.7	5.3 ± 3.3	92.3 ± 6.4
Oral	DCF[d]	86.6 ± 2.2	2.9 ± 0.8	89.5 ± 2.0
Oral	EHNA[e]	61.2 ± 12.8	9.3 ± 1.4	70.5 ± 13.0

[a] A 10-mg/kg (100-μCi/kg) dose of [8-^{14}C]ara-M was administered by the indicated route.

[b] Percentage of radiolabeled dose excreted in the urine during the 0- to 120-h postdose period and in the feces during the 0- to 48-h postdose period. Values are means ± standard deviations (n = 3).

[c] A 50-mg/kg dose of ABT was administered intraperitoneally 19 and 2 h prior to ara-M.

[d] A 3.5-mg/kg dose of DCF was administered intraperitoneally 19 h prior to ara-M, and booster doses (0.35 mg/kg) were administered at 2 h predose and at 2 and 5 h postdose.

[e] A 50-mg/kg dose of EHNA was administered intraperitoneally 2 h prior to ara-M and at 2 and 5 h postodose.

Pharmacokinetic analysis. The ara-M and ara-H plasma concentration-time data were analyzed by noncompartmental methods (11, 21). The peak concentration (C_{max}) and the time to C_{max} were observed values. The elimination rate constant (k) was obtained by log-linear regression analysis of the plasma concentration-time data. The half-life ($t_{1/2}$) was calculated by using the relationship $t_{1/2} = 0.693/k$. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule by extrapolation to infinity by using k. Total body clearance (CL) of an intravenous dose was calculated by dividing dose by AUC and was expressed as total clearance per kilogram. Bioavailability was calculated as the ratio of the oral and intravenous AUCs expressed as a percentage, under the assumption that CL was constant from animal to animal and from day to day. The steady-state volume of distribution (V_{ss}) of an intravenous dose was calculated by the following equation: V_{ss} = CL × (AUMC/AUC), where AUMC is the area under the first moment of the concentration-time curve.

RESULTS

Absorption and metabolism in rats. Urinary excretion and fecal excretion of radioactivity during a 120-h period following oral or intravenous dosing of rats with [8-^{14}C]ara-M are shown in Table 1. The mean urinary excretion of an intravenous dose was 99.1%, and that of an oral dose was 87.7%; the mean fecal excretions of the doses were 0.5 and 2.1%, respectively. Therefore, the mean total recovery of radioactivity derived from [8-^{14}C]ara-M in rats was nearly complete, with 99.6% of an intravenous dose and 89.8% of an oral dose being recovered. Pretreatment and/or concurrent treatment of orally dosed rats with the cytochrome P-450 inhibitor ABT or the ADA inhibitor DCF did not affect the absorption of the radiolabeled drug (based on urinary dose excretion), while concurrent treatment with the ADA inhibitor EHNA appeared to have decreased its absorption (Table 1). This effect with EHNA was confirmed in a second experiment (data not shown) but was not investigated further. The total dose recovered from the EHNA-treated rats (approximately 70%) was lower than that recovered from the other groups (approximately 90 to 100%). This result may, in part, reflect

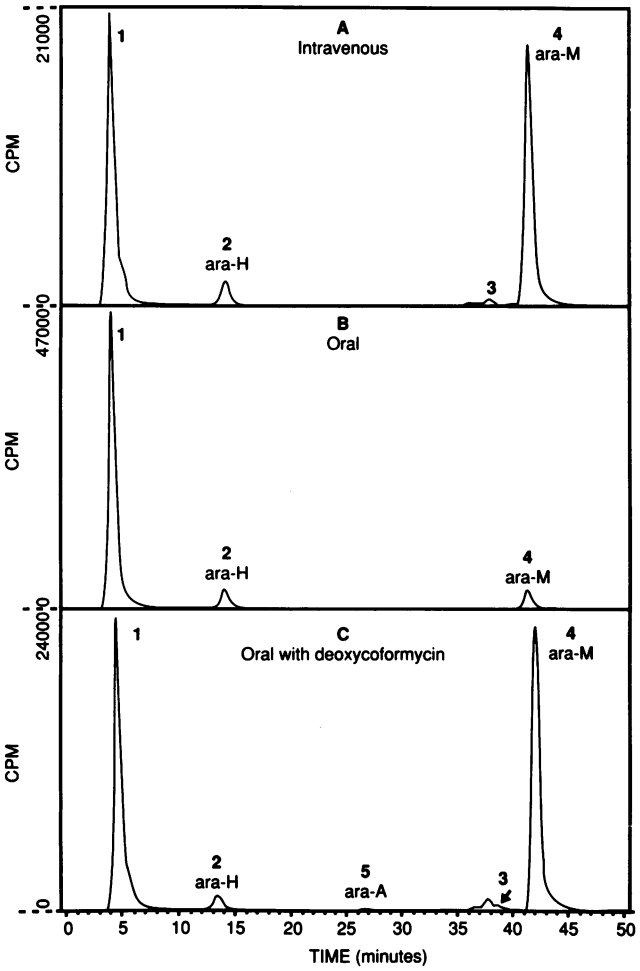

FIG. 1. HPLC radioactivity profiles of 24-h urine samples from Long Evans rats given [8-^{14}C]ara-M (10 mg/kg; 100 μCi/kg) intravenously via a jugular cannula (A), orally (B), or orally with concurrent DCF (C). The radioactivity peaks correspond to allantoin, uric acid, xanthine, and hypoxanthine (breakthrough radioactivity) (1); ara-H (2); 6-methoxypurine (3); ara-M (4); and ara-A (5). The minor metabolites (Met-A and Met-B) described in Results are not shown. Note that the *y* axes (cpm) are scaled differently.

the incomplete extraction of radioactivity from the feces, since the extraction efficiency was not determined.

In all dose groups, the majority of the urinary radioactivity (90 to 95%) was recovered in the first 24-h period after dosing, followed by a gradual washout of radioactivity thereafter. In HPLC radioactivity profiles of the 24-h urine samples (Fig. 1), the amount of intact ara-M recovered after an intravenous dose (43% of the radioactivity; Fig. 1A) was approximately 10-fold greater than that recovered after an oral dose (4.6%; Fig. 1B). The amount of unchanged drug present in the 24-h urine samples following oral administration was dramatically increased by concurrent treatment with DCF (37%; Fig. 1C) or EHNA (69%; profile not shown). Pretreatment with ABT had little effect on the metabolism of orally administered drug, with ara-M accounting for only 7% of the 24-h urinary radioactivity (profile not shown). The overall recoveries of intact ara-M on a percentage-of-dose basis are given in Table 2.

The most significant urinary metabolites of [8-^{14}C]ara-M were ara-H and those eluting as breakthrough radioactivity (hypoxanthine, xanthine, uric acid, and allantoin; not resolved by the HPLC gradient) (Fig. 1 and Table 2). In four of the five dose groups, breakthrough radioactivity accounted for 50% or more of the radiolabeled dose excreted in the urine. ara-H accounted for less than 5% of the dose in all dose groups. The remaining urinary radioactivity was distributed among three minor metabolites: 6-methoxypurine and two unknowns (designated Met-A and Met-B) (Table 2). The metabolite with a retention time corresponding to that of 6-methoxypurine was detected in all dose groups except for the ABT-treated orally dosed rats. The unidentified metabolites, Met-A and Met-B, eluted just prior to 6-methoxypurine with retention times of 36.5 and 37.5 min, respectively. Another minor metabolite, with a retention time similar to that of ara-A, was observed only in the 24-h urine samples from the DCF-treated rats and accounted for approximately 0.3% of the radiolabeled dose (Fig. 1C). This metabolite was a substrate for ADA and was converted to ara-H, as was authentic ara-A.

The HPLC radioactivity profiles of homogenates of feces collected from the rats 0 to 48 h postdose resembled those of the respective urine samples. Breakthrough radioactivity and ara-H accounted for the majority of the dose recovered in the feces. Unchanged ara-M was detected in the feces from animals that had received concurrent treatment with DCF or EHNA. However, peaks of radioactivity corre-

TABLE 2. Urinary metabolites of [8-^{14}C]ara-M in Long Evans rats

Route of administration[a]	Concurrent treatment	% of the dose excreted in the urine as[b]:					
		Breakthrough radioactivity[c]	ara-H	Metabolite Met-A[d]	Metabolite Met-B[d]	6-Methoxypurine	ara-M
Intravenous	None	54.2 ± 1.9	3.6 ± 0.3	0.4 ± 0.1	ND[e]	0.6 ± 0.2	39.7 ± 2.1
Oral	None	78.8 ± 7.2	4.8 ± 2.4	ND	ND	0.1 ± 0.1	3.8 ± 0.2
Oral	ABT[f]	76.2 ± 8.5	4.4 ± 1.5	ND	ND	ND	6.0 ± 0.9
Oral	DCF[g]	50.0 ± 7.5	2.8 ± 0.8	0.3 ± 0.1	1.1 ± 0.4	0.4 ± 0.2	31.3 ± 5.5
Oral	EHNA[h]	18.0 ± 8.6	1.0 ± 0.3	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	40.8 ± 9.0

[a] A 10-mg/kg (100-μCi/kg) [dose of [8-^{14}C]ara-M was administered by the indicated route.
[b] Expressed as a percentage of the radiolabeled dose excreted in the urine 0 to 120 h postdose. Values are means ± standard deviations (n = 3).
[c] Breakthrough radioactivity represents hypoxanthine, xanthine, uric acid, and allantoin (not resolved by the HPLC gradient).
[d] Unidentified metabolite.
[e] ND, not detected.
[f] A 50-mg/kg dose of ABT was administered intraperitoneally 19 and 2 h prior to ara-M.
[g] A 3.5-mg/kg dose of DCF was administered intraperitoneally 19 h prior to ara-M, and booster doses (0.35 mg/kg) were administered at 2 h predose and at 2 and 5 h postdose.
[h] A 50-mg/kg dose of EHNA was administered intraperitoneally 2 h prior to ara-M and at 2 and 5 h postdose.

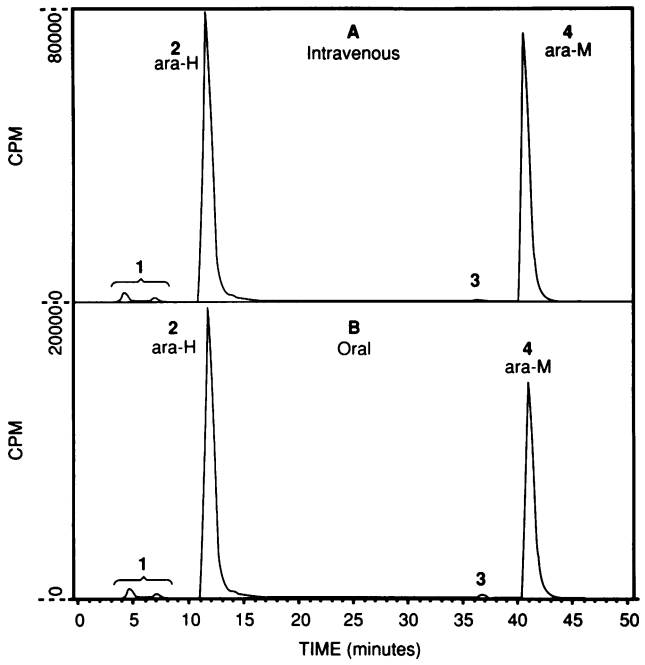

FIG. 2. HPLC radioactivity profiles of representative urine samples collected from cynomolgus monkeys given [8-^{14}C]ara-M (10 mg/kg; 400 μCi/kg) either intravenously (A) or orally (B). The radioactivity peaks correspond to uric acid, xanthine, and hypoxanthine (breakthrough radioactivity) (1); ara-H (2); 6-methoxypurine (3); and ara-M (4). The minor metabolites (Met-A, Met-C, and Met-D) described in Results are not shown. Note that the *y* axes (cpm) are scaled differently.

sponding to the minor urinary metabolites (6-methoxypurine, Met-A, Met-B, or ara-A) were not detected in any of the fecal samples.

Metabolism in monkeys. ara-H was the major metabolite of [8-^{14}C]ara-M in monkeys, accounting for 53% ± 8% of the radioactivity in the 0- to 8-h urine samples collected after intravenous dosing (Fig. 2A) and 61% ± 4% in those collected after oral dosing (Fig. 2B). The majority of the remaining urinary radioactivity was accounted for by unchanged ara-M (42% ± 9% and 33% ± 4%, respectively) and breakthrough radioactivity (hypoxanthine, xanthine, and uric acid; 3.2% ± 1.0% and 3.4% ± 0.3%, respectively) (Fig. 2). Identification of ara-H was confirmed by treatment with *E. coli* PNP, which converted 93% of the isolated peak to hypoxanthine. Tested simultaneously, ara-M isolated from monkey urine was also a substrate for *E. coli* PNP, converting 90% to its base. 6-Methoxypurine accounted for 0.3% ± 0.1% of the intravenous urinary radioactivity and 0.5% ± 0.1% of the oral urinary radioactivity (Fig. 2).

Three minor metabolites were detected in monkey urine samples. One, with a retention time identical to that of the rat metabolite Met-A, accounted for 0.3% ± 0.1% and 0.9% ± 0.1% of the urinary radioactivity after intravenous and oral doses of [8-^{14}C]ara-M, respectively. The other minor metabolites (designated Met-C and Met-D) were identified as glucuronides of ara-M by treatment with β-glucuronidase in the absence and presence of a glucuronidase inhibitor. Metabolite Met-C, with a HPLC retention time of 26 min, accounted for approximately 0.3% of the urinary radioactivity after both intravenous and oral doses of [8-^{14}C]ara-M; similarly, metabolite Met-D, with a retention time of 33 min, accounted for approximately 0.4% of the urinary radioactivity. Neither ara-A nor rat metabolite Met-B was detected in these monkey urine samples.

In vitro metabolism in rat liver microsomes. In a single experiment, [8-^{14}C]ara-M was incubated with viable rat liver microsomes and the formation of ara-H over a 2-h period was monitored by HPLC. The levels of ara-H generated were very low (0.2 to 0.4% of the total radioactivity). The remainder of the radioactivity in the reaction mixtures was accounted for by unchanged ara-M (99.2 to 99.6%) and 6-methoxypurine (0.2 to 0.4%). No significant differences were observed between incubations with ara-M alone, ara-M and DCF, or ara-M and ABT.

Protein binding. Protein binding of ara-M was determined by comparison of the ara-M concentration in plasma ultrafiltrates with that in aqueous standards. The percentage of binding in rat plasma was 43.8% ± 5.3% (ranging from 37.7 to 47.5%) and appeared to be concentration dependent, with the highest percentage of binding occurring at the lowest concentration (3.5 μM). The percentage of binding in monkey plasma was 7.5% ± 1.2% (ranging from 6.0 to 9.0%) and was independent of concentration. HPLC radioactivity profiles of rat plasma ultrafiltrates and aqueous standards (data not shown) confirmed that there had been no in vitro metabolism or degradation of ara-M during incubation. In addition, there was no indication of nonspecific binding to the filter. Therefore, the observed decrease in drug concentration in plasma after ultrafiltration was, in fact, due to protein binding.

Pharmacokinetics in rats. The levels of ara-M and ara-H in the plasma of rats were determined by HPLC analysis of the supernatants obtained after precipitation of plasma proteins with acetonitrile (Fig. 3). The reported ara-M concentrations approximated the total plasma concentrations, since 95% recovery of ara-M, an estimate based on spiked plasma standards, was achieved by this method of deproteination. The reported ara-H concentrations were not the total plasma concentrations, since the estimated recovery of ara-H in spiked plasma standards was approximately 80%. The lower limit of detection for the analysis was 400 cpm/100 μl of plasma extract, or 0.6 μM.

The plasma concentration-time profile for intravenously administered ara-M revealed a rapid disappearance of the drug, with a $t_{1/2}$ of 29 ± 2 min (Fig. 3A). The mean CL and V_{ss} of an intravenous dose were 39.0 ± 0.4 ml/min/kg and 1.4 ± 0.1 liter/kg, respectively. The metabolite ara-H reached an observed mean C_{max} of 2.1 ± 0.1 μM at 40 min postdose and had a $t_{1/2}$ of 44 ± 10 min (Fig. 3A). The levels of ara-M in plasma resulting from an oral dose were only detectable from 20 min to 2 h postdose, with an average C_{max} of 1.5 ± 0.1 μM at 40 min postdose (Fig. 3B). The corresponding levels of ara-H were similar, with a mean (n = 2) C_{max} of 1.5 μM at 1.5 h postdose (Fig. 3B). The limited number of data points in the postabsorption phase for ara-M and ara-H precluded further pharmacokinetic evaluation. Coadministration of EHNA significantly reduced the metabolism of orally administered ara-M, thereby changing its pharmacokinetic profile (Fig. 3C). The C_{max} increased approximately twofold, and a mean concentration of 2.6 ± 0.6 μM (ranging from 1.0 to 3.5 μM; n = 27) was maintained from 40 min to 6 h postdose. No ara-H was detected in the plasma of rats that had received ara-M and EHNA simultaneously.

Pharmacokinetics in monkeys. Because of the low binding of ara-M to monkey plasma proteins, the levels of ara-M and ara-H in the plasma of cynomolgus monkeys were determined by HPLC analysis of plasma ultrafiltrates (Fig. 4). On

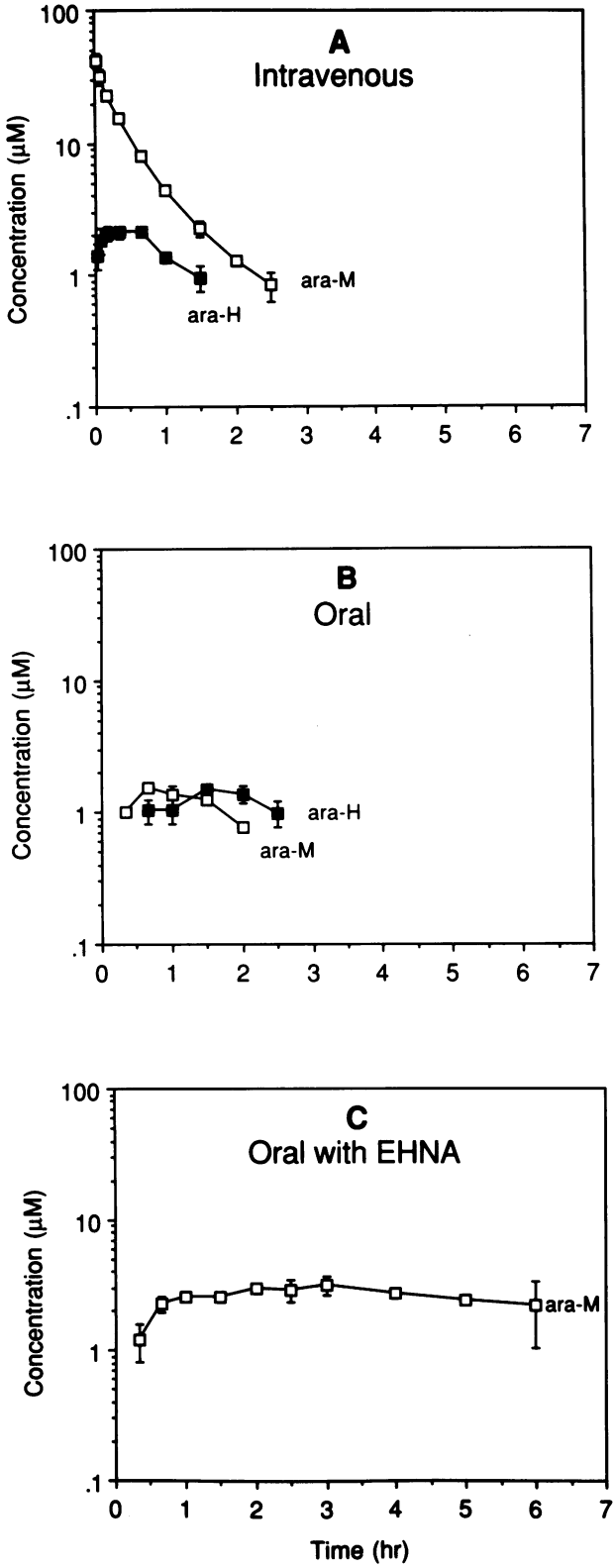

FIG. 3. Mean levels of ara-M and ara-H in the plasma of Long Evans rats. [8-^{14}C]ara-M (10 mg/kg; 100 μCi/kg) was administered intravenously (A), orally (B), or orally with concurrent EHNA (C). The levels were determined by HPLC analysis of acetonitrile extracts of plasma samples (radioactivity detection limit, 400 cpm/100 μl of extract, or 0.6 μM). The error bars indicate standard deviations (n = 3).

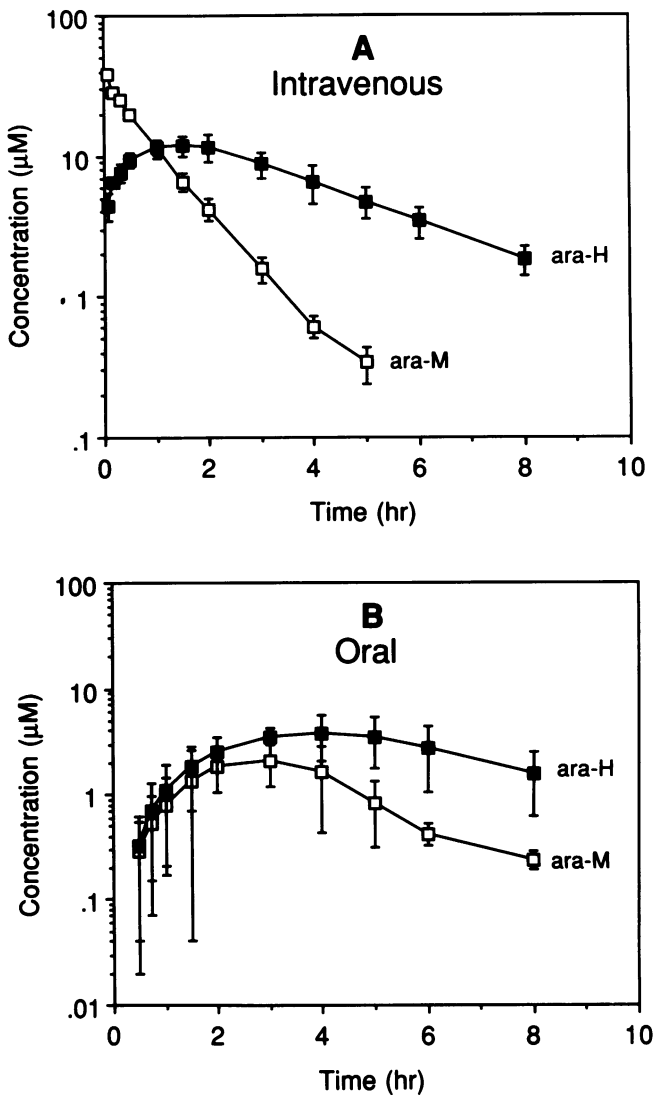

FIG. 4. Mean levels of ara-M and ara-H in the plasma of cynomolgus monkeys. [8-^{14}C]ara-M (10 mg/kg; 400 μCi/kg) was administered intravenously (A) or orally (B). The levels were determined by HPLC analysis of plasma ultrafiltrates (radioactivity detection limit, 400 cpm/100 μl of ultrafiltrate, or 0.2 μM). The error bars indicate standard deviations (n = 3).

the basis of radioactivity measurements obtained by liquid scintillation counting, the recovery of total plasma radioactivity in plasma ultrafiltrates was 97.0% ± 6.1% (n = 32). Therefore, the reported concentrations of ara-M and ara-H approximated the total plasma concentrations. The lower limit of detection for the analysis was 400 cpm/100 μl of plasma ultrafiltrate, or 0.2 μM.

The pharmacokinetic data derived from the plasma concentration-time profiles are presented in Table 3. After an intravenous dose, there was a rapid decline in unchanged ara-M from 38 ± 1 μM at 5 min to 0.15 ± 0.13 μM at 6 h postdose, with a $t_{1/2}$ of 45 min (Fig. 4A). The mean CL and V_{ss} of an intravenous dose were 18 ± 2 ml/min/kg and 1.1 ± 0.1 liter/kg, respectively. The major radiolabeled metabolite of ara-M in plasma, as in urine, was ara-H. Detectable at all sampling times, ara-H was present in plasma at a concentration of 4.5 ± 1.0 μM at 5 min and reached an observed C_{max}

TABLE 3. Pharmacokinetic data for ara-M and ara-H in cynomolgus monkeys following an intravenous or oral dose of [8-^{14}C]ara-M[a]

Compound	Route of ara-M administration	T_{max}[b] (h)	C_{max} (μM)	AUC (μM · h)	$t_{1/2}$ (h)
ara-M	Intravenous			32.7 ± 2.9	0.75 ± 0.06
	Oral	2.7 ± 1.0	2.5 ± 0.6	8.4 ± 1.3	1.3 ± 0.3
ara-H	Intravenous	1.7 ± 0.3	11.9 ± 2.2	58.7 ± 12.3	2.3 ± 0.1
	Oral	3.3 ± 0.6	4.2 ± 1.4	26.3 ± 9.9	2.8 ± 0.3

[a] [8-^{14}C]ara-M was administered at 10 mg/kg (400 μCi/kg). Values are means ± standard deviations (n = 3).
[b] Time to C_{max}.

of 12 ± 2 μM at 1.5 to 2 h postdose. It disappeared slowly, with a $t_{1/2}$ of 2.3 h. By 8 h postdose, the concentration of ara-H (1.8 ± 0.4 μM) was at least 10-fold higher than that of ara-M (<0.2 μM). The AUC for ara-H was approximately twice the AUC for ara-M. After an oral dose, the C_{max} for ara-M (ranging from 1.8 to 3.0 μM) was observed at 1.5 to 4 h postdose and the C_{max} for ara-H (ranging from 2.8 to 5.6 μM) was observed at 3 to 4 h postdose (Fig. 4B). The apparent $t_{1/2}$ values for ara-M and ara-H derived from orally administered [8-^{14}C]ara-M (1.3 and 2.8 h, respectively) were similar to those seen when the drug was administered intravenously. However, after oral administration, the AUC for ara-H was approximately threefold higher than that for ara-M. The mean oral bioavailability of ara-M in monkeys was 26% ± 4%.

DISCUSSION

In both rats and monkeys, poor oral bioavailability of ara-M resulted from extensive metabolism, although nearly complete gastrointestinal absorption of the drug was observed in rats. ara-M was initially metabolized to ara-H. In rats, ara-H was further metabolized to the end products of purine degradation: hypoxanthine, xanthine, uric acid, and allantoin (Fig. 5). However, ara-H was the major metabolite of ara-M in monkeys, with little further metabolism. This difference in metabolism may reflect differences between the specificity and/or level of PNP in rats and monkeys.

The biotransformation of ara-M to ara-H presumably occurs in a single demethoxylation step mediated by ADA. Pretreatment of rats with DCF or EHNA, potent inhibitors of ADA (1, 9, 10, 22), resulted in a marked decrease in the metabolism of orally administered ara-M. In addition, enzyme studies have shown that ara-M is a substrate for ADA, yielding ara-H as its product (3). High levels of ADA in the intestines of rats, as well as other animals (14), would expose ara-M to this enzyme during absorption and, therefore, could explain the marked difference in the extent of ara-M metabolism after intravenous and oral dosing. The presystemic metabolism of ara-M most likely occurs predominantly

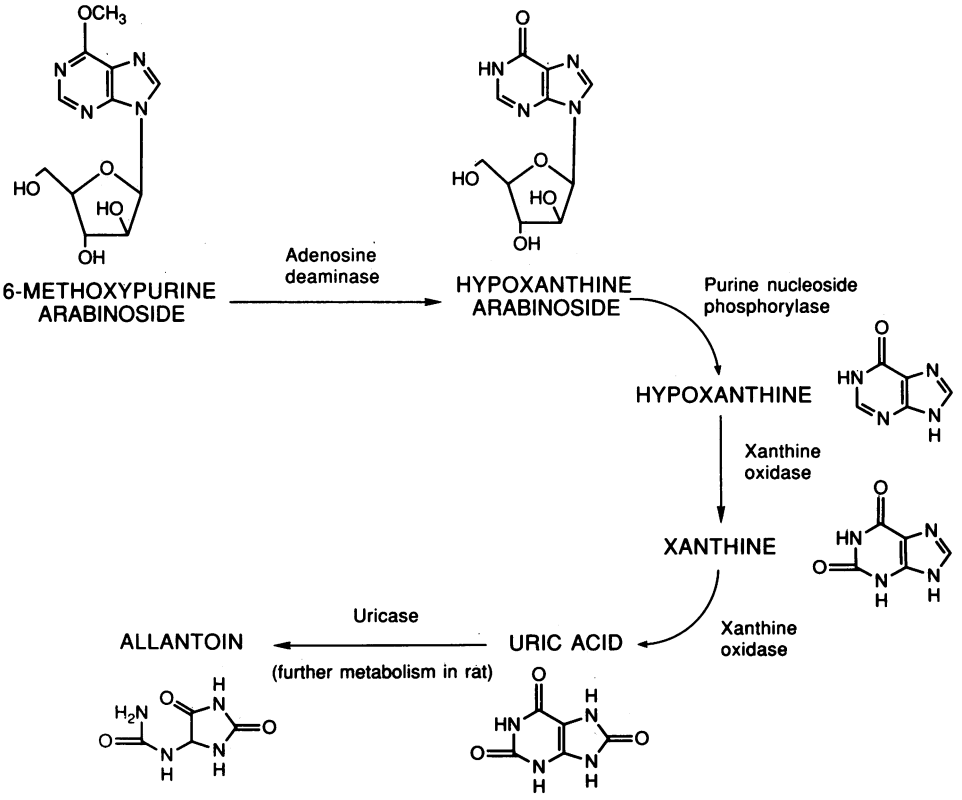

FIG. 5. Metabolism of ara-M in Long Evans rats and cynomolgus monkeys.

in the small intestine, where ADA levels are six- to sevenfold higher than in the liver (14). The prevalence of ADA in a diversity of tissues (14) suggests that this enzyme may play a major role in the systemic metabolism of ara-M as well. In contrast, pretreatment of rats with ABT and SKF 525-A (data not shown), potent inhibitors of cytochrome P-450 (20, 23), had no effect on the metabolism of orally administered ara-M. The lack of ara-M metabolism in rat liver microsomes confirmed that hepatic microsomal enzymes contribute little, if any, to the in vivo metabolism of ara-M.

The metabolic disposition of ara-M in rats and monkeys is similar to that of ara-A (12); however, there was no indication of ara-A formation as a result of ara-M metabolism, except when rats were pretreated with the ADA inhibitor DCF. On a percentage-of-dose basis, the amount of ara-A excreted by rats following coadministration of ara-M and DCF was similar to that previously demonstrated with coadministration of ara-H and DCF but was dramatically smaller than that demonstrated with coadministration of ara-A and DCF (17). These results suggest that ara-M is not a prodrug for ara-A and that ara-A is not a direct metabolite of ara-M, yet it is a minor metabolite of ara-H. The reamination of ara-H to form ara-A could occur via the nucleotide-interconverting pathway, in which ara-H is initially phosphorylated to its monophosphate by deoxycytidine kinase (15). ara-H monophosphate could then be converted to ara-AMP by the adenylosuccinate synthetase and lyase enzymes (25, 26). Dephosphorylation of ara-AMP would result in the formation of ara-A.

The pharmacokinetic profiles in rats and monkeys further demonstrated extensive metabolism of ara-M. Following intravenous administration in rats and monkeys, ara-M was rapidly eliminated. During the first hour after dosing, the disappearance of ara-M from plasma occurred largely via its biotransformation to ara-H. In monkeys, the drug was essentially completely eliminated from plasma by 6 h after dosing. In rats, concurrent administration of the ADA inhibitor EHNA significantly reduced ara-M metabolism, resulting in levels of ara-M in plasma within the 50% inhibitory concentration range for VZV (0.5 to 3 μM) (2) for at least 6 h postdose, with no detectable levels of ara-H.

In summary, these disposition studies with Long Evans rats and cynomolgus monkeys have shown that ara-M is a nucleoside analog demonstrating good oral absorption but undergoing extensive metabolism primarily because of a significant first-pass effect. The potent and selective in vitro anti-VZV activity of ara-M (2) points to the potential of ara-M as a treatment for herpes zoster in humans, but the metabolic disposition of ara-M in rats and especially monkeys indicates the need for the development of analogs or prodrugs that reduce or circumvent the extensive presystemic metabolism.

ACKNOWLEDGMENTS

We acknowledge the contributions of David J. Reynolds, Steven S. Good, Stephen R. Tibbels, Roy L. Hawke, and Stephen Weller to this work.

REFERENCES

1. **Agarwal, R. P.** 1982. Inhibitors of adenosine deaminase. Pharmacol. Ther. **17**:399–429.
2. **Averett, D. R., G. W. Koszalka, J. A. Fyfe, G. B. Roberts, D. J. M. Purifoy, and T. A. Krenitsky.** 1991. 6-Methoxypurine arabinoside as a selective and potent inhibitor of varicella-zoster virus. Antimicrob. Agents Chemother. **35**:851–857.
3. **Averett, D. R., J. N. Steinberg, G. W. Koszalka, T. Spector, and T. A. Krenitsky.** Submitted for publication.
4. **Biron, K. K., P. de Miranda, T. C. Burnette, and T. A. Krenitsky.** Submitted for publication.
5. **Blanchard, J.** 1981. Evaluation of the relative efficacy of various techniques for deproteinizing plasma samples prior to high-performance liquid chromatographic analysis. J. Chromatogr. **226**:455–460.
6. **Campbell, C. D., and C. W. Rees.** 1969. Reactive intermediates. Part I. Synthesis and oxidation of 1- and 2-aminobenzotriazole. J. Chem. Soc. C **1969**:742–747.
7. **Clarke, M. J., R. L. Hawke, and R. M. Welch.** 1986. Separation of hydroxylated metabolites of fatty acids (C_{10}-C_{18}) on a μPorasil silica column using an isocratic HPLC system. J. Liq. Chromatogr. **9**:1711–1725.
8. **de Miranda, P., T. C. Burnette, K. K. Biron, R. L. Miller, D. R. Averett, and T. A. Krenitsky.** Submitted for publication.
9. **Frienden, C., L. C. Kurz, and H. R. Gilbert.** 1980. Adenosine deaminase and adenylate deaminase: comparative kinetic studies with transition state and ground state analogue inhibitors. Biochemistry **19**:5303–5309.
10. **Geiger, J. D., J. L. Lewis, C. J. MacIntyre, and J. I. Nagy.** 1987. Pharmacokinetics of 2′-deoxycoformycin, an inhibitor of adenosine deaminase, in the rat. Neuropharmacology **26**:1383–1387.
11. **Gibaldi, M., and D. Perrier.** 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, Inc., New York.
12. **Glazko, A. J., T. Chang, J. C. Drach, D. R. Mourer, P. E. Borondy, H. Schneider, L. Croskey, and E. Maschewske.** 1975. Species differences in the metabolic disposition of adenine arabinoside, p. 111–133. *In* D. Pavan-Langston, R. A. Buchanan, and C. A. Alford (ed.), Adenine arabinoside: an antiviral agent. Raven Press, New York.
13. **Hall, W. W., and T. A. Krenitsky.** 1990. Purine nucleoside phosphorylase: purification using an ether-linked formycin B/Sepharose 6B resin with unusual properties. Prep. Biochem. **20**:75–85.
14. **Ho, D. H. W., C. Pincus, C. J. Carter, R. S. Benjamin, E. J. Freireich, and G. P. Bodey, Sr.** 1980. Distribution and inhibition of adenosine deaminase in tissues of man, rat, and mouse. Cancer Treat. Rep. **64**:629–633.
15. **Krenitsky, T. A., J. V. Tuttle, G. W. Koszalka, I. S. Chen, L. M. Beacham III, J. L. Ridout, and G. B. Elion.** 1976. Deoxycytidine kinase from calf thymus. J. Biol. Chem. **251**:4055–4061.
16. **Lambe, C. U., and D. J. Nelson.** 1982. Pharmacokinetics of inhibition of adenosine deaminase by erythro-9-(2-hydroxy-3-nonyl)adenine in CBA mice. Biochem. Pharmacol. **31**:535–539.
17. **Lambe, C. U., A. Resetar, T. Spector, G. W. Koszalka, and D. J. Nelson.** Submitted for publication.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Omura, T., and R. Sato.** 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. **239**:2370–2378.
20. **Ortiz de Montellano, P. R., B. A. Mico, J. M. Mathews, K. L. Kunze, B. T. Miwa, and A. Y. H. Lu.** 1981. Selective inactivation of cytochrome P_{450} isozymes by suicide substrates. Arch. Biochem. Biophys. **210**:717–728.
21. **Perrier, D., and M. Mayersohn.** 1982. Noncompartmental determination of the steady-state volume of distribution for any mode of administration. J. Pharm. Sci. **71**:372–373.
22. **Schaeffer, H. J., and C. F. Schwender.** 1974. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. J. Med. Chem. **17**:6–8.
23. **Schenkman, J. B., B. J. Wilson, and D. L. Cinti.** 1972. Diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A)—in vivo and in vitro effects of metabolism by rat liver microsomes—formation of an oxygenated complex. Biochem. Pharmacol. **21**:2373–2383.
24. **Sophianopoulos, J. A., S. J. Durham, A. J. Sophianopoulos, H. L. Ragsdale, and W. P. Cropper.** 1978. Ultrafiltration is theoretically equivalent to equilibrium dialysis but much simpler

to carry out. Arch. Biochem. Biophys. **187**:132–137.
25. **Spector, T.** 1977. Mammalian adenylosuccinate lyase. Participation in the conversion of 2′-dIMP and β-D-arabinosyl-IMP to adenine nucleosides. Biochim. Biophys. Acta **481**:741–745.
26. **Spector, T., and R. L. Miller.** 1976. Mammalian adenylosuccinate synthetase. Nucleotide monophosphate substrates and inhibitors. Biochim. Biophys. Acta **445**:509–517.
27. **Upton, R. A.** 1975. Simple and reliable method for serial sampling of blood from rats. J. Pharm. Sci. **64**:112–114.
28. **Wassilew, S. W.** 1987. Natural history of zoster and a review of current therapy. Res. Clin. Forums **9**:23–28.
29. **Whitlam, J. B., and K. F. Brown.** 1981. Ultrafiltration in serum protein binding determinations. J. Pharm. Sci. **70**:146–150.