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Association between Liver Copper Concentration and Subclinical Hepatitis in Doberman Pinschers

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The prevalence of subclinical hepatitis was investigated in a group of 106 randomly selected 3-year-old Doberman Pinschers. Histopathologic examination of liver samples from 65 dogs (52 dogs with high bile acids, alkaline phosphatase activity, or alanine aminotransferase activity or with copper granules in hepatocytes in a liver aspirate and 13 normal dogs) revealed subclinical hepatitis in 22 dogs (19 females and 3 males). Liver copper concentrations measured by instrumental neutron activation analysis was significantly higher (mean ± SD; 419 ± 414 μg/g dry matter) in dogs with hepatitis than those without liver disease (197 ± 113 μg/g; $P = .0008$). At 2.6 ± 0.6 years hepatitis persisted in 5 of 16 dogs available for examination. One dog with a high copper concentration but normal liver subsequently developed subclinical hepatitis after 3 years. During the follow-up period, the average copper concentration of the 6 dogs with persistent subclinical hepatitis was 939 ± 299 μg/g and had continued to rise significantly ($P = .02$). The hepatitis in these dogs was associated with apoptotic hepatocytes and copper-laden Kupffer cells in centrolobular regions. The results of this study suggest that there is a relationship among copper storage, hepatocellular damage, and hepatitis in Doberman Pinschers.

Key words: Copper toxicosis; Dogs; Etiology

The pathogenesis of chronic hepatitis in Doberman Pinschers, also known as Doberman hepatitis, is unknown.[1–8] The disease might be hereditary and is seen predominantly in female dogs. In the clinical stage, it is most commonly characterized by anorexia, depression, weight loss, gastrointestinal signs, polydipsia, polyuria, and icterus.[1–8] Affected Doberman Pinschers at this stage have micronodular cirrhosis with histologic features comparable to chronic hepatitis in man.[2–8] Copper concentrations are high in the majority of dogs, with some authors concluding that the increased copper concentration is the result of concurrent cholestasis.[2,4,5,8] However in 1983, Thornburg et al[3] described 2 Doberman Pinschers with subacute hepatitis without any signs of cirrhosis or chronic cholestasis. He concluded that there was evidence that the cause of the liver disease was copper toxicosis in these 2 dogs. Recently, Thornburg[8] described a group of Doberman Pinschers with chronic hepatitis in a precirrhotic stage, of which 30 of 35 had high copper concentrations. He concluded that the increased copper was incidental and not the cause of disease because the histopathology was comparable in all dogs and because 5 dogs did not have high copper concentrations.

Fifty-five dogs from a group of 626 apparently healthy Doberman Pinschers of various ages had high plasma activity of liver-derived enzymes.[6,7] Twenty-one of 23 dogs examined had subclinical hepatitis and 19 dogs had high liver concentrations. It was concluded that liver copper concentration could be used as a diagnostic criterion.[7] Thus, it can be concluded from all these studies that the role of copper in the pathogenesis of Doberman hepatitis is unclear.

There is a consensus that clinical hepatitis in Dobermans is more common in female dogs aged 5–7 years. If the clinical disease is indeed the end product of an already existing subclinical form of hepatitis, it should be possible to identify cases at 3 years of age and follow them over time to analyze the course of the disease. Early recognition of the preclinical cases might also provide better insight in the pathogenetic role of copper because high liver copper concentrations at that stage might indicate a primary role for copper. In this study, we investigated a random sample of Dutch Dobermans at 3 years of age by blood examination, histologic examination of liver biopsies, and quantitative liver copper measurements. Follow-up studies of all dogs with subclinical hepatitis were performed over a period of 2–4 years.

Materials and Methods

Dogs

To investigate the prevalence of subclinical hepatitis in 3-year-old Doberman Pinschers, a number of dogs were randomly drawn from a group of 967 Dutch Doberman Pinschers (150 litters). All dogs were born between August 1, 1995, and July 31, 1996, in the Netherlands. One male and 1 female dog were selected from each litter born in this period. Their respective owners were then asked to participate in the study. If an animal had died or an owner failed to respond, then another animal was selected from the same litter.

Procedure

Owners were requested to withhold feed from the animal 12 hours before the study. A clinical history was recorded, and a physical examination was performed. A jugular blood sample and 2 or 3 fine-needle aspirations from the liver were obtained from all animals. The aspirates were taken in the right 9th or 10th intercostal space at approximately midthoracic height. The aspirates were smeared on a glass microscope slide, air-dried, and stained with rubeanic acid stain to examine for the presence of hepatic copper granules.[9] Plasma bile acid concentrations, alkaline phosphatase activity (AP), and alanine aminotransferase (ALT) activity were measured from heparinized plasma.

If 1 or more of the blood variables was increased or there were copper granules visible on cytologic examination of the liver aspirate, then permission was requested from the owner for their dog to have a percutaneous liver biopsy by the Menghini aspiration technique.[10] If

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Table 1. Results of the 65 dogs from which liver tissue was examined.

	Normal		Subclinical Hepatitis		
	Males	Females	Males	Females	Total
No abnormalities in blood tests or FNA	6	6	—	1	13
Abnormalities in blood tests only	1	5	—	2	8
Abnormalities in FNA only	9	9	3	12	32
Abnormalities in blood tests and FNA	4	3	—	5	12
Total number of dogs	20	23	3	19	65

FNA, fine-needle aspirate.

histologic examination did not reveal abnormalities, then the owner was asked whether the dog could be used as a control. Four biopsies 2–3 cm long were taken with a 16-gauge Menghini needle: 2 for histopathologic examination and 2 for quantitative copper analysis. The quantitative copper analysis was by instrumental neutron activation analysis via the determination of ^{66}Cu.[11]

Tissue for histologic examination was fixed in 10% neutral buffered formalin, routinely dehydrated, and embedded in paraffin. Microscope slides (4 μm) were stained with hematoxylin-eosin (HE), van Gieson's stain, reticulin stain according to Gordon and Sweet, and rubeanic acid. Liver samples were semiquantitatively scored on a scale of 0–5 (0, no copper detectable; 1, solitary liver cells or reticuloendothelial system cells containing some copper-positive granules; 2, small groups of liver cells or reticuloendothelial system cells containing small to moderate numbers of copper-positive granules; 3, larger groups or areas of liver cells or reticuloendothelial system cells containing moderate numbers of copper-positive granules; 4, large area of liver cells or reticuloendothelial cells with many copper-positive granules; 5, diffuse presence of liver cells or reticuloendothelial cells with many copper-positive granules).[5] The liver samples for the quantitative copper analysis were put in a small copper-free plastic container, freeze-dried, and stored until they were analyzed.

At this stage, an animal could, on the basis of the histopathologic examination, be classified as normal or as having subclinical hepatitis. If an animal could not be classified in either of these 2 groups (undecided), the animal was re-examined after 6 months.

All dogs that were found to have subclinical hepatitis or to have a high copper concentration of >400 μg/g dry matter were requested to come back for examination at regular intervals of 6–12 months after the initial liver biopsy. The concentration of 400 μg/g dry matter was chosen on the basis of historical data from large numbers of dogs referred to our clinic during the last 20 years.

During the follow-up period, owners were asked not to alter the diet and to record any medication administered. During the 1st 6 months after recognition, all dogs diagnosed with subclinical hepatitis participated in a placebo-controlled study investigating the efficacy of nandrolone laureate as a therapeutic drug. This drug did not alter the disease process. No other medication was administered to the dogs.

Statistics

Statistical evaluation was implemented with the software package Statistix 8.0 for Windows.[a] Descriptive statistics was used for general data. Copper data were analyzed by a Wilcoxon signed rank test. The level of significance was $P < .05$.

Results

Clinical Findings

One-hundred and six dogs (47 males and 59 females) met our criteria and were used for evaluation.

Physical examination of all 106 dogs was normal. Three females were in heat, 2 showed signs of pseudopregnancy, 4 animals were incontinent, and 1 animal was diagnosed earlier with hypothyroidism.

Plasma bile acids concentration was high in 6 dogs (median value 11 μmol/L, range 11–27 μmol/L, reference value up to 8 μmol/L), ALT in 12 dogs (median value 135 U/L, range 94–226 U/L, reference range 23–90 U/L), and AP in 2 dogs (median value 142 U/L, range 135–148 U/L, reference range 25–117 U/L). Biopsies were performed on 8 of these 20 dogs.

Copper granules were found on cytologic examination in 50 of 106 dogs. Twelve of these dogs had high liver enzyme activities, bile acids, or both. Forty-four of 50 had biopsies performed for histologic evaluation and quantitative copper measurement.

Forty-eight out of 106 dogs did not have abnormalities on either blood examination or cytology. Of these dogs, 13 underwent liver biopsy for histologic evaluation and quantitative copper measurement.

Histopathology

Percutaneous Menghini liver biopsies were obtained in 65 of 106 dogs. Nineteen dogs (3 males and 16 females) were diagnosed at 1st examination as having subclinical hepatitis. In all except 1 female, the results of the screening examinations (blood and cytology) were abnormal. A total of 31 dogs had no histopathologic abnormalities. Changes in 15 dogs were borderline (minor nonspecific changes at histopathology), and owners were asked to return their dogs after 6 months. After 6 months, 3 additional dogs (all females) were diagnosed with subclinical hepatitis. The remaining 12 dogs were scored as normal (Table 1).

The histopathologic abnormalities were subtle in all dogs. The majority demonstrated centrolobular copper-laden hepatocytes and, on occasion, an apoptotic hepatocyte associated with activated pigmented Kupffer cells, lymphocytes, plasma cells, and scattered neutrophils.

Copper Analysis

The amount of liver tissue was sufficient for a quantitative copper analysis in 64 of 65 dogs. Copper concentration in 22 dogs with hepatitis (mean ± SD; 419 ± 414 μg/g dry matter) was significantly higher ($P = .0008$) than in dogs without histologic abnormalities (197 ± 113 μg/g dry matter). However, not all dogs with subclinical hepatitis had a high copper concentration (Fig 1). There was no difference between genders in copper concentration ($P = .2$). The females (n = 19) had a mean concentration of 449 ±

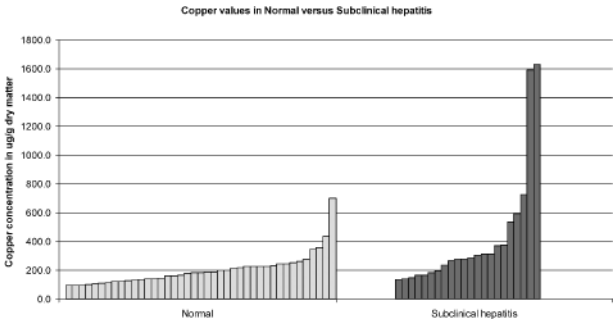

Fig 1. Liver copper concentrations of dogs; displayed are, at left, the dogs without any histologic abnormalities and, at right, the dogs with subclinical hepatitis. There is significant ($P = .0008$) difference between these 2 groups. Each bar represents a value for 1 dog.

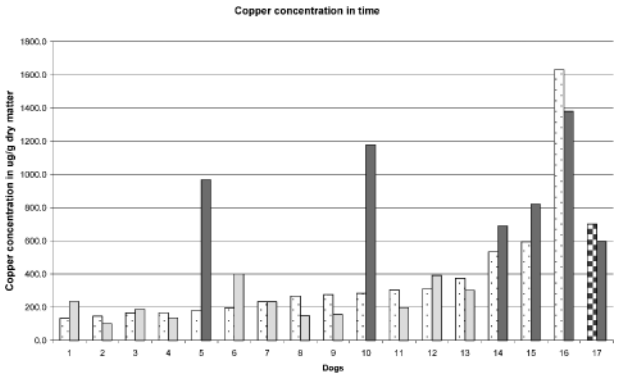

Fig 2. Copper concentrations after a follow-up period of 2.6 ± 0.6 years (mean ± SD). The 1st bars are the copper concentrations at time of the initial examination. Dog 17 (chessboard filling) had at that time only a high copper concentration without any histopathologic abnormality. The 2nd bars are the copper concentrations at the end of the follow-up period. Dogs with dark bars still had subclinical hepatitis.

438 μg/g dry matter compared with the males (n = 3), with a mean of 229 ± 88 μg/g dry matter.

Two dogs without histologic abnormalities had liver copper concentration >400 μg/g dry matter. The others were below this concentration. One of these dogs was excluded from this study. It lived on a pig farm and was known to eat pig food that contained high concentrations of copper.

Follow-Up after 2 Years

Eighty-two of 106 dogs were without abnormalities after 2 years. All 82 owners were asked to return their animal if any clinical signs of hepatitis developed. After a follow-up period of 2 years, none of the owners had returned. Owners of the 22 dogs diagnosed with subclinical hepatitis and the owner of the dog with liver copper concentration >400 μg/g dry matter were requested to present their dog for regular (6–12 month) re-examination and transcutaneous liver biopsies. Seven of 23 dogs were lost for follow-up before the term of 2 years for reasons not related to their subclinical hepatitis status. Seventeen dogs, 16 with subclinical hepatitis and 1 without, remained. For some dogs, we were able to obtain liver biopsies up to 4 years after the initial examination. The average follow-up period was 2.6 ± 0.6 years after the 1st examination. Of the 16 dogs diagnosed with subclinical hepatitis, 9 dogs had no histopathologic abnormalities at their last 2 examinations, and 2 dogs had a nonspecific reactive hepatitis. In 5 of the 16 dogs, the subclinical hepatitis persisted at each examination. The dog with a copper concentration >400 μg/g dry matter but otherwise had no evidence of hepatic disease did develop histologic evidence of subclinical hepatitis after 3 years; the changes have been persistent. All 6 dogs had a histologic copper grade of at least 2–3+, and in all dogs, apoptotic hepatocytes were found associated with copper-laden Kupffer cells centrolobularly with a slight infiltration of lymphocytes, plasma cells, and scattered neutrophils around the hepatic veins.

The copper concentrations after the follow-up period in the groups of dogs with persistent hepatitis (939 ± 299 μg/g dry matter) and without persistent hepatitis (227 ± 100 μg/g dry matter) are significantly different ($P = .02$). The dogs in which the hepatitis disappeared during the follow-up period all had copper concentrations <400 μg/g dry matter (Fig 2).

Discussion

In a group of 106 healthy Doberman Pinschers at 3 years of age, 22 dogs (21%) appeared to have subclinical hepatitis on the basis of histopathology. Subclinical hepatitis in Doberman Pinschers has been previously described by Speeti and others.[6,7] They found that, in a large group of 626 Doberman Pinschers, 55 dogs (8.8%) had a high ALT and 21 dogs (3.4%) had subclinical hepatitis. The percentage of subclinical hepatitis in our study was far higher than in the study of Speeti et al.[6] There are several possible explanations. First, unlike our study, the dogs studied by Speeti et al[6] were not a random selection of a 1-year cohort. The inclusion criterion used by Speeti et al[6] was that the ALT should be 3 times higher than the upper reference value. Our criteria were inclusion of any dog with high liver enzyme activities or bile acids or the presence of copper granules in hepatocytes. For that reason, it seems logical to conclude that not all these dogs with subclinical hepatitis would later develop Doberman hepatitis. Speeti et al[6] found that 19 of 21 dogs with subclinical hepatitis had a high copper concentration. By the time their results were published, 6 dogs had died of Doberman hepatitis. In our study, we found 21% of dogs had subclinical hepatitis, with both normal and increased copper concentrations. This result might indicate that, apart from copper, other etiologic factors (eg, infections, deficiencies, toxins, deficient immune status, or an immune-mediated mechanism) might be the cause of the subclinical hepatitis.[12–15] Although not significant, the majority of dogs with subclinical hepatitis in our study turned out to be female, which could imply, for instance, an immune-mediated etiology because immune-mediated diseases are more common in females[16] and are especially important in hepatitis.[17,18]

The role of copper in the pathogenesis of Doberman hepatitis has been the subject of discussion for many years. In several breeds, such as Bedlington Terriers,[19,20] West Highland White Terriers,[21,22] Skye Terriers,[23] Dalmatians,[24] and Anatolian Shepherd dogs,[25] copper storage has been described as an etiologic factor for the development of hepatitis. In the Doberman Pinscher, its role is unclear. Earlier

studies reported normal hepatic copper concentrations up to 500 μg/g dry matter in dogs.[19,21,26,27] However in a study by Thornburg et al,[28] copper concentrations in 623 purebred and mixed-breed dogs ranged from <100 μg/g dry matter to 6,800 μg/g dry matter. In the same study, hepatic copper values were measured in 20 healthy Doberman Pinschers (without histopathologic changes) and were found to range from 150 to 1,500 μg/g dry matter (mean ± SD, 413 ± 298 μg/g dry matter). In a more recent study, Thornburg[8,29] argued against the role of copper because dogs with copper concentrations ≤1,500 μg/g dry matter were found without any sign of hepatitis. Also, Speeti et al[7] argued that an increased copper concentration can only be used as a discriminatory factor for diagnosis of Doberman Pinscher hepatitis. In our study, the subclinical hepatitis at 1st examination had a significantly higher copper concentration compared with normal dogs. However, both normal and high copper concentrations were seen.

The data of this study indicate copper concentrations in normal Doberman Pinschers are <400 μg/g dry matter (197 ± 113 μg/g dry matter). In the follow-up study on dogs with subclinical hepatitis, 10 of 17 dogs ''recovered''; that is, hepatitis was morphologically absent for at least 2 examinations. Furthermore, the copper concentration had never been >400 μg/g dry matter at any stage in the study. The 6 Doberman Pinschers (5 females and 1 male) in which the initial and final copper concentration was >400 μg/g dry matter or who developed pathologic copper concentrations during follow-up had persistent hepatitis. They all showed morphologic copper storage centrolobularly with copper-laden Kupffer cells, lymphocytes, plasma cells, scattered neutrophils, and, on occasion, apoptotic hepatocytes. This suggests that there is a relation among copper storage, hepatocellular damage, and hepatitis in Doberman Pinschers.

This study indicated that normal hepatic copper concentrations in Doberman Pinschers are <400 μg/g dry matter. Subclinical hepatitis was seen in 22 dogs, which persisted in only 6 dogs with increased copper concentrations, suggesting a role for copper in the pathogenesis of Doberman hepatitis.

Footnotes

[a] Analytical Software, P.O. Box 12185, Tallahassee, FL 32317-2185

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References

1. Meyer DJ, Iverson WO, Terrell TG. Obstructive jaundice associated with chronic active hepatitis in a dog. J Am Vet Med Assoc 1980;176:41–44.

2. Johnson GF, Zawie DA, Gilbertson SR, et al. Chronic active hepatitis in Doberman Pinschers. J Am Vet Med Assoc 1982;180: 1438–1442.

3. Thornburg LP, Rottinghaus G, Koch J, et al. High liver copper levels in two Doberman Pinschers with subacute hepatitis. J Am Anim Hosp Assoc 1983;20:1003–1005.

4. Crawford MA, Schall WD, Jensen RK, et al. Chronic active hepatitis in 26 Doberman Pinschers. J Am Vet Med Assoc 1985;187: 1343–1350.

5. van den Ingh TSGAM, Rothuizen J, Cupery R. Chronic active hepatitis with cirrhosis in the Dobermann Pinscher. Vet Q 1988;10: 84–89.

6. Speeti M, Ihantola M, Westermarck E. Subclinical versus clinical hepatitis in the Dobermann: Evaluation of changes in blood parameters. J Small Anim Pract 1996;37:465–470.

7. Speeti M, Eriksson J, Saari S, et al. Lesions of subclinical Doberman hepatitis. Vet Pathol 1998;35:361–369.

8. Thornburg LP. Histomorphological and immunohistochemical studies of chronic active hepatitis in Doberman Pinschers. Vet Pathol 1998;35:380–385.

9. Teske E, Brinkhuis BG, Bode P, et al. Cytological detection of copper for the diagnosis of inherited copper toxicosis in Bedlington terriers. Vet Rec 1992;131:30–32.

10. Lettow E. Lebererkrankungen beim hund. Tierarztl Prax 1974; 2:321–343.

11. Bode P. Automation and quality assurance in the neutron activation facilities in Delft. J Radioanal Nucl Chem 2000;245:127–132.

12. Gocke DJ, Morris TQ, Bradley SE. Chronic hepatitis in the dog: The role of immune factors. J Am Vet Med Assoc 1970;156:1700–1705.

13. Dill-Macky E. Chronic hepatitis in dogs. Vet Clin North Am Small Anim Pract 1995;25:387–398.

14. Rothuizen J, van den Ingh TSGAM. Hepatitis in dogs; a review. Tijdschr Diergeneeskd 1998;123:246–252.

15. Twedt DC. Reactive hepatopathies and chronic hepatitis in the dog. Vet Q 1998;20(Suppl 1):S46–S47.

16. Beeson PB. Age and sex associations of 40 autoimmune diseases. Am J Med 1994;96:457–462.

17. McFarlane IG, Heneghan MA. Autoimmunity and the female liver. Hepatol Res 2004;28:171–176.

18. Strettell MD, Thomson LJ, Donaldson PT, et al. HLA-C genes and susceptibility to type 1 autoimmune hepatitis. Hepatology 1997; 26:1023–1026.

19. Twedt DC, Sternlieb I, Gilbertson SR. Clinical, morphologic, and chemical studies on copper toxicosis of Bedlington Terriers. J Am Vet Med Assoc 1979;175:269–275.

20. Sternlieb I. Copper and the liver. Gastroenterology 1980;78: 1615–1628.

21. Thornburg LP, Shaw D, Dolan M, et al. Hereditary copper toxicosis in West Highland White Terriers. Vet Pathol 1986;23:148–154.

22. Thornburg LP, Rottinghaus G, Dennis G, et al. The relationship between hepatic copper content and morphologic changes in the liver of West Higland White Terriers. Vet Pathol 1996;33:656–661.

23. Haywood S, Rutgers HC, Christian MK. Hepatitis and copper accumulation in Skye Terriers. Vet Pathol 1988;25:408–414.

24. Webb CB, Twedt DC, Meyer DJ. Copper-associated liver disease in Dalmatians: A review of 10 dogs (1998–2001). J Vet Intern Med 2002;16:665–668.

25. Bosje JT, van den Ingh TSGAM, Fennema A, et al. Copper-induced hepatitis in an Anatolian Shepherd dog. Vet Rec 2003;152: 84–85.

26. Rolfe DS, Twedt DC. Copper-associated hepatopathies in dogs. Vet Clin North Am Small Anim Pract 1995;25:399–417.

27. Keen CL, Lonnerdal B, Fisher GL. Age-related variations in hepatic iron, copper, zinc and selenium concentrations in Beagles. Am J Vet Res 1981;42:1884–1887.

28. Thornburg LP, Rottinghaus G, McGowan M, et al. Hepatic copper concentrations in purebred and mixed-breed dogs. Vet Pathol 1990; 27:81–88.

29. Thornburg LP. A perspective on copper and liver disease in the dog. J Vet Diagn Invest 2000;12:101–110.