Case Report Rapport de cas

Asparaginase-associated pancreatitis in a dog

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Abstract — A dog with lymphosarcoma was evaluated for vomiting, lethargy, and abdominal pain 48 h after treatment with L-asparaginase. Based on drug administration, clinical signs, bloodwork, and elevated canine pancreatic lipase immunoreactivity, L-asparaginase-associated pancreatitis was diagnosed. This is an acknowledged toxicity; however, its pathophysiology and incidence rate in veterinary patients are unknown and sparsely documented.

Résumé — Pancréatite associée à l'asparaginase chez un chien. Un chien avec un lymphosarcome a été évalué pour des vomissements, de l'abattement et des douleurs abdominales 48 heures après le traitement avec L-asparaginase. En se fondant sur l'administration des médicaments, les signes cliniques, le bilan sanguin et l'immunoréactivité lipase pancréatique canine élevée, une pancréatite associée à L-asparaginase a été diagnostiquée. Il s'agit d'une toxicité reconnue; cependant, sa pathophysiologie et son taux d'incidence chez les patients vétérinaires sont inconnus et peu documentés.

(Traduit par Isabelle Vallières)

Can Vet J 2011;52:1009–1012

L-asparaginase is a naturally occurring enzyme that catalyzes the hydrolysis of L-asparagine (a nonessential amino acid) to L-aspartic acid and ammonia, and to a lesser extent, glutamine to glutamic acid. Pharmacologic quantities of L-asparaginase are isolated from *Escherichia coli* and *Erwinia carotovora* and sold as 99.9% pure, endotoxin-free lyphophilized powder. A pegylated form of the enzyme exists as well. After reconstitution and administration this enzyme results in a rapid and complete depletion of L-asparagine in the plasma. In the dog, negligible levels of plasma L-asparagine are noted by day 7 and then rebound within a few weeks. The plasma half-life of L-asparaginase is 12 to 40 h (median 14 h), which does not appear to be influenced by dose, age, sex, body surface area, renal or hepatic function, or extent of neoplastic disease (1,2). Loss of plasma L-asparagine leads to a decrease in protein synthesis and apoptosis in cells that lack significant intracellular L-asparagine synthetase, an enzyme needed to synthesize L-asparagine from the components remaining in the plasma (1).

The enzyme's role in cancer treatment exploits a true metabolic difference between normal versus neoplastic cell populations. L-asparagine synthetase is present in many tissues, especially the liver, pancreas, and brain; however, lymphoproliferative neoplasms notably lack asparagine synthetase and are thus susceptible to the rapid depletion of circulating L-asparagine (2). In human oncology, L-asparaginase is a key component to remission induction in acute lymphoblastic leukemia, and a component of therapy for some forms of non-Hodgkin's lymphoma and acute myelogenous leukemia (3). In veterinary practice, L-asparaginase, administered IM or SQ, is indicated for the treatment of canine and feline lymphosarcoma and lymphoid leukemias (1,4).

Resistance to L-asparaginase in neoplastic cell populations appears to develop rapidly in most patients. The mechanisms of resistance can be attributed to preferential selection of cells that up-regulate L-asparagine synthetase activity, formation of neutralizing antibodies by the host, and defective apoptotic pathways in the neoplastic cells (1,2). Due to the rapid development of resistance, and its debated role in induction protocols, repeat dosing with L-asparaginase is often avoided until the rescue phase of therapy (4–6).

L-asparaginase's toxicity profile can be divided into 2 main groups: those attributed to immunologic sensitization to a foreign protein, and those resulting from inhibition of protein synthesis. Toxicity seen in human patients includes decreased pro- and anticoagulant clotting factors leading to thrombosis and hemorrhage, hypoalbuminemia, hyperglycemia (via decreased circulating insulin), hypersensitivity reactions, anaphylaxis,

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serum sickness, cerebral dysfunction, elevated liver enzymes, leukopenias, and pancreatitis (1,3,7). The most common toxicity seen in veterinary patients is a hypersensitivity reaction, although other side effects including pancreatitis have been anecdotally described. The hypersensitivity reaction usually occurs within 60 min but may appear as late as 4 to 6 h post administration. Affected animals may demonstrate vomiting, diarrhea, urticaria, edema, pruritus, dyspnea, restlessness, hypotension, and rarely, collapse. H1 receptor blockers or glucocorticoids or both are given prior to L-asparaginase administration to decrease the likelihood of this occurrence (2,4).

L-asparaginase-associated pancreatitis (AAP) is a less common toxicity and in the human literature the incidence ranges from 0.7% to 18% (3,7). In veterinary oncology, the incidence of AAP is not known, is extremely low, and is not well-documented. A case of hemorrhagic pancreatitis diagnosed on necropsy 2 h after drug administration was reported, and along with other findings, was attributed to systemic vascular collapse secondary to a hypersensitivity reaction (8). Other reports may list pancreatitis as a possible side effect seen, but the diagnosis is made based on clinical signs and history (9). A recent study attempted to discern the incidence of clinical and subclinical pancreatitis after L-asparaginase administration in canine patients with lymphoma by prospectively evaluating canine pancreatic lipase immunoreactivity (cPLI) and clinical signs. No dogs receiving L-asparaginase alone showed evidence of clinical pancreatitis and or a statistically significant change in cPLI concentrations pre and post L-asparaginase administration. Furthermore, dogs demonstrating clinical signs compatible with pancreatitis after chemotherapy treatment did not have a cPLI $> 400/\mu g/L$, which is the value corresponding to a positive test for pancreatitis. These results may indicate the clinical signs noted in these patients were secondary to post chemotherapy induced gastritis. However, it was demonstrated in the study that 14% of dogs receiving both L-asparaginase and vincristine concurrently had elevations in post cPLI measurements approaching statistical significance. With only 52 dogs in this study, investigation with larger sample sizes is needed to validate the findings and define incidence rates. The authors of this study advised against concurrent administration of vincristine and L-asparaginase to canines with lymphoma and predisposing factors to pancreatitis such as presentation in substage b (10).

Pancreatitis results from auto digestion of the pancreatic tissue by premature activation of zymogen within the acinar cells. Early events are abnormal fusion of the lysosomes and zymogen granules, providing an environment consisting of lysosomal proteases and low pH, favoring zymogen activation. The lysosomal proteases activate pancreatic zymogens, and the low pH hinders the normal protective mechanisms provided by substances such as pancreatic secretory trypsin inhibitor (11,12). The inciting cause of this chain reaction is often unknown, but more than 50 drugs and drug classes have been implicated in humans although proof of a causal relationship is often not present (11). Drugs used in veterinary medicine suspected, but not proven, to cause pancreatitis include L-asparaginase, azathioprine, estrogen, furosemide, potassium bromide, salicylates, sulfonamides, tetracyclines, thiazide diuretics, and vinca alkaloids (11–13).

Making the diagnosis of confirmed pancreatitis can be difficult. Clinical signs in the dog can be vague and are not well-documented. Abdominal ultrasound is highly specific for pancreatitis when stringent criteria are applied, but ultrasonographic changes are not always present. Historically, serum amylase and lipase activities were used to diagnose canine pancreatitis, but have been shown to lack specificity. Recently, the use of serum cPLI has been shown to be the most sensitive and specific diagnostic test for canine pancreatitis (11).

Case description

A 15-year-old, male castrated, American Eskimo dog weighing 13 kg was presented for further evaluation and treatment of cytologically confirmed lymphosarcoma. Initial staging included complete blood (cell) count (CBC), serum chemistry panel, urinalysis, abdominal ultrasound, thoracic and abdominal radiographs, flow cytometry for immunophenotyping, and bone-marrow aspirate to confirm a IVa mixed phenotype lymphosarcoma. The patient was placed on the 25-week University of Wisconsin-Madison chemotherapy protocol including induction with L-asparaginase (14). A complete remission based on physical examination was achieved by the fourth week of the protocol. The protocol was continued with no noted side effects or dose reductions.

At week 15 of 25 in the protocol, physical examination revealed enlarged mandibular lymph nodes with no other abnormalities. Cytologic confirmation of recurrent lymphosarcoma was obtained via fine-needle aspiration of the mandibular lymph nodes. Rescue therapy was initiated using the MOPP protocol (15) with substitution of melphalan (Melphalan, Alkeran; Glaxo-Smith-Kline, Research Triangle Park, Durham, North Carolina, USA) at 1.5 mg/m^2 given orally on days 0 to 14 for procarbazine. The patient was presented on day 7 of rescue therapy for the next administration of vincristine and mechlorethamine with a history of mild lethargy and decreased appetite. Physical examination revealed a decrease in size of the mandibular lymph nodes by 50% and no other abnormalities. A CBC revealed a normocytic hypochromic anemia of 30.8% [reference range (RR): 41% to 60%], a leukopenia with 3900 cells/μL (RR: 5.1 to 14 $\times$ 10^3/μL) and mature neutrophils at 1760 cells/μL (RR: 2.65 to 9.8 $\times$ 10^3/μL) with no bands, and a thrombocytosis of 614 000 cells/μL (RR: 147 to 423 $\times$ 10^3/μL). Due to neutropenia, in lieu of the planned injectable drugs L-asparaginase was given at 10 000 U/m^2 IM following premedication with diphenhydramine (Diphenhydramine HCL injection; Baxter Healthcare, Deerfield, Illinois, USA) at 2 mg/kg body weight (BW) IM. The owner was instructed to discontinue the melphalan and return in 5 to 7 d for continued chemotherapy.

Forty-eight hours following L-asparaginase therapy, the dog was presented for anorexia, shaking, labored breathing, and an episode of vomiting. Physical examination revealed a normal temperature, pulse, respiration, and capillary refill time and hydration status. All lymph nodes were of normal size (complete clinical remission) and abdominal palpation elicited no pain. A CBC and serum biochemistry showed resolution of the neutropenia and were otherwise unremarkable. Due to the

development of gastrointestinal symptoms after administration of L-asparaginase, serum was submitted for a cPLI test. Empiric therapy for the pancreatitis and or gastrointestinal upset consisted of 500 mL of 0.9% NaCl and 0.6 mg/kg BW of dolasetron (Dolasetron injection; Sanofi-Aventis, Kansas City, Missouri, USA) subcutaneously. Prednisone (Prednisone; Westward, Eatontown, New Jersey, USA) was discontinued and the patient was discharged on dolasetron (0.6 mg/kg BW once daily). The owners were instructed to restrict food intake for the remainder of the day and initiate feeding small amounts of a low fat diet starting in 24 h.

In less than 24 h the dog was presented with progressive vomiting and appeared painful to the owner. Physical examination revealed tachycardia of 200 beats per min, a grade III/VI left systolic murmur, clear lung fields, weak thready pulses, pale mucous membranes with a capillary refill time of 2 s, estimated 7% dehydration, and pain was elicited on abdominal palpation. The systolic blood pressure measured 180 mmHg on 3 readings. A CBC revealed a leukocytosis of 27 500 wbc/μL with 17 330 mature neutrophils/μL, 7700 bands/μL with mild toxic changes noted, and a 32.9% hematocrit. Serum biochemistry panel revealed an alkaline phosphatase of 640 U/L (RR: 15 to 109 U/L), alanine aminotransferase of 102 U/L (RR: 21 to 81 U/L), blood urea nitrogen of 10 mmol/L (RR: 3.2 to 8.6 mmol/L), glucose of 18.8 mmol/L (RR: 4.8 to 6.1 mmol/L), and cholesterol of 2.8 mmol/L (RR: 3.6 to 8.4 mmol/L). Electrolyte analysis revealed sodium at 139 mEq/L (RR: 146 to 153 mEq/L), potassium at 3.2 mEq/L (RR: 3.6 to 5.1 mEq/L), and chloride at 93 mEq/L (RR: 110 to 117 mEq/L). Prothrombin time was measured at 7 s (RR: 6.8 to 8.7 s), and a partial thromboplastin time was 16.3 s (RR: 14.5 to 25.6 s). Thoracic radiographs and abdominal ultrasound showed no significant abnormalities other than small pulmonary vessels suggestive of hypovolemia, in agreement with physical examination findings. The serum cPLI was 517 μg/L (RR: 0 to 400 μg/L), consistent with pancreatitis.

Based on the clinical symptoms of anorexia, vomiting, and abdominal pain, in conjunction with an elevated cPLI, a diagnosis of pancreatitis was made and therapy was instituted as follows. The dog received IV fluids (Normosol R with 15 mEq KCl/L), 0.6 mg/kg BW, IV dolasetron, 2 units of fresh frozen plasma, fentanyl (Fentanyl; Hospira, Lake Forest, Illinoid, USA)/lidocaine (Lidocaine injection; Hospira) constant rate infusion (CRI), and 10 mg/kg BW metronidazole (Metronidazole; Barr Labs, Pamona, New York, USA). After 3 d of treatment, the patient was discharged. The dog continued to receive chemotherapy, minus prednisone and L-asparaginase, for another 9 m.

Discussion

The mechanism behind AAP and which individuals are at higher risk for developing pancreatitis are unknown. While the incidence in humans is relatively low, the severity of some cases (fatality or discontinuation of therapy) warrants further investigation into the phenomenon (3,7). Post L-asparaginase pancreatitis in humans occurs 7 to 26 d after treatment, depending on the source of the enzyme (*Erwinia* versus *E. coli* versus pegylated) and its half-life ($t_{1/2}$). Pancreatitis post *E. coli* sourced L-asparaginase occurred at a mean of 12 d after L-asparaginase administration in humans (3). Clinical signs attributable to pancreatitis in the dog in this case were apparent within 2 d. The shortened time frame could be explained by a difference in plasma half-lives. The median $t_{1/2}$ for *E. coli* L-asparaginase is 14 h in the dog versus 26 h in humans (2).

A second mechanism proposed for AAP is the synergistic effect of medications used in consort. L-asparaginase is often given in conjunction with other medications, including prednisone and vinca alkaloids that have suspected associations with pancreatitis (3,7,10,11,16,17). In fact, in 1 human study, AAP was more common in patients who received L-asparaginase in combination with prednisone versus those receiving dexamethasone in conjunction with treatment (3). The dog in this case report was receiving prednisone at the time of L-asparaginase administration which could have predisposed him to the development of the disease. However, a recent study on the effects of L-asparaginase alone on rat pancreatic tissue demonstrated significantly increased levels of pancreatic amylase, trypsin, and pancreatic secretory trypsin inhibitor, as well as histologic damage to the pancreatic acinar cells following administration of 1000 IU/kg of L-asparaginase compared to controls (7). Also, corticosteroids have been removed from the list of drugs that may induce pancreatitis in humans, and a recent study in dogs did show that cPLI concentrations were not affected by long-term administration of prednisone (11,18).

Another proposed explanation for AAP is that the disruption in protein synthesis brought about by the pharmacologic effects of the enzyme may create an imbalance between activated zymogens, such as trypsin, and protease inhibitors in the systemic circulation and in the pancreas itself. How susceptible the pancreas is to the decrease in protein synthesis depends on the inherent activity of L-asparagine synthetase in that organ (3). The activity of L-asparagine synthetase in the dog pancreas per gram of tissue is 1539.0 versus 163.0 in man (19). The higher activity of this enzyme in the canine pancreas may be the reason the incidence of AAP in veterinary medicine is so rare compared to what is seen in humans. Asparagine-associated pancreatitis may also occur more frequently in human patients due to the higher frequency of administration. Commonly in human medicine *E. coli* asparaginase is administered on an alternating day schedule for 6 to 9 doses versus the typical one time administration given to veterinary patients (1–4). To the authors' knowledge this report is the first to document by way of drug association and timing of administration, elevated serum PLI, clinical signs, and hematologic data, a confirmed case of AAP in the dog. While this patient did receive other medications with suspected links to pancreatitis (vincristine and prednisone), the clinical signs developed in a timely manner associated with the administration of the L-asparaginase alone and with no occurrence of an associated hypersensitivity reaction (3,7,10,11,16,17). How much of a role the other medications had in a synergistic effect is not known, although for the remaining 9 mo of treatment the patient received other courses of vincristine without a recurrence. It was decided to not re-challenge the patient with L-asparaginase because of the severity of this episode. Also, in AAP in human patients, it was noted that only 2 of 26 patients

with diagnosed AAP had another episode of pancreatitis following further administration of L-asparaginase. The decision to re-challenge human patients is often based on the severity of their first occurrence (3).

Lymphosarcoma is one of the most common canine malignancies for which owners seek treatment for their pets. L-asparaginase has a role in the treatment of this neoplasia as a chemotherapy agent with a relatively low toxicity profile. While pancreatitis is an uncommon toxicity seen in veterinary patients receiving L-asparaginase, its incidence rate and risk factors are not defined. Further investigation is needed to address these issues. CVJ

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