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Review
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Hepatic Fibrosis in Dogs

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Hepatic fibrosis is commonly diagnosed in dogs, often as a sequela to chronic hepatitis (CH). The development of fibrosis is a crucial event in the progression of hepatic disease that is of prognostic value. The pathophysiology of hepatic fibrosis in human patients and rodent models has been studied extensively. Although less is known about this process in dogs, evidence suggests that fibrogenic mechanisms are similar between species and that activation of hepatic stellate cells is a key step. Diagnosis and staging of hepatic fibrosis in dogs requires histopathological examination of a liver biopsy specimen. However, performing a liver biopsy is invasive and assessment of fibrotic stage is complicated by the absence of a universally accepted staging scheme in veterinary medicine. Serum biomarkers that can discriminate among different fibrosis stages are used in human patients, but such markers must be more completely evaluated in dogs before clinical use. When successful treatment of its underlying cause is feasible, reversal of hepatic fibrosis has been shown to be possible in rodent models and human patients. Reversal of fibrosis has not been well documented in dogs, but successful treatment of CH is possible. In human medicine, better understanding of the pathomechanisms of hepatic fibrosis is leading to the development of novel treatment strategies. In time, these may be applied to dogs. This article comparatively reviews the pathogenesis of hepatic fibrosis, its diagnosis, and its treatment in dogs.

Key words: Antifibrotic; Chronic hepatitis; Fibrogenesis; Hepatic stellate cell.

Hepatic fibrosis is characterized by progressive accumulation of fibrillary extracellular matrix (ECM) components in the liver. With persisting inflammation, the collagen profile of the liver changes, with increasing relative amounts of collagen types I and III accompanied by modification and cross-linking of ECM components.[1–3] In a fibrotic liver, the total collagen content is 3- to 10-fold higher than normal.[3] Development of hepatic fibrosis is an important step in the progression of many liver diseases, and it has been shown to have prognostic implications in human patients.[4,5] As is the case with human patients, hepatic fibrosis can contribute to the development of portal hypertension and acquired portosystemic collateral blood vessels in dogs.[6] The true prevalence of hepatic fibrosis in dogs is not known. However, in a study of 200 dogs undergoing necropsy for any reason, 12% were found to have histological changes consistent with chronic hepatitis (CH), a disease for which hepatic fibrosis is a defining feature.[7] Therefore, hepatic fibrosis is likely to be a common finding in dogs. Although the etiology of CH in dogs is different from the disease in humans, the histological appearance and progression of hepatic fibrosis is similar in both species.[8,9] Therefore, hepatic fibrosis is of importance to small animal veterinarians.

Abbreviations:

ACE	angiotensin-converting enzyme
ALT	alanine transaminase
CH	chronic hepatitis
CTGF	connective tissue growth factor
ECM	extracellular matrix
ET	endothelin
HSC(s)	hepatic stellate cell(s)
IL-10	interleukin-10
MMP	matrix metalloproteinase
NOX	nicotinamide adenine dinucleotide phosphate oxidase
PDGF	platelet-derived growth factor
RAS	renin angiotensin system
ROS	reactive oxygen species
TGFβ-1	transforming growth factor beta-1
TIMP	tissue inhibitor of metalloproteinase
αSMA	alpha-smooth muscle actin

A considerable amount of research into the pathogenesis of hepatic fibrosis has been performed by studying models of induced liver disease in rodents and naturally occurring liver disease in human patients. As this process is better understood, novel strategies for its treatment are being developed for use in human patients. Hepatic fibrosis in dogs with naturally occurring CH may prove to be a valuable nonrodent model to study the efficacy of these agents.

The aims of this article are to comparatively review the pathogenesis of hepatic fibrosis, its diagnosis, as well as existing and novel strategies for its treatment in dogs. The Medline database was searched for articles relating to hepatic fibrosis. Priority was given to articles published within the last 5 years and those addressing this process in dogs. The reference lists of the articles identified in this search were used to find other pertinent articles.

Pathogenesis

Hepatic Fibrosis and Myofibroblasts

Hepatic fibrosis is a wound healing response to chronic injury and inflammation in which there is an

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imbalance between ECM deposition and removal, leading to excess ECM accumulation.[10] In the normal liver, fibril-forming collagens (type I, type III, type V, and type XI collagens) can be found in the capsule, in large vessels, and the portal regions.[3] Only small amounts of type I and type III collagens are present in the subendothelial space. Additional components of the normal ECM include glycosaminoglycans and proteoglycans (hyaluronan, fibronectin, tenascin, or laminin) and other collagens (types VI, XIV, and XVIII).[3] In human patients and rodent models of liver disease, early deposition of ECM components takes place along the subendothelial space.[2,3] In humans, von Willebrand's factor expression is used as a marker of this process and expression of von Willebrand's factor, varying in distribution from diffuse to periportal, also was found in 69% of dogs with chronic liver disease.[11] The main mechanism of fibrogenesis is believed to be the activation of myofibroblast precursor cells, which results in the progressive deposition of ECM. The fibrotic liver contains increased amounts of fibrillary collagens (types I, III, and V), nonfibrillary collagens (types IV and VI), and glycosaminoglycans and proteoglycans (eg, fibronectin, tenascin and laminin, perlecan, decorin, aggrecan, and fibromodulin).[1,3] During ECM accumulation, cross-linking of matrix proteins occurs. In advanced fibrosis, this feature has been proposed to render the ECM more resistant to degradation.[12,13]

Several cells have been reported to be sources of ECM production during hepatic fibrosis: hepatic stellate cells (HSCs, Ito cells), liver resident fibroblasts (portal or centrilobular), epithelial cells that undergo epithelial-to-mesenchymal transition, bone marrow-derived fibrocytes, and smooth muscle cells that surround blood vessels.[1,14] In human patients, HSCs generally are believed to be the main source for myofibroblasts in CH, whereas portal fibroblasts are considered to play an important fibrogenic role in cholestatic liver disease.[15,16] Evidence exists that perisinusoidal HSCs also are involved in the pathogenesis of hepatic fibrosis in dogs.[17]

The transdifferentiation of quiescent HSCs to myofibroblasts is a multi-step process that involves cytokines, chemokines, growth factors, reactive oxygen species (ROS), and apoptotic bodies derived from hepatocytes (Fig 1).[3,18–20] In the early phase of activation, the HSC acquires responsiveness to cytokine stimuli by exposure to fibronectin or apoptotic bodies derived from damaged hepatocytes.[18] In the next phase, cytokines and growth factors, produced by neighboring cells such as liver resident macrophages (Kupffer cells), hepatocytes, endothelial cells, lymphocytes, and platelets, bind to specific receptors on the HSC membrane. Stimulation of intracellular signaling pathways results in altered gene expression and a phenotypic change in the HSC.[3] The last phase is the maintenance of activation, which involves paracrine and autocrine mechanisms.[18]

Myofibroblast Precursor Cells

Hepatic stellate cells (Fig 2) have a dendritic morphology and are located in the perisinusoidal space in close contact with hepatocytes and sinusoidal endothelial cells. They are the main location for vitamin A storage in the healthy liver. Upon activation, HSCs change from their quiescent vitamin A-rich state to a highly fibrogenic (myofibroblastic) phenotype. This is characterized by diminution of vitamin A droplets, enlargement of the rough endoplasmic reticulum, a ruffled nuclear membrane, and the appearance of contractile filaments.[1,3,18,21] They acquire increased ability for proliferation, chemotaxis, contractility, and ECM production. Activated HSCs can further promote their myofibroblastic phenotype and survival by paracrine and autocrine cytokine cross talk with surrounding cells (eg, the secretion of monocyte chemoattractant protein-1 and chemokine [C-C motif] ligand 5 or the release of tissue inhibitor of metalloproteinase-1 [TIMP-1]).[18]

The increased contractility of activated HSCs is due to the expression of the cytoskeletal protein and alpha-smooth muscle actin (αSMA).[3] Regulators of HSC contractility include endothelin-1 (ET-1), nitric oxide, and angiotensin II.[3,22] In human patients and rodent models of liver disease, the expression of αSMA is used as a marker of HSC activation. However, in the healthy canine liver, αSMA expression also can be found in perisinusoidal HSCs as well as in myofibroblasts and vascular smooth muscle cells within the portal tracts. In dogs with chronic liver disease, an increase in αSMA expression, in perisinusoidal spaces as well as in fibrotic septa, has been demonstrated. A positive correlation between αSMA expression and fibrosis stage was found in some studies, but not in others.[11,17,23,24] Figure 3 shows αSMA immunostaining in liver sections of a healthy dog without fibrosis (A) and a dog with CH and very marked fibrosis (B).

Portal fibroblasts are located in the mesenchyme of the portal tracts. They surround the hepatic bile ducts and are important for the integrity of the portal triads.[25] In biliary fibrosis of humans, portal fibroblasts seem to be the source of myofibroblasts in the portal area.[26] In studies of rodents with bile duct ligated-induced fibrosis, they contribute to >70% of matrix deposition during early injury.[16] However, their contribution during more advanced disease is still controversial, and newer studies suggest that HSCs are the major collagen producing cells in both biliary and nonbiliary fibrosis.[27] Nevertheless, portal fibroblasts seem to have a role in vascular remodeling during advanced fibrosis and cirrhosis.[28,29]

Epithelial-to-mesenchymal transition is a process whereby epithelial cells acquire mesenchymal features.[30] Epithelial-to-mesenchymal transition can occur through hedgehog or transforming growth factor beta-1 (TGFβ-1) signaling pathways, and both cholangiocytes and hepatocytes can undergo this process.[31–34] However, newer studies show that there is no evidence of cholangiocyte or hepatocyte epithelial-to-mesenchymal transition in mouse models of hepatic fibrosis.[35,36] Therefore, epithelial-to-mesenchymal transition is not thought to play a major role in the pathogenesis of hepatic fibrosis.

Table 1 gives a summary of important liver cell types and their roles in hepatic fibrosis.

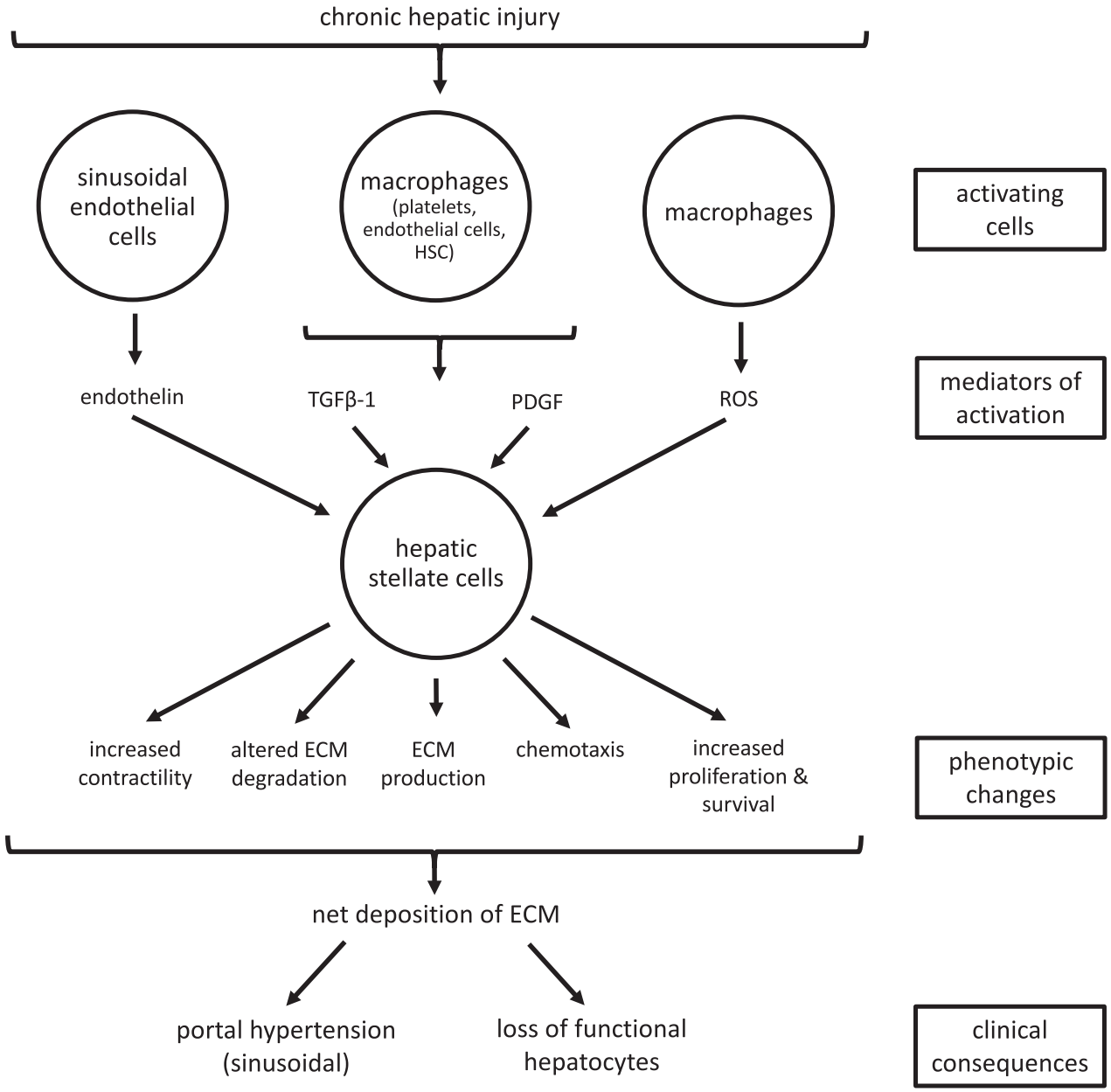

Fig 1. Role of hepatic stellate cells in hepatic fibrosis. The figure shows a simplified representation of the main factors involved in the activation of a hepatic stellate cell and the phenotypic changes after activation. TGFβ-1, transforming growth factor beta 1; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; ECM, extracellular matrix.

Mediators of Myofibroblast Precursor Cell Activation

Platelet-derived Growth Factor

Early ECM changes (eg, production of fibronectin by endothelial cells) and apoptotic bodies from damaged hepatocytes are initiators of HSC activation.[3,18] Hepatic stellate cells acquire responsiveness to further paracrine activation by neighboring cell types by the expression of certain cell surface receptors.[3,18] Platelet-derived growth factor (PDGF) is the most potent factor that induces proliferation of HSCs (Fig 1).[3,37,38] It is released by platelets, but also by sinusoidal endothelial cells, activated liver resident macrophages, and myofibroblasts during ongoing disease (Table 1).[1,3,39] Downstream signaling involves the renin angiotensin system (RAS)/extracellular signal-regulated kinase and phosphoinositol 3-kinase pathways, which enhance proliferation and migration and promote survival of the HSC.[39,40] Additionally, PDGF is a chemoattractant and guides HSCs to the site of injury.[3,41] Increased expression of PDGF mRNA has been demonstrated in liver from dogs with CH.[42]

Transforming Growth Factor Beta-1

Transforming growth factor beta-1 is considered the major factor accelerating hepatic fibrosis.[3,18,43] Hepatocytes, liver resident macrophages, sinusoidal endothelial cells, platelets, and activated HSCs produce this

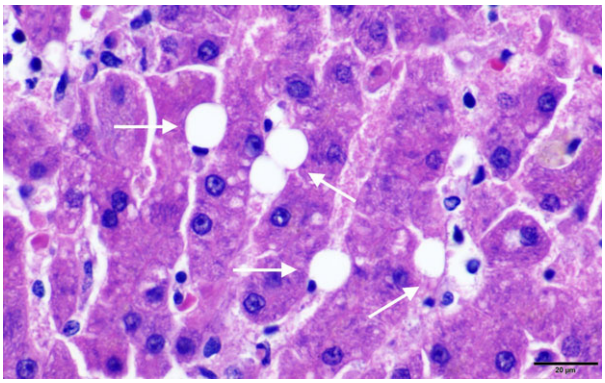

Fig 2. Hepatic stellate cells (Ito cell; hematoxylin and eosin). The stellate cells reside in the sinusoidal space and contain clear non-staining vacuoles (arrows). Courtesy of Randi Gold (Texas A&M University).

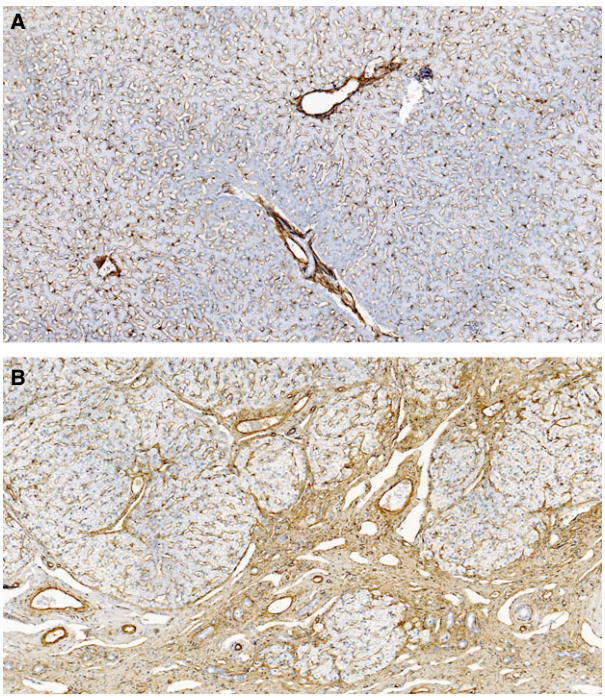

Fig 3. Alpha-smooth muscle actin expression in the canine liver. **A**: healthy dog (absent fibrosis) with mild staining around the portal tracts and perisinusoidal spaces (hepatic stellate cells). **B**: dog with chronic hepatitis (very marked fibrosis) with increased staining around the portal tracts, fibrotic septa, and perisinusoidal spaces.

cytokine (Table 1).[1] Downstream signaling involves phosphorylation and thus activation of the Smad2 and Smad3 proteins.[43] After forming complexes with Smad4 proteins, they are translocated into the nucleus where they interact directly on Smad-binding elements and alter gene expression, for example, by causing upregulation of collagen types I and III, and TIMP-1, and downregulation of MMPs.[14,39,43] The result is increased capability of HSCs to produce ECM components and inhibition of ECM removal (Fig 1). TGFβ-1 also seems to be an important mediator of lysyl oxidase expression.

Table 1. Hepatic cell types and their roles in fibrosis.

Cell Type	Role in Fibrosis
Hepatic stellate cells[3,18]	• Main producer of ECM in early and advanced hepatic fibrosis • Inhibition of ECM degradation • Maintenance of HSC survival • Production of mononuclear cell and neutrophil chemoattractants • Production of growth factors and cytokines
Portal fibroblasts[26,193]	• ECM producer in early cholestatic hepatic fibrosis • Vascular remodeling (activation of endothelial cells by vascular endothelial growth factor) • TIMP-1 and alpha-smooth muscle actin expression upon activation
Bone marrow-derived mesenchymal cells[194–196]	• Differentiation into collagen type I producing hepatic myofibroblasts
Hepatocytes[18,197,198]	• Activation of HSCs by production of fibrogenic lipid peroxides and apoptotic bodies
Cholangiocytes[18,199]	• Activation of portal fibroblasts by production of monocyte chemoattractant protein-1
Macrophages[18,200,201]	• Activation of HSCs (via TGFβ-1, reactive oxygen species, platelet-derived growth factor) • Involved in ECM remodeling and resolution of fibrosis
Sinusoidal endothelial cells[18,202]	• Activation of HSCs by production of fibronectin, endothelin-1, and nitric oxide
Natural killer cells[18,203]	• Involved in fibrosis resolution by TNF-induced ligand-mediated apoptosis of HSCs

HSC, hepatic stellate cell; ECM, extracellular matrix; TIMP-1, tissue inhibitor of metalloproteinase 1; TGFβ-1, transforming growth factor beta 1; TNF, tumor necrosis factor.

Lysyl oxidases are copper-dependent amine oxidases that are important for cross-linking of ECM proteins and further activation of myofibroblast precursor cells.[44] TGFβ-1 and phosphorylated Smad2/3 expression were shown to be upregulated in the liver of dogs with CH, lobular dissecting hepatitis, and cirrhosis.[45] Serum concentrations of TGFβ-1 were increased in dogs with moderate-to-severe hepatic fibrosis.[46] Increased TIMP-1 mRNA expression also has been demonstrated in liver from dogs with CH.[42,47]

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is another fibrogenic signal for HSCs. In addition, CTGF is involved in promoting the adhesion of HSCs to the ECM.[3,48] Expression of CTGF is increased in the fibrotic human liver and in animal models of hepatic fibrosis.[49] CTGF production is considered to be TGFβ-1/Smad2/3-dependent, but other induction ways have been reported (eg, ET-1, angiotensin II).[50–52] Because CTGF is recognized as a profibrogenic mediator, its inhibition is a

potential option for new antifibrotic therapies.[51,53] Additionally, CTGF has been evaluated as a noninvasive biomarker in human patients with CH.[49,54] Patients with advanced disease showed higher serum concentrations of CTGF, and these were linked to stage of fibrosis.[49,54] CTGF mRNA expression was shown to be upregulated in dogs with CH.[a]

Endothelin-1

Endothelin-1 is a vasoactive peptide produced by endothelial cells and by activated HSCs in cirrhotic livers of humans.[18,55] ET-1 acts through 2 receptors: ET-1 receptor type A and ET-1 receptor type B, which can be found on quiescent and activated HSCs.[55] This promoted proliferation, contraction, and the maintenance of the activated state.[18,56] In a recent study of 20 CH dogs, hepatic mRNA expression and plasma concentration of ET-1 were shown to be increased in dogs with CH, and a weak correlation between plasma concentration of ET-1 and splenic pulp pressure has been demonstrated, suggesting a possible role in the development of portal hypertension.[57]

Reactive Oxygen Species

Reactive oxygen species such as superoxide anion, hydrogen peroxide, or hydroxyl radicals are generated by the "respiratory burst" of phagocytic cells of the innate immune system as a first defense mechanism against invading pathogens.[58,59] Excessive production of ROS, however, leads to necrosis of surrounding cells and inflammation. The healthy liver contains several enzymatic and nonenzymatic antioxidant systems to detoxify excessive ROS. During liver disease, these antioxidant systems can become depleted, intensifying inflammation. ROS formation by phagocytic cells (eg, liver resident macrophages) is mainly the result of the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NOX) 2. Besides NOX2, 6 other members of the NOX family have been identified so far: NOX1, NOX3, NOX4, NOX5, dual oxidase 1, and dual oxidase 2. Some of these have been shown to play a role in liver fibrosis: HSCs and hepatocytes express NOX2, NOX1, NOX4, dual oxidase 1, and dual oxidase 2, which may play a role in the maintenance of the activated HSC state.[15,34,59,60] In human patients with cirrhosis, NOX1 and NOX4 proteins are increased.[61] Moreover, NOX4 expression was correlated with stage of fibrosis.[34,62] Knocking down NOX4 in mice has been shown to attenuate HSC activation and reverse the myofibrotic phenotype.[34,61] Although oxidative stress plays an important role in a variety of hepatobiliary diseases of dogs,[63] its role in fibrogenesis has not yet been studied.

Renin Angiotensin System

The local RAS may play a role in the pathogenesis of hepatic fibrosis.[64] A simplified view of the RAS is to describe it in 2 axes: the angiotensin-converting enzyme (ACE)/angiotensin II/angiotensin II receptor type I axis (pathway A), and the ACE/angiotensin (1-7)/angiotensin (1-7) receptor axis (pathway B).[65] The first step in both axes is the cleavage of angiotensin I to angiotensin II by ACE. In pathway A, angiotensin II binds to angiotensin II receptor type I, activating profibrotic mechanisms (eg, the induction of TGFβ-1).[66] In pathway B, a second enzyme (ACE2) cleaves angiotensin II, which results in the formation of angiotensin (1-7). The G protein-coupled Mas receptor has been recognized as the main receptor for angiotensin (1-7). Binding of angiotensin (1-7) to Mas activates a counter-regulatory pathway with antifibrotic, anti-inflammatory, and vasodilatory effects.[65] Cultured activated human HSCs have been shown to express RAS components and synthesize angiotensin II,[67] which has been proposed to be a trigger for the profibrogenic mediator CTGF.[52] In rodent models, treatment with angiotensin (1-7), and thus enhancement of the antifibrotic RAS pathway, has been shown to decrease hepatic fibrosis.[68,69] Furthermore, administration of ACE inhibitors or angiotensin receptor blockers, which leads to inhibition of the profibrotic RAS pathway, has been shown to attenuate hepatic fibrosis in animal models.[64,70,71] A RNA sequencing study did not identify upregulation of the RAS in dogs with CH compared to healthy dogs, but further studies are needed to evaluate whether it has a role or not.[a]

Reversibility of Hepatic Fibrosis

In the past, hepatic fibrosis was thought to be an irreversible process. However, recent studies in humans and rodent models have shown that resolution of fibrosis, even in more advanced disease, is possible.[3,10,12] In contrast, dense cirrhosis with intense ECM cross-linking, nodule formation, and low cell density in "fibrotic scars" still is considered irreversible.[3,12] Several studies have shown that fibrosis regression takes place after specific treatment and removal of the causal agent in human patients.[72] Because fibrosis now is recognized to be a continuous remodeling process, in which either net collagen deposition or resolution takes place,[73] inhibiting mediators of collagen deposition or enhancing mediators of ECM degradation may result in regression of fibrosis. The balance between MMPs and TIMPs seems to play an important role in this regulation.[10] Tissue inhibitor of matrix metalloproteinase-1 overexpression has been shown to accelerate fibrosis by inhibition of metalloproteinases, but also by inhibition of HSC apoptosis. Tissue inhibitor of matrix metalloproteinase-1 activity decreases quickly during fibrosis resolution.[74–78] Monocyte-derived macrophages with a proresolution phenotype seem to be important in the reversal of fibrosis, because they produce MMPs, which degrade the ECM or mediate apoptosis of myofibroblasts.[10,79,80] Additionally, natural killer cells can induce apoptosis of HSCs and thus contribute to the inhibition of fibrosis (Table 1). The main mechanism for the resolution of fibrosis seems to be the apoptosis or senescence of activated myofibroblasts (HSCs), which removes the source of TIMP-1, resulting in increased matrix metalloproteinase activity and the degradation of ECM.

Causes of Hepatic Fibrosis in Dogs

In our experience, the most common cause of hepatic fibrosis in dogs is CH, which is histologically

characterized by hepatocyte apoptosis or necrosis, inflammation, a mononuclear cell infiltrate, and fibrosis (Fig 4).[81] The fibrosis often co-localizes with necrosis and, especially for idiopathic CH, is initially present in the periportal zones of the liver.[81] With more advanced fibrosis, portal-portal or portal-central bridging fibrosis may develop with eventual formation of discrete nodules (Fig 4).[82] In a retrospective study, copper accumulation was the underlying cause in 36% of dogs with CH.[83] When copper accumulation is the primary cause of liver disease, it usually initially accumulates in the centrilobular zones. Centrilobular to bridging fibrosis was reported in Labrador retrievers with copper-associated CH.[84] In >60% of dogs with CH, no underlying cause can be found, and these patients are referred to as having idiopathic CH. Copper-associated[85] and idiopathic CH[86] are reviewed elsewhere. Granulomatous hepatitis is an uncommon form of CH in dogs[83] and may be the result of infectious diseases such as schistosomiasis,[87] histoplasmosis,[88] *Angiostrongylus vasorum* infection,[89] leishmaniasis,[90] or with lymphoma and histiocytosis.[88] Regardless of the underlying cause of CH, chronic inflammation leads to fibrosis.

Lobular dissecting hepatitis is a distinct type of CH that typically (but not always) occurs in young dogs at an average age of 2 years. It is not clear whether lobular dissecting hepatitis is a pattern of liver injury or a distinct disease process. It has been reported in a number of breeds, including the Standard Poodle, Rottweiler, German Shepherd, Golden Retriever, and American Cocker Spaniel. This disease has a rapid clinical course and a poor prognosis with a short survival time.[91] Lobular dissecting hepatitis is histologically characterized by a diffuse infiltrate of inflammatory cells and dissection of the lobular parenchyma with reticulin fibers (type III collagen) surrounding single or small groups of hepatocytes.[92] The cause of lobular dissecting hepatitis is not known. However, the abnormal ECM is mainly composed of laminin and fibronectin.[93]

Extrahepatic bile duct obstruction can result in fibrosis around biliary ducts, presumably because of proliferation of portal myofibroblasts. Causes for extrahepatic bile duct obstruction in dogs include pancreatic or biliary tumors, inflammation, or cholelithiasis.[94]

Cholangitis is less well described in dogs than in cats (but may be underdiagnosed)[95] and with chronicity can lead to biliary fibrosis. Biliary fibrosis can progress to portal-portal bridging fibrosis and biliary cirrhosis (when there is concurrent nodular regeneration).[8] Destructive cholangitis, characterized by loss of bile ducts with accompanying inflammation, also can lead to biliary fibrosis.[8,96] Idiosyncratic drug reactions have been implicated in causing this uncommon disease.[97]

Right-sided heart failure or obstruction of the cranial vena cava leads to increased central venous pressure and passive venous hepatic congestion. Liver perfusion is impaired, and ischemia and necrosis occur.[98] Chronically, this can lead to centrilobular fibrosis. A similar pattern can develop after toxin ingestion.[8]

Ductal plate abnormalities are a diverse group of developmental disorders of the biliary system that can be associated with increased hepatic ECM, portal hypertension, abdominal effusion, and hepatic encephalopathy. The most severe form is called congenital hepatic fibrosis and is characterized by portal-portal bridging fibrosis, multiple small bile ducts, and discontinuous biliary profiles. Ductal plate abnormalities, including congenital hepatic fibrosis, were reported in a series of 30 boxer dogs.[99] Six cases of congenital hepatic fibrosis (in a mixed breed dog and several other breeds) were reported in a separate series.[100,101] These conditions may be misdiagnosed as CH with secondary fibrosis.

Consequences of Hepatic Fibrosis

In humans, hepatic fibrosis is an important event in the progression of liver disease that can proceed to cirrhosis. Although there is no consistently used definition of cirrhosis in small animal medicine, it is generally considered the end stage of liver disease, where the deposited and remodeled ECM is connecting (bridging) and disrupting the functional architecture of the liver.[8,13] In CH, hepatocyte swelling, increased HSC contractility, fibrosis, and the formation of regenerative nodules impede portal blood flow, leading to hepatic (sinusoidal) portal hypertension.[102]

Portal hypertension in dogs is defined as a portal vein pressure >10 mmHg (normal values in anesthetized dog are 6–9 mmHg)[103,104] and is reviewed extensively elsewhere.[104] Direct measurement of portal vein pressure is an invasive technique, which requires direct puncture of the portal vein, and therefore is rarely performed in dogs. An indirect method that has been performed in veterinary patients is catheterization of the splenic pulp. However, values obtained from this measurement seem to be 0.5–1.5 mmHg higher than for the direct measurement of the portal vein pressure.[103,104] In cirrhotic human patients, a hepatic venous pressure gradient >5 mmHg is defined as portal hypertension and a value above 10 mmHg is correlated with development of clinical consequences, including life-threatening gastroesophageal varices.[104–107] In dogs, gastroesophageal varices have been described, but their clinical importance is unclear.[104,108] In addition, portal hypertension can contribute to the development of ascites and can lead to the opening of vestigial blood vessels that bypass the portal circulation (acquired portosystemic collaterals).[104] Ascites is the consequence of a combination of splanchnic arterial vasodilation, decreased cardiac output, and activation of the RAS, which leads to sodium and water retention.[105] In addition, high sinusoidal pressure drives fluid into the interstitial space.[104] Ascites has been shown to be a negative prognostic indicator in dogs with CH.[83,109] Portosystemic shunting often results in hepatic encephalopathy, where abnormal ammonia metabolism acts synergistically with a variety of other factors, such as neurosteroids and inflammatory mediators, to cause astrocyte swelling and neurological dysfunction.[110] Type C hepatic encephalopathy (as a complication of CH) was the

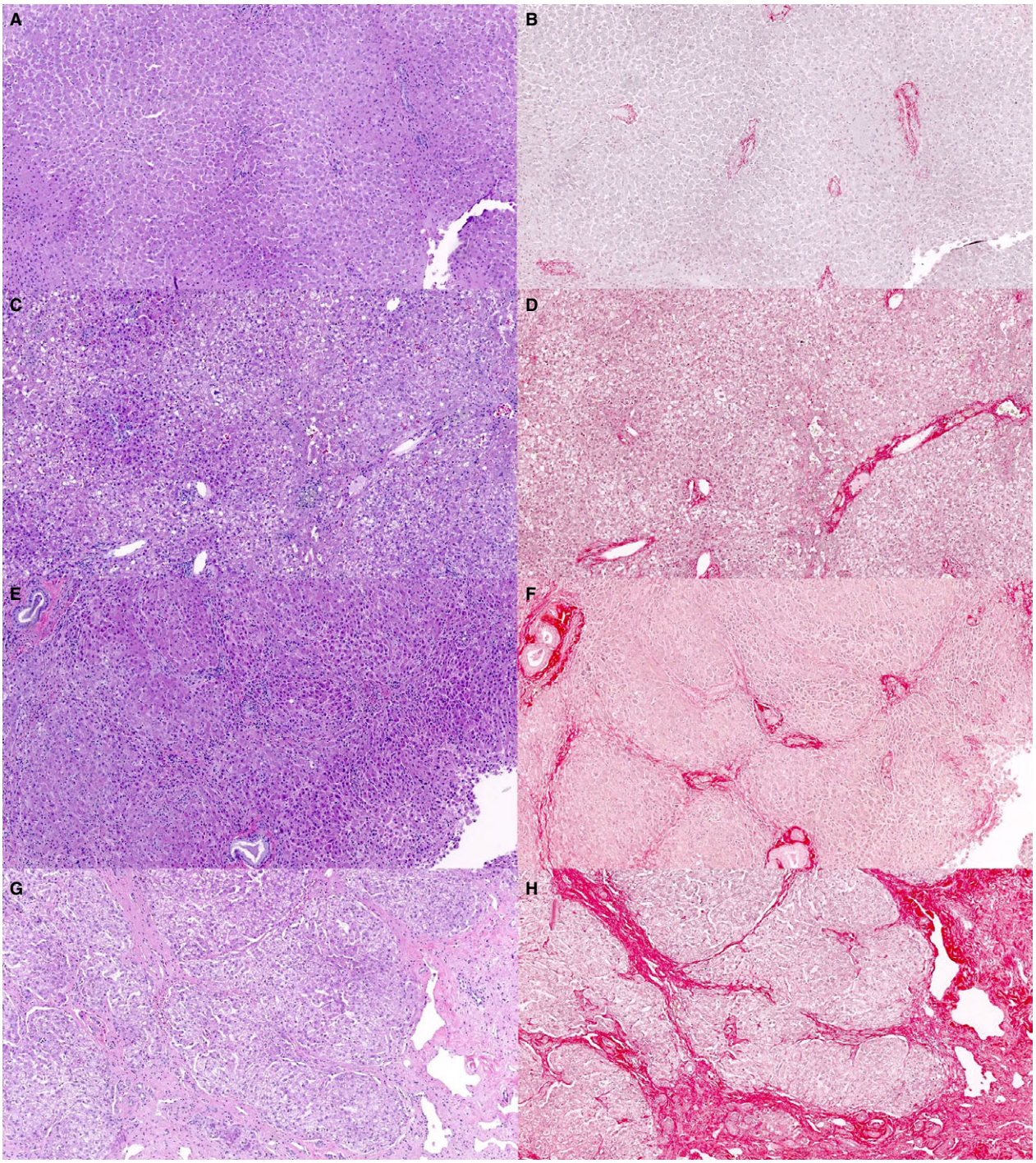

Fig 4. Hepatic fibrosis in dogs (hematoxylin and eosin: **A**, **C**, **E**, **G** and picrosirius red: **B**, **D**, **F**, **H**). Liver sections from dogs with various stages of fibrosis. Note the collagen fibers are more distinct when serial sections are stained with picrosirius red. **A**, **B**: absent/minimal fibrosis; **C**, **D**: moderate fibrosis with fibrous expansion of the portal tracts; **E**, **F**: marked fibrosis with portal-portal bridging; **G**, **H**: very marked fibrosis with discrete nodule formation.

second most common category of hepatic encephalopathy in dogs. Portal hypertensive gastropathy is common in humans and is characterized by mucosal and submucosal vascular ectasia without inflammation.[104,105] To our knowledge, the histological characteristics of the gastrointestinal tract of dogs with portal hypertension have not been well described. However, gastroduodenal ulceration has been reported to be a complication of various hepatic diseases in dogs.[111,112] Hypergastrinemia does not appear to be common in dogs with CH,[113] and thus, mechanisms other than gastric hyperacidity are likely to be important. Hepatorenal syndrome and

spontaneous bacterial peritonitis are other complications of portal hypertension in humans.[105] Hepatorenal syndrome has not been reported to occur secondary to spontaneous liver disease in dogs, and although dogs can develop spontaneous bacterial peritonitis,[114] an association with portal hypertension has not been found.[104] Treatment of portal hypertension in dogs is focused on managing its complications, for example, diuretic therapy and fluid therapy for ascites, administration of lactulose and antimicrobials PO for hepatic encephalopathy, and a sodium-restricted diet to decrease water retention. However, the optimal treatment would be to remove the underlying cause by resolving or decreasing hepatic fibrosis.

Progressive replacement of hepatocytes with fibrous tissue is another consequence of chronic hepatic disease, which can result in hepatic synthetic failure. If this develops, coagulopathies may occur.[115] Dogs with liver disease traditionally were thought to be hypocoagulable because they can have prolonged clotting times (prothrombin and activated partial thromboplastin times), hypofibrinogenemia, and mild-to-moderate thrombocytopenia.[83,116,117] Despite this, spontaneous bleeding is rare.[118] In a recent study, dogs with CH were found to have variable thromboelastography results.[118] In this study of 21 dogs, 5 were hypocoagulable, 9 were normocoagulable, and 7 were hypercoagulable. In a retrospective study of portal vein thrombosis in dogs, hepatic disease was a common concurrent condition, suggesting that hypercoagulability may have clinical consequences in these patients.[119] Interestingly, in humans with CH, thrombin stimulates fibrosis by protease-activated receptor signaling and by leading to microthrombi formation with subsequent local hypoxia.[120–122]

In humans with CH, advanced stages of hepatic fibrosis are associated with decreased survival times.[4,5] The prognostic implications of various stages of hepatic fibrosis (assigned by a histological scoring scheme) have not been well characterized in dogs with CH. However, those with ascites[83,109] or cirrhosis[123] have decreased survival times. In human patients and rodent models, even when hepatic fibrosis is advanced, it potentially can resolve if the underlying cause is successfully treated.[12]

Diagnosis of Hepatic Fibrosis

Histopathology

Although the presence of increased numbers of spindle cells and mast cells on cytological evaluation of the liver was reported to diagnose hepatic fibrosis with reasonable accuracy,[124] histopathologic examination of liver biopsy specimens is required for definitive diagnosis in dogs. However, liver biopsy is expensive and associated with a risk of hemorrhage and other complications (eg, postbiopsy pain, peritonitis, shock, or complications related to general anesthesia).[125,126] In small animal medicine, the following liver biopsy techniques are used: ultrasound-guided percutaneous needle biopsy, laparoscopic biopsy, and surgical biopsy during laparotomy. No matter which technique is used, only a small portion of the organ is sampled. Because many lesions (including fibrosis) are heterogeneously distributed throughout the hepatic parenchyma, liver biopsy is susceptible to sampling error.[127,128] Substantial variation can occur in the distribution of lesions among liver lobes, and therefore, it is important to collect samples from several lobes.[128] In dogs undergoing necropsy, histological diagnoses were in agreement with those from wedge samples in 66% of needle samples, 60% of cup samples, and 69% of punch samples, but these proportions were not significantly different from each other. The authors of this study concluded that the histopathologic interpretation of a liver biopsy specimen in the dog is unlikely to vary whether it contains at least 3–12 portal triads.[129] However, it is recommended that pathologists be presented with specimens containing at least 11 portal triads.[130,131] Evaluation of samples with fewer portal triads results in underestimation of fibrosis stage in human patients.[127,132]

In human patients, histological scoring schemes are widely used to provide a more objective assessment in patients with CH. They assess hepatic necrosis and inflammation (grade), which gives an indication of disease activity, and fibrosis (stage), which indicates the chronicity of the disease.[133] These schemes include the Ishak scheme[9] and the simpler METAVIR scheme.[134] In general, schemes with fewer levels are more clinically applicable because there usually is better interobserver agreement when using them.[133] Several studies have used a scoring scheme adapted from the Ishak scheme[82] to stage hepatic fibrosis in dogs with CH.[135,136] When 6 board-certified veterinary pathologists used this scheme to stage hepatic fibrosis from picrosirius red-stained sections in 50 dogs, their agreement was interpreted as only being fair.[137] However, it is our hope that the scheme can be refined to improve interobserver agreement.

Although fibrosis may be apparent on hematoxylin and eosin (H&E)-stained sections (Fig 4), other histological stains differentially stain collagen fibers and allow subjectively more accurate assessment of fibrosis. These include Masson's trichrome that stains type I collagen fibers, picrosirius red (Fig 4) that stains type I and III collagen fibers, and reticulin that stains reticulin fibers (type III collagen).[126] Interestingly, there was no difference in fibrosis scores assigned to serial sections of liver stained with H&E and picrosirius red.[137]

Computerized image analysis has been used to provide quantification of hepatic fibrosis in humans.[138,139] The histological section then is digitized, and image analysis software is used to calculate the fibrotic proportion of the section. This technique may allow a more objective quantification of hepatic fibrosis. In dogs, there was a positive correlation between the median fibrosis score assigned to each section and the fibrotic proportion.[140] This technique does not detect key features in the progression of fibrosis, such as the development of bridging fibrosis. Therefore, it should not be considered to be a direct replacement for the histologic assessment of fibrosis.[138]

Another innovative approach is to perform gene expression analysis on hepatic fine needle aspirates. Investigators showed upregulation of collagen and other fibrosis-related genes in livers of dogs with CH. The upregulation in gene expression for PDGF, TGFβ-1, TIMP-1, MMP2, and collagen type I and III, for example, showed a significant positive correlation with the severity of fibrosis.[42]

Serum Biomarkers

Because of the disadvantages of liver biopsy described above, serum markers of hepatic fibrosis have been developed for use in humans. In general, biomarkers of hepatic fibrosis can be divided into direct and indirect markers.[141] Direct markers are proteins and other molecules involved in the pathogenesis pathways of fibrosis (eg, TGFβ-1, tumor necrosis factor-α, angiotensin II) or those involved in the degradation or remodeling of the ECM (eg, hyaluronic acid, procollagen peptides, MMPs, TIMPs, chitinase-3-like protein 1). Using such markers, the diagnosis of advanced fibrosis stages is possible.[141,142] Hyaluronic acid appears to be the most promising,[141] and in a meta-analysis of hepatitis C patients, the sensitivity and specificity for diagnosing cirrhosis were 82 and 89%, respectively.[143] In human patients, these direct markers of fibrosis are not specific for hepatic fibrosis and may be increased when fibrosis of other organs is present.[144] Some of these serum markers have been evaluated in dogs. Serum hyaluronic acid concentration is increased in dogs with hepatic disease, especially cirrhosis, and therefore holds some promise as a biomarker.[145,146] Serum concentrations of TGFβ-1,[46] the 7S fragment of type IV collagen,[147] and procollagen type III N-terminal peptide[148] also have been found to be increased in dogs with hepatic fibrosis. Another study did not find a positive correlation between hepatic fibrosis and serum concentrations of hyaluronic acid, procollagen type III N-terminal peptide, or TIMP-1.[149] Even in the studies that did detect a difference between groups of dogs, concentrations from the advanced liver fibrosis groups either overlapped with those from dogs with milder fibrosis or there was only a separation of concentrations for dogs with the most advanced stage of hepatic fibrosis.

Indirect markers are measurement of variables that indicate liver damage, liver function impairment, and portal hypertension, such as liver enzyme activities, albumin and bilirubin concentration, and platelet counts or a combination of these. Two commonly used combinations in human medicine are the aspartate transaminase-to-platelet ratio index and FibroTest[b] (FibroSURE[c] in the United States). The latter combines age, sex, and results for serum haptoglobin, alpha 2-macroglobulin, apolipoprotein A1, gamma-glutamyltransferase, and bilirubin analyses into a single index.[150,151] Recently, an index for the assessment of hepatic fibrosis was developed for use in dogs.[d] This combines patient age, sex, and several biochemical variables in a proprietary algorithm to create a fibrosis score. In 1 study, this index had a negative predictive value for the diagnosis of moderate fibrosis of 90–100% and distinguished dogs with clinically relevant fibrosis with a positive predictive value of 90–100%.[e]

MicroRNAs are small noncoding RNAs that have a distinct expression profile depending on the liver disease.[152,153] Liver concentrations of hepatocyte-derived microRNAs seem to correlate with serum concentrations.[153,154] In human patients with hepatic fibrosis, the expression of miR-29 and miR-652 is decreased, whereas the expression of miR-571 is increased.[155,156] A recent study evaluated whether serum miRNA biomarkers hold promise for distinguishing among several hepatobiliary diseases in dogs. Two miRNAs were found to be increased in hepatobiliary disease: miR-200c in the hepatocellular carcinoma group (6 dogs) and miR-126 in the CH group (6 dogs).[157] Measurement of microRNAs in serum potentially could be used to assess hepatic fibrosis in dogs. However, further studies with greater sample sizes are needed to evaluate the sensitivity and specificity of these markers.

Elastography

Elastography is a medical imaging method to measure soft tissue elasticity (stiffness). Liver stiffness reflects the accumulation of ECM and has been shown to correlate with fibrosis stage. Transient elastography, real-time shear wave elastography, and acoustic radiation force impulse are new ultrasound-based, reliable, and reproducible methods to assess liver fibrosis in humans.[158,159] These techniques are noninvasive and allow a large area of the hepatic parenchyma to be sampled, thus decreasing sampling error. For example, transient elastography has been shown to measure a volume that is 100 times larger than a typical needle biopsy specimen.[160] Methods such as shear wave elastography and acoustic radiation force impulse also have been shown to be useful in patients with ascites or in obese patients.[160,161] Magnetic resonance elastography quantitatively measures acoustic shear waves in liver tissue. This method also detects early fibrosis stages with a much higher sensitivity and specificity (98 and 99%) than does transient elastography. A disadvantage of this method is higher cost compared to ultrasound-based techniques.[144] To our knowledge, the utility of elastography for the diagnosis of hepatic fibrosis in dogs has not been evaluated.

Current Treatment Options for Hepatic Fibrosis

The optimal way to stop the progression of or resolve hepatic fibrosis is to identify and treat its underlying cause. This approach is applicable in human medicine, where the underlying cause for the chronic hepatic disease usually is known.[3] For example, in human patients that were treated with direct-acting antiviral agents against hepatitis C, fibrosis resolved.[162]

Treatment of the Underlying Cause

In dogs with copper-associated CH, it often is possible to address the underlying cause of hepatic fibrosis

by a combination of chelation with D-penicillamine and feeding a low copper diet. In a study of 43 Labrador retrievers, despite improved copper scores, histologic fibrosis scores were not significantly different before and after treatment with D-penicillamine. However, the majority of dogs in this study did not have hepatic fibrosis at the time of diagnosis (median fibrosis score: 0 of 4 [absent fibrosis]), presumably because of early diagnosis.[136] This decreased the likelihood of detecting a treatment effect. In our experience, hepatic fibrosis can improve after chelation with D-penicillamine. Even if fibrosis does not resolve, chelation therapy is thought to be beneficial in these patients, although the criteria for which patients to chelate are somewhat controversial.[85]

Immunomodulatory Therapy

For dogs with idiopathic CH, treatment with prednisolone or another immunomodulatory drug often is initiated, especially if there is histologic evidence of active inflammation. Glucocorticoids bind to glucocorticoid receptors in the cytoplasm. These complexes are translocated to the nucleus, where they act on glucocorticoid response elements and initiate the transcription of anti-inflammatory and immunomodulatory protein coding genes (eg, IL-10).[163] Inflammatory genes are under transcriptional control of nuclear factor-kappa B and activator protein-1. Glucocorticoids inhibit the effects of these transcription factors.[164,165] The response of dogs with idiopathic CH to glucocorticoids seems to be quite variable. In a retrospective study of 20 dogs with idiopathic CH that were treated with prednisolone at a dosage of 1 mg/kg PO q24h for at least 6 weeks, fibrosis resolved in 5 dogs, improved in 4 dogs, and worsened in 5 dogs, but a statistically significant difference in histological fibrosis scores before and after treatment was not found.[166] However, in an older retrospective study that did not separate dogs with copper-associated CH from those with idiopathic CH, prednisolone treatment was associated with longer median survival times.[167] The effect of prednisolone on fibrosis was not evaluated in this study. In an uncontrolled study, 35 of 46 dogs (76%) with idiopathic CH achieved remission (normalization of serum ALT activity) after treatment with cyclosporine.[f] The efficacy of prednisolone and other immunomodulatory medications for the treatment of idiopathic CH in dogs needs to be further evaluated, ideally with randomized controlled clinical trials.

Antioxidant Treatment

Antioxidant drugs have a cytoprotective effect by scavenging ROS or increasing tissue concentrations of antioxidant enzymes or proteins such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, or metallothionein.[168] Oxidative stress occurs in a variety of liver diseases and contributes to the development of hepatic fibrosis in rodent models and humans. Therefore, although there is no direct evidence that antioxidants decrease hepatic fibrosis or improve clinical outcome for most hepatobiliary diseases in dogs, there is a rationale for using them.[169] Antioxidants commonly used to treat hepatobiliary disease in dogs include S-adenosylmethionine, vitamin E, and silymarin (milk thistle extract). Silymarin also may inhibit hepatic fibrosis by decreasing HSC DNA synthesis, proliferation and migration, and decreasing hepatic collagen expression as well having anti-inflammatory effects.[170–172] Ursodeoxycholic acid is a nontoxic bile acid that has choleretic effects. It displaces hydrophobic bile acids from the circulating pool and therefore is used to treat cholestatic liver disease in dogs.[173] There is evidence in other species that ursodeoxycholic acid also may have antiapoptotic properties.[174] The cytoprotective and antiapoptotic effects of ursodeoxycholic acid on hepatocytes have been proposed to be a result of unspecific binding to steroid receptors and the upregulation of cellular antioxidant systems, such as glutathione and superoxide dismutase.[175,176] Because hepatocyte-derived apoptotic bodies are a mediator of HSC activation, ursodeoxycholic acid seems to be a reasonable treatment to inhibit fibrogenesis. Ursodeoxycholic acid is used to treat primary biliary cirrhosis in humans, but resolution of fibrosis does not consistently occur and other treatments may be needed for advanced disease.[177] Case reports describe the use of ursodeoxycholic acid in dogs with hepatobiliary disease, but to our knowledge, there are no studies that critically evaluate its effectiveness in this species.[96,173,178,179]

Colchicine

Colchicine, a plant extract from *Colchicum autumnale* that acts as a microtubule assembly inhibitor, has been shown to decrease hepatic fibrosis in rodent models[180] and also in some human patients with hepatic fibrosis.[181] The suggested mechanism is the inhibition of microtubule-associated transport of procollagen and the enhancement of collagenase activity.[182,183] However, there is insufficient evidence to support its use in humans with liver fibrosis or cirrhosis and it is commonly associated with adverse effects.[182] A few case reports describe its use in dogs,[184,185] but because of the lack of proven efficacy and relatively common occurrence of adverse effects (gastrointestinal tract, central nervous system), we do not recommend its use in this species.[186]

Novel Treatment Strategies for Hepatic Fibrosis

There is considerable interest in developing novel treatments specifically aimed to manage hepatic fibrosis in humans. Extensive research performed to elucidate the pathogenesis of hepatic fibrosis supports the achievement of this goal. These therapeutic strategies can be divided into those that decrease myofibroblast activation, those that induce apoptosis of activated myofibroblasts, and those that induce ECM degradation and are reviewed elsewhere.[21]

Appealing drugs to evaluate for antifibrotic activity in dogs are those that block the RAS: ACE inhibitors

(eg, enalapril, benazepril) and angiotensin receptor blockers (eg, losartan, telmisartan). Targeting the RAS with ACE inhibitors, angiotensin receptor blockers, and angiotensin (1-7) receptor agonists has been shown to attenuate liver fibrosis in rodent models[64,187] and to downregulate fibrogenic and NADPH oxidase genes in human patients with chronic hepatitis C and fibrosis.[188] However, in a cohort of human patients with hepatitis C, ACE inhibitors and angiotensin receptor blockers were not shown to have a beneficial effect.[189] These drugs are used to treat proteinuria[190] and generally are well tolerated in dogs. However, involvement of the RAS in hepatic fibrosis has not yet been demonstrated in dogs and so clinical trials assessing efficacy of these drugs for this purpose are premature.

Pirfenidone is an antifibrotic drug that is licensed in Europe and Japan to treat idiopathic pulmonary fibrosis in humans. It acts by inhibiting nuclear factor-kappa B and its downstream profibrogenic mediators, including PDGF, TGFβ-1, and interferon alpha, resulting in decreased HSC activation and ECM deposition. It has been shown to decrease hepatic fibrosis and inflammation in humans with chronic hepatitis C when given for 2 years.[191] This drug has been reported to cause hepatoxicity, which may limit its use in patients with preexisting liver disease. Nevertheless, in the studies described above, adverse effects were minor. We are not aware of any reports of this drug being used in dogs with CH, although its pharmacokinetics were studied in healthy beagles.[192]

Conclusion

Hepatic fibrosis has been extensively studied in rodent models and in human patients. Its pathogenesis appears to be similar in dogs, but further research is needed to fully confirm or refute this supposition. Histologic assessment of a liver biopsy specimen is required for the diagnosis of hepatic fibrosis in dogs. The development and widespread institution of a practical, well-validated, clinically relevant scheme for the histologic scoring of hepatic fibrosis (and necroinflammatory activity) in dogs with CH would be useful in both clinical and research settings and should be a priority for the veterinary community. Investigators are attempting to develop serum markers of hepatic fibrosis for use in dogs, and some of these have been shown to have some limited discriminating ability. Elastography is a useful technique for the diagnosis of hepatic fibrosis in humans and is worthy of evaluation in dogs. Even if such noninvasive tests of hepatic fibrosis are successfully developed for use in dogs, in our opinion, they are unlikely to replace biopsy, because histologic evaluation and copper quantification play a large role in the diagnosis and subcategorization of liver disease in dogs. They could, however, prove useful for monitoring response to treatment in both clinical and research settings. In small animal medicine, fully evaluating the efficacy and optimal use of existing treatments for CH, such as glucocorticoids or cyclosporine, should be a priority. A deeper understanding of the pathogenesis of hepatic fibrosis in dogs eventually may lead to the development of new medications that specifically target this process.

Footnotes

[a] Eulenberg VM, Lawrence YA, Suchodolski JS, et al. High-throughput RNA sequencing and differential gene expression analysis in dogs with chronic hepatitis. J Vet Intern Med;31:1307. (abstract)

[b] FibroTest, BioPredictive, Paris, France

[c] FibroSure, LabCorp, Burlington, NC

[d] FibroVet, Echosens, Paris, France

[e] Lecoindre A, Lecoindre P, Chevallier M, et al. A new combination of blood parameters for accurate non-invasive diagnosis of liver fibrosis in dogs. J Vet Intern Med 2015;29:1197. (abstract)

[f] Cyclosporine in the treatment of canine chronic hepatitis. Ullal T, Twedt DC, Webster CL et al. Proceedings of the 2017 European College of Veterinary Internal Medicine Conference, St. Julian's, Malta, Sept 2017

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