Canine Copper-Associated Hepatitis

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KEYWORDS

• Dog • Liver • Bedlington terrier • Labrador retriever • Wilson disease • *ATP7A* • *ATP7B* • *COMMD1*

KEY POINTS

- Canine copper-associated hepatitis shares similarities with human copper accumulation disorders.
- Copper-associated hepatitis is recognized in several dog breeds and differences exist in causal genes and inheritance patterns between breeds.
- Clinical signs are usually noted late in disease stage when severe liver damage due to hepatic copper accumulation is already present.
- D-Penicillamine (DPA) is the most commonly used chelator to treat hepatic copper accumulation and treatment is most effective in early stages of disease.
- A low-copper/high-zinc diet can help to prevent accumulation or reaccumulation of hepatic copper in dogs with complex forms of copper-associated hepatitis.

INTRODUCTION: PATHOPHYSIOLOGY OF COPPER HOMEOSTASIS AND CELLULAR COPPER METABOLISM

Copper Homeostasis

Copper is an essential trace element necessary for many vital functions in the body. Free copper is toxic, however, due to the potential to create reactive oxygen species. Therefore, copper uptake, distribution, and excretion are tightly regulated.[1] Dietary copper is predominantly absorbed in the small intestine. Copper uptake by the enterocyte is mainly mediated by copper transporter 1 (CTR1), a high-affinity copper transporter. The copper transporter ATPase copper transporting alpha (ATP7A) is located at the basal membrane of the enterocytes and facilitates copper transport into the portal circulation. In the portal blood, copper is predominantly bound to albumin and is delivered to the hepatocellular cytosol via apically located CTR1. The liver is the most important organ in copper metabolism and is responsible for copper storage,

Disclosure Statement: The authors have nothing to disclose.
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Vet Clin Small Anim 47 (2017) 631–644
http://dx.doi.org/10.1016/j.cvsm.2016.11.011 **vetsmall.theclinics.com**

redistribution to other tissues and organs, and excretion of excess copper via the biliary system. The kidneys excrete a small proportion of excess body copper.

Cellular Copper Metabolism

After copper enters the hepatocytes, it is immediately bound by proteins to prevent oxidative damage (Fig. 1). Copper scavengers, including the small proteins metallothionein (MT) and glutathione (GSH), are the first to bind and store copper. Special delivery proteins, the copper chaperones, ensure safe handover of copper to their destination molecules.[2] Cyclooxygenase (COX)17 is the copper chaperone for cytochrome C oxidase (CCO), which resides in the inner mitochondrial membrane. CCO is the terminal enzyme in the mitochondrial respiratory chain and thus plays a crucial role in aerobic energy metabolism. The copper chaperone for superoxide dismutase (CCS) shuttles copper to superoxide dismutase (SOD1), which is an important protein in the defense against oxidative stress. Antioxidant 1 copper chaperone (ATOX1) is the copper chaperone for the copper transporters, ATP7A and ATPase, copper transporting, beta (ATP7B). Both ATPases reside in the trans-Golgi network (TGN) under normal copper conditions. When intracellular copper concentrations are rising, they move away from the TGN to their respective destinations. In the TGN, ATP7B loads 6 copper atoms onto the ferroxidase ceruloplasmin (CP), which is secreted into the circulation.[3] CP is the main copper transport protein in the blood. Under elevated copper conditions, ATP7B traffics to a lysosomal or apical membrane-associated cellular component and facilitates excretion of excess copper into the bile.[4] Previously, the main role of ATP7A was presumed to be copper uptake in the intestines, but recently hepatocellular ATP7A was demonstrated to have an important role in mobilizing and redistributing hepatic copper stores in case of peripheral copper deficiency.[5] The copper metabolism (Murr1) domain containing 1 (COMMD1) protein interacts with the amino

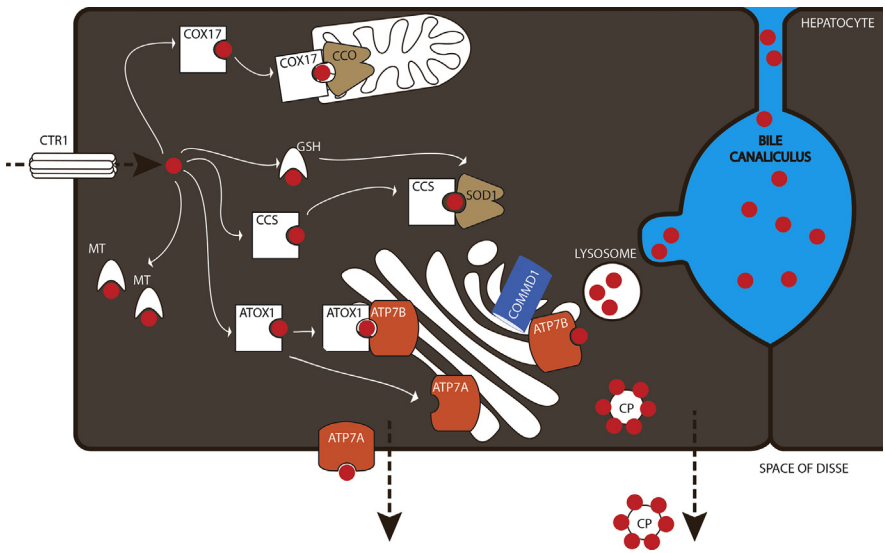

Fig. 1. Hepatocellular copper metabolism. Copper enters the cell via CTR1 and is immediately bound by MTs and/or GSH to prevent oxidative stress. The chaperones COX17, CCS, and ATOX1 transfer copper to their respective destination molecules CCO, SOD1, and ATP7A/ATP7B. ATP7A and ATP7B function in the export of copper to the blood (ATP7A and ATP7B) or to the bile (ATP7B). COMMD1 interacts with both ATPases.

terminus of ATP7B and presumably facilitates biliary excretion of copper. In addition, COMMD1 has a role in the stability and quality control of both ATPases.[6]

COPPER METABOLISM DISORDERS IN HUMANS

Wilson Disease

Wilson disease is an autosomal recessive disorder in humans in which copper accumulates in liver and neuronal tissues. The disease manifests as hepatopathy and/or neurologic or psychiatric symptoms. Wilson disease can result from several mutations in the copper transporter ATP7B. Because of its role in the incorporation of copper into CP, this may lead to low serum CP concentrations, which is one of the diagnostic criteria. Furthermore, urinary copper excretion may be increased in patients with Wilson disease. Conventional treatment consists of lifelong copper chelation with DPA.[7]

Non-Wilsonian Forms of Copper Toxicosis

Other copper storage disorders in humans in which the causative genes have not yet been identified include Indian childhood cirrhosis,[8] endemic Tyrolean infantile cirrhosis,[9] and idiopathic copper toxicosis.[10] In these diseases, a predominant hepatic presentation is observed. A hereditary predisposition in combination with increased (dietary) exposure to copper is thought to be responsible for the observed disease symptoms.

Menkes Disease

Mutations in the copper transporter ATP7A result in X-linked, recessive copper deficiency due to impaired dietary intestinal copper uptake. Patients suffer from severe neurologic impairment and failure to thrive in early childhood, and the disease is often lethal despite parenteral copper supplementation.[11]

HEREDITARY COPPER-ASSOCIATED HEPATITIS IN DOGS

Bedlington Terrier

Historically, the Bedlington terrier was the first dog breed in which canine copper-associated hepatitis was studied extensively and where a causal mutation was identified. The disease is characterized by liver cirrhosis induced by massive, centrolobular copper accumulation (**Fig. 2**A, D). Hepatic copper may be as high as 10,000 mg copper per kg dry weight liver (dwl). The causal mutation is a large deletion in the second exon of the *COMMD1* gene, leading to autosomal recessive copper toxicosis.[12] Due to the development of a DNA test, the disease frequency in the Bedlington terrier population has been drastically reduced. Recently, cases of non–*COMMD1*-related copper toxicosis were observed in Bedlington terriers. Variations in the metal transporter *ABCA12* were found to be associated with non-*COMMD1* copper toxicosis; however, convincing functional data proving involvement of this gene were lacking.[13]

Labrador Retriever

The Labrador retriever was the second breed in which part of the hereditary background of copper-associated hepatitis was elucidated. In this breed, the disease follows a complex inheritance pattern and genetic as well as dietary factors[14–18] play a role in pathogenesis. A recently performed genome-wide association study showed a significant association of increased hepatic copper concentrations with a mutation in the Wilson disease gene (*ATP7B*). Concurrent presence of a mutation in the Menkes disease gene (*ATP7A*) seemed to attenuate hepatic copper accumulation without resulting in a copper-deficient phenotype. Approximately 12% of total heritability can be explained by the 2 identified mutations. Missing heritability may be explained

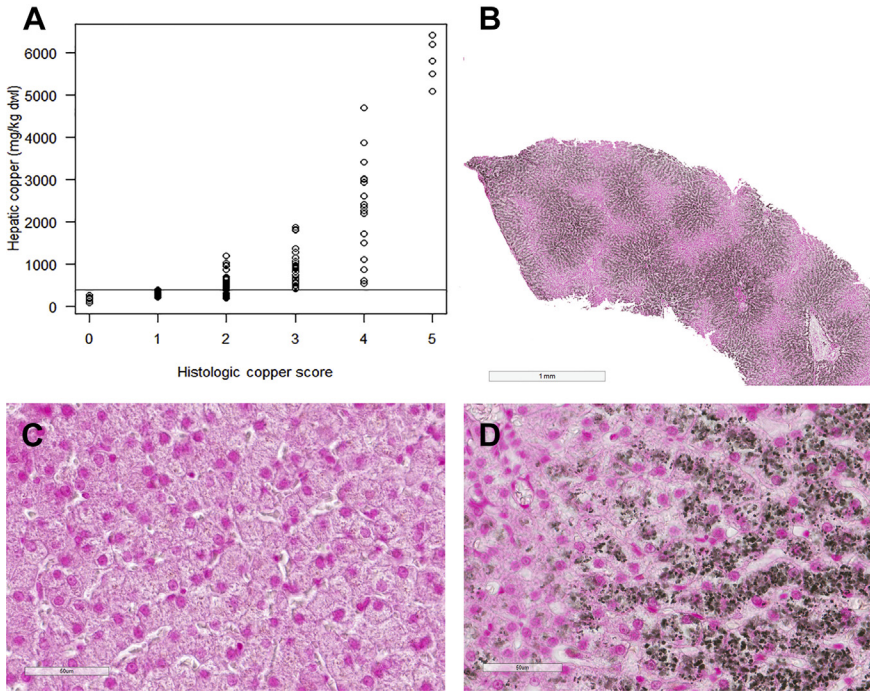

Fig. 2. (*A*) Relation between histologic hepatic copper scores (X axis) and quantitative copper measurements (Y axis) in 109 canine liver samples collected at the Faculty of Veterinary Medicine, Utrecht University, between 2010 and 2016. The horizontal line indicates the cut-off level for normal hepatic copper (400 mg/kg dry weight liver [dwl]). (*B*) Centrolobular copper distribution is clearly visible in a liver biopsy of a *COMMD1*-deficient dog (rubeanic acid stain). (*C*) Copper score 0 (rubeanic acid stain) in a Labrador retriever (quantitative copper concentration 146 mg/kg dwl). (*D*) Copper score 5 (rubeanic acid stain) in a Bedlington terrier (quantitative copper concentration 6540 mg/kg dwl).

by environmental factors or as-yet unidentified genetic mutations. Functional assays in cell lines showed that the *ATP7B* mutation in the conserved arginine resulted in an aberrant retention of the protein in the endoplasmic reticulum in high copper circumstances. The *ATP7A* mutation, located in a conserved phosphorylation site, did not affect trafficking of the protein yet led to a decrease of copper efflux in dermal fibroblasts, indicating a functional impairment of the protein.[19]

Other Breeds

Copper-associated hepatitis with a suspected hereditary background has been described in several other dog breeds including the Dobermann,[20] the West Highland white terrier,[21] and the Dalmatian.[22] Case reports of dogs diagnosed with copper-associated hepatitis include the Skye terrier,[23] Anatolian shepherd,[24] Pembroke Welsh corgi, Cardigan Welsh corgi,[25,26] and the clumber spaniel.[26] More extensive reviews of hepatic copper concentrations in dogs diagnosed with primary hepatitis suggest that there are many more breeds, including crossbreeds, in which copper-associated hepatitis is present.[27,28] The authors think it is unlikely that environmental factors, such as dietary composition, could explain copper accumulation and associated hepatitis in genetically healthy dogs and anticipate that most of the reported

breeds have some form of hereditary dysfunction in their copper metabolism. Genetic studies are needed to elucidate the affected genes in these dogs.

DIAGNOSIS

Signalment

The rate of hepatic copper accumulation and development of associated clinical signs depends on genetic predisposition and dietary copper intake and varies between breeds and between individuals within a breed. In Labrador retrievers, the age at which dogs present with clinical signs can range from 2 years old to 12 years old, but most dogs are middle aged (median age of 6 years). Bitches in the postpartum period may be at increased risk for development of clinical signs. A strong female predisposition is noted in the Labrador retriever[29] and the Dobermann,[30] whereas in other dog breeds both genders are usually represented equally.

Clinical Signs

The subclinical phase in dogs with inherited copper-associated hepatitis is usually long for 2 reasons. First, hepatic copper accumulation precedes the development of histologic changes in the liver. In Bedlington terriers, it has been shown that copper starts accumulating between 6 months and 12 months of age without overt histologic signs of hepatitis.[31] Second, clinical signs only develop when a large portion of liver parenchyma is affected. Because the liver has an enormous reserve capacity, this is usually in the end stage of the disease when chronic hepatitis or cirrhosis becomes overt. Initially, clinical signs are nonspecific and may include anorexia, lethargy, nausea, vomiting, and weight loss. When the disease becomes more progressive, more specific signs pointing toward hepatic failure, such as ascites, hepatic encephalopathy, polyuria/polydipsia, and icterus, can be noticed. Although rare, in the Bedlington terrier an acute hemolytic crisis due to the massive release of copper into the bloodstream has been reported.[32]

Clinical Pathology

The most commonly used biochemical indicators for hepatocellular injury are alanine aminotransferase (ALT) and alkaline phosphatase (ALP).[33] In the subclinical stage of copper accumulation, however, extensive hepatocellular injury is not necessarily present. The sensitivities of ALP for detection of acute hepatitis, chronic hepatitis, and nonspecific reactive hepatitis in a group of 191 clinically healthy Labrador retrievers that were admitted to the Faculty of Veterinary Medicine, Utrecht University, between 2003 and 2015, were all below 35%. For ALT, sensitivities for the detection of acute hepatitis, chronic hepatitis, and nonspecific reactive hepatitis were 45%, 71%, and 5%, respectively. This group of dogs included 131 Labrador retrievers with hepatic copper accumulation (Fieten and Dirksen, unpublished data, 2016). In affected Bedlington terriers, hepatocellular injury became visible between 12 and 18 months of age, whereas an increase in ALT and ALP was only detected at 24 months and 18 months of age, respectively.[31] Both observations underline that ALT and ALP are not useful for screening for subclinical copper-associated hepatitis. In a more advanced stage of the disease, an increase of ALT and ALP can be noticed, and a slight decrease in albumin concentration may be observed as well, although concentrations still may be within the reference range.[22,29,34] Other laboratory indicators include an increase in bile acids, ammonia, bilirubin, prothrombin time, and activated partial thromboplastin time and a decrease in fibrinogen and packed cell volume.[29,34] Serum/plasma ALT and ALP activity as well as bile acid concentration are useful parameters for detecting liver disease but neither is specific for copper-associated liver

disease. In dogs, serum copper concentrations do not correlate with hepatic copper concentrations. Decreased CP is a diagnostic hallmark for human Wilson disease. It would be interesting to investigate serum CP concentrations in Labrador retrievers with copper-associated hepatitis due to *ATP7B* mutations.

Fanconi Syndrome

Comparable to observations in humans with Wilson disease, Fanconi syndrome has been recognized in dogs with copper-associated hepatitis.[26,35,36] In these dogs, proximal tubular dysfunction was present due to copper accumulation in the proximal tubular epithelium. Dogs presented with low urinary specific gravity, proteinuria, and normoglycemic glucosuria. The observed abnormalities were reversed by DPA chelation therapy.

Cytology

Fine-needle aspiration and cytology of hepatocytes stained with a specific copper stain (ie, rubeanic acid) may be used as a noninvasive way to get an indication of the presence of copper in individual hepatocytes.[20,37] Limitations of this technique are the impossibility of evaluating zonal copper distribution, degree of hepatocellular injury, and the determination of the exact amount of copper (needed for determination of treatment duration). Further studies into the negative predictive value are necessary, because copper distribution in the liver lobules is zonal and theoretically a negative sample could be obtained from a dog affected with copper-associated hepatitis.

Liver Histopathology

Histologic distribution of copper

A histologic biopsy remains the gold standard for diagnosing copper-associated hepatitis. Samples can be obtained via laparotomy, via laparoscopy or, percutaneously with a needle (14–16 gauge) using ultrasound guidance. Because copper is not visible on routine hematoxylin-eosin stains, additional staining for copper (rubeanic acid[38] or rhodanine[39]) is necessary for the diagnosis. In cases of primary copper-associated hepatitis, copper typically starts to accumulate in the centrolobular regions of the hepatic lobule (zone 3; **Fig. 2**B).[28,29,40] Copper-loaded hepatocytes trigger the emergence of an inflammatory infiltrate, which can be mononuclear or mixed. Because excess copper is excreted into bile, an increase of hepatic copper, especially in the periportal areas of the liver lobules, could be anticipated in dogs with cholestatic diseases. In many cases of cholestatic liver disease, however, no periportal copper accumulation is detectable and the interpretation of rare cases with periportal copper accumulation is not totally clear.[41] In end-stage liver disease, where severe cirrhosis, massive necrosis, and lobular collapse are present, it may be difficult to distinguish the different zones of the liver lobule. Furthermore, in an advanced stage of the disease necrotic hepatocytes have released their copper burden, and newly formed hepatocytes, which arise during the regeneration process, do initially not contain copper,[21,42] neither does scar tissue, further diluting total copper content in the transition to end-stage disease. Mainly because of these unevenly distributed histologic changes, results of both the histologic examination (distribution and scoring) and quantitative copper determination always have to be considered in hepatic copper assessment.

Histologic scoring of copper

A semiquantitative grading system to determine hepatic copper content can be applied on copper-stained sections of liver tissue (see **Fig. 2**A).[40] Scoring is based on zonal location and number of hepatocytes and macrophages containing copper granules. In a grading scale of 0 to 5, copper scores of 2 or higher are considered abnormal

(see Fig. 2A, C, D). However, each semiquantitative score includes a wide range of quantitative copper concentrations, with overlap between scores (see Fig. 2A).

Quantitative copper determination

Hepatic copper concentrations can also be assessed quantitatively by the irradiation of small pieces of liver and the measurement of the induced copper radioactivity.[43] Therefore, an additional biopsy specimen of at least 5 mg is needed, which is freeze dried before analysis to determine dry weight copper. Other methods for quantification are spectrophotometric methods, including atomic absorption spectrometry or inductively coupled plasma emission spectrometry. Normal hepatic copper concentrations in dogs are considered below 400 mg/kg dwl.[44] In dogs affected with copper-associated hepatitis, hepatic copper concentrations are usually above 800 mg/kg dwl but can reach 10,000 mg/kg dwl. In this respect, dogs are markedly different from humans where normal copper concentrations lie in the range of 50 mg/kg dwl, and patients with Wilson disease usually have hepatic copper concentrations in the range of 500 mg/kg dwl.

Digital estimation of copper concentrations

Digital microscopic scanning of copper-stained liver sections has shown to be more accurate than qualitative copper scoring but still allows assessment of zonal histologic lesions.[45] This technique can be applied for histologic slides of liver biopsies where no additional sample for copper quantification is available.

Biomarkers

Because currently the only way to diagnose and monitor copper-associated hepatitis is by (repeated) histologic assessment of liver biopsies, the development of a noninvasive biomarker for copper status from samples of blood or urine is warranted. Such a biomarker could help identify at risk dogs to prevent clinical illness by institution of early treatment and to prevent breeding of affected individuals. Moreover, it would be easier to monitor copper concentrations during treatment using a serum or urine biomarker. The urinary copper/zinc ratio was significantly associated with hepatic copper concentration in Labrador retrievers, but the diagnostic value was limited due to overlap between normal and affected dogs.[46] Copper/zinc SOD1 and its chaperone (CCS) have both been studied as biomarkers in humans and animals with copper deficiency and overload. Erythrocyte CCS and CCS/SOD1 ratio was significantly decreased in a pilot study in Labrador retrievers with copper-associated hepatitis, suggesting promise for future clinical use. Other biomarkers under investigation include microRNAs, which are small noncoding RNAs that regulate gene expression.[47] The hepatocyte-derived microRNA-122 was significantly increased in Labrador retrievers with high hepatic copper concentrations compared with Labrador retrievers with normal copper concentrations, both without histological abnormalities, likely reflecting early copper-induced hepatocellular damage.[48] MicroRNA-122 is not copper-specific and new copper-specific microRNAs should be identified.

TREATMENT

General Recommendations

The goal of treatment in dogs with copper toxicosis is to create a negative copper balance. This can be achieved by restricting copper intake and by increasing urinary copper excretion using copper chelators (Table 1). Because treatment has the best outcome early in the disease when hepatocellular injury is limited, it is important that treatment is initiated as soon as possible and ideally in the subclinical phase.

Table 1
Medication for copper-associated hepatitis

Drug	Dose	Adverse Effects	Remarks
DPA	10–15 mg/kg po bid, separate from meals	Anorexia, vomiting, and possibly immune-mediated reactions	Most commonly used Possibly immunomodulatory and antifibrotic properties Prediction model for treatment duration available for Labrador retrievers
Trientene (2,2,2-tetramine)	15 mg/kg po bid	None reported in dogs	
2,3,2-Tetramine	15 mg/kg po bid	None reported in dogs	Not commercially available
Ammonium TTM	Unknown	Anorexia, vomiting	High risk of severe copper deficiency resulting in bone marrow depression
Zinc salts • Zinc acetate • Zinc gluconate • Zinc sulfate	5–10 mg/kg elemental zinc po bid	Generally well tolerated, but gastrointestinal side effects may occur	Should not be the sole therapy in clinical cases Slow onset of action Monitoring of plasma zinc concentrations necessary

Increased hepatic copper concentrations may induce oxidative stress and in this way contribute to progression of hepatocellular injury. It is currently unknown at exactly what concentration of copper this process starts and when chelation therapy should be initiated. For dogs, usually a hepatic copper level of greater than 400 mg/kg dwl is considered increased, which is already high, for example, compared with normal hepatic copper levels in humans (50 mg/kg dwl). In general, clinically ill dogs or dogs with overt hepatocellular injury and increased hepatic copper levels should be treated with a copper chelator and treatment should ideally be monitored by follow-up liver biopsies.

In dogs without clinical signs, with normal hepatic enzymes and moderately increased hepatic copper levels (ie, 400–600 mg/kg dwl), changing to a low-copper/high-zinc diet may be sufficient in normalization of hepatic copper levels. Individual variation in response to diet was noted, however, and continuing copper accumulation may occur despite feeding a low-copper/high-zinc diet.[16] Because it is currently not possible to predict response to diet in individual dogs, a second biopsy is always necessary 6 months after initial diagnosis and dietary change.

D-Penicillamine

DPA is a highly soluble degradation product of penicillin that is excreted by the kidneys. It binds 1 copper atom at its sulfhydryl group and facilitates excretion of copper into the urine.[49] DPA is one of the most potent copper chelators and is also able to form lower avidity complexes with other metals like zinc and iron.[50,51] Besides chelating properties, DPA may have additional favorable immunomodulatory and

antifibrotic activities.[52,53] It has been shown to be effective in the treatment of canine copper-associated hepatitis and is the most commonly used chelator.[29,54–56] The recommended dose for use in dogs is 10 mg/kg to 15 mg/kg orally twice daily. To increase bioavailability and to maximize plasma concentrations, DPA should not be given with food.[57] Side effects in dogs are usually limited to gastrointestinal signs, such as anorexia and vomiting.[55,57] Gastrointestinal side effects are easily manageable by temporarily decreasing the dose or by giving antiemetics an hour prior to DPA administration.[55,57]

In humans, DPA may induce immunologic side effects, but these have not been regularly reported in dogs. The authors and editor are aware of 2 cases in which immunologic effects were presumed related to DPA administration. A 4-year-old, female neutered English springer spaniel developed severe protein-losing glomerulonephropathy, resulting in hypoalbuminemia and ascites approximately 4 months after initiation of DPA therapy. Proteinuria and hypoalbuminemia resolved completely within 2 weeks after cessation of DPA. Furthermore, a West Highland white terrier presented with severe dermatologic lesions shortly after starting DPA, which quickly and completely resolved after DPA cessation. Although in both cases a causal link remains difficult to prove, there was a suspicion of DPA-related immunologic side effects.

Recently a model has been published that can be used as a guideline for the calculation of necessary duration of DPA treatment depending on hepatic copper concentrations in Labrador retrievers (**Box 1**).[55] Most likely, this model can also be used for other dog breeds with complex forms of copper-associated liver disease and copper concentrations in a similar range. Treatment should be continued until normal hepatic copper concentrations are achieved.

Continuous DPA therapy may lead to copper and zinc deficiency due to enhanced urinary excretion of these metals.[46,55] Despite 1 case-report of an affected Bedlington terrier developing DPA-induced copper deficiency,[58] affected Bedlington terriers usually need lifelong continuous chelation therapy. In many of these dogs, normalization of hepatic copper concentrations does not occur despite therapy,[54] but progression of disease is precluded. In other dog breeds, lifelong therapy is not recommended. An intermittent treatment regime with recheck biopsies every 1 to 2 years prevents copper (re-) accumulation and concurrently avoids copper and zinc deficiencies.

Trientene (2,2,2-Tetramine)

Trientine is a tetramine chelator that was originally introduced for humans who developed adverse reactions to DPA. Like DPA, trientene is an effective promoter of urinary copper excretion although it may act through a different pool of body copper.[59] In humans, fewer side effects are reported than for DPA,[60] whereas in dogs no side effects have been reported.[61] Studies in dogs, however, are limited. The recommended dose in dogs is 15 mg/kg twice daily. At the time of writing, the cost of trientine in the United States prohibits its use for veterinary patients.

Box 1
Formula to calculate necessary treatment duration with D-penicillamine in Labrador retrievers

$$CuQ_1 = -81.5 + 0.99 \times CuQ_0 + 51.0 \times T - 3.92 \times T^2 - 0.16 \times CuQ_0 \times T + 0.92 \times 10^{-2} \times CuQ_0 \times T^2$$

Abbreviations: CuQ_0, quantitative copper at start of treatment; CuQ_1, quantitative copper at certain period of treatment; T, treatment duration.

2,3,2-Tetramine

2,3,2- Tetramine is another tetramine chelator but was reported to produce a 4-fold to 9-fold greater urinary copper excretion than trientene.[61] 2,3,2-Tetramine therapy was studied in 5 Bedlington terriers with copper toxicosis.[62] After 200 days of treatment, hepatic copper concentrations decreased 55%, without the development of adverse reactions. Besides this study, few data are available and 2,3,2-tetramine is not commercially available.

Ammonium Tetrathiomolybdate

Ammonium tetrathiomolybdate (TTM) is a strong copper chelator that forms a tripartite complex with copper and protein in the intestines, plasma, and liver tissue. It decreases MT-bound hepatic copper by excretion of copper TTM complexes into the bile and blood.[63,64] Due to its extensive decoppering effects, it has antiangiogenic properties, which also make it a candidate for cancer treatment in humans and dogs.[65,66] To date, TTM has not been used for the treatment of copper-associated hepatitis in dogs. One study, conducted in healthy dogs, showed that TTM administration (1 mg/kg) resulted in a significant increase in serum copper concentration, underlining possible potential as a future therapeutic agent.[67]

Zinc

The oral administration of zinc salts (zinc acetate, zinc gluconate, and zinc sulphate) interferes with copper uptake in the enterocytes. Zinc oxide has a limited bioavailability. Increased intestinal zinc concentrations are believed to induce up-regulation of intestinal MT, which has a high affinity for copper. With high levels of copper bound to MT, less copper is available for serosal transfer and passage into the portal circulation is blocked.[68,69] Presumably, zinc may also attenuate the toxicity of copper by inducing MT up-regulation in hepatocytes.[70] In human copper storage diseases, long-term effectivity is similar to that of DPA, but zinc is generally tolerated better.[71,72] Zinc acetate, zinc gluconate, and zinc sulfate have all been used in dogs with copper toxicosis.[14,73,74] Acetate and gluconate salts may be better tolerated than sulfate, but individual differences in response exist. Normal plasma zinc concentrations range from 90 μg/dL to 120 μg/dL. To suppress gastrointestinal copper uptake, a minimal plasma zinc concentration of 200 μg/dL is needed.[73] The recommended dose to achieve a plasma concentration between 200 μg/dL and 300 μg/dL is 5 to 10 mg/kg elemental zinc twice daily or 200 mg of elemental zinc per day, in divided doses. To be effective, zinc salts should not be given with any food. Plasma zinc concentrations exceeding 1000 μg/dL may result in hemolysis. Therefore, plasma zinc concentrations should be monitored during treatment. Because a minimum of 3 months of administration is required to obtain a therapeutic response, zinc therapy is not recommended as the sole treatment in clinical cases. Those cases require more aggressive therapy with copper chelators.

Dietary Management

Dietary intake has a significant impact on hepatic copper accumulation[15,18] and an adjusted diet (low copper and high zinc) may be valuable in the management of dogs with copper-associated hepatitis. A low-copper/high-zinc diet may be beneficial to prevent or postpone reaccumulation of copper in dogs that were initially treated with a copper chelator.[14,17] Another role for dietary management could be in subclinical dogs with moderate copper accumulation. In 1 study, hepatic copper concentrations could be normalized with dietary intervention alone in approximately 50% of

subclinical Labrador retrievers with increased hepatic copper.[16] Some individuals, however, continued to accumulate copper, even when fed a low-copper/high-zinc diet. Individual variation in response to diet may be influenced by hereditary factors.[19] Because variation in response to low-copper/high-zinc diets occurs, hepatic copper concentrations should be evaluated by repeat biopsy with copper staining and quantification.

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