Pharmacologic evaluation of ammonium tetrathiomolybdate after intravenous and oral administration to healthy dogs

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OBJECTIVE
To evaluate pharmacokinetics of ammonium tetrathiomolybdate (TTM) after IV and oral administration to dogs and effects of TTM administration on trace mineral concentrations.

ANIMALS
8 adult Beagles and Beagle crossbreds (4 sexually intact males and 4 sexually intact females).

PROCEDURES
Dogs received TTM (1 mg/kg) IV and orally in a randomized crossover study. Serum molybdenum and copper concentrations were measured via induc
tively coupled plasma mass spectrometry in samples obtained 0 to 72 hours after administration. Pharmacokinetics was determined via noncompartmen
tal analysis.

RESULTS
For IV administration, mean ± SD terminal elimination rate constant, maximum concentration, area under the curve, and half-life were 0.03 ± 0.01 hours⁻¹, 4.9 ± 0.6 μg/mL, 30.7 ± 5.4 μg/mL•h, and 27.7 ± 6.8 hours, respec
tively. For oral administration, mean ± SD terminal elimination rate constant, time to maximum concentration, maximum concentration, area under the curve, and half-life were 0.03 ± 0.01 hours⁻¹, 3.0 ± 3.5 hours, 0.2 ± 0.4 μg/mL, 6.5 ± 8.0 μg/mL•h, and 26.8 ± 8.0 hours, respectively. Oral bioavailability was 21 ± 22%. Serum copper concentrations increased significantly after IV and oral administration. Emesis occurred after IV (2 dogs) and oral administration (3 dogs).

CONCLUSIONS AND CLINICAL RELEVANCE
Pharmacokinetics for TTM after a single IV and oral administration was determined for clinically normal dogs. Absorption of TTM after oral adminis
tration was variable. Increased serum copper concentrations suggested that TTM mobilized tissue copper. Further studies will be needed to evaluate the potential therapeutic use of TTM in copper-associated chronic hepatitis of dogs. (Am J Vet Res 2015;76:445–453)
which has led some authors to recommend coadministration of the drug with a meal.\textsuperscript{11} However, this practice results in markedly decreased drug absorption.\textsuperscript{12} Although \(\beta\text{-penicillamine}\) remains an effective drug for many dogs with CACH, these limitations warrant evaluation of alternative agents.

Ammonium tetrathiomolybdate has potential value as a chelating agent for CACH. It is unique as a copper chelator because of its additive actions in the intestinal tract, plasma, and hepatic tissue.\textsuperscript{13} When used for Wilson disease, it has superior therapeutic efficacy, compared with the efficacy for trientine or \(\beta\text{-penicillamine}.\textsuperscript{2,14,15}

It is specifically indicated for humans with neurologic manifestations of Wilson disease because other treatments often lead to worsening and sometimes irreversible clinical signs.\textsuperscript{2,14,15} In rodents with experimentally induced Wilson disease, intraperitoneal administration of a single dose of TTM rapidly reduces clinical signs, total hepatic copper concentrations, and liver enzyme activities.\textsuperscript{16} Given the success of TTM for treating copper overload in other species, critical evaluation of this agent is needed in dogs.

To the authors’ knowledge, use of TTM has not been reported in dogs with CACH. The only report of TTM use in dogs was a small study\textsuperscript{17} of dogs with metastatic neoplasia in which the drug was administered for its antiangiogenic properties. The pharmacokinetics of TTM have been reported in sheep and rats, with discordant results.\textsuperscript{18,19} Dosages and frequency of administration for the treatment of copper storage disease also differ widely across species.\textsuperscript{3,14,17,20,21} Prior to clinical evaluation of TTM in dogs with CACH, pharmacological evaluation of the drug is warranted. The purposes of the study reported here were to determine pharmacokinetics after oral and IV administration of TTM to healthy dogs and to evaluate effects of TTM administration on serum trace mineral concentrations. We hypothesized that serum copper concentrations would increase after TTM administration.

\section*{Materials and Methods}

\subsection*{Animals}

Eight laboratory dogs (4 sexually intact male and 4 sexually intact female Beagles or Beagle crossbreds) were used in the study. Mean \(\pm\) SD body weight was 14.4 \(\pm\) 1.7 kg, and mean age was 2.1 \(\pm\) 1.1 years. All dogs were considered healthy on the basis of results of physical examination, a CBC, serum biochemical analysis, and urinalysis. Dogs were housed in large runs, fed a standard commercial diet,\textsuperscript{6} and allowed ad libitum access to water. The live-animal portion of the study was conducted at the Michigan State University College of Veterinary Medicine Vivarium. The study protocol was reviewed and approved by the Michigan State University Institutional Animal Care and Use Committee.

\subsection*{Drug preparation}

Powdered TTM\textsuperscript{b} (purity, 99.97\%) was used for compounding by the Michigan State University Veterinary Teaching Hospital pharmacy. Doses for oral administration were placed into capsules; capsules contained a dose of 1 mg/kg for each dog. Microcrystalline cellulose was used as an excipient for the orally administered doses. Doses for IV administration involved creation of a stock solution (5 mg/mL) in sterile saline (0.9\% NaCl) solution that was passed through a 0.2-\(\mu\)m filter and placed into sterile vials. Aliquots of the stock solution were placed into separate vials that contained a dose of 1 mg/kg for each dog. Analysis of randomly selected capsules and solutions by use of ICP-MS established that the capsules and solutions were prepared to a mean \(\pm\) SD of 94.0 \(\pm\) 10.0\% and 100 \(\pm\) 0\% of the intended target, respectively. On the basis of the syringes used to measure each dose of TTM for IV administration, SD of the individual doses should have been \(\leq\) 5\%.\textsuperscript{22,23} All capsules and IV solutions were stored under nitrogen gas at 21.1\°C and used within 6 weeks after preparation.

\subsection*{Study design}

The study was conducted in accordance with a randomized crossover design. Dogs (numbered 1 through 8) were assigned by means of a computerized random number procedure to initially receive TTM via oral or IV administration. After completion of the initial treatment, there was a 10-day period for drug washout and recovery of blood volume before the dogs received TTM via the other route of administration.

Food was withheld from all dogs for 12 hours before drug administration, but water was available ad libitum. All dogs were fed 8 hours after drug administration.

An 18-gauge catheter was aseptically placed in a cephalic vein of each dog on the morning of drug administration. For oral administration, a capsule containing TTM (1 mg/kg) was administered. If a dog vomited after administration, all sampling was discontinued and oral administration was repeated after a 10-day washout period. If a dog vomited after the second attempt at oral administration, there was another 10-day washout period. Then, maropitant\textsuperscript{6} (1 mg/kg, SC) was administered 1 hour before the third attempt to orally administer the dose of TTM. For IV administration, a 20-gauge catheter was aseptically placed in the contralateral cephalic vein of each dog. The TTM solution (dose of 1 mg/kg) was administered IV over a 5-second period through the 20-gauge catheter; that catheter was removed after TTM administration.

\subsection*{Sample collection and processing}

For oral administration, blood samples (3 mL) were collected immediately before (time 0) and 15, 30, 45, 60, 90, 120, 150, 180, and 210 minutes and 4, 8, 12, 24, 48, and 72 hours after TTM administration. For IV administration, blood samples (3 mL) were collected immediately before (time 0) and 1, 5, 15, 30, 60, 120, and 180 minutes and 4, 8, 12, 24, 48, and 72 hours after TTM administration.
TTM administration. After the blood sample was collected at 4 hours after TTM administration (both oral and IV administration), the 18-gauge catheter was removed; remaining samples were obtained via cephalic or jugular venipuncture. All blood samples were placed immediately into trace nutrient analysis serum collection tubes. After clots had formed, samples were centrifuged at 1,200 × g for 10 minutes at 4°C. Serum then was harvested and stored in cryovials at −80°C. Samples were analyzed within 3 months after collection.

Sample analysis

Serum concentrations of molybdenum, copper, cobalt, iron, manganese, selenium, and zinc were measured by use of ICP-MS. The assay had been validated previously at the Michigan State University Diagnostic Center for Population and Animal Health. Briefly, 5 mL of diluent consisting of 0.5% EDTA, 1% ammonium hydroxide, 0.5% Triton-X 100, 2% propanol, and 20 μg/L for each internal standard (scandium, rhodium, indium, and bismuth) was prepared. Samples were mixed in a vortexer. If solids were still visible in the diluent, it was centrifuged at 2,800 × g for 5 minutes, and the supernatant was harvested and mixed with 200 μL of serum. Samples were placed in the auto-sampler rack and entered in the sample queue. An inductively coupled plasma mass spectrometer was used for analyses. The machine was adjusted to yield a minimum sensitivity of 8,000 counts/s for 1 μg of yttrium/kg, < 1.0% oxide as determined by the 156-to-140 mass ratio, and < 2.0% double-charged ions as determined by the 70-to-140 mass ratio. Standards were prepared for instrument calibration at the following concentrations for each trace mineral: cobalt, manganese, and molybdenum at 0, 0.0005, 0.005, and 0.025 μg/mL; selenium at 0, 0.005, 0.05, and 0.25 μg/mL; copper at 0, 0.02, 0.2, and 1 μg/mL; and zinc and iron at 0, 0.05, 0.5, and 2.5 μg/mL. Each trace mineral was calibrated on a 4-point linear curve of the analyte-to-internal standard response ratio. Samples typically were diluted and reassayed if responses exceeded the listed calibration range or were reported as less than the lowest nonzero calibrator below the calibration range.

Three modes were used to minimize spectral interferences for the analyses. Copper, zinc, and cobalt were analyzed in helium mode, selenium and iron were analyzed in hydrogen mode, and manganese and molybdenum were analyzed in nongas mode. The calculated lower limit of quantitation for each trace mineral was < 0.5 ng/mL, which was well below the lowest nonzero calibration point for each element and therefore below their individual reportable limits.

Validation of a thiomolybdate assay

The TTM was quantitated by determination of the molybdenum concentration and reported as the number of micrograms per milliliter. For interassay comparisons, conversion of molybdenum to TTM requires multiplication of results by 2.33, given that the molecular weight of the TTM anion (as MoS₄²⁻) is 224. The application of standards to trace mineral quantitation has been validated in typical matrices of serum and whole blood. The determined limit of quantitation for molybdenum in serum was 0.0001 μg/mL, which was reported as < 0.0005 μg/mL (the lowest nonzero calibrator used for molybdenum in the assay). The ICP-MS assay for molybdenum was linear for the concentration range of 0.0001 to 5 μg/mL, and R² for calibration curves ranged between 0.998 and 0.999. Quality control was maintained by determination of acceptable results for 2 concentrations of an in-house certified serum control sample measured every 12th sample; accuracy of this serum control sample was confirmed by comparison to a certified reference material. Assay precision (percentage relative SD) for molybdenum standards across the range of 0.0005 to 0.05 μg/mL was as follows: 0.05 μg/mL, 0.99%; 0.025 μg/mL, 1.00%; 0.005 μg/mL, 0.89%; and 0.0005 μg/mL, 1.20%.

Analysis of drug stability

A set of TTM capsules and IV solutions used to assess stability were prepared identically to those used in the present study and stored similarly under nitrogen gas at 21.1°C until analysis was performed 420 days after preparation. Solutions of TTM (5.0 mg/mL in saline solution) or the solid (capsule) formulation (1 mg/5 g of powder, in cellulose) were analyzed on a spectrophotometer. Instrument performance was verified by scanning a didymium filter, and the instrument was calibrated by use of air as a blank. The TTM solutions and capsule contents were diluted or dissolved in deionized water to achieve a concentration of 10 μg/mL so that they were within the absorbance scale of the instrument. Scans were made across the range of 300 to 800 nm.

Molybdenum and sulfur were analyzed by use of inductively coupled plasma-atomic emission spectrometry. An aliquot (30 mg) of capsule contents was used in the analyses. One milliliter of concentrated nitric acid was added to each sample, and samples were digested overnight at 95°C. Appropriate blanks and standards were used. Standard solutions of 157μa tomato leaves and multi-element quality control were used as quality control samples. Digested capsule contents were diluted appropriately to create solutions that were within the expected linear dynamic range of the instrument.

Pharmacokinetic analysis

Similar to previous studies, the serum molybdenum concentration was used as the surrogate marker for serum TTM concentration. Data were evaluated by use of noncompartmental analysis with publicly available software to determine standard pharmacokinetic parameters, including terminal elimination rate constant, Cmax, Tmax, clearance rate, apparent distribution volume, and volume of distribution at steady state, and AUC from time 0 to infinity.
The program assumed first-order kinetics on the basis of linearity of the terminal portion of the semilogarithmic concentration-time plots. Bioavailability for each dog was calculated by use of the following standard equation: (AUCoral•100)/AUCIV, where AUCoral and AUCIV are the AUC for the oral and IV administrations, respectively. Terminal elimination rate constant was determined from linear regression of the logarithmic serum concentration-time curve during the elimination phase. Total AUC was estimated by use of the trapezoidal rule.

**Statistical analysis**

Data were reported as mean ± SD. The effect of time on serum molybdenum and copper concentrations after oral or IV administration of TTM was determined by use of repeated-measures ANOVA with a Greenhouse-Geisser correction. When a significant effect for time was detected by use of the Greenhouse-Geisser-corrected repeated-measures ANOVA, a Dunnett 1-tailed post hoc test was performed to compare values for postadministration time points with the preadministration (time 0) value. Statistical analysis was performed with publicly available software; differences were considered significant at P ≤ 0.05.

**Results**

**Drug stability**

All TTM preparations, whether IV solutions or capsule contents, had mean ± SD UV maxima at 468 ± 0.27 nm. This was fortuitous in that it effectively excluded substantial accumulation of oxygenated species of TTM, specifically MoOS$_3^{2–}$ (diagnostic UV maximum, 398 nm), MoO$_2S_2^{2–}$ (diagnostic UV maximum, 394 nm), and MoO$_5S_2^{2–}$ (diagnostic UV maximum, 390 nm).\(^\text{26}\) Comparison of results for freshly prepared solutions and capsules with results for samples prepared 420 days earlier revealed that capsule contents diminished in potency (ie, decrease in absorbance at 468 nm) at the rate of 0.08%/d, whereas IV solutions diminished at the rate of 0.14%/d. At these rates, capsule contents should diminish only 2.4% and solutions only 4.2% if used within 30 days after preparation, which would provide 97.6% and 95.8% of the expected potencies.

An even more encouraging picture of long-term stability of properly stored TTM powder was determined by inductively coupled plasma-atomic emission spectrometry$^\text{a}$ examination of capsule contents. The molar ratio of sulfur to molybdenum in fresh (< 1 week) TTM capsules was 3.98, which was reasonably close to the expected ratio of 4.0, whereas the mean ± SD of 420-day-old capsules was 3.83 ± 0.01. This indicated minor degradation, presumably by oxidation, of only 3.8% over 420 days (rate of 0.009%/d).Capsules used within 30 days after preparation should only degrade by 0.27%, which would provide 99.7% of the expected dose by this determination.

**Adverse effects**

Three dogs vomited after oral administration of TTM. For these 3 dogs, 1 required 2 doses of TTM to complete oral administration, and 2 required 3 doses for completion. The 2 dogs that required 3 doses of TTM (dogs No. 7 and 8) were administered maropitant (1 mg/kg, SC) 1 hour before the third attempt to orally administer the dose of TTM. Neither dog vomited after maropitant and TTM administration. Two dogs vomited after the first IV administration (one of which also vomited after oral administration); neither of these dogs vomited after a 10-day washout period and the second IV administration of TTM. No other adverse effects were detected.

**Pharmacokinetic and trace mineral analysis**

Pharmacokinetic parameters for each route of drug administration were summarized ([Tables 1 and 2](#)). Overall mean ± SD AUC after oral administration (6.46 ± 7.96 μg/mL•h) was substantially less than that after IV administration (30.7 ± 5.38 μg/mL•h; Figure 1). Intersubject drug absorption after oral administration was variable, with AUC and Cmax ranging from 1.64 to 22.9 μg/mL•h and 0.077 to 1.11 μg/mL, respectively (Figure 2). The 2 dogs

### Table 1—Mean ± SD pharmacokinetic parameters for TTM after IV and oral administration (1 mg/kg) to 8 dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg/mL•h)</td>
<td>30.7 ± 5.4</td>
<td>6.5 ± 8.0</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>—</td>
<td>21.0 ± 22.0</td>
</tr>
<tr>
<td>Clearance (mL/kg•h)</td>
<td>32.6 ± 6.1</td>
<td>—</td>
</tr>
<tr>
<td>CI/F (mL/kg•h)</td>
<td>—</td>
<td>154.8 ± 194.0</td>
</tr>
<tr>
<td>Cmax (μg/mL)</td>
<td>4.9 ± 0.6</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>ke (1/h)</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Mean residence time (h)</td>
<td>33.4 ± 6.7</td>
<td>32.6 ± 12.7</td>
</tr>
<tr>
<td>t$_{1/2}$ (h)</td>
<td>27.7 ± 6.8</td>
<td>26.8 ± 8.0</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.02 ± 0.00</td>
<td>3.00 ± 3.50</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>1.0 ± 0.2</td>
<td>—</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>1.0 ± 0.1</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$CI/F = Apparent clearance rate. ke = Terminal elimination rate constant. t$_{1/2}$ = Elimination half-life. Vd = Volume of distribution. Vdss = Volume of distribution at steady state. — = Not determined.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without maropitant (n = 6)</th>
<th>With maropitant Dog 7</th>
<th>With maropitant Dog 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg/mL•h)</td>
<td>4.3 ± 2.5</td>
<td>22.9 ± 18.3</td>
<td></td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>14.0 ± 7.8</td>
<td>59.5 ± 59.1</td>
<td></td>
</tr>
<tr>
<td>CI/F (mL/kg•h)</td>
<td>310.6 ± 179.0</td>
<td>43.6 ± 54.7</td>
<td></td>
</tr>
<tr>
<td>Cmax (μg/mL)</td>
<td>0.2 ± 0.2</td>
<td>1.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>ke (1/h)</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Mean residence time (h)</td>
<td>36.9 ± 14.2</td>
<td>29.1 ± 27.4</td>
<td></td>
</tr>
<tr>
<td>t$_{1/2}$ (h)</td>
<td>26.1 ± 9.0</td>
<td>22.1 ± 19.4</td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>5.0 ± 3.4</td>
<td>0.8 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>
pretreated with maropitant attained the 2 largest values for Cmax and AUC. During initial unsuccessful attempts to complete oral administration for these 2 dogs, blood samples were collected during the first 30 minutes after TTM administration (the dogs vomited between 30 and 45 minutes after drug administration). The Cmax and AUC through 30 minutes for these initial samples in the 2 pretreated dogs were far larger than the mean Cmax and AUC of the corresponding initial samples for the other 6 dogs. However, Cmax and AUC through 30 minutes in dogs 7 and 8 were far larger when these 2 dogs received maropitant and TTM, compared with values when they did not.

Serum copper concentrations increased after oral administration of TTM from a mean ± SD preadministration value of 0.65 ± 0.09 μg/mL to a mean Cmax of 0.76 ± 0.19 μg/mL (Figure 3). Serum copper concentrations increased after IV administration from a mean ± SD preadministration value of 0.61 ± 0.09 μg/mL to a mean Cmax of 0.94 ± 0.12 μg/mL. The increases in serum copper concentrations were significant for both routes of administration. Mean ± SD Tmax after oral and IV administration was 12.8 ± 24.2 hours and 6.0 ± 3.0 hours, respectively.

The 3 dogs that had the highest serum molybdenum concentrations did not have the 3 highest serum copper concentrations after oral or IV administration. Treatment order had no effect on pharmacokinetic parameters.

Mean ± SD serum concentrations of iron, zinc, cobalt, manganese, and selenium after oral and IV administration of TTM were graphically displayed because they were part of a comprehensive trace mineral panel (Figure 4). These results were not statistically evaluated for patterns relevant to TTM administration.
compound with dietary copper and proteins. It is its absorption of intestinal copper by forming an inert bound copper to prevent cellular uptake, and it inhibits from the liver, it complexes with plasma albumin–removes intracellular metallothionein-bound copper because of its additive actions in 3 locations: it rapidly considered particularly effective as a copper chelator from 2 to 4 days. In contrast, human dosing intervals range from 3 to 6 times/d, both with and without food. The 1 mg/kg dose used in the study reported here was extrapolated from previous human reports and a published canine report. The bioavailability of 21% was surprising, considering that food withholding increases intestinal drug absorption in other species. On the basis of the calculated bioavailability, an orally administered dose would likely need to be 5 times as great as an IV administered dose to achieve similar serum concentrations. However, TTM has high affinity for hepatocytes in other species, and a high first-pass effect may be responsible for the low relative drug exposures in the present study. If the low bioavailability was caused by a high first-pass effect, a larger dose for oral administration, compared with the dose for IV administration, may be unnecessary because the liver is the target organ for dogs with CACH. Elimination characteristics described in the present report suggested that a long dosing interval may be appropriate. Given the various dosages and dosing regimens among species, additional pharmacokinetic and pharmacodynamic investigations are needed to determine the appropriate dose, dosing interval, and route of administration for dogs.

Significant increases in serum copper concentrations were detected after both IV and oral administration, which suggested that TTM mobilized tissue copper in dogs. This also has been reported in sheep, and rats. Ammonium tetrathiomolybdate tissue concentrations and the amount of copper mobilized from that tissue are directly related to intracellular copper concentrations, and it is possible that dogs with CACH would have greater increases in serum copper concentrations than would clinically normal dogs. However, TTM-mobilized copper is exported into both the bloodstream and biliary system. In fact, most of the chelated copper is excreted via the biliary system, and only a small amount undergoes urinary elimination in rats and sheep. This contrasts with β-penicillamine and trientine in which nearly all chelated copper undergoes urinary elimination. Although we found in the present study that TTM increases serum copper concentrations, both the origin of the mobilized copper and its ultimate fate after mobilization are unknown. Most dogs affected with CACH are suspected to have an underlying heritable disorder of hepatic copper metabolism, and it is unknown the effect, if any, this would have on elimination of TTM-mobilized copper.

To the authors’ knowledge, serum copper concentrations after β-penicillamine or trientine administration have not been measured in dogs. In humans receiving orally administered β-penicillamine for 2 to 15 years, mean ± SD serum copper concentrations were 0.4 ± 0.2 μg/mL. In humans receiving daily doses of trientine, serum free copper concentrations were 0.3 μg/mL after 1 week of administration. In young chickens receiving a single SC dose of β-penicillamine, serum copper concentration reached a Cmax of ap-

**Figure 4**—Mean ± SD serum concentration of iron (inverted triangles), zinc (diamonds), selenium (squares), manganese (circles), and cobalt (triangles) before (time 0) and after IV (A) and oral (B) administration of TTM (1 mg/kg) to 8 dogs. Notice that the intervals on the x-axis represent increments of 2 hours to the left of the axis break and increments of 20 hours to the right of the axis break. These data were not statistically evaluated for any patterns relevant to TTM administration.

**Discussion**

Tetrathiomolybdate is a simple molecule composed of a molybdenum atom and 4 sulfhydryl groups. The ammonium salt is the most commonly used form of thiomolybdate in human and veterinary medicine. Each sulfhydryl group increases the molecule’s copper-binding capacity; therefore, TTM has the greatest copper-binding capacity among the thiomolybdates. It is considered particularly effective as a copper chelator because of its additive actions in 3 locations: it rapidly removes intracellular metallothionein-bound copper from the liver, it complexes with plasma albumin-bound copper to prevent cellular uptake, and it inhibits absorption of intestinal copper by forming an inert compound with dietary copper and proteins. It is currently used as a chelating agent for copper toxicity in sheep and Wilson’s disease in humans. Given TTM’s ability to suppress inflammation, fibrosis, autoimmunity, and angiogenesis, therapeutic potential exists for many diseases. Accordingly, TTM has been investigated as a therapeutic agent for use in Alzheimer’s disease, pulmonary fibrosis, multiple sclerosis, and neoplasia.

Treatment efficacy has been reported for sheep with copper toxicosis in which dosing intervals range from 2 to 4 days. In contrast, human dosing intervals range from 3 to 6 times/d, both with and without food. The 1 mg/kg dose used in the study reported here was extrapolated from previous human reports and a published canine report. The bioavailability of 21% was surprising, considering that food withholding increases intestinal drug absorption in other species. On the basis of the calculated bioavailability, an orally administered dose would likely need to be 5 times as great as an IV administered dose to achieve similar serum concentrations. However, TTM has high affinity for hepatocytes in other species, and a high first-pass effect may be responsible for the low relative drug exposures in the present study. If the low bioavailability was caused by a high first-pass effect, a larger dose for oral administration, compared with the dose for IV administration, may be unnecessary because the liver is the target organ for dogs with CACH. Elimination characteristics described in the present report suggested that a long dosing interval may be appropriate. Given the various dosages and dosing regimens among species, additional pharmacokinetic and pharmacodynamic investigations are needed to determine the appropriate dose, dosing interval, and route of administration for dogs.

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proximately 0.2 μg/mL 30 minutes after injection.\textsuperscript{35} In mice receiving orally administered α-penicillamine for 5 days, serum free copper concentrations reached a Cmax of approximately 1.2 μg/mL.\textsuperscript{34} Given the variability among species, dose regimens used, and copper excretion patterns for α-penicillamine, trientine, and TTM, it is difficult to determine whether the serum copper concentrations attained in the present study can be considered comparable to those attained with α-penicillamine or trientine. Additionally, treatment of Wilson disease is considered more effective when the serum free copper concentrations are lower, thus implying that more copper is protein-bound.\textsuperscript{2}

There was marked intersubject variability for Cmax, Tmax, and AUC after oral administration of TTM. This could have important clinical implications, especially if copper chelation efficacy is directly related to drug absorption and subsequent serum and tissue drug concentrations. If this is true, treatment dose and treatment duration could differ considerably among dogs.

The 2 dogs pretreated with maropitant had a Cmax and bioavailability greater than those of the remaining 6 dogs. Measurements of serum molybdenum concentrations in those same 2 dogs when maropitant had not been administered (for the first 30 minutes after TTM administration until emesis occurred) also revealed increased molybdenum concentrations, compared with concentrations of the remaining 6 dogs; independent of maropitant administration, those 2 dogs absorbed larger amounts of TTM. Although the magnitude of the increase appeared more pronounced with concurrent maropitant administration, it is difficult to draw conclusions because samples were collected only for the initial 30 minutes after administration, because the dogs vomited prior to the next scheduled sample collection time. Some dogs had profound changes in serum molybdenum concentrations over short periods. As such, it is possible that serum molybdenum concentrations were even higher at the time of emesis and were similar to the concentrations attained when the 2 dogs were administered maropitant. It is also possible that these dogs consistently absorbed a larger fraction of the administered dose, but the differences reflected some degree of typical intrasubject variability in absorption characteristics. Regardless, those 2 dogs could represent an important subset of the population that absorbs more TTM.

Those 2 dogs had even greater increases in molybdenum concentrations through 30 minutes after TTM administration when they were pretreated with maropitant; therefore, an alternate hypothesis is that pretreatment with maropitant influenced drug exposure. If maropitant actually increases the bioavailability of TTM, it could be useful in preventing emesis as well as improving TTM efficacy. Both maropitant and TTM are highly protein-bound substances and are hepatically metabolized and eliminated.\textsuperscript{13,14} The Tmax and half-life for maropitant in dogs are 0.75 ± 1.11 hours and 8.84 hours, respectively;\textsuperscript{1} so plasma concentrations of maropitant would have been almost maximal at the time of TTM administration. The authors are not aware of any studies that have been conducted to evaluate whether maropitant affects plasma concentrations of concomitantly used drugs,\textsuperscript{4} but no problems have been reported after concurrent use of maropitant and various antimicrobials, crystalloid fluids, gastroprotectants, antiparasitics, and dexamethasone.\textsuperscript{35} Currently, it is still unclear the effect, if any, maropitant has on plasma concentrations of other highly protein-bound drugs. This intriguing variable warrants further evaluation.

Ammonium tetrathiomolybdate can cause adverse effects, most notably anemia, leukopenia, and gastrointestinal tract upset.\textsuperscript{27} Hematologic effects are related to copper deficiency associated with chronic drug administration. In the only reported study\textsuperscript{17} in dogs in which investigators evaluated dogs with metastatic neoplasia, there was at least 5 weeks of daily administration before the development of anemia in some dogs. During the course of that study,\textsuperscript{17} no dogs became leukopenic, and no significant biochemical abnormalities were detected. Dogs with increased total body copper content would presumably be less susceptible to copper deficiency, as was seen in some dogs from the aforementioned report.\textsuperscript{17} Follow-up hematologic examinations were not performed in the dogs of the present study because of the short study duration.

Vomiting was observed in 2 dogs and 3 dogs after IV and oral administration of TTM, respectively. Although the potential for gastrointestinal tract upset was known, food was withheld from the dogs to theoretically maximize TTM absorption. Human patients with Wilson disease usually are given several small doses throughout a day. It is possible that this strategy would avoid TTM-induced emesis in dogs while not altering drug efficacy. Neither dog pretreated with maropitant vomited, which suggested that antiemetics may also have a role in the prevention of TTM-induced vomiting. Furthermore, avoidance of the gastrointestinal tract via parenteral administration might reduce the incidence of vomiting. All of these hypotheses require additional evaluation.

Limitations of the present study included the complex and variable pharmacokinetic patterns for TTM and the small sample size in the context of TTM’s highly variable pharmacokinetics. The authors modeled the TTM pharmacokinetics after a first-order kinetic pattern on the basis of linearity of the terminal portion of the elimination profile; however, TTM’s elimination profile is more complex than a simple first-order pattern (Figure 1). It is likely that an elimination saturation phenomenon was present that influenced the elimination profile to follow a zero-order kinetic pattern when TTM concentrations were greater than the saturation point. If so, this would have impacted calculation of the terminal elimination rate constant.

The reported serum concentrations of iron, zinc, selenium, manganese, and cobalt were part of a compre-
hensive trace nutrient profile that also included copper and molybdenum, which were the main focus of our report. Because these other mineral concentrations were not related to our objectives, the data were not statistically evaluated. Although TTM has not been shown to chelate zinc, cadmium, or iron from the body in rodents, it is unknown if any effect could occur in other species or on other trace minerals.29,36 This preliminary data could be of interest to researchers in related fields. As such, the authors elected to report these findings herein.

In the present study, we determined the pharmacokinetics of TTM in healthy dogs after oral and IV administration. Increases in serum copper concentrations were encouraging findings that support the need to further evaluate TTM as a possible therapeutic agent for CACH. Vomiting occurred in some dogs shortly after TTM administration, and development of alternative dosing strategies to avoid this may be needed. Results of the present study should provide an important framework for future pharmacological and clinical evaluations of TTM.

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Footnotes

a. Pro Plan Chicken and Rice Adult Maintenance Food, Nestlé Purina PetCare Co, St Louis, Mo.
b. Sigma-Aldrich Corp, St Louis, Mo.
d. 7500ce inductively coupled plasma mass spectrometer, Agilent Technologies, Santa Clara, Calif.
f. UV160U spectrophotometer with CPS 240A cell positioner, Shimadzu, Kyoto, Japan.
g. Varian Vista Pro with radial aligned torch, Agilent Technologies, Santa Clara, Calif.
h. Avantor Performance Materials, Center Valley, Pa.
k. PKSolver, version 2.0, China Pharmaceutical University, Nanjing, China.
l. Prism, version 6.00 for Windows, GraphPad Software Inc, La Jolla, Calif.

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