Use of calcium folinate in the management of accidental methotrexate ingestion in two dogs

Daniel H. Lewis, MA, VetMB; Dominic M. Barfield, BVSc; Karen R. Humm, MA, VetMB, DACVECC; Robert A. Goggs, BVSc, DACVECC

Case Description—2 English Pointers were suspected of having consumed toxic doses of methotrexate, a dihydrofolate reductase inhibitor frequently used in human and veterinary chemotherapeutic protocols.

Clinical Findings—Potentially toxic plasma concentrations of methotrexate were detected in both dogs. Results of physical examination, a CBC, blood gas analysis, and serum biochemical analysis were predominantly unremarkable, although 1 dog had mild hyponatremia (137.2 mmol/L; reference range, 140 to 153 mmol/L) and mild hypocalcemia (1.03 mmol of ionized calcium/L; reference range, 1.13 to 1.33 mmol of ionized calcium/L).

Treatment and Outcome—Point-of-care determination of plasma methotrexate concentrations was not available; thus, palliative care was provided. Emesis was induced in both dogs by SC administration of apomorphine, and 3 doses of a suspension of activated charcoal with sorbitol were administered orally over a 6-hour period. Fluid diuresis was initiated in both dogs by administration of a compound sodium lactate solution, and N-acetylcysteine was administered IV to both dogs as a hepatoprotectant. A solution of calcium folinate (also known as leucovorin) was administered IV to both dogs to mitigate the effects of ingested methotrexate. No adverse effects associated with calcium folinate administration were identified, and no clinical or pathological evidence of methotrexate intoxication was detected.

Clinical Relevance—IV administration of calcium folinate appeared to prevent the pathological sequelae of methotrexate intoxication without adverse effects. Administration of calcium folinate is recommended for the treatment of dogs with suspected or confirmed methotrexate overdose. (J Am Vet Med Assoc 2010;237:1450–1454)

A 25-month-old sexually intact male English Pointer (dog 1) and a 4-year-old sexually intact male English Pointer (dog 2), both of which had the same owner, were concurrently evaluated at the Royal Veterinary College Queen Mother Hospital for Animals because of suspected ingestion of the owner’s methotrexate tablets. Neither dog had any previous relevant medical history, and they were both reportedly fit and healthy. On the day of admission, the owner observed dog 1 playing with the lid from a medicine bottle that had contained approximately forty 2.5-mg tablets of methotrexate. The owner found no tablets in the surrounding environment. Dog 2 was also present in the room. Neither of the dogs had been seen for several hours prior to the discovery of dog 1 with the lid of the bottle. Approximately 30 minutes later, the dogs were examined by a referring veterinarian who induced emesis by administration of a compound sodium lactate solution, but no tablets were evident in the emesis. The dogs were then referred to our veterinary medical hospital and were admitted approximately 4.5 hours after the suspected ingestion.

From the Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire AL9 7TA, England.

The authors thank Matthew Jordinson for assistance with the methotrexate assays.

Address correspondence to Dr. Lewis (dhlewis@rvc.ac.uk).

ABBREVIATIONS

DHFR Dihydrofolate reductase
NAC N-acetylcysteine

At admission, both dogs were bright, alert, and responsive. Dog 1 (body weight, 20.7 kg [45.5 lb]) had a heart rate of 72 beats/min with synchronous metatarsal pulses, respiratory rate of 32 breaths/min, and rectal temperature of 38.2°C (100.7°F). Dog 2 (body weight, 21.2 kg [46.6 lb]) had a heart rate of 120 beats/min with synchronous metatarsal pulses, respiratory rate of 24 breaths/min, and rectal temperature of 38.8°C (101.8°F). Mucous membranes of both dogs were pink with a capillary refill time within the reference range. Thoracic auscultation did not reveal cardiac or thoracic abnormalities in either dog. No abnormalities were detected in dog 1 or 2 during abdominal palpation.

Blood samples were collected from each dog via jugular venipuncture and submitted for a CBC, serum biochemical analysis, and blood gas analysis. Blood was also collected into tubes containing 3.2% sodium citrate for assay of the methotrexate concentration. The sodium citrate–containing samples were centrifuged at 11,000 X g for 5 minutes, and the citrated plasma then was removed and frozen at −26°C pending analysis.

The PCV of dog 1 was 45%, and the total protein concentration (measured via a refractometer) was 6.5 g/dL. The
venous blood gas analysis and electrolyte and metabolite profile were obtained by use of a point-of-care analyzer. The acid-base status of dog 1 was unremarkable, although dog 1 was mildly hypernatremic (137.2 mmol/L; reference range, 140 to 153 mmol/L) and had mild hypocalcemia (1.03 mmol of ionized calcium/L; reference range, 1.13 to 1.33 mmol of ionized calcium/L). Blood glucose and blood lactate concentrations were within the respective reference ranges. The PCV of dog 2 was 31%, and the total protein concentration was 6.4 g/dL. Results of venous blood gas, electrolyte, and metabolite analyses for dog 2 were unremarkable.

For dog 1, results of a CBC revealed that all cell counts and calculated indices were within the respective reference intervals. Serum biochemical analysis for dog 1 identified mild total hypercalcemia (2.81 mmol of calcium/L; reference range, 2.13 to 2.70 mmol of calcium/L), but all other results were unremarkable. Results of a CBC and serum biochemical analysis for dog 2 were also unremarkable.

Emesis was induced in both dogs by SC administration of apomorphine® (40 µg/kg [18 µg/lb]), but no methotrexate tablets were recovered. Three doses of a suspension of activated charcoal with sorbitol® were administered (1.5 g/kg [0.68 g/lb], PO) over a 6-hour period. Following insertion of a cannula into a cephalic vein of each dog, fluid diuresis was initiated by administration of a compound sodium lactate solution a at a rate of 5 mL/kg/h (2.3 mL/lb/h). In addition, NAC® was administered IV (140 mg/kg [63.6 mg/lb] once; then 70 mg/kg [31.8 mg/lb], q 6 h for 8 doses) to both dogs as a hepatoprotectant. A nonproprietary formulation of calcium folinate was obtained from a local human hospital pharmacy and administered IV (200 mg/m², q 6 h for 8 doses) to both dogs.

Diuresis was continued for 48 hours. Dipstick analyses were conducted on midstream-catch urine samples obtained from both dogs during the second day of hospitalization. Urine of dog 1 had a pH of 7 and trace protein concentration. All other dipstick values were within expected limits, and results of urine sediment examination were unremarkable. Urine of dog 2 also had a pH of 7 and trace protein concentration, but all other dipstick values were within expected limits; results of urine sediment examination were unremarkable.

Both dogs remained bright, alert, and responsive and had good appetites throughout hospitalization. Results of serial venous blood gas, electrolyte, and metabolite analyses of samples obtained from both dogs were unremarkable. Both dogs were discharged 2 days after admission. Blood samples were collected from both dogs and placed in tubes containing sodium citrate on the day of discharge; citrated plasma was harvested as described previously and again stored at −26°C.

Plasma methotrexate concentrations were assayed via a fluorescence polarization immunoassay. The lower limit of detection of the assay was 0.01 µmol/L. The samples obtained from dogs 1 and 2 at the time of admission to our veterinary medical hospital had a methotrexate concentration of 0.22 and 0.05 µmol/L, respectively. The samples obtained from dogs 1 and 2 on the day of discharge from our hospital had concentrations below the lower limit of detection (ie, < 0.01 µmol/L). A negative control sample of canine plasma also had a methotrexate concentration < 0.01 µmol/L.

The dogs were scheduled for an examination at 10 days after discharge from our veterinary medical hospital, but they were not returned at that time. Both dogs were returned to the hospital and examined 25 days after discharge. The owner reported that both dogs were well. Results of physical examinations were unremarkable. Results of a serum biochemical analysis and a CBC for dog 1 were unremarkable, except for mild hyperglobulinemia (19.8 g/L; reference range, 21 to 41 g/L), a mild reduction in creatinine concentration (89 µmol/L; reference range, 98 to 163 µmol/L), and monocytopenia (0.084 × 10⁹ cells/L; reference range, 0.15 × 10⁹ cells/L to 1.5 × 10⁹ cells/L), all of which were of questionable clinical relevance. Dog 2 had a mild reduction in creatinine concentration (85 µmol/L); all other results of a serum biochemical analysis and a CBC were unremarkable. During a follow-up telephone conversation with the client 4 months after suspected methotrexate ingestion, the dogs were reported to be healthy.

**Discussion**

Methotrexate is an S-phase–specific antimetabolite chemotherapy agent commonly used to treat neoplasia, psoriasis, and rheumatoid arthritis in humans and lymphoma and myeloproliferative disorders in dogs. Accidental overdose and management of overdoses in humans has been reported. However, despite the widespread use of methotrexate in chemotherapy protocols for dogs, management of overdose in dogs has not been reported to our knowledge.

Methotrexate enters cells via the reduced folate carrier and undergoes polyglutamation catalyzed by folylpolyglutamate synthetase. Methotrexate polyglutamates block de novo nucleotide synthesis primarily by depleting cells of reduced tetrahydrofolate cofactors through inhibition of DHFR. Dihydrofolate reductase appears to have a much greater affinity for methotrexate than for either folate or dihydrofolate, which potentially enhances the chemotherapeutic effects of methotrexate. Methotrexate also leads to the production of dihydrofolate polyglutamates, which further inhibit enzymes involved with folate-dependent nucleotide synthesis. Because methotrexate inhibits DNA synthesis, rapidly proliferating cells (such as neoplasms, bone marrow, and gastrointestinal tract epithelium) are most sensitive to the drug’s effects. Methotrexate is associated with myelosuppression (with a nadir of 5 to 10 days), gastroenterocolitis, hepatotoxicosis, and nephrotoxicosis. Blood concentrations and methotrexate-associated toxicosis vary greatly among individuals receiving the same dose (as determined on the basis of body weight or body surface area). Toxic effects depend on plasma drug concentration and duration of exposure. Exposure to high plasma concentrations (> 10 µmol/L) for minutes to hours may cause nephrotoxicosis, hepatotoxicosis, and CNS damage, whereas exposure to methotrexate concentrations as low as 0.005 to 0.01 µmol/L for > 24 hours may result in bone marrow and gastrointestinal epithelial toxicosis. The dogs reported here had plasma concentrations of 0.22
µmol/L (dog 1) and 0.05 µmol/L (dog 2) 5 hours after suspected ingestion of the methotrexate tablets; thus, without treatment, they would likely have had bone marrow and gastrointestinal toxicoses. We were unable to determine a lack of myelosuppression at the expected nadir time for methotrexate intoxication because the dogs were not returned for reexamination until 25 days after discharge from the hospital; however, no clinical signs related to bone marrow or gastrointestinal tract effects were reported by the owner. In humans, methotrexate is absorbed from the gastrointestinal tract by a saturable active transport system, which leads to dose-dependent variability in absorption. Peak plasma concentrations are evident 1 to 2 hours after oral administration of the drug. The half-life of methotrexate in plasma appears variable but is likely to be from 5 to 9 hours in humans, although it may be 10 to 12 hours in dogs. Terminal half-life also appears dependent on methotrexate dose, route of administration, duration of exposure, and renal function. Methotrexate is secreted into the biliary tract and undergoes enterohepatic recirculation. Although < 10% of methotrexate is excreted via the fecal route, in patients with diminished renal function, enterohepatic recirculation may become a more important factor in methotrexate intoxication, which may warrant administration of activated charcoal for binding of the drug in the intestines. Oral administration of neomycin may also be of use in reducing methotrexate absorption from the gastrointestinal tract; however, neomycin was not administered to the dogs described here because a suitable preparation was not available.

The administration of crystalline sodium carbonate, although commonly used as an emetic, can be associated with caustic damage to the orofacial tissues, pharynx, and esophagus; therefore, we do not recommend its use. In addition, as was the case in these 2 dogs, crystalline sodium carbonate is often ineffective, which potentially necessitates use of additional emetic drugs. Given the questionable efficacy of this compound and uncertainty regarding the timing of ingestion in these dogs, we believed it prudent to induce emesis with apomorphine. Although methotrexate is rapidly absorbed from the gastrointestinal tract, we thought the potential benefit of removing any undigested tablets outweighed the risks associated with apomorphine administration.

Depending on the dose, 40% to > 90% of methotrexate is cleared by renal excretion of the metabolized drug. Methotrexate-induced renal dysfunction is believed to be mediated by the precipitation of the drug and its metabolites in the renal tubules or via a direct toxic effect of methotrexate on the renal tubules. Methotrexate and its metabolites are poorly soluble at an acidic pH. An increase in urine pH from 6.0 to 7.0 results in a 5- to 8-fold increase in solubility of methotrexate and its metabolites. This property can be exploited in patients by providing fluid diuresis and urine alkalinization by the IV administration of sodium bicarbonate (0.5 to 1 mEq/kg [0.23 to 0.45 mEq/lb]/300 mL of fluid). Sodium bicarbonate was not administered to these dogs, but the use of lactate-containing alkalinizing fluids would likely have aided methotrexate excretion because the urine pH of both dogs was 7. Both hemodialysis and peritoneal dialysis are ineffective in removing substantial quantities of methotrexate, although these techniques may be useful for stabilization and treatment of patients with methotrexate-induced renal failure. Charcoal hemoperfusion techniques have been successfully used in humans to remove methotrexate from whole blood. These techniques are not widely available, however, and hemoperfusion may lead to thrombocytopenia as a result of adherence of platelets to the charcoal column.

Several drugs that interfere with methotrexate excretion have been associated with increased toxic effects when administered concurrently with methotrexate. These agents, which include penicillins, ciprofloxacin, and NSAIDs, compete with methotrexate for renal tubular secretion, and their use should be avoided in patients with methotrexate intoxication. This becomes particularly important in patients with methotrexate-induced myelosuppression that may require prophylactic administration of antimicrobials while neutropenic.

Methotrexate-induced hepatotoxicosis is more commonly associated with chronic treatment, rather than with acute intoxication in both dogs and humans. Patients receiving high-dose methotrexate treatment frequently develop increased serum activities of alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase. No such increases were detected in the 2 dogs reported here, but this may have been because the severity of increases in liver enzyme activities appears to be related to the number of methotrexate doses received. Because the exact biochemical basis for methotrexate hepatotoxicosis is unknown, we chose to treat the 2 dogs with the hepatoprotectant drug NAC. N-acetylcysteine is a glutathione precursor and reactive oxygen–species scavenger; thus, as a hepatoprotectant drug, it is likely to be most effective against drugs such as acetaminophen, which induce oxidative injury. The role of oxidative injury in methotrexate hepatotoxicosis is unclear; therefore, the effect of NAC in these dogs is uncertain. However, major adverse effects associated with NAC administration are extremely rare.

Calcium folinate (also known as folic acid, leucovorin, or 5-formyl tetrahydrofolate) is an active form of folic acid. Leucovorin is capable of directly restoring intracellular concentrations of reduced folate cofactors without the action of DHFR, thereby reversing or preventing antifolate toxicosis even in the presence of methotrexate. Leucovorin is activated via an unrelated series of enzymatic steps to 5-methyl tetrahydrofolate in hepatocytes and gastrointestinal epithelia. Following this conversion, tetrahydrofolate enters the reduced folate cycle distal to the site of methotrexate inhibition of DHFR, bypassing the enzymatic block.

Leucovorin administration is most frequently used as a rescue treatment in association with high-dose methotrexate administration. Leucovorin negates most of the acute toxic effects of methotrexate; this enables the administration of higher doses of methotrexate and facilitates drug distribution into large solid tumors, overcomes intrinsic drug resistance of tumor cells, and prevents emergence of methotrexate-resistant tumor clones. Leucovorin is most effective when given within 24 to 48
hours after methotrexate ingestion.\textsuperscript{34} The dose required is dependent on the plasma methotrexate concentration. Suggested dosages range from 25 to 200 mg/m\textsuperscript{2} every 6 hours, which is administered until plasma methotrexate concentrations decrease to < 0.01 µmol/L.\textsuperscript{21} In the United Kingdom, licensed drug information for leucovorin preparations suggests a dose based on measured plasma methotrexate concentrations, with 15 mg/m\textsuperscript{2} for methotrexate concentrations < 1.5 µmol/L, 30 mg/m\textsuperscript{2} for methotrexate concentrations of 1.5 to 5 µmol/L, and 100 mg/m\textsuperscript{2} for methotrexate concentrations > 5 µmol/L.\textsuperscript{35} An alternative strategy has been suggested that involves the administration of a dose of leucovorin at least equal to the consumed dose of methotrexate.\textsuperscript{36} Given a potential worst-case scenario, the maximal ingested dose in each of the dogs described here was 133 mg/m\textsuperscript{2}; however, the maximum recommended leucovorin dose of 200 mg/m\textsuperscript{2} was used in these dogs because plasma methotrexate concentrations were unknown, underdosing of leucovorin is undesirable, and there was no concern about reducing the antineoplastic efficacy of the methotrexate. We were unable to ascertain plasma methotrexate concentrations in sufficient time to guide leucovorin administration; thus, we treated the dogs for 48 hours, which equated to approximately 5 half-lives of methotrexate. In conjunction with urinary alkalization, fluid diuresis, and administration of activated charcoal, we believed this amount of time was likely to allow complete elimination of the drug.

Carboxypeptidase G2, a bacterial enzyme that cleaves methotrexate and has a high affinity for methotrexate but not for reduced folates, has been investigated as a rescue treatment for high-dose methotrexate administration.\textsuperscript{37} Although carboxypeptidase G2 (also known as glucarpidase) may be of benefit in patients with methotrexate intoxication in the future, it is not currently commercially available.\textsuperscript{38}

Two methods are commonly used for methotrexate assays: 1 is based on drug-antibody interactions, and the second involves binding to DHFR. The DHFR-binding assays are sensitive and do not cross-react with methotrexate metabolites; however, they are time consuming to perform and cannot be automated. The fluorescence polarization immunoassay used to assess the concentrations in the 2 dogs described here was sensitive and rapid, although there may be some cross-reactivity with the methotrexate metabolite 2,4-diamino-N\textsubscript{6}-methylpteroylglutamic acid that can yield falsely increased values for the parent compound.\textsuperscript{21} It is unlikely that the measured methotrexate concentrations in samples obtained at the time of admission to our veterinary medical hospital would have been falsely increased because substantial amounts of 2,4-diamino-N\textsubscript{6}-methylpteroyl acid are only generated 24 to 48 hours after oral administration of high doses of methotrexate and are only considered clinically relevant after this period.\textsuperscript{21} The results of the methotrexate assay were available 48 hours after sample submission. Although a methotrexate assay may be of use in determining the necessary duration of treatment, it is unlikely that results of methotrexate assays will be available soon enough after methotrexate ingestion to assist in the decision to administer leucovorin.\textsuperscript{34}

In the 2 dogs reported here, timely intervention with gastrointestinal decontamination, IV administration of fluids to induce diuresis, and high-dose leucovorin treatment prevented methotrexate toxicosis following accidental ingestion of the owner's medication. Veterinarians should be aware of the likely toxicoses associated with methotrexate overdose and of the potential treatments available to minimize absorption, maximize excretion, and prevent toxic effects. Various methotrexate assays are available in human hospitals (typically at large or regional toxicology centers) that can measure plasma methotrexate concentrations to assist clinicians when making treatment decisions. In patients in which methotrexate overdose is likely but concentrations cannot be ascertained with sufficient speed to guide treatment, presumptive administration of leucovorin appears to be both safe and effective for the prevention of toxic effects.

References

10. Borsa JD, Moe PJ. A comparative study on the pharmacokinetics of methotrexate in a dose range of 0.5 g to 33.6 g/m\textsuperscript{2} in children with acute lymphoblastic leukemia. Cancer 1987;60:5–13.
Effects of intracameral administration of α-chymotrypsin on intracapsular lens extraction and postoperative outcome in clinically normal dogs

David J. Maggs et al

Objective—To assess the intraocular and postoperative clinical effects and histologic effects of intracameral administration of α-chymotrypsin in clinically normal dogs undergoing standard intracapsular lens extraction (ICLE).

Animals—6 young adult male dogs without evidence of systemic or ocular disease.

Procedures—All dogs underwent bilateral ICLE 7 minutes following injection of 75 U of α-chymotrypsin or an identical volume (0.5 mL) of a commercially available balanced saline solution (BSS) into the posterior chamber of the eye. Ease of lens extraction was subjectively assessed and intraoperative intraocular hemorrhage and fibrin accumulation scored. For 27 days after surgery, ocular hyperemia and discharge, chemosis, corneal edema, hyphema, and aqueous flare were scored, and intraocular pressure (IOP) was measured. Thirty days after surgery, histologic evidence of anterior synechia, collapse of and inflammation within the iridocorneal angle, and iritis were scored.

Results—In 5 of 6 dogs, the surgeon was able to correctly identify the eye treated with α-chymotrypsin on the basis of ease of lens extraction. Mean intraoperative intraocular hemorrhage and fibrin scores for BSS-treated eyes were significantly higher than for α-chymotrypsin–treated eyes. Postoperatively, there were no significant differences between treatments for any clinical variables, including IOP. Histologic scores were not significantly different between treatments for any variable. Vision was lost as a result of glaucoma in 1 α-chymotrypsin–treated eye and 1 BSS-treated eye.

Conclusions and Clinical Relevance—Intracameral administration of 75 U of α-chymotrypsin 7 minutes before ICLE facilitated lensectomy without apparent adverse effects in clinically normal dogs.