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# Long-term Management of Copper-associated Hepatic Cirrhosis with D-penicillamine, SAMe, and DBB in a Dog

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**Abstract :** A 4-year-old intact female American Cocker Spaniel presented with lack of appetite, shivering, and abdominal distension. It was initially diagnosed with chronic hepatitis with cirrhosis, by serum chemistry, radiography, ultrasonography, and histopathologic examination following liver biopsy. Abundant copper granules were detected in most hepatocytes with rhodanine stain, with hepatic copper concentration at 1460 ppm (reference range: < 400 ppm). Based on these findings, copper-associated hepatitis with cirrhosis was diagnosed and successfully managed with long-term D-penicillamine, s-adenosylmethionine, biphenyl-dimethyl-dicarboxylate and supportive care. The spaniel died 35 months after diagnosis.

***Key words*** **:** American Cocker Spaniel, canine, copper-associated hepatopathy, hepatic cirrhosis.

## Introduction

Hepatic cirrhosis is the end stage of chronic hepatitis and is characterized by hepatocellular apoptosis and necrosis, mononuclear or mixed cell inflammation, fibrosis, and ductular proliferation and regeneration (18). The cause is usually unknown, although some cases have been associated with infection, toxins, and hepatic copper accumulation (1,11). Hepatic copper accumulation can result from excessive intestinal absorption of copper, a primary metabolic defect in hepatic copper metabolism; or from altered secretion of copper in bile (15). Genetically determined excessive copper accumulation is well known in Bedlington Terriers, whereby a deletion of the COMMD1 gene causes accumulation of copper in hepatocytes, resulting in chronic hepatitis (17). This primary copper storage and associated hepatitis has also been reported in West Highland White Terriers, Skye Terriers, Doberman Pinschers, Dalmatians, and Labradors (3,4,10,16). Although the American Cocker Spaniel is predisposed to early onset of chronic hepatitis that quickly progresses to cirrhosis, it has not been determined whether copper accumulation is primary or secondary to chronic inflammation, fibrosis, and cholestasis or cholate-stasis (20). However, there is little information of the prognosis with and without therapy in copper- associated end stage of hepatopathy. Most clinically affected dogs with hepatic cirrhosis die within a month of diagnosis. In this report, a case of copper-associated hepatic cirrhosis successfully managed with D-penicillamine, s-adnosylmethionine (SAMe), biphenyl dimethyl dicarboxylate (BDD) with supportive care in a young American Cocker Spaniel is presented.

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## Case

A 4-year-old intact female weighing 9.2 kg American Cocker Spaniel was referred for further evaluation of anorexia, shivering, and abdominal distension. The dog fed a commercial diet. On the physical examination, the dog was afebrile (rectal temperature 38.7°C), mildly dehydrated and the abdomen was distended. Complete blood cell count results were within the reference range, while abnormal serum chemistry results included elevated ALT (466 U/L; reference range: 3-50 U/L), AST (64 U/L; reference range: 10-43 U/L), ALP (186 U/L; reference range: 8-100 U/L), and GGT (22 U/L; reference range: 0-8 U/L) activity, and low BUN (5 mg/dl; reference range: 8-30 mg/dl), glucose (75 mg/dl; reference range: 80-120 mg/dl), albumin (2 g/dl; reference range: 2.8-4.5 g/dl), and total protein (4.9 g/dl; reference range: 5.0-7.5 g/dl). Cystocentesis and abdominocentesis were performed to collect urine and ascites. No abnormal findings were not detected on the urinalysis. Abdominal effusion was consistent with transudate (total nucleated cell counts: 300/μl, specific gravity: 1.006, total protein: 0.1 g/dl, predominant cell types: macrophages and neutrophils). Additional diagnostic tests showed significantly elevated postprandial bile acid levels (fasting, 29 μmol/l; reference range, < 10 μmol/l, postprandial, 78 μmol/l; reference range: < 20 μmol/l). There is no clinical significance to a decreased

PTT and thrombin time. The prothrombin time was elevated (prothrombin time, 9.9 sec; reference range, 5.8-7.9 sec, thrombin time, 3.1 sec; reference range, 4.2-7.0 sec, activated partial thromboplastin time, 11.1 sec; reference range, 13.1-17.4 sec).

Abdominal ultrasound revealed that the liver was diffusely hypoechoic with a coarse echotexture and irregular and nodular margins.

To rule out the neoplasia, fine needle aspirates were obtained from liver by sono-guide, and the smears were stained with Diff-Quik. The cytologic preparations were highly cellular, with clusters of hepatocytes showing moderate cytoplasmic rarefaction, anisocytosis, anisokaryosis, and frequent binucleation. Inflammatory cells such as neutrophils, hemosiderin laden macrophages, small lymphocytes, and plasma cells were also found along with many red blood cells (RBCs) and nucleated RBCs (nRBCs).

Exploratory surgery was conducted and by the wedge biopsy three samples were taken from multiple lobes in a size of 1 $cm^3$. Abundant free fluid (1300 ml) was aspirated and the liver showed multiple small surface nodules as well as decreased volume (Fig 1A). Liver tissues were submitted to Antech Diagnostics, Inc. (Memphis, USA) for histological evaluation and copper analysis. Samples were used for aerobic and anaerobic bacteria culture. Pending histologic evaluation and bacterial culture, supportive care for hepatic failure was initiated with administration of ursodeoxycholic acid (UDA) (7.5 mg/kg, PO, bid), biphenyl-dimethyl-dicarboxylate (BDD, Lefotil, 25 mg/day, PO, bid), S-adenosylmethionine (SAMe, 20 mg/kg PO, sid), spironolacton (1 mg/kg, PO, bid) and Vitamin E (400 IU/day, PO, sid) for 1 month. At this time, changes in serum chemistry panels were unremarkable, but anemia [RBC count; $3.09 \times 10^6$ cells/μl; packed cell volume (PCV 19%)] was found. Supportive care included whole blood transfusion (120 ml), fluid therapy, and Vitamin $K_1$ (1 mg/kg SC, bid). Three days later, RBC count had increased ($4.38 \times 10^6$ cells/μl) and PCV was slightly increased (25%).

Histopathology on the biopsy sample indicated end-stage hepatopathy with nodular hyperplasia. The changes were chronic, severe and no normal functioning hepatic parenchyma existed. Microscopically, the hepatic parenchyma was severely collapsed with scattered hyperplastic nodules. The regions of collapsed parenchyma exhibited prominent biliary duct hyperplasia with variable arteriole hyperplasia and mild to moderate stromal and bridging fibrosis. Hemosiderin laden macrophages forming aggregates and a few scattered lymphocytes were noted as well as extramedullary hematopoiesis in some areas (Fig 2A, B). Rhodanine staining of the liver biopsy sample showed high accumulations of copper positive granules in most hepatocytes within the parenchyma and the hyperplastic nodules (Fig 2C, D). Hepatic copper concentration was determined as 1460 ppm (reference range: < 400 ppm, (1). The culture of liver tissue was negative for both aerobic and anaerobic bacteria. Based on these results, hepatic copper accumulation was considered to be the most likely cause for the hepatic failure in this case. D-penicillamine (10 mg/kg, PO, bid) was prescribed as a copper chelator and was administered 1 hr before feeding. The patient was examined bi-weekly, and the owner was asked to report adverse effects such as vomiting, lethargy, nausea, fever, and skin problems. The clinical signs, including anorexia, shivering, and ascites, gradually diminished, although changes in serum chemistry profiles were not significant. The patient managed well with D-penicillamine, SAMe, and BDD twice a day until it died 35 months after diagnosis. At necropsy, the liver tissue was submitted to Antech Diagnostics, Inc. (Memphis, USA) for histologic evaluation and copper analysis. Necropsy revealed gross macronodular cirrhosis (Fig 1B). Microscopic findings included marked, diffuse, and chronic-active portal hepatitis with infiltration of moderate numbers of lymphocytes, plasma cells, and neutrophils in and around portal triads. There was mild fibrosis and marked hyperplasia of bile duct epithelial cells and vascular endothelial cells. Most hepatocytes were mildly to moderately swollen due to cytoplasmic granulization. No neoplasia or infectious organisms were seen (Fig 3). However, rhodanine stain did not reveal a significant amount of hepatocellular copper.

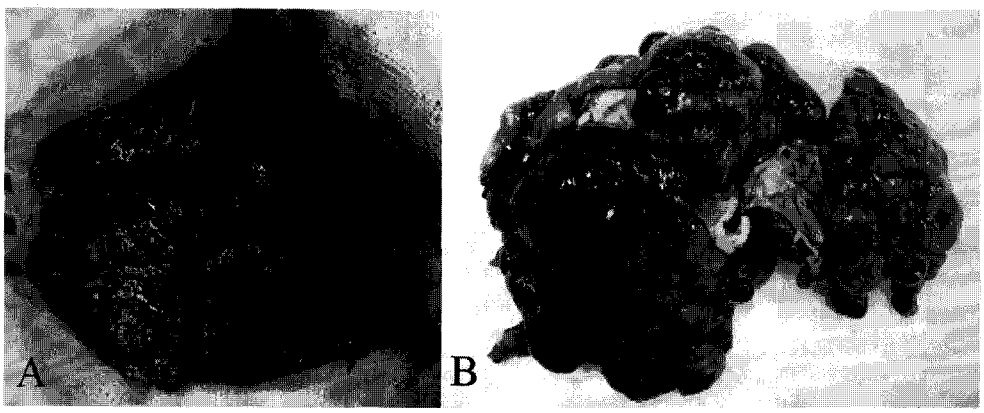


**Fig 1.** Gross appearance of liver of a 4 year old intact female American Cocker Spaniel before (A) and after (B) treatment. Note the small multiple nodules on the surface and the decrease in volume at the time of exploratory surgery (A). Necropsy showed macronodular cirrhosis after 35 months of treatment with D-penicillamine, SAMe and DDB (B).

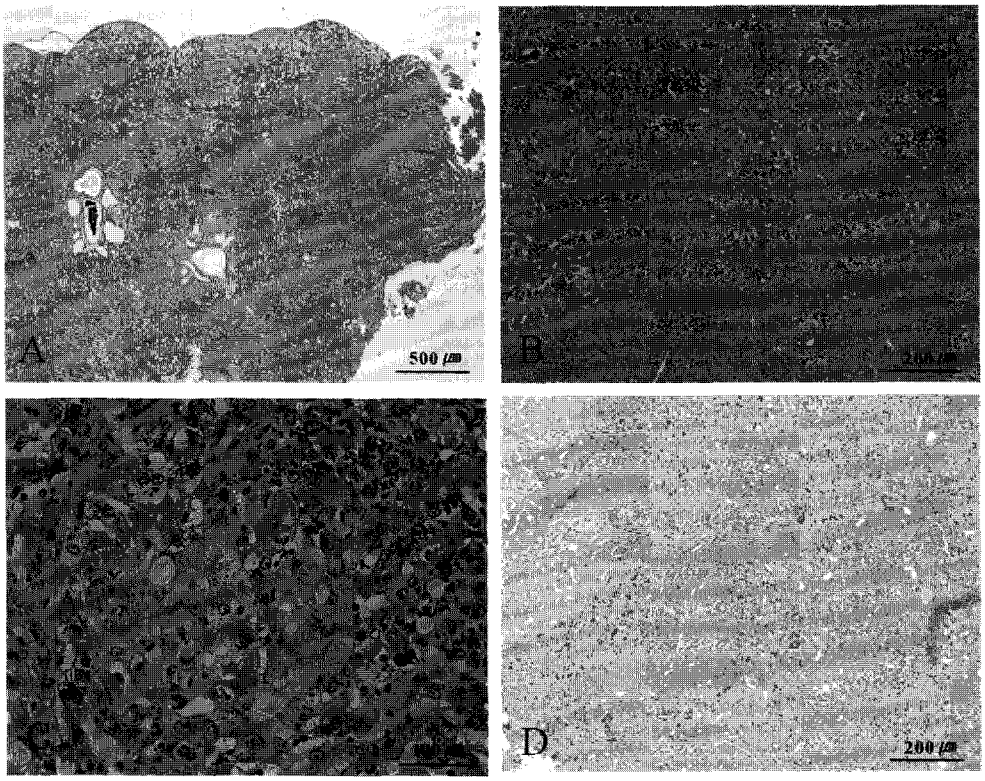


**Fig 2.** Liver biopsy of an American Cocker Spaniel with copper related hepatic cirrhosis. Note the severely collapsed hepatic parenchyma with fibrosis and hemosiderin laden macrophages forming aggregates. HE (A and B). Most hepatocytes showed evidence of accumulated copper positive granules within the parenchyma and the hyperplastic nodules. Copper granules are stained red-brown. Rhodanine stain for copper (C and D).

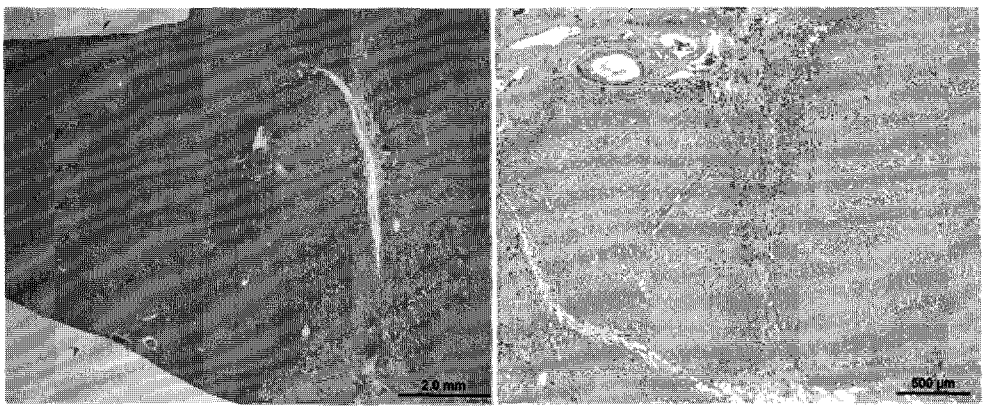


**Fig 3.** Necropsy sample of liver from an American Cocker Spaniel with copper related hepatic cirrhosis treated with D-penicillamine, SAMe and DDB for 35 months, revealed marked, diffuse, and chronic-active portal hepatitis. HE.

## Discussion

Hepatic copper accumulation was considered to be the most like causes for the hepatic failure in this dog for the following reasons: there was no history of prior hepatotoxic injury or any suspicious medication. Also there was no evidence of cholestasis on the result of hepatic biopsy. The substantial increases in ALT activities even though the total bilirubin levels were remained in normal range can be the evidence of toxic hepatocellular injury. Moreover, the copper concentration of the liver biopsy sample was 1460 ppm and additional histochemical staining (rhodanine stain) was revealed abundant copper positive granules in most hepatocytes within the parenchyma and hyperplastic nodules. When hepatic copper was assessed by a semi-quantitative histochemical grading system in a previous report (4), the score was 4+ in this case. Semi-quantitative copper measurements were correlated with quantitative analysis in other breeds, in which semi-quantitative copper scores of 2-3+

corresponded to quantitative copper concentrations of 300-2000 ppm in Doberman Pinschers, and semi-quantitative scores of 3-5+ corresponded to quantitative concentrations of 1500-4000 ppm in Bedlington Terriers (6,19). The hepatic copper concentration in healthy dogs with a normal liver is below 400 ppm, and levels of hepatic copper lower than 1000 ppm are likely secondary to hepatic disease such as cholestasis. Concentrations greater than 2000 ppm are likely primary causes of hepatic disease, as in inherited copper storage disease in the Bedlington Terrier, and concentrations greater than 1000 ppm are possibly primary causes in other breeds (12,20). However hepatic copper levels in breeds with primary copper storage disease vary among breeds and individuals. The copper level in Bedlington Terriers with a mutation of the COMMD1 gene for primary copper storage disease is 850-12000 ppm. Other breeds in which the molecular background is uncertain are: West Highland White Terrier, 1000-3000 ppm; Skye Terrier, 800-2200 (3). Doberman Pinscher, 1000-2000 ppm and Labrador Retriever, 750-2000 ppm (4,10,16). The copper level in the liver of the patient in the present study was determined as 1460 ppm, which is within the reported range of affected Bedlington Terriers and other breeds with primary copper storage disease. However, to the author's knowledge, there have been no reports of a range of copper concentrations in Cocker Spaniels with copper related hepatopathy although this breed knows as one of the breeds predisposed to copper accumulation.

Copper is mostly accumulated centrolobularly (zone 3) in primary copper storage disorders such as Bedlington terrier copper toxicosis and Wilson's disease in humans (13). In other breeds including West Highland white terrier, Skye terrier, Dalmatian and Labrador retriever, copper accumulates in hepatocytes, starting in the centrolobular regions, and with progressive accumulation, results in hepatocellular necrosis, inflammation with copper-laden macrophages and finally chronic hepatitis and cirrhosis (18). In this case, observations of copper positive granules were within hepatocytes in the parenchyma and in the scattered hyperplastic nodules. Localization of copper granules between the centrololular (zone 3) and periportal (zone 1) areas could not be investigated due to the severe chronic changes with essentially no normal functioning hepatic parenchyma.

The prognosis in dogs with advanced chronic hepatitis and cirrhosis is guarded. A study showed that mean survival times in chronic hepatitis dogs with therapy was ranged 6 to 16 months. In this study also identified that dogs with hypoalbuminemia, hypoglycemia and coagulopathies had very guarded prognostic factor and many died within 1 week of diagnosis (14). In this case, the dog lived for 35 months with long-term D-penicillamine, SAMe, and BDD combined therapy. D-penicillamine, which can enhance urinary copper excretion, is the copper chelator of choice. However, reduction in hepatic copper concentration may take months to years, and adverse effects include anorexia, nausea, and vomiting (12). To prevent progression of copper generated hepatic damage exacerbated by increased oxidative stress, we administered anti-oxidant stress medications including SAMe and BDD. Adenosylmethionine is a precursor of hepatic glutathione, an important endogenous hepatic antioxidant that becomes depleted in liver diseases. Biphenyl dimethyl dicarboxylate (BDD) is a synthetic analog of Schizandrin C, a compound isolated from *Fructus shizandrae*, a traditional medicine in China (8). Experimental evidence suggests that BDD has a hepato-protective function as a potent antioxidant, and it has proven valuable in the treatment of chronic viral and chemically induced hepatitis (2,5,9). A pharmacological study has shown BDD to increase liver protein and glycogen synthesis and have an inducing effect on the cytochrome P-450 enzyme system (9). In addition, BDD inhibits vitamin C/NAPDH induced lipid peroxidation in rat liver microsomes and appears to be more effective than vitamin E at the same concentration (7). However, the effect of BDD on damaged liver cells was not determined in the present study because of the multi-drug treatment protocol. The dog was managed with combined D-penicillamine, SAMe, and BDD without clinical signs. The liver enzyme level decreased and serum albumin and total protein increased gradually over the course of 35 months. While liver cirrhosis did not improve, and necropsy showed macronodular cirrhosis, staining did not reveal a significant amount of hepatocellular copper. The results of semi-quantitative staining for copper concentrations suggest that the long-term treatment with D-penicillamine, SAMe, and BDD was successful in eliminating copper from the hepatocytes.

Probable copper storage disease in this American Cocker Spaniel demonstrates the need for further report in regards to the disease prevalence in the breed, pathogenesis, and mode of inheritance. Also, the efficacy of combination therapy with D-penicillamine, SAMe, and BDD in copper-associated hepatic disease in dogs could be considered as a therapeutic option.

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# 개에서 발생한 구리 축적성 간경화에 D-penicillamine, SAMe, DBB로 병용 치료하여 장기간 생존한 1례

**서경원 · 이영흔 · 방동하 · 안진옥 · 고예린 · 황철용 · 김대용 · 윤화영[1]**

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**요 약** : 4살령의 아메리칸 코카 스파니엘이 식욕부진, 몸떨림 그리고 복부 팽만증세로 내원하였다. 혈청학적 검사, 방사선 검사, 초음파 검사 그리고 간생검을 통한 조직병리학적 검사를 통해 경화로 진행된 만성 간염으로 진단할 수 있었으며. 특수 염색법을 통해 대부분의 간세포내에 구리가 축적되어 있는 것을 확인할 수 있었으며, 구리 농도는 1460 ppm으로 매우 높음을 알 수 있었다. 이러한 검사 결과를 바탕으로 구리 축적으로 인한 간염이 간경화까지 진행된 말기 병변으로 진단하고 D-penicillamine, s-adenosymethionine, biphenyl-dimethyl-dicarboxylate과 대증 치료로 본 환자를 치료 하였으며, 본 환자는 진단 후 35개월을 생존하는 것에 성공하였다.

**주요어** : 아메리칸 코카 스파니엘, 개, 구리 축적성 간경변, 간경화