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Copper-Related Hepatotoxicity

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INTRODUCTION

Although copper is an essential element for many of the body's metabolic processes, excessive accumulation of copper in hepatic tissues can lead to oxidative damage further progressing to hepatocellular damage and ultimately cirrhosis of the liver.[16] The liver is a prime target for copper accumulation since it receives most of the copper that is absorbed and is the primary copper storage organ for the body.[9] Primary copper hepatotoxicity, as seen in Bedlington terriers, has been thoroughly described due to its similarity to a human disease, known as Wilson disease;[3,16,17] however, research over the last decade has revealed other affected breeds as well as more information concerning secondary copper.[8,9] The purpose of this report is to describe the presentation, diagnostic plan, treatment, and outcome in a patient that presented with icterus associated with a gallbladder mucocele and secondary copper hepatotoxicity.

HISTORY AND PRESENTATION

Sadie, a 7-year-old female spayed poodle/terrier mix, presented to her primary care veterinarian on June 24, 2016, for a two-week history of concentrated, foul-smelling urine. Her owner also reported that Sadie had exhibited trouble urinating on a few occasions.

On initial examination, Sadie appeared alert and responsive with mild lethargy. Severe icterus was noted. Capillary refill time was less than 2 seconds, and mucous membranes appeared moist and icteric. Thoracic auscultation was within normal limits, but Sadie was guarded on abdominal palpation.

Based on Sadie's presentation, her veterinarian recommended initial diagnostic testing including a chemistry profile, complete blood count, coagulation profile, abdominal radiographs,

and an abdominal ultrasound. A complete blood count (CBC) revealed a mild neutrophilia of 13.98 cells/µL (3-12 cells/µL). A chemistry profile revealed moderate hypoalbuminemia 1.7 g/dL (2.5-4.4 g/dL), moderately elevated alkaline phosphatase 1662 U/L (20-150 U/L), moderately elevated alanine transferase 617 U/L (10-118 U/L), markedly elevated total bilirubin of 11.3 mg/dL (0.1-0.6 mg/dL), and a moderately low blood urea nitrogen of 4 mg/dL (7-25 mg/dL). A coagulation profile revealed normal prothrombin and partial thromboplastin times.

Abdominal radiographs revealed a small liver and intestinal gas distention. The radiographs were otherwise unremarkable. Abdominal ultrasound showed an enlarged gallbladder with evidence of a dilated bile duct. The ultrasonographic findings of the gallbladder were suspicious for a gallbladder mucocele; however, the gallbladder did not exhibit the typical "kiwi fruit" appearance frequently described with ultrasound views of mucoceles.[1] The liver and other abdominal organs appeared within normal limits on abdominal ultrasound.

After reviewing the diagnostic results, Sadie's veterinarian discussed the likelihood of a gallbladder mucocele with her owner and recommended an immediate exploratory laparotomy with a likely cholecystectomy; however, due to financial constraints, Sadie's owner opted to attempt medical management. Sadie was discharged on June 24, 2016, with ursodiol (9 mg/kg PO q24h) and was instructed to return for follow-up care on June 28, 2016.

On June 28, 2016, Sadie returned for further examination. Her owner reported that Sadie was mildly improved at home. Sadie again appeared markedly icteric, and her physical exam was unchanged from her previous visit. A CBC revealed a mild neutrophilia of 13.76 cells/µL (3-12 cells/µL). A chemistry profile revealed an improved albumin 2.1 g/dL (2.5-4.4 g/dL) mildly improved alkaline phosphatase 1483 U/L (20-150 U/L), mildly improved alanine transferase 448 U/L (10-118 U/L), improved total bilirubin of 7.6 mg/dL (0.1-0.6 mg/dL), and an improved

blood urea nitrogen of 6 mg/dL (7-25 mg/dL). Sadie's veterinarian again discussed recommendation for surgery; however, the decision was made to continue with medical management and add metronidazole (25 mg/kg PO q12h) to her medical management regimen with continued ursodiol administration.

Sadie returned again on July 11, 2016, and her owner reported that Sadie had been doing well at home. She again appeared unchanged from previous examinations, and her complete blood count and chemistry profile showed no improvement from previous studies. Surgery was recommended; however, Sadie's owner decided to continue medical management and return for a second opinion to be obtained as part of Mississippi State University College of Veterinary Medicine's Dr. Alyssa Sullivant's on-site consultation program, "Strategies to Improve Case Outcome When Referral Is Not Affordable".

On July 29, 2016, Sadie returned for her second opinion with Dr. Sullivant. After physical examination and an abdominal ultrasound, Dr. Sullivant confirmed gallbladder mucocele and recommended an immediate cholecystectomy and antibiotic therapy with enrofloxacin (4.5 mg/kg PO q12h). At that time, Sadie's owners consented to schedule her for surgery.

On August 2, 2016, Sadie returned for surgery, and a cholecystectomy was performed. Upon entering the abdominal cavity, Sadie's veterinarian noted an abnormal appearance to the liver, and after completing the cholecystectomy, he obtained a liver biopsy using the guillotine method. The liver biopsy was submitted for histopathology, and culture and sensitivity was submitted from the gallbladder contents.

The liver histopathology report revealed diffuse, chronic cholangiohepatitis with fibrosis, hemosiderosis, and evidence supportive of increased copper levels. The changes in the liver parenchyma supported chronic irritation in the portal triad regions and included areas of increased fibrosis, increased cellular infiltration with lymphocytes and plasma cells, and focal necrotic hepatocytes. These changes could be due to chronic inflammation ascending up the biliary tree or could also be the result of fibrosis and degeneration occurring secondarily to copper buildup. The copper level appeared to be markedly increased based on pigment uptake in hepatocytes. Tissue mineral analysis revealed the hepatic copper concentration to be 2400 ppm (<400 ppm).

Sadie was hospitalized for two days following surgery, and her pain was controlled using buprenorphine (0.04 mg/kg SC q12h), tramadol (2.5 mg/kg PO q6h), and gabapentin (6 mg/kg PO q6h). On August 3. 2016, blood work was performed again, and a coagulation profile showed a prolonged prothrombin time of 21.8 sec (14-19 sec) and a prolonged partial thromboplastin time of 134 sec (75-105 sec). A CBC revealed a leukocytosis of 25.99 cells/µL (6-17 cells/µL) and increased neutrophilia of 23.08 cells/µL (3-12 cells/µL). Her chemistry profile was unchanged from previous studies.

On August 4, 2016, Sadie was discharged with metoclopramide (0.2 mg/kg PO q8-12h) and amoxicillin/clavulanic acid (13.75 mg/kg PO q12h), as well as her previously prescribed enrofloxacin, ursodiol, tramadol, and gabapentin. Her antibiotic usage was discontinued after the liver culture and sensitivity report revealed no growth.

PATHOPHYSIOLOGY

Copper is an essential trace element that, like other trace elements, serves as a cofactor for antioxidant enzymes. Because copper can transition between an oxidized and reduced state, copper is able to effectively function with a wide variety of enzymes including cytochrome-c-oxidase, superoxide dismutase, ceruloplasmin, and others. Free copper ions in their reduced, unstable state are able to catalyze the formation of hydroxyl radicals, which are toxic and create oxidative damage.[9,16]

This oxidative damage is of primary importance in the liver. As the major copper storage organ, the liver is at increased risk of the damage to proteins, lipids, and nucleic acids caused by toxic free radicals. This oxidative stress leads to inflammation of the liver and activation of profibrotic cytokines in macrophages and other cells that induce collagen formation.[9]

Copper is normally present in a variety of foods including meats, fish, vegetables, and fruit.[9] Roughly 40-60% of ingested copper is absorbed in the upper small intestine, and once absorbed into the bloodstream, copper is bound to circulating proteins such as albumin and ceruloplasmin.[9] From there, copper circulates to the liver and kidneys; in the liver, copper is bound to intracellular chaperones that guide the copper to specific target molecules.[9] After approximately four hours, the copper is exported from the liver by the ATPase, *ATP7A*, and transported to other organs.[9]

Normal liver tissue typically contains copper concentrations between 200 to 400 ppm. Patients with copper hepatotoxicity may have hepatic copper concentrations ranging from 600 to upwards of 2200 ppm. Copper concentrations in the blood are typically unaffected, even with severe copper hepatotoxicity.[16] Copper initially accumulates in hepatic lysosomes, but as the excess copper overwhelms the lysosome, it overflows to the cytosol where it affects protein function, damages other organelles, and compromises the cell membrane.[9] This process is

ultimately seen histologically as hepatocellular necrosis surrounding the central veins.[9] Other histologic evidence may vary from focal hepatitis to cirrhosis depending on the stage of disease.[15]

DIAGNOSTIC APPROACH/CONSIDERATIONS

Liver biopsy with subsequent staining is necessary for appropriate evaluation of copper content. Traditionally, Wright-Giemsa stain has been used to detect copper; however, recent research suggests that rhodanine staining with subsequent grading may be diagnostically superior.[10,13] Staining should always be followed by quantitative analysis of hepatic tissue by dry weight basis. Ideally, the liver biopsy should be obtained by other means than a needle-core biopsy, as this method frequently does not obtain a large enough tissue sample for adequate mineral detection.[10]

There are three understood methods by which excessive hepatic copper accumulation occurs: primary copper hepatotoxicity, secondary copper hepatotoxicity, and excessive copper ingestion.[9] Primary copper-associated hepatotoxicity is a breed-specific, autosomal recessive disease first described in Bedlington terriers.[3] Affected patients inadequately excrete copper through their biliary tract due to a mutation of the *ATP7B* gene.[3] This gene codes for the *COMMD1* (copper metabolism domain containing 1) protein responsible the vesicular excretion of copper at the canalicular membrane of hepatocytes.[3] *COMMD1* was previously referred to as *MURR1*.[3] When *COMMD1* does not function appropriately or is absent, copper is not adequately excreted, and excessive accumulation occurs. Recent research suggests that there may be other genes that can code for and effect mutation of *COMMD1*; however, these other factors are not fully understood at this time.[3] Histologically, primary copper storage in dogs is associated with centrilobular accumulation, but copper also is found in other hepatocytes and within regenerative

nodules.[9] Genetic testing is commercially available to assess Bedlington terriers for this autosomal recessive disease.[9] These patients typically present with clinical signs of liver disease between two and four years of age, although presentations as early as six months of age have been rarely reported.[5] Clinical signs vary but may include no signs of illness, lethargy, anorexia, vomiting, melena, and icterus. The most commonly reported lab work abnormality is elevated alanine transferase; however, alkaline phosphatase, aspartate transaminase, and other liver associated values may be affected.[2,5,15,17]

Breed-related copper hepatotoxicity has been described in multiple other breeds, including Labrador retrievers, West Highland terriers, Skye terriers, and Doberman Pinschers, among others.[11,14] However, the pathology in these breeds varies from that of Bedlington terriers in that evidence points to chronic cholestasis as a more likely culprit than a mutation of *ATP7B.*[11,14] The *COMMD1* protein is not indicated as a contributing factor in these patients.[11,14] Clinical signs and presentation are similar to that of the Bedlington terrier, but age of presentation and severity of clinical signs may vary.[5,8,9]

Secondary copper-associated hepatopathy is becoming more commonly recognized, and may affect patients of any age, breed, or gender. As copper is excreted through the biliary tree, any disruption of that bile flow may cause cholestasis and copper accumulation. Histologically, secondary copper accumulation is restricted to areas adjacent to cellular injury.[9] Associated diseases of the liver include chronic hepatitis, gallbladder mucocele, and canalicular diseases.[1,2] These patients may present with clinical signs based on the inciting cause of the cholestasis. Signs may include weight loss, anorexia, vomiting, diarrhea, polyuria/polydipsia, jaundice, and even hepatic encephalopathy and ascites in severely affected patients. These patients often present with similar blood work to that of primary copper hepatotoxicity patients, and all liver-

associated values may be affected including hyperbilirubinemia, hypoalbuminemia, hypoglycemia, hypocholesterolemia, and low blood urea nitrogen.[1,2]

Prognosis for both primary and secondary copper hepatotoxicity is dependent on the level of cirrhosis and liver dysfunction evident prior to treatment. Inflammation due to/along with copper accumulation may be addressed with appropriate therapy, but fibrosis and cirrhosis of the liver is a permanent effect. Prognosis of secondary copper hepatotoxicity is also be affected by the inciting cause of the cholestasis with conditions such as gallbladder mucoceles having perioperative mortality rates of 20-40%.[1,12]

TREATMENT/MANAGEMENT

Treatment for both primary and secondary copper-associated hepatotoxicity is aimed at minimizing free radical oxidation of tissues and decreasing inflammation. Available treatments include chelating agents, elemental zinc, ursodiol, glucocorticoids, S-adenosylmethionine (SAM-e) and vitamin E, as well as dietary modifications.[4,5,6,7] Supportive care is also recommended, such as gastroprotectants, fluid therapy, prescription liver diets, appetite stimulation, and antibiotic therapy.

Chelating agents, such as D-penicillamine, chelate copper in the blood and tissues and allow it to be excreted through the urine[4,5,7]. These agents are considered the first line of defense, particularly D-penicillamine, as it also prevents cross-linking of collagen fibers and exerts

immunosuppressive effects.[4,5,7] D-penicillamine does have significant potential side effects including vomiting, nausea, and anorexia. D-penicillamine is most effective when the patient has been fasted and administration is recommended to be separated from meals.[4] Chelating agents may rarely lead to copper deficiency with signs such as central nervous system dysfunction, anemia, and abnormal ossification.[4,5,7]

Elemental zinc induces synthesis of intestinal mucosal metallothionein, which has a high affinity for copper and, thus, binds dietary copper.[5,7] The copper is then excreted via the feces. Elemental zinc should not be administered with chelating agents, as the chelating agents will bind the zinc and minimize efficacy.[5,7]

Ursodiol is a hydrophilic bile acid that competes with other bile acids in the body, shifting the bile acids to less toxic hydrophilic forms. It also carries immune-modulating effects, acts as an antioxidant, and may help increase copper excretion.[9] Glucocorticoids are indicated if the biopsy reveals lymphocytic plasmacytic inflammation and may also decrease hepatic fibrosis, while SAM-e and vitamin E are used as antioxidants.[9]

Ideally, those patients with copper hepatotoxicity should be fed copper-restricted diets. Research has shown that some patients may be effectively managed with dietary restriction alone when chelating agents are not an option.[6] Low copper, high zinc diets are commercially available through veterinary prescription brands.[6] Additional medications for liver failure or neutrophilic inflammation may be indicated based on results of hepatic function testing and liver histopathology, respectively.

CASE OUTCOME

Sadie was diagnosed with a gallbladder mucocele with secondary copper-associated hepatotoxicity. Post-operatively, Sadie continued to recover, and after learning the results of the liver biopsy, she was initially maintained on elemental zinc (10 mg/kg PO q24h), Denamarin (18 mg/kg PO q24h), and a copper-restricted diet of Hill's Science Diet L/D. Due to financial constraints, elemental zinc was discontinued, and D-penicillamine (10 mg/kg PO q12h) was initiated.

At the time of this report, nine months after presentation, Sadie has continued to improve. Her lab work shows consistent decreases in her alanine transferase as she continues to trend toward normal. Her icterus has almost completely resolved, and she continues to be maintained on D-penicillamine, Denamarin, and Hill's L/D.

CONCLUSION

While Bedlington terriers have become synonymous with copper hepatotoxicity, it is important to remember that all patients with sustained liver disease are at risk for excessive copper accumulation. Copper hepatotoxicity is not a benign process, and the resulting inflammation and damage to hepatocytes can be treated in an effort to avoid cirrhosis and liver failure All patients presenting with cholestatic liver disease or chronic hepatic inflammation should be considered for increased copper concentrations. If a liver biopsy is performed for histopathology, a second sample should be considered for quantitative analysis, particularly in breeds of increased risk, since appropriate diagnosis and treatment may mitigate or reverse some of the copper-associated changes.[9,15]

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