Toxicity, Elimination, and Metabolism of 10-Ethyl-10-deazaaminopterin in Rats and Dogs


Clinical Pharmacology Laboratory [M. P. F., J. J. K., C. H., F. F., C. W. Y.], Laboratory for Molecular Therapeutics [L. L. S., F. M. S.], Pharmacology Laboratory [M. P. F., T-C. C., P. M. F.], Pathology Department [S. S. S.], and Bio-statistics Laboratory [D. N.], Memorial Sloan-Kettering Cancer Center, New York, NY 10021, and Stanford Research Institute, Menlo Park, California 94025-3493 [J. I. D.]

ABSTRACT

10-Ethyl-10-deazaaminopterin (10-EdAM) is an antifolate compound with greater therapeutic activity than methotrexate against transplanted tumors in mice. When given weekly for 3 weeks, the 10% lethal dose in rats was 125 mg/kg (i.p.) and in dogs it was 2.5 mg/kg (i.v.). The major histopathological findings in intoxicated animals were damage to the mucosa of the gastrointestinal tract in rats and dogs and hypopcellularity of the marrow in rats. The elimination of 50 mg/kg of 10-EdAM from the plasma of rats was triexponential with a terminal phase t0.01 of 18.5 h but a mean residence time of 0.7 h. The primary route of elimination in rats was biliary secretion of parent compound and eventual excretion of the parent compound and the deglutamate metabolite in the feces; the 7-hydroxy metabolite was also present in plasma, bile, and feces. Biliary elimination was independent of dose over a 5-fold range. The elimination of 10-EdAM from the plasma of dogs was also triexponential with a mean terminal phase t0.01 of 9.1 h and a mean residence time of 2.5 h; nonrenal clearance was the primary route of elimination. The pharmacokinetic parameters were independent of dose over the range of 0.25 to 5.0 mg/kg. High tissue concentrations of 10-EdAM were observed initially in liver, kidney, and small intestine of rats, while concentrations in bone marrow were low. Some polyglutamate formation was observed in these tissues as early as 0.5 h after drug administration but declined over 72 h.

INTRODUCTION

Many structural analogues of folic acid have been evaluated for the therapy of cancer since 1947, but only MTX1 has proven clinically useful (1). Recent studies of the kinetics of the membrane bound folate transport system responsible for entry of MTX into cells and of the biochemistry of MTX polyglutamate formation have provided insight into the selectivity of this drug for neoplastic cells (2-5). Importantly, there is now evidence for a difference in both membrane transport and in polyglutamylation of MTX between sensitive tumor cell lines and normal proliferating cells (6, 7). These differences in membrane transport and in polyglutamylation appear to favor greater accumulation of MTX as MTX polyglutamates in sensitive tumor cell lines when compared with normal proliferating tissues.

Recently, DeGraw et al. (8) have synthesized a series of 10-alkyl-10-deazaaminopterins which are transported more efficiently into sensitive murine tumor cells than into normal murine proliferative cells when compared with MTX (9). Although 10-dAM was the first to enter clinical trials (10), a newer analogue, 10-EdAM, is more effective than 10-dAM or MTX against transplanted murine tumors and against human tumor xenografts in athymic mice (11, 12). The greater therapeutic effectiveness of 10-EdAM was due to greater accumulation in tumor cells compared with MTX or 10-dAM, whereas uptake into normal cells was lower (9). Higher concentrations of polyglutamates were found in many tumor cell lines after exposure to 10-EdAM compared with MTX, whereas lower concentrations of 10-EdAM polyglutamates were found in normal proliferating cells (9). Based on these favorable in vitro and in vivo data, 10-EdAM was selected for further toxicological and pharmacological study in rats and dogs.

MATERIALS AND METHODS

Chemicals and Radiochemicals. 10-EdAM, anhydrous molecular weight of 467, was synthesized as described previously (8) and was 98.4% pure by spectral determination. [3,5,9-3H]10-EdAM was obtained from Moravek Biochemicals, Brea, CA. After purification by HPLC, the specific activity was 7.0 Ci/mmol and the radiochemical purity was 97.5%. The chromatographic standards, 10-PdAM and DGL-10-EdAM, were synthesized by Dr. Joseph I. DeGraw. 7-OH-10-EdAM was synthesized enzymatically by rabbit liver aldehyde oxidase according to the method of Johns and Loo (13). 10-EdAM with 1, 2, and 3 additional glutamate residues was supplied by M. G. Nair of the University of South Alabama, Mobile, AL.

Experimental Animals. The animals used for the toxicity and pharmacological studies were male CD rats (Charles River Breeding Laboratories, Wilmington, MA) and adult mongrel dogs (Quaker Farm Kennels, Warrentown, PA). The dogs were received as conditioned animals that had been immunized against distemper, hepatitis, and leptospirosis, had been found free of intestinal parasites by stool examination, and had been treated to remove ectoparasites. All animals were maintained in air-conditioned quarters with controlled temperature and humidity; lighting of rooms cycled regularly between 12 h of “day” and 12 h of “night.” Rats were weighed when 3 weeks old. They were observed for 2 to 3 weeks before study and were used only if their growth matched laboratory standards for weight gain.

Experimental Animals. The animals used for the toxicity and pharmacological studies were male CD rats (Charles River Breeding Laboratories, Wilmington, MA) and adult mongrel dogs (Quaker Farm Kennels, Warrentown, PA). The dogs were received as conditioned animals that had been immunized against distemper, hepatitis, and leptospirosis, had been found free of intestinal parasites by stool examination, and had been treated to remove ectoparasites. All animals were maintained in air-conditioned quarters with controlled temperature and humidity; lighting of rooms cycled regularly between 12 h of “day” and 12 h of “night.” Rats were weighed when 3 weeks old. They were observed for 2 to 3 weeks before study and were used only if their growth matched laboratory standards for weight gain.

Toxicology Studies. EdAM was administered weekly for 3 weeks i.p. to rats and weekly for 3 weeks i.v. to dogs. The dose ranges tested were:

- For rats, 25 to 400 mg/kg/week, and for dogs, 1.25 to 10 mg/kg/week.
- Dose escalation was by doubling. Rats were randomly assigned to treatment and control groups; 10 rats and 3 dogs were used at each dose level.

The amount of drug weighed was corrected for purity and dissolved in isotonic sodium chloride USP by addition of 2 molar equivalents of 1 N NaOH; the pH of this solution was between 7 and 8. Solutions were used after filtration through a sterile 0.22-μm filter. Injections in rats were in the constant volume of 0.01 ml/g of body weight.

Received 6/25/86; revised 1/20/87; accepted 1/27/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, 1275 York Ave., New York, NY 10021.

2 The abbreviations used are: MTX, methotrexate; 10-dAM, 10-deazaaminopterin; 10-EdAM, 10-ethyl-10-deazaaminopterin; 7-OH-10-EdAM, 7-hydroxy-10-ethyl-10-deazaaminopterin; DGL-10-EdAM, 2,4-diamino-C6 methylpyrrolic acid; 10-EdAM-G1-3, 10-EdAM polyglutamates with 1-3 additional glutamate residues; 10-PdAM, 10-propyl-10-deazaaminopterin; HPLC, high performance liquid chromatography; LD100, 10% lethal dose; PBS, phosphate-buffered saline.
When under study, rats and dogs were weighed daily during injection periods and for at least 1 week after injection or until all signs of toxicity had resolved. Thereafter, they were weighed at least once per week until 4 weeks after the final injection. All animals were watched closely for signs of acute or chronic changes in behavior, posture, and locomotor activity. Stools were examined for evidence of diarrhea and/or blood. Additionally, the following daily measurements were carried out with dogs: body weight, anal temperature, food and water consumption, and urine volume, specific gravity, and protein content. At intervals before and during experiments, venous blood samples were obtained from unanesthetized dogs for hematological and biochemical experiments. The LD₅₀ for each species was estimated by the median effect equation (14).

Autopsies were performed on 5 intoxicated rats and 3 intoxicated dogs approximately 96 h after the second or third dose of 10-EdAM. Samples of the following organs were examined microscopically: buccal mucosa, salivary gland, cervical and mesenteric lymph nodes, spleen, marrow, thymus, trachea, thyroid, myocardium, lungs, gastric and duodenal mucosa, ileum, pancreas, adrenals, kidneys, bladder mucosa, prostate, spleen, liver, colonic mucosa, testis and seminal vesicles, and skeletal muscle.

Elimination and Metabolism Studies. Plasma concentration of 10-EdAM and metabolites were measured in rats at different times over 72 h after bolus i.v. injection of 50 mg/kg. The drug was injected into the femoral veins of anesthetized rats through small incisions which were closed with metal clips. Plasma was obtained from blood drawn from the dorsal aorta of animals under ether anesthesia.

The urinary and fecal elimination of [²H]-10-EdAM and metabolites over 72 h was measured in rats placed individually in metabolism cages without food but provided with a drinking solution of 0.3% NaCl in 5% dextrose. Urine and feces were collected in 24-h aliquots. Homogenates of the fecal collections (10:1, v/w) were prepared in PBS using a Polytron (Brinkman Instruments, Westbury, NY).

Biliary excretion of 10-EdAM was studied after insertion of plastic catheters into the common bile ducts of rats anesthetized with pentobarbital sodium. About 1 h after insertion of the catheter, when bile was flowing freely, the animals were given injections i.v. and bile was collected continuously in 1-h aliquots for up to 4 h thereafter.

The plasma elimination and renal clearance of 10-EdAM and metabolites was measured over 72 h in individual dogs receiving 0.25, 1.0, or 5.0 mg/kg of 10-EdAM by i.v. bolus injection. Animals were maintained in metabolism cages where they were permitted free access to food and water. Dog plasma was obtained from blood sampled from brachial arteries. Urine was collected from bladder sampled from brachial veins through an indwelling catheter and heparin lock; urine was collected into ice-cold containers in 24-h aliquots.

Binding to rat and canine plasma protein was measured at concentrations of 10, 100, and 1000 nM 10-EdAM by ultrafiltration using a 5,25,000 exclusion filter (Amicon Co., Danvers, MA).

Distribution of [²H]-10-EdAM Radioactivity into Rat Tissues. The distribution of radioactivity into rat tissues was determined under administration of 10 mg (165 μCi/kg) [²H]-10-EdAM. The percentage of total drug in tissues that was accounted for by metabolites and by 10-EdAM polyglutamates was measured after i.v. injection of 50 mg/kg of 10-EdAM. After sacrifice, organs were removed, minced in ice-cold PBS, pH 7.5, rinsed and weighed. The intestines were first opened lengthwise and rinsed three times in ice-cold PBS to remove intraluminal contents. Bone marrow was collected by forcing 0.5 ml of ice-cold PBS through each femoral shaft. Homogenates (3:1, v/w) were prepared in ice-cold PBS using a Polytron, then centrifuged at 17,000 x g for 15 min. The pellets were first opened lengthwise and rinsed three times in ice-cold PBS to remove intraluminal contents. Bone marrow was collected by forcing 0.5 ml of ice-cold PBS through each femoral shaft.

Homogenates (3:1, v/w) were prepared in ice-cold PBS using a Polytron, then centrifuged at 17,000 x g for 15 min. The pellets were homogenized twice more using the same volumes of buffer, and the supernatants were pooled. The recovery of radioactivity was >98%; this was confirmed by measuring the radioactivity in the residual pellets after incubation at 40°C with 1 N NaOH for 24 h.

Formation of 10-EdAM Polyglutamates in Rat Tissues. The concentrations of 10-EdAM and 10-EdAM polyglutamates in rat tissues were measured after i.v. injection of 50 mg/kg of 10-EdAM. After sacrifice, organs were removed as described above. Homogenates (3:1, v/w) were prepared in ice-cold PBS and boiled at 100°C for 15 min, as described by Krakower and Kamen in their study of in vivo MTX polyglutamate formation in rat tissues (15).

Chromatography. 10-EdAM and its metabolites were extracted from samples of plasma, urine, and bile and from homogenates of tissue and feces by elution from bonded phase extraction columns (Waters Associates, Milford, MA), as described previously (6). Polyglutamate derivatives of 10-EdAM were also extracted from tissue homogenates using this method (16). An internal standard, 10-PdAM, was added to each sample prior to extraction. The recovery of 10-EdAM and the internal standard was 100 ± 5%.

The compounds were measured by high pressure liquid chromatography (HPLC) and a fluorescence detector as described previously (16), except that 0.1 M ammonium formate was used as the mobile phase buffer. Fig. 1 shows a chromatogram of known standards. The linear range for the assay is 1-100 nM for the known standards with a coefficient of variation of <7%.

Measurement of Radioactivity. Radioactivity was measured in a Packard Tri-Carb Model 3775 liquid scintillation spectrophotometer using Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ). Counting efficiencies were determined for each sample by addition of an internal standard, 1,3H₂O (New England Nuclear, Boston, MA), to a duplicate sample.

Pharmacokinetic Calculations. The plasma concentration (C) versus time (t) data were fit to the equation

\[ C_t = \sum \frac{a_i}{\lambda_i} e^{-\lambda_i t} \]

using NONLIN 84 (Statistical Consultants Inc., Lexington, KY), an iteratively reweighted nonlinear least squares regression program (17). The method of residuals was used to select the number of exponential terms which best fit the concentration versus time data (18). The elimination half-lives were calculated from the equation

\[ t_{1/2} = \frac{\ln 2}{\lambda} \]

The area under the concentration versus time curve from time of administration to infinity (\(\text{AUC}_{\text{to}}\)) was calculated from

\[ \text{AUC}_{\text{to}} = \sum \frac{a_i}{\lambda_i} \]

and the area under the moment curve from time of administration to infinity (\(\text{AUMC}_{\text{to}}\)) was calculated from

\[ \text{AUMC}_{\text{to}} = \sum \frac{a_i}{\lambda_i (\lambda_i)^2} \]

The total body clearance (\(\text{Cl}_b\)), the renal clearance (\(\text{Cl}_r\)), the mean residence time (MRT) and the volume of distribution at steady state (\(V_{ds}\)) were calculated from the \(\text{AUC}_{\text{to}}\) and \(\text{AUMC}_{\text{to}}\) using equations found in Ref. 18.

Fig. 1. HPLC fluorescence chromatogram of 3 pmols of 10-EdAM, 10-EdAM-polyglutamates (G₁-G₅), 7-OH-10-EdAM, and DGL-10-EdAM standards. 10-PdAM was added as an internal standard. Elution conditions are outlined in "Materials and Methods" and in Ref. 16.
RESULTS

Toxicity of 10-EdAM in Rats and Dogs. The LD_{50} in rats was 125 mg/kg/week for 3 weeks i.v.; no toxicity was observed in rats receiving 25 or 50 mg/kg/week. Intoxicated animals appeared ruffled and had retardation of growth, diarrhea, melena, and dehydration. The findings at autopsy were marked atrophy of cervical and mesenteric lymph nodes, thymus, and spleen, and petechial hemorrhages in the mucosa of the terminal ileum. On microscopic examination, there was hypoplasia of the bone marrow and of the hematopoietic elements and lymphoid follicles of the spleen. The villi of the small intestine were blunted and irregular with atypia of the epithelial cells. Many of the crypts were atrophic or ectatic and the remaining crypt cells had swollen nuclei.

Dogs were more sensitive to the toxic effects of 10-EdAM. The LD_{50} in dogs was estimated to be 2.5 mg/kg/week for 3 weeks i.v. No immediate reactions were observed in any of the animals. All animals had vomiting 24–72 h after each dose, decrease in food and water consumption, and weight loss. Animals given doses of 2.5 mg/kg or greater developed hyperemia and ulcerations of the buccal mucosa, bloody diarrhea, and dehydration. Animals with gastrointestinal bleeding had anemia. Reticulocytopenia and lymphocytopenia were present in all dogs, but neutropenia was only seen at the 10-mg/kg dose. Biochemical abnormalities attributable to dehydration were also observed in dogs given 5.0 and 10.0 mg/kg.

The findings at autopsy were erythema of the buccal mucosa, enlarged hemorrhagic mesenteric lymph nodes, and hemorrhagic streaks in the mucosa of the terminal ileum and colon. Microscopic examination revealed hyperemia and inflammatory cells within the buccal mucosa, flattening of the villi of the ileum, and ectasia of the small and large intestine. The cells lining the remaining crypts showed atypia and swelling of the nuclei. There were areas of mucosal hemorrhage and chronic inflammation. There was congestion of the mesenteric lymph nodes and of the marrow vasculature, but the marrow cellularity was normal.

Elimination and Metabolism of 10-EdAM in Rats. The concentrations of 10-EdAM in plasma obtained over 72 h from rats after an i.v. bolus injection of 50 mg/kg were summarized in Fig. 2. 10-EdAM declines 4 logs in concentration over 18 h and was not detectable in plasma beyond 72 h. The elimination data were best fit by a triexponential equation (f = 3) with the sum of squares iterations weighted by 1/(concentration)^2; the pharmacokinetic parameters are summarized in Table 1. 10-EdAM was eliminated from the plasma of rats with a terminal phase t_{1/2} of 18.5 h but with a MRT of only 0.7 h. The volume of distribution for 10-EdAM was approximately twice the volume of total body water. Rat plasma also contained minor amounts of 7-OH-10-EdAM and DGL-10-EdAM. The binding of 10-EdAM at concentrations of 10, 100, and 1000 nm to plasma proteins was 30 ± 2%.

The recovery of radioactivity in the urine and feces of two rats receiving 10 mg (165 μCi/kg) of [H]-10-EdAM is shown in Table 2. Greater than 86% of the administered radioactivity was recovered in the urine and feces over 72 h. Excretion in the feces was the primary route of elimination with the 0- to 24-h fecal collection alone containing 62% of the dose. HPLC analysis of homogenates of the 0- to 24-h fecal collection revealed that 10-EdAM accounted for only 23% of the recovered radioactivity. The majority of the recovered radioactivity was accounted for by DGL-10-EdAM (63%) and by 7-OH-10-EdAM (14%). A minor radioactive peak (0.5%) was present at the expected retention time of DGL-7-OH-10-EdAM.

The recovery of radioactivity from cannulated common bile ducts of two rats given 10 mg (165 μCi/kg) of [H]-10-EdAM accounted for 49.1 and 51.4% of the administered dose in 4 h, with >90% of the radioactivity recovered in the first hour. The biliary elimination of 10-EdAM and metabolites was also measured in three rats by HPLC-fluorescence assay after a dose of 50 mg/kg; the total recovery of drug in bile was 51 ± 4% of the administered dose. 10-EdAM accounted for 85% of the drug recovered in the bile with the remainder due to 7-OH-10-EdAM. DGL-10-EdAM was not detected in bile.

Approximately 18% of the dose of [H]-10-EdAM administered to rats was recovered in the urine over 72 h; 95% of the recovered radioactivity was found in the 0- to 24-h collection. 10-EdAM accounted for >98% of the recovered radioactivity with <2% of the radioactivity as DGL-10-EdAM.

Distribution of 10-EdAM into Tissues of Rats. The concentrations of radioactivity in tissues and plasma of rats receiving 10 mg (165 μCi/kg) [H]-10-EdAM are compared in Table 3. The distribution ratios relative to plasma were 4 to 8 in liver, kidney, and small intestine at 0.5 h after injection, and remained elevated in these organs and in spleen and lung for up to 24 h. The distribution ratios in the skeletal muscle compartment were 0.3 to 0.9, whereas concentrations in bone marrow and brain were a small fraction of simultaneous plasma concentrations and varied little during the course of the experiment.

Concentration of 10-EdAM and Polyglutamates in Rat Tissues. The concentrations of 10-EdAM and polyglutamates in rat tissues after a dose of 50 mg/kg was determined by an HPLC-fluorescence assay and is presented in Table 4. 10-EdAM polyglutamates (G_1–G_3) were observed as early as 0.5 h after drug administration in kidney, liver, and small intestine, but declined rapidly and were not detectable in kidney and small intestine after 18 h. Polyglutamates (G_1–G_3) were not detectable in rat tissues after a dose of 50 mg/kg.
Table 1 Pharmacokinetic parameters for 10-EdAM

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose (mg/kg)</th>
<th>t(_{1/2}), (min)</th>
<th>t(\beta), (h)</th>
<th>t(\alpha), (h)</th>
<th>AUC(_{0-\infty}), (nm-h)</th>
<th>V(d_{\infty}), (liters/kg)</th>
<th>Cl(d_{\infty}), (liters/kg/h)</th>
<th>CI, (liters/kg/h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>50.00</td>
<td>6.3</td>
<td>0.8</td>
<td>18.5</td>
<td>64,932</td>
<td>1.09</td>
<td>1.55</td>
<td>-</td>
<td>0.7</td>
</tr>
<tr>
<td>Dog 1</td>
<td>0.25</td>
<td>2.9</td>
<td>0.6</td>
<td>4.8</td>
<td>1,090</td>
<td>0.16</td>
<td>0.46</td>
<td>0.11</td>
<td>2.5</td>
</tr>
<tr>
<td>Dog 2</td>
<td>0.25</td>
<td>5.2</td>
<td>1.0</td>
<td>7.8</td>
<td>1,260</td>
<td>1.16</td>
<td>0.40</td>
<td>0.10</td>
<td>2.9</td>
</tr>
<tr>
<td>Dog 3</td>
<td>1.00</td>
<td>3.9</td>
<td>0.7</td>
<td>10.0</td>
<td>3,030</td>
<td>1.44</td>
<td>0.66</td>
<td>0.14</td>
<td>2.6</td>
</tr>
<tr>
<td>Dog 4</td>
<td>1.00</td>
<td>3.1</td>
<td>0.6</td>
<td>10.6</td>
<td>3,440</td>
<td>1.82</td>
<td>0.58</td>
<td>0.03</td>
<td>3.1</td>
</tr>
<tr>
<td>Dog 5</td>
<td>5.00</td>
<td>2.7</td>
<td>0.4</td>
<td>6.7</td>
<td>10,800</td>
<td>1.58</td>
<td>0.94</td>
<td>0.10</td>
<td>1.7</td>
</tr>
<tr>
<td>Dog 6</td>
<td>5.00</td>
<td>4.4</td>
<td>0.7</td>
<td>14.4</td>
<td>16,200</td>
<td>1.68</td>
<td>0.63</td>
<td>0.07</td>
<td>2.7</td>
</tr>
</tbody>
</table>

- not done.

Table 2 Recovery of radioactivity in urine and feces of rats

<table>
<thead>
<tr>
<th>Collection time (h)</th>
<th>% of administered radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>18.2*</td>
</tr>
<tr>
<td>24-48</td>
<td>0.2</td>
</tr>
<tr>
<td>48-72</td>
<td>0.1</td>
</tr>
</tbody>
</table>

- Animals received 10 mg, 165 \(\mu\)Ci [^3H]-10-EdAM/kg.
- Average of two animals which differ by less than 10%.

Table 3 Distribution of radioactivity into tissues of rats receiving [^3H]-10-EdAM

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (nmol/g tissue or ml plasma) at Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
</tr>
<tr>
<td>Plasma</td>
<td>3.48</td>
</tr>
<tr>
<td>Liver</td>
<td>16.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>26.37</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.57</td>
</tr>
<tr>
<td>Lung</td>
<td>2.09</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.98</td>
</tr>
<tr>
<td>Brain</td>
<td>0.81</td>
</tr>
<tr>
<td>Small intestine</td>
<td>13.38</td>
</tr>
<tr>
<td>Colon</td>
<td>1.03</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.14</td>
</tr>
</tbody>
</table>

- Concentration was calculated as if all radioactivity was accounted for by 10-EdAM.

Table 4 Concentrations of 10-EdAM and 10-EdAM polyglutamates in rat tissues

Animals received 10-EdAM 50 mg/kg i.v. and were sacrificed at indicated times.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time (h)</th>
<th>10-EdAM</th>
<th>10-EdAM-G1</th>
<th>10-EdAM-G2</th>
<th>10-EdAM-G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.5</td>
<td>27.40</td>
<td>ND</td>
<td>0.904</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.720</td>
<td>0.003</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>0.586</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>48.0</td>
<td>0.628</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>72.0</td>
<td>0.330</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>0.5</td>
<td>29.210</td>
<td>ND</td>
<td>0.489</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.541</td>
<td>ND</td>
<td>ND</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>0.447</td>
<td>ND</td>
<td>ND</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>48.0</td>
<td>0.577</td>
<td>ND</td>
<td>ND</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>72.0</td>
<td>0.375</td>
<td>ND</td>
<td>ND</td>
<td>0.009</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.5</td>
<td>0.925</td>
<td>0.003</td>
<td>0.039</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.186</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>0.150</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>48.0</td>
<td>0.090</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>72.0</td>
<td>0.072</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

- Polyglutamates were not detectable in homogenates of brain, bone marrow, and spleen.
- ND, not detected.

in homogenates of brain, bone marrow, and spleen. DGL-10-EdAM and 7-OH-10-EdAM were present in kidney, liver, and small intestine, accounting for 7-12% and 3-8% of the total drug, respectively.

Elimination and Metabolism of 10-EdAM in Dogs. The concentrations of 10-EdAM in plasma obtained over 72 h from individual dogs after i.v. bolus injections of 0.25, 1.0, and 5.0 mg/kg are summarized in Fig. 3. 10-EdAM declined 3 logs in concentration in 8 h and was detectable beyond 24 h only in the plasma of animals receiving 5 mg/kg. The elimination data were best fit by a triexponential equation \( i = 3 \) with the sum of squares iterations weighted by 1/concentration: the pharmacokinetic parameters are summarized in Table 1. The mean (range) terminal phase \( t_{\alpha} \) was 9.1 h (4.8-14.4 h). The V\(d_{\infty}\) was 2-3 fold the volume of total body water. Except for Dog 5, the AUC\(_{0-\infty}\) was proportional to dose, with a linear correlation coefficient of 0.999. There was little variation in the MRT with a mean of 2.6 h. Minor amounts of DGL-10-EdAM were also detectable in plasma.

As in the rat, 10-EdAM was eliminated primarily by nonrenal processes; the Cl\(_{d_{\infty}}\) although varying among the animals, in all cases accounted for a fraction of the Cl\(_{d_{\infty}}\). Of the 10-EdAM recovered in the urine over 72 h, >95% was present in the 0-24 h collection. The recovery of DGL-10-EdAM in the urine accounted for <5% of the administered dose; 7-OH-10-EdAM was not detectable.

The binding of 10-EdAM at concentrations of 10, 100, and 1000 nm to plasma proteins was 59 ±2%.

**DISCUSSION**

The weekly for 3 weeks schedule of administration was used to study the toxicology of 10-EdAM because MTX is commonly administered weekly to patients and because a weekly for 3 weeks schedule was well tolerated and efficacious in earlier clinical trials with 10-deazaminopterin (10). The dog, with a LD\(_{10}\) of 2.5 mg/kg/week x 3 weeks, was more sensitive to intoxication with 10-EdAM than was the rat, with a LD\(_{10}\) of
125 mg/kg/week for 3 weeks. 10-EdAM is less toxic than MTX in both species: the single dose LD<sub>50</sub> for MTX was 15 mg/kg in rats and approximately 2 mg/kg in dogs (20).

The signs of intoxication from 10-EdAM observed in rats and dogs correlated with the predominant histopathological finding of damage to the intestinal mucosa. Bone marrow hypoplasia was observed in rats but not in dogs. This is different from MTX which causes damage to both the gut and bone marrow in rats and dogs (20).

The pharmacokinetic parameters we calculated for 10-EdAM in rats and dogs cannot readily be compared with published values for the pharmacokinetics of MTX. The estimates for t<sub>1/2</sub>, clearance, and distribution volume have largely relied on non-specific measurements of total radioactivity from <sup>3</sup>H]MTX in biologic fluids and tissues, with sampling limited to less than eight hours after drug administration. Early studies demonstrated a biexponential plasma elimination curve with a terminal phase t<sub>1/2</sub> of 30 to 40 min in rats and 1.5 to 2 h in dogs (21–23). More recent experiments have suggested the presence of a third phase with an elimination t<sub>1/2</sub> of greater than six hours (24); however, assay methods have not allowed accurate characterization of this component (25).

The half-lives of 10-EdAM and MTX in the plasma have been directly compared only in mice (9). The terminal phase t<sub>1/2</sub> of 10-EdAM was 8 h, identical to that of MTX, when samples up to 24 h after administration of 12 mg/kg s.c. were assayed by dihydrofolate reductase inhibition. We observed a terminal phase t<sub>1/2</sub> for 10-EdAM of 18.5 h in the rat and 9.1 h in the dog, using a sensitive and specific fluorescence assay which allowed analysis up to 72 h after drug administration. However, only a small percentage of the administered dose of 10-EdAM is eliminated during the terminal phase; the MRT, the time required for elimination of 62.3% of the administered dose from the body (26), is 0.7 h for rats and 2.5 h for dogs.

For similar doses of <sup>3</sup>H]-10-EdAM and <sup>3</sup>H]MTX (21), approximately 85% of the administered radioactivity was recovered in the urine and feces of rats over 48 h. For both drugs, biliary elimination accounted for one-half of the administered radioactivity and was not saturable over a wide dose range (21, 25). These data suggest that the clearances of 10-EdAM and MTX in the rat are similar. However, only 30–40% of the administered dose of <sup>3</sup>H]MTX was eventually eliminated in the feces (21), compared with approximately 60% for <sup>3</sup>H]-10-EdAM, suggesting greater absorption of MTX or its metabolites from the lumen of the gut.

Like MTX (13), 10-EdAM is a substrate for hepatic aldehyde oxidase<sup>5</sup>, and small amounts of 7-OH-10-EdAM were also detectable in bile. The major metabolite in feces, DGL-10-EdAM, was formed in the lumen of the gut, probably by bacterial enzymes in a fashion similar to MTX (27).

There is a wide variation in the distribution of <sup>3</sup>H]-10-EdAM radioactivity into the tissue compartments of rats. Like MTX (22, 23), 10-EdAM radioactivity is concentrated in kidney and liver tissue relative to plasma, whereas lower concentrations were observed in bone marrow and brain. With the high concentrations of 10-EdAM in the intestinal lumen, some of it is reabsorbed, resulting in high concentrations of drug in the wall of both the small and large intestine. The volume of distribution for <sup>3</sup>H]MTX, determined by direct measurement of total radioactivity in tissues, has been reported to be approximately total body water (25). This is approximately one-half the V<sub>D</sub><sub>app</sub> calculated for 10-EdAM. The value for MTX includes polyglutamates and metabolites which comprise a substantial percentage of total drug in many tissues (15).

The data in Table 4 show that 10-EdAM polyglutamate formation occurs as early as 0.5 h after drug administration in the kidney, liver, and small intestine; a similar rapid formation of polyglutamates in liver and kidney of rats has been observed by Krakower and Kamen (15) after a single injection of 10 mg/kg of MTX. However, the percentage of MTX as polyglutamates in these organs varied from 33–66%, substantially higher than what we observed for 10-EdAM. The percentage of MTX polyglutamates increased or remained constant over 170 h, in contrast to the declines observed for 10-EdAM polyglutamates. Furthermore, high concentrations of MTX polyglutamates were observed in the brain and bone marrow, where we were unable to detect 10-EdAM polyglutamates. MTX polyglutamates could not be detected in the small intestine, where we found small concentrations of 10-EdAM polyglutamates. The differences between the two drugs in polyglutamate formation and elimination by normal tissues may be important factors in the reduced host toxicity of 10-EdAM.

The elimination and distribution of 10-EdAM and MTX differ substantially in dogs. Henderson et al. (21) found that 70% of the administered <sup>3</sup>H]MTX radioactivity was excreted in the urine over 24 h; the volume of distribution of radioactivity approximated total body water. Our studies indicate that 10-EdAM is eliminated primarily by nonrenal processes and that 10-EdAM has a substantially larger volume of distribution than MTX. Although some variability was observed in the pharmacokinetic parameters, probably due to our use of mongrel dogs, the linear relationship between dose and AUC and the constant MRT indicate that elimination was not saturable over a wide dose range.

One-tenth the LD<sub>50</sub> in the dog, the more sensitive species, was selected as the initial dose for the Phase I study of 10-EdAM, i.e., 5 mg/m<sup>2</sup>/week for 3 weeks. The results of this study indicate that damage to the gastrointestinal tract is likely to be the dose-limiting toxicity for 10-EdAM in humans. Elimination from the plasma of humans is expected to be triphasic with a terminal phase t<sub>1/2</sub> of at least 9 h. Nonrenal elimination is likely to be the major route of drug clearance, which is likely to be independent of dose over a wide range.

REFERENCES


7. Samuels, L. L., Moccio, D. M., and Sirotnak, F. M. Similar differential for total polyglutamylation and cytotoxicity among various folate analogues in
Toxicity, Elimination, and Metabolism of 10-Ethyl-10-deazaaminopterin in Rats and Dogs

Michael P. Fanucchi, James J. Kinahan, Lawrence L. Samuels, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/9/2334

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.