FULL PAPER *Pathology*

Histological and Immunohistochemical Evaluations of Lobular Dissecting Hepatitis in American Cocker Spaniel Dogs

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(Received 6 October 2012/Accepted 17 December 2012/Published online in J-STAGE 28 December 2012)

ABSTRACT. Histological and immunohistochemical evaluations of lobular dissecting hepatitis (LDH) were performed in nine American cocker spaniel dogs. Histological examination showed diffuse fibrosis with weak inflammatory reaction of extensive neutrophils, macrophages, lymphocytes and plasma cells. Immunohistochemical examination revealed that the myofibroblastic cells positive for anti-α-smooth muscle actin and anti-vimentin antibodies produced reticular and collagen fibers and brought about the dissection of hepatic cords and diffuse disappearance of hepatocytes. Reticular fibers invading between hepatocytes and the surrounding small group of hepatocytes were strongly positive for anti-collagen type III and anti-collagen type IV antibodies. The positivity to anti-fibronectin and anti-laminin antibodies was frequently continuous on the basement membrane of the sinusoids of the remaining hepatic cords and between the hepatocytes. Positive findings for anti-E-cadherin antibody were not observed between the hepatocytes showing positive findings for anti-collagen type III and anti-collagen type IV antibodies. These results may explain the expression of fibronectin and laminin that occurs prior to the invasion of reticular fibers between hepatocytes. The present study further suggests that expression of an extracellular matrix mainly containing fibronectin and laminin between the hepatocytes and proliferation of collagen fibers and reticular fibers have a major role in the rupture of the hepatic cords and disappearance of hepatocytes.

KEY WORDS: canine, fibrosis, hepatitis, immunohistochemistry.

doi: 10.1292/jvms.12-0447; *J. Vet. Med. Sci.* 75(5): 597–603, 2013

Lobular dissecting hepatitis (LDH) has been described in individuals and litters in young standard poodle, Rottweiler, German shepherd, golden retriever and American cocker spaniel dogs [2, 13, 20, 23, 32]. The disease usually occurs in an average of two years and has a poor prognosis and short survival time [23, 32]. Clinically, the symptoms of ascites, weight loss, anorexia, diarrhea and acquired portosystemic shunts are exhibited individually. In biochemical blood tests, specific findings in this disease have not been described, although hypoalbuminemia and elevated levels of liver enzymes such as AST and ALP are observed [23, 32].

Regarding the histological characteristics, it has been explained that reticular fibers surrounding an individual or group of hepatocytes divide the hepatic cords and finally destroy the lobular structure. Mild infiltration of neutrophils, lymphocytes, plasma cells and macrophages and necrosis of individual hepatocytes, as well as cholestasis, are observed [2, 13, 20, 23, 32]. Immunohistochemical examination has not yet been conducted for the histogenesis of hepatic fibrosis of LDH. It is assumed that the proliferation of reticular fibers that divide the hepatic cords characteristic of LDH is induced by myofibroblastic cells proliferating around the hepatocytes.

LDH is classified as a form of cirrhosis by the World Small Veterinary Association (WSAVA) [11]. But, to the best of our knowledge, there are no reports that have defined LDH as a form of cirrhosis. We considered further studies to be required in order to define the histopathological identity of LDH. In this study, we examined the mechanism by which fibrosis divides the hepatic cords of American cocker spaniels diagnosed with LDH, using histopathological and immunohistochemical examinations.

MATERIALS AND METHODS

Animals and samples: Samples were obtained from biopsies and postmortem livers of nine American cocker spaniel dogs. The dogs were 2 to 7 years of age, and their average age was 4 years. Subjects included five spayed females and 4 castrated males (Table 1). These dogs showed clinical signs of loss of appetite, weight loss, abdominal distension, urine bilirubin and depression. Clinical examination found ascites, multiple portal shunts and portal hypertension (Table 1). Biochemical blood examination showed elevated levels of liver enzymes (ALT 104 IU/*l* and AST 69 IU/*l*, No. 9) and total bilirubin (1.1 mg/d*l*, No. 3) and decreased total protein (3.8 g/d*l*, No. 3) and albumin (1.6 g/d*l*, No. 3; 1.9 g/d*l*, No. 9). Multiple portal shunts in two dogs (Nos. 3 and 9) were clinically considered to be acquired portosystemic shunts because the dogs had bilirubinuria, ascites or portal hypertension. Microhepatia in 2 dogs (Nos. 2 and 9) was considered to be a secondary change due to fibrosis accompanying

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Table 1. Clinical findings and sampling methods

No	Age	Sex[a)]	Clinical signs	Clinical tests	Biochemical blood examination[b)]		Sampling methods
					Elevated levels of liver enzymes	Decreased albumin	
1	2	M	No	Ascites	○	ND	Postmortem
2	2	M	Weight loss, anorexia, apathy and abdominal distention	Ascites, microhepatia and bilirubinuria	○	○	Tru-cut needle
3	3	F	No	Ascites, multiple portal shunts and bilirubinuria	○	○	Tru-cut needle
4	4	F	Abdominal distention	Ascites	○	ND	Postmortem
5	4	M	Weight loss and anorexia	No	○	○	Laparoscopy
6	6	F	No	Ascites	ND	○	Laparotomy
7	7	F	Abdominal distention	Ascites	○	ND	Laparotomy
8	Adult	F	ND	ND	ND	ND	Laparotomy
9	Adult	M	Weight loss, anorexia and apathy	Multiple portal shunts, portal hypertension, microhepatia and bilirubinuria	○	○	Laparoscopy

a) M: Male. F: Female. b) ○: Found. ND: No data.

Table 2. Primary antibody for immunohistochemistry

Antibody	Antibody type[a)]	Dilution	Antigen retrieval	Source
Vimentin	MM	1:100	Microwave	Dako Denmark A/S, Glostrup, Denmark
α-Smooth muscle actin	MM	1:100	Microwave	Dako Denmark A/S, Glostrup, Denmark
Collagen type I	RP	1:50	Pepsin	Quartett GmbH, Berlin, Germany
Collagen type III	RP	1:50	Pepsin	Quartett GmbH, Berlin, Germany
Collagen type IV	RP	1:500	Pepsin	Abcam plc, Tokyo, Japan
Laminin	RP	1:100	Pepsin	Thermo Fisher Scientific, Fremont, CA, U.S.A.
Fibronectin	RP	1:500	Pepsin	Dako Denmark A/S, Glostrup, Denmark
E-cadherin	MM	1:100	Autoclave	Zymed Laboratories, Carlsbad, CA, U.S.A.
Hepatocyte paraffin-1	MM	1:200	Microwave	Dako Denmark A/S, Glostrup, Denmark
Cytokeratin 7	MM	1:100	Proteinase K	Dako Denmark A/S, Glostrup, Denmark
Cytokeratin 19	MM	1:100	Microwave	NeoMarkers, Fremont, CA, U.S.A.
α-Fetoprotein	RP	Prediluted	Autoclave	Nichirei, Tokyo, Japan

a) MM: mouse monoclonal antibody. RP: rabbit polyclonal antibody.

ascites, portal hypertension or multiple portal shunts.

Control samples were prepared from the livers of three beagle dogs without clinical abnormality.

Histological examination: Liver tissues were fixed in 15% formalin solution and embedded in paraffin. Histological sections with a thickness of 4 μm were prepared from paraffin-embedded tissues and stained with hematoxylin and eosin (HE), silver impregnation stain (Watanabe's method), Masson-trichrome stain, Victoria blue stain, periodic acid-Schiff (PAS) reaction and PAS after diastase digestion, Hall stain, rhodanine stain and Berlin blue stain. Paraffin sections from normal liver tissues of three control dogs were stained the same way and used for comparative histopathology and immunohistochemistry.

Immunohistochemical examination: Using avidin-biotin-peroxidase complex (ABC) stain, immunohistochemical examinations were performed on the paraffin sections. Details of the primary antibodies used are shown in Table 2. To remove endogenous peroxidase, sections were immersed in 0.5% periodic acid solution at room temperature for 15 min. The sections were incubated in primary antibody solution at 4°C for 12 hr and incubated in secondary antibody solution at room temperature for 30 min. After incubation, the sections were reacted in avidin-peroxidase conjugate solution (Vector Laboratories, Burlingame, CA, U.S.A.) for 30 min. Visualization was accomplished using 0.05% diaminobenzidine solution. Mayer's hematoxylin stain was used as a counterstain. As a negative control, sections without the primary antibody were subjected to the same procedures.

RESULTS

Histological findings: Six dogs (Nos. 1, 3, 4, 5, 7 and 8) had histologically both severe and mild lesions. The other three dogs (Nos. 2, 6 and 9) had only severe lesions. Histological findings of severe and mild lesions are summarized in Table 3. In severe lesions, the hepatic lobular structure had completely disappeared, and a few remaining vestiges of the hepatic triad and central vein were recognized in some areas. Most of the hepatic cords had disappeared. The remaining hepatocytes were observed individually or as small groups (Figs. 1 and 2). Mild infiltration of neutrophils,

Table 3. Summarized findings of histological and immunohistochemical examinations of LDH

	Histological findings	Immunohistochemical findings
Severe lesions	·The collapse of the hepatic lobule ·Rupture of hepatic cords ·Increase of reticular fibers and collagen fibers around a small group of hepatocytes ·Proliferation of myofibroblasts and bile duct structures ·Mixed inflammatory cell infiltration	·Collagen fibers of collagen type I, type III, and type IV and extracellular matrix proteoglycan of fibronectin and laminin ·Positive for fibronectin between E-cadherin-negative hepatocytes ·Proliferation of myofibroblasts positive for SMA and vimentin and bile duct epithelial cells positive for CK7 and CK19
Mild lesions	·Retention of some hepatic cords ·Mild proliferation of myofibroblasts in the sinusoids or in Disse's space ·Proliferation of the bile ducts with a slit-shaped structure in the sinusoids or in Disse's space	·Collagen fibers of collagen type IV and extracellular matrix proteoglycan of fibronectin and laminin ·Positive for fibronectin between E-cadherin-positive hepatocytes ·Proliferation of myofibroblasts positive for SMA and vimentin and bile duct epithelial cells positive for CK7 and CK19

lymphocytes, plasma cells, macrophages and macrophages with cytoplasmic brown granules was diffusely observed. Fibrous tissue consisting of spindle-shaped myofibroblastic cells with growth of reticular fibers and collagen fibers was observed surrounding the remaining hepatic triad. Small groups of hepatocytes were circumscribed by thin reticular fibers. Reticular fibers invading between the individual hepatocytes were frequently observed (Fig. 3). In some areas, thick fibrous tissues consisting of collagen fibers, including elastic fibers, exhibited histological features of severe hepatic fibrosis.

In mild lesions, the spindle-shaped myofibroblastic cells slightly proliferated in the sinusoids or in Disse's space along the hepatic cords. However, collagen and reticular fibers were not observed between the individual hepatocytes. In fibrous tissues, short spindle or oval-shaped epithelial cells with a small, pale, clear cytoplasm had formed tubular or slit-shaped lumen structures. The unequally sized remaining hepatocytes contained cytoplasm with vacuolar, fatty, hydropic and glycogen degeneration. Hepatocytes with cytoplasm containing blue-gray or brown granules were occasionally found in other areas. Nuclei of hepatocytes were small or large in size and irregular. Neither piecemeal necrosis nor massive necrosis was observed in the liver. Apoptosis (single cell necrosis) of hepatocytes was observed occasionally. Bile thrombus was observed sporadically in the bile canaliculi in the remaining lobules. Nuclear or cytoplasmic inclusion bodies suggesting viral infection were not observed.

In four cases (Nos. 1, 4, 8 and 9), nodular hyperplasia of hepatocytes compressing the surrounding tissues was accompanied by a boundary of thin fibrous tissue. In addition, extensions of the lymphatic vessels and interlobular vein were observed in the hepatic triad of some cases. Significant proliferation of the interlobular bile duct was also observed.

Immunohistochemical findings: In severe lesions as summarized in Table 3, fibrous tissues were strongly positive for anti-collagen type I, anti-collagen type III and anti-collagen type IV antibodies. The spindle-shaped cells were positive for anti-α-smooth muscle actin (α-SMA) and anti-vimentin antibodies (Fig. 4). The reticular fiber invading between the hepatocytes and surrounding the small groups of hepatocytes was strongly positive for anti-collagen type III and anti-collagen type IV antibodies. A positive reaction to anti-fibronectin and anti-laminin antibodies was observed between the hepatocytes of the small groups, but a positive reaction for anti-E-cadherin antibody was not observed (Fig. 5).

In mild lesions as summarized in Table 3, the spindle-shaped cells proliferating in the lumen of sinusoids or Disse's space along the remaining hepatic cords showed strong positive reactions to anti-α-SMA and anti-vimentin antibodies. Fine fibrils showing positive reactions to anti-collagen type IV antibody were also found in the same area, but no fibrils were observed between the individual hepatocytes (Fig. 6). Positive reactions to anti-fibronectin and anti-laminin antibodies were frequently continuous at the basement membrane of the sinusoids of the remaining hepatic cords and between the individual hepatocytes (Fig. 7). Positive reactions to the anti-E-cadherin antibody were not observed in places showing positive reactions to anti-fibronectin and anti-laminin antibodies (Fig. 8).

All remaining hepatocytes showed strong positive reactions to anti-hepatocyte paraffin-1 (HepPar-1) antibody. A few hepatocytes were also positive for anti-α-fetoprotein (AFP) antibody. Short spindle-shaped or oval epithelial cells showing a slit-shaped lumen or small tubular structure in the fibrous tissue were positive for anti-cytokeratin 7 and anti-cytokeratin 19 antibodies, but were not positive for anti-HepPar-1 and anti-AFP antibodies.

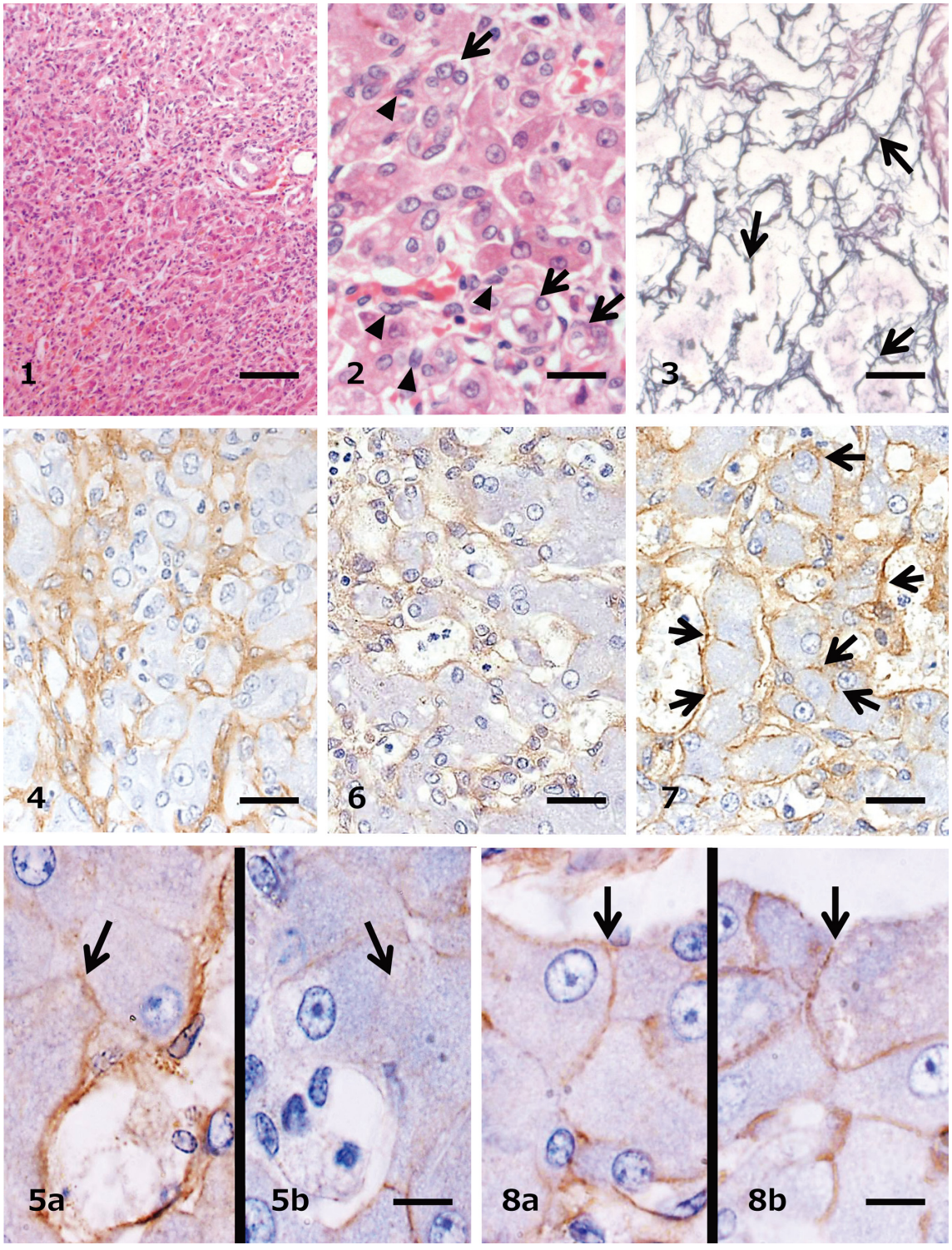
In control tissue, the fibrous tissues of hepatic triads showed immunopositive reactions to anti-collagen type I, anti-collagen type III and anti-collagen type IV antibodies. On the other hand, positive reactions to anti-fibronectin, anti-laminin and anti-collagen type IV antibodies were found on the basement membrane of the sinusoids along hepatic cords. Positive reactions to anti-E-cadherin antibody were found between hepatocytes and between bile duct epithelial cells.

DISCUSSION

Pathological examinations in the present study showed diffuse fibrosis with a weak inflammatory reaction of extensive neutrophils, macrophages, lymphocytes and plasma cells, which has been reported in lobular dissecting hepatitis in dogs [2, 13, 20, 32]. Mild extensive infiltration of inflammatory cells; solitary hepatocellular necrosis suggesting apoptosis; and vacuolar, glycogen and hydropic degenera-

tion were different from those of massive necrotizing hepatitis [2, 13, 20, 21, 32]. It was considered that the proliferating fibrous tissue consisting of collagen and reticular fibers brought about the dissection of the hepatic cords and disappearance of the hepatocytes.

In hepatic fibrosis, myofibroblastic cells derived from stellate cells are known to play a major role [6, 10, 14, 20, 21]. Extracellular matrix proteoglycan (such as fibronectin,



laminin and hyaluronan) and collagen fibers (type I, type III and type IV) produced by myofibroblastic cells are deposited in the walls of sinusoids and progress along the sinusoids. The distribution of fibrosis in the liver reflects the pathogenesis of the responsible necroinflammatory disease [19, 22, 24, 28, 29, 33, 35]. It was considered that spread of the diffuse fibrosis in the present case irregularly dissected the lobular structure.

In human medicine, the pattern of fibrosis distribution is biliary fibrosis, postnecrotic scarring, diffuse hepatic fibrosis or periacinar fibrosis [4, 12]. Fibrosis of the liver generally arises in the periportal areas. Biliary fibrosis is observed in biliary disease, including cholangitis [4, 12, 24]. Postnecrotic scarring after massive necrosis is observed as an irregular zone of fibrosis resulting in broad, irregular bands of scar tissue with a variable irregular area of parenchymal regeneration interspersed [4, 12]. Diffuse hepatic fibrosis is the outcome of chronic parenchymal injury, such as prolonged inflammation or multiple episodes of zonal necrosis [4, 12]. Diffuse hepatic fibrosis usually forms the lobules bridges connective tissue in portal areas and hepatic venules and often observed in chronic hepatitis including hepatitis B and C virus infection in human beings or infectious disease with piecemeal necrosis [4, 12, 29, 33, 35]. Periacinar fibrosis is often observed in copper poisoning, pyrrolidine alkaloid poisoning and congestion of the hepatic vein [4, 12, 18]. Pericellular fibrosis is usually observed in neonatal hepatitis and alcoholic hepatitis in humans. This fibrosis is recognized to result in rupture of the hepatic cords and creation of a small group of hepatocytes with diffuse inflammation [17, 30]. A pattern of fibrosis similar to that in humans frequently occurs in domestic animals [20, 25], such as in the case of biliary disease, copper poisoning, pyrrolidine alkaloid poisoning and congestion of the hepatic vein [20, 25]. In dogs, copper-associated hepatitis in Bedlington terriers and chronic hepatitis in Doberman pinscher have been reported many times [8, 20, 27, 28, 31]. Copper-associated hepatitis is observed as centrilobular hepatocyte necrosis and periacinar fibrosis. In the Doberman pinscher, chronic hepatitis is observed as various degrees of periportal fibrosis and bridging fibrosis [20, 28].

In the present study, fibrous tissue surrounding the small group of hepatocytes was made up of reticular fibers positive for anti-collagen type III and anti-collagen type IV antibodies. Furthermore, the reticular fibers infiltrated between the individual hepatocytes in mild lesions. These findings suggest that pericellular fibrosis may have occurred in the dogs [17, 30]. On the other hand, fibronectin- and laminin-positive findings were observed extensively between individual hepatocytes in the lesions where invasion of reticular fibers was observed. The findings suggest that the expression of fibronectin and laminin occurs prior to the invasion of reticular fibers between the hepatocytes.

In the areas with expression of fibronectin and laminin found between the hepatocytes, E-cadherin-positive findings were not observed. These findings were not observed in the liver tissue of the control. Cadherin is the main adhesion protein holding epithelial cells together in a sheet arrangement [7]. E-cadherin appearing along cell surfaces is responsible for maintenance of the sheet arrangement of hepatocytes [9, 15]. We think that disappearance of E-cadherin induced by expression of fibronectin and laminin would indicate the loss of maintenance of the sheet arrangement of hepatocytes. Proliferation of reticular fibers and deposition of intercellular matrix between the hepatocytes may be responsible for the isolation of hepatocytes and dissection of hepatic cords [5, 16, 34].

Spindle-shaped cells were myofibroblastic cells immunohistochemically positive for anti-α-SMA and anti-vimentin antibodies. In normal human livers, hepatic stellate cells usually show negative reactions to anti-α-SMA antibody. Activated hepatic stellate cells have been explained as differentiating into myofibroblastic cells with the expression of α-SMA [6, 10, 12]. However, hepatic stellate cells usually express α-SMA in the normal liver of the dog [10]. The role of hepatic stellate cells concerned with fibrosis can be estimated easily by the proliferation of myofibroblastic cells [21]. Significant proliferation of α-SMA-positive myofibroblastic cells in LDH suggests that hepatic fibrosis progressed by persistent activation of myofibroblastic cells

Fig. 1. LDH in dog No. 1. The hepatic lobular structure and most of the hepatic cord have completely disappeared. HE. Bar=100 μm.

Fig. 2. LDH in dog No. 1. Hyperplastic bile ducts (arrows) and proliferative myofibroblastic cells (arrowheads) can be seen around the hepatocytes. HE. Bar=25 μm.

Fig. 3. LDH in dog No. 4. Proliferated reticular fibers can be seen around the small group of hepatocytes. Fine reticular fibers (arrows) are between the individual hepatocytes. Silver method for reticulum. Bar=25 μm.

Fig. 4. LDH in dog No. 1. Spindle cells surrounding small groups of hepatocytes are positive for anti-α-SMA antibody. ABC method with hematoxylin counterstain. Bar=25 μm.

Fig. 5. LDH in dog No. 4. A mirror image of immunohistochemical staining of the severe lesions in the liver. Positive expression for anti-fibronectin (Fig. 5a, arrow) can be seen between hepatocytes. Positive expression for anti-E-cadherin antibody (Fig. 5b, arrow) cannot be seen between hepatocytes. ABC method with hematoxylin counterstain. Bar=10 μm.

Fig. 6. LDH in dog No. 4. Fine reticular fibrils surrounding small groups of hepatocytes are positive for anti-collagen type IV antibody. ABC method with hematoxylin counterstain. Bar=25 μm.

Fig. 7. LDH in dog No. 4. Positive expression for anti-fibronectin antibody can be seen around the small groups of hepatocytes and sometimes between the individual hepatocytes (arrows). ABC method with hematoxylin counterstain. Bar=25 μm.

Fig. 8. LDH in dog No. 4. A mirror image of immunohistochemical staining of the mild lesions in the liver. Positive expressions for anti-fibronectin antibody (Fig. 8a, arrow) and anti-E-cadherin antibody (Fig. 8b, arrow) can be seen simultaneously between hepatocytes. ABC method with hematoxylin counterstain. Bar=10 μm.

derived from stellate cells. The increased number of small bile ducts observed in hepatic fibrosis indicates repair from hepatic tissue injury involving the rupture of hepatic cords and the disappearance of hepatocytes [1].

Accumulation of copper and iron observed in the cytoplasm of hepatocytes in some cases has been described in LDH [32]. Although the cytoplasmic accumulation of copper indicates the possibility of copper-related liver disease, copper was not always observed in all samples and the central lobules [3, 8, 26, 27, 31].

LDH may develop in young litters, and the involvement of a genetic predisposition has been suspected, but the cause of LDH remains unknown [2, 13, 20, 32]. The present study suggests that expression of an extracellular matrix mainly consisting of fibronectin and laminin between the hepatocytes and proliferation of collagen fibers and reticular fibers have a major role in formation of the rupture of the hepatic cords and disappearance of the hepatocytes.

REFERENCES

1. Alvaro, D., Mancino, M. G., Glaser, S., Gaudio, E., Marzioni, M., Francis, H. and Alpini, G. 2007. Proliferating cholangiocytes: a neuroendocrine compartment in the diseased liver. *Gastroenterology* **132**: 415–431. [Medline] [CrossRef]
2. Bennett, A. M., Davies, J. D., Gaskell, C. J. and Lucke, V. M. 1983. Lobular dissecting hepatitis in the dog. *Vet. Pathol.* **20**: 179–188. [Medline]
3. Bexfield, N. H., Andres-Abdo, C., Scase, T. J., Constantino-Casas, F. and Watson, P. J. 2011. Chronic hepatitis in the English springer spaniel: clinical presentation, histological description and outcome. *Vet. Rec.* **169**: 415. [Medline] [CrossRef]
4. Bissell, D. M. and Mahaer, J. J. 1996. Hepatic fibrosis and cirrhosis. pp. 502–525. *In*: Hepatology: A Textbook of Liver Disease, 3rd ed. (Zakim, D. and Boyer T.D. eds.), Sauders, Philadelphia.
5. Chen, L. B., Burridge, K., Murray, A., Walsh, M. L., Copple, C. D., Bushnell, A., McDougall, J. K. and Gallimore, P. H. 1978. Modulation of cell surface glycocalyx: studies on large, external, transformation-sensitive protein. *Ann. N. Y. Acad. Sci.* **312**: 366–381. [Medline] [CrossRef]
6. Friedman, S. L. 2000. Molecular regulation of hepatic fibrosis: an integrated cellular response to tissue injury. *J. Biol. Chem.* **275**: 2247–2250. [Medline] [CrossRef]
7. Han, S. P. and Yap, A. S. 2012. The cytoskeleton and classical cadherin adhesions. *Subcell. Biochem.* **60**: 111–135. [Medline] [CrossRef]
8. Haywood, S., Rutgers, H. C. and Christian, M. K. 1988. Hepatitis and copper accumulation in Skye terriers. *Vet. Pathol.* **25**: 408–414. [Medline] [CrossRef]
9. Ihara, A., Koizumi, H., Hashizume, R. and Uchikoshi, T. 1996. Expression of epithelial cadherin and alpha- and beta-catenins in nontumoral livers and hepatocellular carcinomas. *Hepatology* **23**: 1441–1447. [Medline]
10. Ijzer, J., Roskams, T., Molenbeek, R. F., Ultee, T., Penning, L. C., Rothuizen, J. and van den Ingh, T. S. 2006. Morphological characterisation of portal myofibroblasts and hepatic stellate cells in the normal dog liver. *Comp. Hepatol.* **5**: 7. [Medline] [CrossRef]
11. Jan, R. and Susan, E. B. jenny, A. C., John M. C., Valeer, J. D., Viktor, S., David, C. T., Ted, S. G. A. M. van den Ingh., Tom, V. W. and Robert, J. W. 2006. WSAVA Liver Standardization Group: Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Disease. Saunders Elsevier, Edinburgh, UK.
12. Jay, H. L. 2010. SCHEUER'S Liver Biopsy Interpretation, 8th ed., Saunders, Phiadelphia.
13. Jensen, A. L. and Nielsen, O. L. 1991. Chronic hepatitis in three young standard poodles. *Zentralbl. Veterinärmed. A.* **38**: 194–197. [Medline] [CrossRef]
14. Kisseleva, T. and Brenner, D. A. 2006. Hepatic stellate cells and the reversal of fibrosis. *J. Gastroenterol. Hepatol.* **21** (*Suppl*) 3: S84-87.
15. Kozyraki, R., Scoazec, J. Y., Flejou, J. F., D'Errico, A., Bedossa, P., Terris, B., Fiorentino, M., Bringuier, A. F., Grigioni, W. F. and Feldmann, G. 1996. Expression of cadherins and alpha-catenin in primary epithelial tumors of the liver. *Gastroenterology* **110**: 1137–1149. [Medline] [CrossRef]
16. Kurkinen, M., Vaheri, A., Roberts, P. J. and Stenman, S. 1980. Sequential appearance of fibronectin and collagen in experimental granulation tissue. *Lab. Invest.* **43**: 47–51. [Medline]
17. Lefkowitch, J. H. 2005. Morphology of alcoholic liver disease. *Clin. Liver. Dis.* **9**: 37–53. [Medline] [CrossRef]
18. Lefkowitch, J. H. and Mendez, L. 1986. Morphologic features of hepatic injury in cardiac disease and shock. *J. Hepatol.* **2**: 313–327. [Medline] [CrossRef]
19. Lotersztajn, S., Julien, B., Teixeira-Clerc, F., Grenard, P. and Mallat, A. 2005. Hepatic fibrosis: molecular mechanisms and drug targets. *Annu. Rev. Pharmacol. Toxicol.* **45**: 605–628. [Medline] [CrossRef]
20. Margaret, J. Stalker. and M. A. Hayes. 2007. Liver and biliary system pp. 297–388. *In*: Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Vol. 2, 5th ed. (M, Grant. Maxie. ed.), Saunders, Philadelphia.
21. Mekonnen, G. A., Ijzer, J. and Nederbragt, H. 2007. Tenascin-C in chronic canine hepatitis: immunohistochemical localization and correlation with necro-inflammatory activity, fibrotic stage, and expression of alpha-smooth muscle actin, cytokeratin 7, and CD3+ cells. *Vet. Pathol.* **44**: 803–813. [Medline] [CrossRef]
22. Nouchi, T., Tanaka, Y., Tsukada, T., Sato, C. and Marumo, F. 1991. Appearance of alpha-smooth-muscle-actin-positive cells in hepatic fibrosis. *Liver* **11**: 100–105. [Medline]
23. Poldervaart, J. H., Favier, R. P., Penning, L. C., van den Ingh, T. S. and Rothuizen, J. 2009. Primary hepatitis in dogs: a retrospective review (2002–2006). *J. Vet. Intern. Med.* **23**: 72–80. [Medline] [CrossRef]
24. Rubio, C. A. 2000. Qualitative and quantitative differences between bile ducts in chronic hepatitis and in primary biliary cirrhosis. *J. Clin. Pathol.* **53**: 765–769. [Medline] [CrossRef]
25. Rutgers, H. C., Haywood, S. and Kelly, D. F. 1993. Idiopathic hepatic fibrosis in 15 dogs. *Vet. Rec.* **133**: 115–118. [Medline] [CrossRef]
26. Schultheiss, P. C., Bedwell, C. L., Hamar, D. W. and Fettman, M. J. 2002. Canine liver iron, copper, and zinc concentrations and association with histologic lesions. *J. Vet. Diagn. Invest.* **14**: 396–402. [Medline] [CrossRef]
27. Smedley, R., Mullaney, T. and Rumbeiha, W. 2009. Copper-associated hepatitis in Labrador Retrievers. *Vet. Pathol.* **46**: 484–490. [Medline] [CrossRef]
28. Speeti, M., Eriksson, J., Saari, S. and Westermarck, E. 1998. Leions of subclinical doberman hepatitis. *Vet. Pathol.* **35**: 361–369. [Medline] [CrossRef]
29. Takahara, T., Nakayama, Y., Itoh, H., Miyabayashi, C., Watanabe, A., Sasaki, H., Inoue, K., Muragaki, Y. and Ooshima, A. 1992. Extracellular matrix formation in piecemeal necrosis: im-

munoelectron microscopic study. *Liver* **12**: 368–380. [Medline]
30. Torbenson, M., Hart, J., Westerhoff, M., Azzam, R. K., Elgendi, A., Mziray-Andrew, H. C. and Kim, G. E. 2010. Neonatal giant cell hepatitis: histological and etiological findings. *Am. J. Surg. Pathol.* **34**: 1498–1503. [Medline] [CrossRef]
31. Twedt, D. C., Sternlieb, I. and Gilbertson, S. R. 1979. Clinical, morphologic, and chemical studies on copper toxicosis of Bedlington Terriers. *J. Am. Vet. Med. Assoc.* **175**: 269–275. [Medline]
32. van den Ingh, T. S. and Rothuizen, J. 1994. Lobular dissecting hepatitis in juvenile and young adult dogs. *J. Vet. Intern. Med.* **8**: 217–220. [Medline] [CrossRef]
33. Vaquer, P., Canet, R., Llompart, A., Riera, J., Obrador, A. and Gayá, J. 1994. Histological evolution of chronic hepatitis C. Factors related to progression. *Liver* **14**: 265–269. [Medline]
34. Wierzbicka-Patynowski, I. and Schwarzbauer, J. E. 2003. The ins and outs of fibronectin matrix assembly. *J. Cell. Sci.* **116**: 3269–3276. [Medline] [CrossRef]
35. Yano, M., Kumada, H., Kage, M., Ikeda, K., Shimamatsu, K., Inoue, O., Hashimoto, E., Lefkowitch, J. H., Ludwig, J. and Okuda, K. 1996. The long-term pathological evolution of chronic hepatitis C. *Hepatology* **23**: 1334–1340. [Medline] [CrossRef]