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Pathophysiology of liver fibrosis and the methodological barriers to the development of anti-fibrogenic agents

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ABSTRACT

Liver fibrosis and cirrhosis resulting from long-standing liver damage represents a major health care burden worldwide. To date, there is no anti-fibrogenic agent available, making liver transplantation the only curative treatment for decompensated cirrhotic liver disease. Liver fibrosis can result from different underlying chronic liver disease, such as chronic viral infection, excessive alcohol consumption, fatty liver disease or autoimmune liver diseases. It is becoming increasingly recognised that as a result from different pathogenic mechanisms liver fibrosis must be considered as many different diseases for which individual treatment strategies need to be developed. Moreover, the pathogenic changes of both liver architecture and vascularisation in cirrhotic livers, as well as the lack of "true-to-life" in vitro models have impeded the development of an effective anti-fibrogenic drug. Thus, in order to identify an efficient anti-fibrogenic compound, novel in-vitro models mimicking the interplay between pro-fibrogenic cell populations, immune cells and, importantly, the extracellular matrix need to be developed.

1. General aspects/introduction

Liver fibrosis is a chronic liver condition that develops as a result of a chronic wound healing response following long-standing liver injury. During hepatic fibrogenesis, the liver parenchyma undergoes fundamental remodelling characterized by progressive accumulation of fibrillar extracellular matrix (ECM) associated with nodular regeneration of the liver parenchyma. If untreated, liver fibrosis develops into cirrhosis and results in progressive loss of the normal liver function, which can lead to liver failure and death [1,2].

In Europe, liver cirrhosis is the fourth most common cause of death with a prevalence of 76.3 per 100,000 aged over 25 in 2001 in the United Kingdom, and is more likely to occur in men [3]. The development of liver cirrhosis is driven by several different risk factors, the frequency of which varies regionally. Thus, in western countries excessive alcohol consumption, hepatitis C virus (HCV) infection and fatty liver disease are most common, whereas chronic hepatitis B virus (HBV) infection is the main risk factor in Asia [4,5]. Furthermore, liver cirrhosis can evolve from a chronic immune-mediated damage in the context of autoimmune liver disease (AILD), such as primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) [6–8]. Other less common risk factors include Wilson's disease (copper overload), haemochromatosis (iron overload) and α1-antitrypsin deficiency, while some cases are cryptogenic [9,10].

Although liver fibrosis has historically been considered as one disease, it has become clear that the pathophysiology of liver cirrhosis varies depending on the underlying aetiology, which has not only changed the perception of liver cirrhosis, but also created new challenges in treating cirrhosis.

Preventing the progression to cirrhosis and even attempting a regression of the fibrogenic process is based on treating the underlying cause of disease, as the progression of liver fibrosis, and even cirrhosis, can be attenuated when the harmful agent or stimulus is removed [11,12]. Hence, antiviral treatment in HCV and HBV infection, immunosuppression in autoimmune hepatitis, abstinence from alcohol in alcoholic liver disease, weight loss and lifestyle change in fatty liver disease, venesection for haemochromatosis and copper chelating agents or zinc in Wilson's disease have been established as means to stabilize and possibly even reverse disease progression [10,13–15]. Nevertheless, the possibility of approaching established fibrosis and even cirrhosis with an effective anti-fibrotic strategy would immensely change the prognosis and the overall management of patients with advanced liver fibrosis and cirrhosis. For this reason, extensive investments have been made in the past 20–30 years for the development of anti-fibrotic drugs exploring different therapeutic approaches and routes of drug delivery.

Importantly, despite the deeper knowledge of the pathophysiology and advances in treating liver cirrhosis, this condition still represents the main indication for over 5000 liver transplantations in Europe per year [4,10], which is the only curative treatment for end-stage,

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decompensated liver cirrhosis at present. This is further aggravated by the fact that liver transplantation is not eligible to all cirrhotic patients and there is a severe lack of donor organs, stressing the need for novel and high impact therapeutic strategies. This article summarises the current knowledge on the mechanisms of liver fibrogenesis and attempts an analysis on the methodological barriers to the development of anti-fibrotic agents to be tested in preclinical studies.

2. Hepatic fibrogenesis: general mechanisms

The development of liver fibrosis and subsequent cirrhosis is driven by ongoing liver injury through multiple mechanisms, and can be considered as an excessive wound healing response fuelled by a pathogenic vicious circle of hepatocyte necrosis, inflammation and excessive ECM deposition [1,16]. Progression from healthy liver tissue to cirrhosis occurs after approximately 15–20 years of chronic hepatocellular damage [16], by when the cirrhotic liver contains up to six times more ECM than a normal liver [13]. Long-term chronic exposure to toxic agents such as hepatitis viruses, alcohol or bile acids can induce hepatocyte damage and apoptosis. In response, a repair reaction is triggered, which is characterized by ECM deposition and inflammation and results in liver fibrosis, when not only the exposure to toxic agents, but also the repair reaction is chronic [1]. The main ECM producing cell type in the liver are hepatic stellate cells (HSCs) which develop into hyper proliferative, ECM secreting myofibroblasts upon activation [1,17]. Although HSCs are the main source of myofibroblasts in the liver [18,19], other cell types contribute to the pool of fibrogenic myofibroblasts in liver disease. Portal myofibroblasts are located around bile ducts and play a role for the development of biliary fibrosis [20,21]. Moreover, bone marrow derived myofibroblasts are thought to contribute to the development of liver fibrosis [22], although their contribution in murine fibrosis has shown to be minimal [23].

Activation of HSCs is stimulated by damaged and apoptotic hepatocytes through two main routes: release of damage-associated reactive oxygen species (ROS) and other fibrogenic mediators [24,25] and recruitment of immune cells, which in turn mediate HSC activation and stimulate collagen secretion through release of cytokines and chemokines [26,27]. Following the initial activation of HSCs, cytokines secreted by HSCs in an autocrine manner, as well as immune cell derived cytokines, provide signals that maintain HSC activation and survival and the associated ECM deposition [17]. As a result, a vicious circle emerges, in which mutual stimulation between inflammatory and pro-fibrogenic cells drives hepatic fibrogenesis [28,29].

Besides affecting the quantity of ECM, liver fibrosis also results in changes in the quality and topographic distribution of different ECM components. In the healthy liver, the ECM in the space of Disse, the space between endothelial cells and hepatocytes, mainly consists of collagen IV and VI. During fibrosis development, ECM is replaced by fibrillary collagens, such as collagen I and III, as well as fibronectin, leading to so-called capillarization of the sinusoids [30]. When fibrosis is established and chronic liver diseases has evolved from fibrosis to cirrhosis, major structural changes including extensive capillarization of the liver sinusoids and formation of intrahepatic vascular shunts, as well as functional abnormalities, such as endothelial dysfunction, occur. Endothelial dysfunction results from decreased endothelial synthesis of vasodilators, such as nitric oxide, as well as increased secretion of vasoconstrictors, such as thromboxane A2 and endothelin [31,32].

Such structural and functional changes result in the development of portal hypertension (PH), the major complication of liver cirrhosis, which in turn gives rise to other clinically relevant complications of cirrhosis, including ascites, variceal bleeding, hepatic encephalopathy and renal failure [1,10]. Moreover, liver cirrhosis is the major risk factor for the development of hepatocellular carcinoma (HCC), as more than 80% of HCCs develop on a fibrotic or cirrhotic background [16,33]. The high risk of HCC development represents a major healthcare issue, as HCC is the fifth most common solid tumour and the second leading cause for cancer deaths worldwide with a rising incidence in Europe and the United States of America [34,35].

3. Cirrhosis or cirrhoses?

Liver fibrosis can result from many different conditions, in which liver damage shows characteristic patterns of injury [2]. Along these lines, liver fibrosis shows different morphological patterns according to the underlying aetiology. Thus, viral hepatitis is associated with interface hepatitis and portal-central vein bridging fibrosis, whereas alcoholic fibrosis and non-alcoholic steatohepatitis (NASH) are characterized by perisinusoidal or pericellular fibrosis showing a so-called chicken wire pattern. In biliary cirrhosis, bile duct and portal myofibroblast proliferation result in the formation of portal-portal fibrotic septa [2,36,37]. Moreover, some pathophysiological mechanisms contributing to hepatic fibrogenesis are distinct between different aetiologies (Fig. 1), whereas other mechanisms are shared across aetiologies, highlighting the need of individual concepts for the therapy of liver fibrosis and cirrhosis.

3.1. Alcoholic liver disease (ALD)

Resulting from long-standing excessive alcohol consumption, ALD can range from hepatic steatosis to acute alcoholic hepatitis to the development of liver fibrosis and cirrhosis on the basis of which HCC can develop [38]. Fibrosis development in ALD is driven by hepatocyte apoptosis and formation of ROS induced by the toxic effect of ethanol and its metabolite acetaldehyde [39,40]. Moreover, several mechanisms specific to excessive alcohol intake stimulate HSC activation and thereby drive ECM deposition and inflammation. Thus, acetaldehyde can directly activate HSCs and stimulate collagen I expression [41]. Furthermore, bacteria derived lipopolysaccharide (LPS), which is translocated from the gut to the liver due to increased gut permeability in ALD [42], can stimulate HSC activation directly via Toll-like-receptor (TLR) 4 ligation [43,44]. Along these lines, LPS acts indirectly on HSC activation via stimulation of Kupffer cells, which in turn secrete HSC activating cytokines [45]. Ethanol furthermore inhibits the function of natural killer (NK) cells, which can contribute to fibrosis resolution through IFNγ secretion and killing of activated HSCs, thereby suppressing the anti-fibrotic effects of NK cells [46].

3.2. NAFLD/NASH

Non-alcoholic fatty liver disease (NAFLD) and its more severe form NASH occur in the context of the metabolic syndrome and are characterized by hepatic steatosis which can lead to the development of fibrosis and cirrhosis over time [47]. The formation of ROS and resulting oxidative stress induced by a mitochondrial overflow of free fatty acids is thought to be a critical factor in fibrosis development in NAFLD/NASH though several pathways. Oxidative stress hinders the replication of mature hepatocytes, thus leading to an accumulation of immature progenitor cells [48] originating from the Canals of Hering. Proliferation of such progenitor cells results in the formation of small ductules. This so-called ductular reaction has been linked to the development of fibrosis in NAFLD/NASH as the newly formed ductular cells secrete pro-inflammatory cytokines [49], and epithelial-mesenchymal transition of cholangiocytes to fibrogenic myofibroblasts can occur [50]. Moreover, hepatic steatosis is accompanied by an inflammatory reaction with elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα, mediated by activation of the Ikk-β/NF-κB signalling pathway in the liver [51]. Free fatty acids can directly activate the Ikk-β/NF-κB signalling pathway in hepatocytes [52] and it has been shown that cytokine production by hepatocytes is a critical factor for the progression of steatosis to NASH [53].

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