Pathogenesis of Biliary Fibrosis

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Abstract

Chronic cholestatic liver diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are associated with active hepatic fibrogenesis, and, ultimately, to the development of cirrhosis. However, the precise relationship between cholestasis, in its broad meaning, and liver tissue fibrosis is still poorly defined. Fibrogenesis is currently viewed as a dynamic process that appears strictly related to the extent and duration of parenchymal injury. This relationship is clearly evident in the presence of reiterative hepatocellular necrosis due to viral infection or alcohol abuse. It appears that "pure" intralobular intrahepatic cholestasis secondary to biliary secretory failure of the hepatocyte, in absence of hepatocellular damage, lobular inflammation and bile duct damage and/or proliferation, is not associated with marked and/or progressive liver tissue fibrosis. In contrast, marked and progressive liver tissue fibrosis always follows liver diseases characterized by chronic inflammatory bile duct damage as seen in PBC and PSC or chronic mechanical obstruction of the biliary tree. Overall, the fibrogenic process in these clinical conditions appears to be related to a more complex interaction between immune/inflammatory mechanisms, cytokine networks and the derangement of the homeostasis between epithelial and mesenchymal cells. The elucidation of these mechanisms is indeed crucial for the identification of potential diagnostic and therapeutic targets.

Key Words: Cholestasis, liver fibrosis, PBC, PSC

Introduction

Chronic liver diseases affecting bile ducts, namely primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), are characterized by the development of the so-called biliary fibrosis. In both conditions, biliary fibrosis originates from portal tracts and tends to expand with a portal-to-portal pattern. In more advanced stages, fibrosis extends to the lobular area and eventually leads to the development of biliary-type cirrhosis. It is important to stress that, in both PBC and PSC, the fibrogenic process is consequent to the involvement of small bile ducts within portal areas. In PBC, bile ducts are infiltrated by immune/inflammatory cells causing structural damage (Figure 1-A), ductopenia (Figure 1-B) and peri-biliary stromal expansion. In PSC the early features are represented by a peri-biliary inflammatory reaction. The duct epithelium may show various degrees of atrophy and might even disappear, leaving a characteristic fibro-obliterative scar. Major bile ducts are often inflamed, ulcerated or dilated. These features are associated with concentric "onion-like" fibrosis (Figure 1-C) with a progressive obliteration of the bile ducts (Figure 1-D). In both PBC and PSC, the progressive structural damage of the intrahepatic biliary three leads to cholestasis.

The histopathological features typical of PBC, including the extent of fibrotic development, have been proposed for the disease staging according to Ludwig [**1**]: Stage I is characterised by bile duct injury and portal inflammation with minimal fibrosis (Figure 1-C); Stage II by expansion of portal tracts, periportal fibrosis and further inflammation; Stage III by fibrous septa, bridging fibrosis, and progressive ductopenia; Stage IV by cirrhosis (Figure 1-E, F). Recently, Nakanuma et al. [2], have proposed a new grading and staging system for PBC. In addition to the fibrosis, other two items (bile duct loss and deposition of orcein positive granules, which are characteristic of both PBC and PSC) constitute the basis of this new staging

system. Although the Ishak system (designed primarily to assess disease grade and/or stage in chronic hepatitis), the Ludwig and Nakannuma systems have been used to grade and stage histologic disease severity in PSC [3], at present there is no specific histologic scoring system for PSC.

Cholestasis and liver fibrogenesis

The progressive structural damage of the intrahepatic biliary three leads to cholestasis, which has been traditionally considered an important pro-fibrogenic factor. Indeed, bile salts are classically regarded as "cytotoxic" and their accumulation in liver tissue is thought to play a key role in determining liver cell necrosis and, then, in sustaining the development of liver fibrosis. Indeed, according to this paradigm, fibrogenesis in chronic cholestatic liver diseases could be seen as a classic chronic wound healing reaction similar to that observed in other fibrogenic chronic liver diseases. However, the concept of bile acid toxicity is at least in part based on studies performed on cultured hepatocytes by employing concentrations of bile salts in general largely exceeding the pathophysiological range. In addition, some studies have suggested that the accumulation of bile salts in liver tissue is responsible for a certain degree of hepatocellular apoptosis rather than necrosis [**4**].

The possibility that bile acids could directly affect the pro-fibrogenic phenotype of hepatic stellate cells (HSC) and other extracellular matrix (ECM) producing cells, thus leading to a mechanism of direct fibrogenesis, i.e. independent of the wound healing reaction, has been suggested by one study [**5**]. This study shows that bile acids bind to the epidermal growth factor receptor on HSC activating the protein kinase C/extracellular signal-regulated

kinase/p70S6K-dependent pathway thereby causing cell proliferation. However, this possibility remains controversial since other studies performed more recently and focused on nuclear receptors, FXR in particular, suggest that bile acids could modulate HSC activity by restoring peroxisome proliferator-activated receptor γ (PPARγ) signalling and by FXR-SHP-dependent inhibition of AP-1 signalling on downstream pro-fibrogenic targets [**6**].

Overall, it appears that “pure” intrahepatic cholestasis in the absence of hepatocellular damage, lobular inflammation and bile duct damage and/or proliferation, is not associated with marked and/or progressive liver tissue fibrosis. Although it could be argued that most causes of intrahepatic cholestasis are generally short-lasting (i.e. drug-induced), or not continuous (i.e. benign recurrent cholestasis), it is quite evident that a significant fibrogenic reaction occurs only when the pathological process involves the structure of the bile duct and the cellular phenotype of cholangiocytes.

Fibrogenic cells in biliary fibrosis

In conditions of chronic liver damage, as well as following prolonged culture on plastic, HSC undergo a process of activation from the quiescent "storing" phenotype to the highly proliferative "myofibroblast-like" phenotype. However, the simple paradigm that fibrosis and tissue scarring are only due to the activation and progressive hyperplasia of HSC does not fully satisfy the features of biliary fibrosis. Indeed, it has become increasingly clear that pro-fibrogenic myofibroblasts are a heterogeneous population of cells and do not derive entirely from quiescent HSC [**7,8**]. Portal fibroblasts (PF) located in the connective tissue around portal tracts appear to be of particular importance in ischemic and cholestatic liver disease and are different from HSC with regards to expression of marker proteins and response the pro-

fibrogenic and mitogenic stimuli [**9**]. Therefore, it is reasonable to suggest that PF could represent the "first responders" in biliary fibrosis, later to be superseded by activated HSC when the pathological process involves the lobular area [**10**]. Overall, PF constitute the mesenchymal component of the intraportal bile ducts and it is possible that they undergo a phenotypical modulation similar to that of HSC in situation characterised by a derangement of the epithelial-mesenchymal interface typical of PBC and PSC. A better understanding of the differences between HSC and PF as regards their relative contribution to fibrosis and their molecular regulation could have significant implications for the development of antifibrotic therapies and even more for the discovery of disease-specific biomarkers.

Role of bile acid and membrane lipid peroxidation in the fibrogenic evolution of chronic cholestatic diseases

While the direct pro-fibrogenic role of bile acids is uncertain, consolidated experimental evidence suggests the involvement of reactive oxygen species (ROS) and free radical reactions in the pathogenesis of cholestatic liver injury [**11-13**]. Along these lines, it is of particular relevance to demonstrate lipid peroxidation by-products in liver tissue obtained from patients with PBC and such with cholestasis induced by extrahepatic obstruction of the biliary tree [**14]**. As suggested by pioneer *in vitro* studies, ROS and reactive aldehydes originating from the lipid peroxidation process may exert direct fibrogenic effects on activated HSC [**15-17**]. It is therefore possible that bile salts may indirectly affect ECM production by inducing lipid peroxidation in liver tissue as a consequence of their excessive accumulation.

Liver tissue fibrosis occurring in chronic cholestatic diseases is characterised by different key features likely depending on the aetiology and the mechanisms of the disease. The main pathophysiological settings are illustrated in Figure 2.

Liver Fibrosis associated with bile duct damage/ductopenia. In PBC, interlobular bile ducts are surrounded and infiltrated by an inflammatory process occupying the portal tract. At later stages, inflammation extends to the lobule causing the formation of fibrous septa and finally the full blown picture of cirrhosis with nodular regeneration. The pattern of ECM accumulation in PBC is characterized by an increased expression of several collagenous and noncollagenous ECM components. Particularly, increased levels of mRNA encoding for collagens type I, III and IV have been demonstrated in mesenchymal cells of expanding portal tracts, fibrous septa and sinusoids of liver tissue obtained from patients with PBC [**18-19**]. The coexistence of a large inflammatory infiltrate with evident bile duct damage is consistent with the "inflammatory" nature of this disease: inflammation (lympho-mononuclear cells) likely represents the primary effector of bile duct injury. Infiltrating inflammatory cells, through the secretion of metalloproteinases (gelatinase), contribute to the degradation of the basal membrane like-ECM surrounding bile ducts which represent a potent stimulus for the activation of PF and HSC. In this context, the release of inflammatory growth factors and cytokines by inflammatory cells provide the "fuel" for the proliferation of ECM-producing cells thus leading to the deposition of excessive fibrillar ECM. In other words, in PBC liver tissue fibrosis is a direct consequence of the primary inflammatory disturbance. In more advanced stages of the disease, the extension of fibrogenic process involves damaged and non-damaged bile ducts as well as the periportal (or periseptal) sinusoidal system. This leads to marked cholestasis and progressive hepatocellular damage with further inflammatory infiltration, this time as a part of the tissue repair process leading to progressive scarring and nodular regeneration. Indeed, in the advanced stage of the disease, the mechanisms leading to scar tissue formation greatly overlap with those involved in the pathogenesis of cirrhosis due to chronic hepatocellular damage. Along these lines, it is of interest the predominant

expression of tissue inhibitor of metalloproteinase I (TIMP-1) mRNA when compared to that of matrix metalloproteinase I (collagenase I; MMP-1) in liver tissue obtained from patients with chronic cholestatic liver diseases [**20**].

These aspects, which appear pathognomonic for PBC, are likely to occur, at least in the early phases, in PSC, where there is a clear derangement of the homeostasis of the biliary epithelium. However, due to the usual late diagnosis of PSC, these features are overtaken by the typical concentric peribiliary fibrosis.

Liver fibrosis associated with bile duct proliferation. Cholestasis secondary to biliary tree obstruction, either intra- or extrahepatic, is usually associated with progressive liver fibrosis. Two key features appear to be relevant in this process: the proliferation of bile ducts and the direct or indirect contribution of bile salts to the fibrogenic process. The proliferation of bile ducts at the periphery of portal tracts that always follows biliary tree obstruction is attributed to proliferation of pre-existing ductules and/or differentiation of liver cell plates into biliary type structures, i.e. “ductular metaplasia” or more commonly “ductular reaction”. Ductular reaction is a dynamic, multicellular reparative system that includes mesenchymal and inflammatory cells accompanying the expansion of the epithelial cells lining the smallest ramifications of the biliary tree, in continuity with the canals of Hering, which is the niche where the hepatic progenitor cells (HPC) is thought to reside. Expansion of the HPC compartment is a compensatory mechanism of liver repair, activated when proliferative ability of hepatocytes is compromised because of a severe liver damage [**21,22**]. Ductular reaction accompanied changes in the surrounding mesenchymal stroma comprise, among others, activation and proliferation of HSC and active deposition of fibrillar ECM occur [**18,23**]. It is still unclear whether the changing epithelial phenotype directly induces an alteration of

the immediately adjacent mesenchymal cell biology and ECM, or whether a more fibrotic ECM induces the epithelial shift in differentiation. It is possible that this constitutes a two-way mechanism with relative predominance in different albeit similar conditions: biliary duct proliferation consequent to primary obstruction of the biliary tree is then followed by ECM changes (necessary to allow parenchymal "invasion" of newly formed bile duct), whereas bile duct neoformation present in fibrous septa of cirrhotic liver, independent of the etiology, is consequent to the remarkable changes in ECM composition observed in this condition. Regardless, it is evident that ductular cells play a key role in the re-arrangement of the epithelial-mesenchymal interface. Indeed, cholangiocytes express a "reactive" phenotype (ductular reactive cells, DRC) and acquire the ability to produce cytokines, chemokines, growth factors, and angiogenic factors and to express a rich repertoire of receptors typically displayed by ductal plate cells in the early stages of liver development [**24**]. These phenotypic changes typical of DRC lead to the establishment of powerful paracrine communications with multiple stromal cell types, including PF and HSC, inflammatory cells, and Kupffer and endothelial cells [**25**].

The possibility that DRC are able to directly contribute to fibrogenesis through epithelial-to-mesenchymal transition (EMT) has been suggested on the basis of the expression of mesenchymal cell markers. These phenotypical changes are sustained by the need to acquire intercellular independence, motility and achieve a high degree of cell plasticity. DRC move from the HPC niche towards the site of damage by interacting with other inflammatory and mesenchymal cell elements, thus building up the ductular reaction [**26**]. However, DRC lack the ability to actively secrete ECM components, such as type I or type IV collagen, and must cooperate with other effector cells by stimulating their pro-fibrotic activities [**27,28**].

Concentric "onion-like" peribiliary fibrosis in PSC. The development of liver fibrosis in PSC presents a very peculiar pattern characterised by concentric "onion-like" fibrosis with a progressive obliteration of the bile ducts. Interestingly, this type of fibrotic development suggests the contribution of a pro-fibrogenic focus originating from the cholangiocyte layer leading to the concentric recruitment and activation of pro-fibrogenic cells from the portal stroma and from the neighboring lobular area. It is of interest that the scarring typical of PSC has been compared to atherosclerosis [**29**] and the paracrine profibrogenic potential of activated cholangiocytes (there is not significant DRC in advanced PSC) compared to that of the activated endothelium in atherosclerosis. It is important to observe that a similar concentric "onion-like" fibrotic development is typical also of kidney and lung vascular strictures of scleroderma [**30**] where the pathogenesis involves the presence of peri-arterial vasculitis and a consequent ischemia of the vessel wall. Along these lines the potential role of vascular injury with ischemia of bile duct epithelium cells in the development of sclerosing cholangitis is supported by animal models of endothelial cell injury showing close morphological similarities with human PSC [**31**].

In conclusion, the available knowledge indicates that the development of biliary fibrosis is complex and its understanding requires the analysis of several coordinated cellular and biochemical mechanisms. The biology of cholangiocytes in health and disease is becoming more and more fascinating and the search of therapeutic targets and biomarkers even more challenging. Although, rapid progresses have been made in recent years, biliary fibrosis is definitely one of the areas of high demand in Hepatology.

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FIGURE LEGENDS

Figure 1. **Photomicrographs of characteristic histopathological features of chronic biliary disease**. **A**. Cytokeratin 7 immunostaining showing florid duct lesions in early PBC (Ludwig stage I): damaged CK7+ interlobular bile ducts (arrows) are infiltrated and surrounded by dense CK7- inflammatory cells. **B**. Cytokeratin 7 immunostaining showing ductopenia: no CK7+ native bile ducts are seen within the portal tract (PT); CK7 positivity highlights instead the marginal ductular reaction (large arrows), closely associated with numerous CK7+ periportal cells (small arrows) with an intermediate hepatobiliary phenotype. **C**. Early PSC showing an interlobular bile duct surrounded by a cuff of concentric fibrous tissue with "onion-skin" appearance (arrow). **D**. Late PSC showing a fibro-obliterative scar (FS) replacing the bile duct in a large portal tract. **E.** Biliary cirrhosis (Ludwig stage IV) showing regenerative nodules (RF) surrounded by fibrous septa (FS) with typical peripheral halo (arrows). F. Orcein stain showing biliary cirrhosis with characteristic prominent deposition of coarsely granular dark brown pigment representing copper-associated protein within the periseptal hepatocytes.

Figure 2. Main pathophysiological settings in the pathogenesis of biliary fibrosis. EMT: epithelia-mesenchymal transition; DRC: ductular reactive cells.