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REVIEW ARTICLE

New approaches for fibrosis regression in alcoholic cirrhosis

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Abstract Liver fibrosis is a dynamic process of fibrinogenesis and fibrinolysis. It is sequelae of recurrent injury and inflammation to the liver. Only recently has there been significant progress in understanding the pathophysiology behind liver fibrosis. This has allowed for the development of identifiable targets for potential therapies. In this article we will discuss the underlying general cellular mechanisms that play a key role in the pathway of fibrinogenesis and fibrinolysis and then focus on the mechanisms that are key in alcohol-induced liver fibrosis. Challenges in formulating potential fibrosis therapies as well as current potential targets for liver fibrosis will be reviewed as well.

Keywords Fibrosis · Liver · Inflammatory

Development of fibrosis

There are variations of the inflammatory response to liver injury due to underlying genetic variability and difference in environmental exposures [1]. Thus as it is important to block the downstream effect of extracellular matrix deposition by the hepatic stellate cells, it is also equally important to remove the trigger of liver inflammation and injury [1].

The cellular pathways that are involved in fibrinogenesis cause both qualitative and quantitative changes in the extracellular matrix [1]. Losses of hepatocytes, vascular changes, or fluctuations within the liver cell population are all possible changes that can occur [1]. Among the multitude of cells that play a significant role in fibrosis, activated hepatic stellate cells (HSC) are key in the formation of myofibroblasts (MF) and deposition of extracellular matrix proteins [2, 3]. Their interaction with immunologic cells—such as macrophages and natural killer cells—can lead to either fibrinogenesis or fibrinolysis [4].

The liver has a robust adaptive immune system that involves multiple immunologic cells and cytokine production [5]. Each of the immune cell types and their respective related cytokines have all been evaluated as potential targets for anti-fibrolytic therapy [1].

Macrophages

Kupffer cells are the macrophages that line the walls of the sinusoids and are a part of reticuloendothelial system [6]. When liver injury occurs there is activation of the $Ly6C^{hi}$ monocytes [7]. This causes a release of potent cytokines such as TNFα, IL 1b, TGFβ1 and PDGF BB and chemokines CCL2, −3, −5, −7, and −8 [4]. These cytokines and chemokines then trigger myofibroblasts formation and thus fibrinogenesis [4]. However macrophages can also demonstrate a different phenotypic expression promoting fibrinolysis. Sometimes after removal of the inciting insult they can switch to $CD11b^{hi}/F4/80^{In}$ $tLY6C^{low}$, which enhances matrix metalloproteinases to degrade excess extracellular matrix and induce apoptosis of HSC and MF [4]. Potential therapies aimed at blocking the cytokines such as TGF1β with an antibody (lerdelimumab) or with blocking peptides or blocking CCR5, CXCR4, CXCR have shown anti-fibrotic effects [8–10]. The difficulty is that many of

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these pathways are commonly shared in angiogenesis and cell differentiation and blocking them may lead to deleterious side effects [11].

T-helper 1 cells and T-helper 2 cells

T helper cells also secrete cytokines that can promote fibrinogenesis or anti-fibrosis [12, 13]. TH1 cells secrete IFN1 and IL12 cytokines that are fibrolytic whereas TH2 cells secrete IL4 and IL13 that are fibrogenic [13, 14]. Efforts to block these cytokine pathways have been difficult since they play multiple different roles in the liver (Fig. 1).

Natural killer cells (NK)

Natural killer cells also play a large role in fibrinogenesis [15]. Natural killer cells can induce apoptosis in HSC cells by inducing cell cycle arrest, whereas natural killer T cells can induce fibrinogenesis through an unclear pathway [15]. As discussed above, blocking these cells or promoting them can be difficult given their multiple functions throughout the body.

Liver sinusoidal endothelial cells (LSEC)

LSEC are in close proximity to HSC cells and are thought to regulate portions of intrahepatic portal hypertension via release of cytokines that can induce vasoconstriction and decrease expression of vasodilators [16]. It can contribute to fibronectin and collagen I and IV deposition into the extracellular matrix via activation of chemokine receptors CXCR7 and CXCR4 [17, 18]. Interestingly, when an acute insult occurs the CXCR7 activates transcription factor Id1 which is fibrolytic [19]. When chronic injury occurs this pathway is dampened and there is upregulation of CXCR4 which is profibrotic [19]. Potential targets that have been identified at LSEC cells are inhibitors of VEGF, sorafenib and sutinib [20, 21].

Platelets

Platelets have also been shown to demonstrate fibrinogenic activity [22]. Activated platelets can lead to the release of PDGFBB and TGF B1, which have been shown to induce fibrosis [22, 23]. Certain studies have focused on the role of antibodies in blocking PDGF BB release vs. the use of aspirin to prevent platelet activation to prevent release of these fibrinogenic cytokines [1, 24].

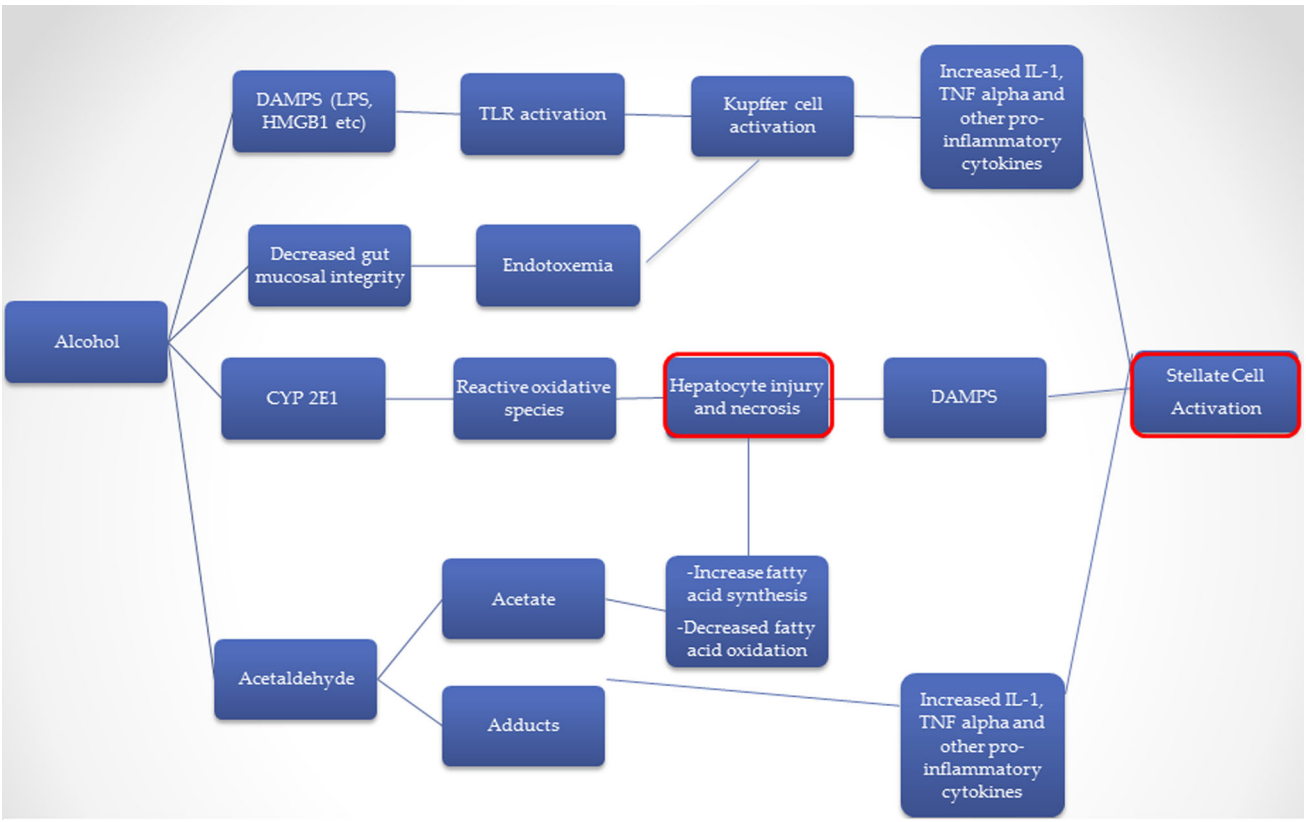

Fig. 1 A number of pathways are activated by alcohol and converge on activation of hepatic stellate cells. These present a number of therapeutic opportunities. The two in *red* are thought to be key steps in the initiation of fibrogenesis

Extracellular matrix

The extracellular matrix is composed notably of collagen types I, III, V, VI, basement membrane types IV, XV, XVIII, XIX, glycoproteins, and proteoglycans [25, 26]. Increase in collagen type I and III, laminins and proteoglycans can lead to crosslinking between collagen [26]. When the crosslinking occurs it can lead to resistance to fibrinolysis. Matrix metalloproteinases (MMP), which help break down the crosslinks between the collagen, can be inhibited by TIMPs [27]. This makes TIMPs and MMPs attractive targets for anti-fibrotic therapy. Recently the family of lysyl oxidase has been of major interest in the study of fibrosis. It is a class of enzymes that crosslink fibrillary collagen at the non-triple helical ends and has been seen to be upregulated in Wilson's disease, primary biliary cirrhosis, and pulmonary fibrosis [28, 29]. Simtuzumab, an antibody that blocks LOXL2, is currently being studied in liver fibrosis in patients with primary biliary cirrhosis or non-alcoholic steatohepatitis [30].

Alcohol-induced liver fibrosis

Approximately 48 % of cirrhosis deaths in 2007 have been linked to heavy alcohol intake [31, 32]. Alcohol can cause several stage of liver injury from simple steatosis to steatohepatitis to cirrhosis and has been shown to accelerate liver fibrosis in patients with already underlying liver disease [32]. Alcohol can act as a modifying factor for patients with already underlying liver disease by possibly affecting viral replications, potentiating virus cytotoxicity, increasing oxidative stress and inflammation [32]. In fact, individuals who have obesity and excessive alcohol intake can have a two-fold increase risk of developing cirrhosis whereas those with hereditary hemochromatosis can have a nine-fold increase risk of developing cirrhosis with heavy alcohol use [33, 34].

Although there have been studies that show that consumption of equal to or more than 60 g of alcohol per day in men and 40 g in women can lead to significant fibrosis, there are new studies that show that fibrosis can occur even at 25 g/day in certain individuals [32, 35]. Variability in histological response to alcohol exposure suggests that there are genetic differences and environmental factors that play a role in fibrosis [36]. For example, genetic variations in the production of alcohol's toxic metabolite acetaldehyde have been shown as a possible explanation for varying degrees of injuries with the same amount of alcohol exposure [37]. Similarly individuals that have genetic polymorphisms that decrease expression of superdioxide dismutase and gluthathione transferase have also been shown to be at risk of severe alcoholic liver disease [38, 39]. It has also been noted that the host's oral and intestinal microbiota contribute significantly to metabolizing alcohol [40]. Chronic alcohol intake can induce intestinal bacterial overgrowth leading to local inflammation and increase in gut permeability [41]. Chronic alcohol intake can also kill NK cells that induce HSC apoptosis and thus decreasing fibrosis [15]. The translocation of gut bacteria can then upregulate CD14 and TLR and induce lipoprotein saccharide exposure causing inflammation [42]. Due to the different intestinal microbiota in individuals the metabolism of alcohol varies, leading to either a more or less inflammation response [42].

The cellular mechanism of alcohol injury and fibrosis involves multiple pathways that trigger an inflammatory response. The toxic metabolite acetaldehyde and its adducts–malondialdehyde, 4-hydroxynonenal and malondialdehyde–acetaladehyde can promote fibrinogenesis by directly acting on HSC cells and stimulating collagen I production [43–45]. Chronic inflammation and fibrosis can also occur from the generation of reactive oxidative species and lipid peroxidation [46]. In addition the release of cytokines such as IRAK-M, IL1-β, IL-1 receptor antagonists, IL-2, IL-6 and IL-10, TNF alpha, TGF beta, CCL2 have all been shown to trigger an inflammatory response [47–49]. Studies thus far on potential target for therapy include potential chemokines such as PPAR α which regulate lipid homeostasis and inflammation response [46]. It was shown in a mouse model study that using PPAR α agonist downregulated TNFα, TGFβ1 and a chemoattractant molecule OPN which then activates PI3 K and downregulates MMP2 and MMP9 that stimulate fibrinogenesis [46]. In another mouse model study IL22 expression was noted to reduce liver fibrosis by activating the STAT3-transcription three dependent mechanism that induces HSC sensencence [50].

Alcohol has also been shown to impact gut permeability and lipopolysaccharide (LPS) levels in the blood [51]. Alcohol increases gut permeability by affecting the tight junction proteins via direct effects or acetaldehyde mediated inhibition of tight junction protein expression [51]. In addition to affecting the tight junctions, alcohol also effects the composition of the gut microbiome [51]. The combination of increased gut permeability and change in gut microbiome has been associated with increased LPS and pro-inflammatory cascade [51]. This upregulation of inflammatory cascade has been associated with steatosis and inflammation, and systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis [51].

These mechanistic insights have resulted in the initiation of some novel therapeutic strategies. The concept behind these is that the inflammation and liver injury seen in alcoholic hepatitis is the key driver to alcohol driven liver

fibrosis. The increase in gut permeability with translocation of LPS is thought to be a key factor and a clinical trial of hyperimmune bovine colostrum (IMM 124-E) with LPS immunoglobulin G (IgG) antibodies is currently underway [52]. Reduction in intestinal bacterial translocation by modifying the microbiome is being attempted with Lactowell, a white cheese based product from which whey has been removed, with early results showing a trend towards improvement in ALT and LPS levels [53]. The microbiome is also being manipulated by using *Lactobacillus rhamnosus* and a number of antibiotics with and without steroids [54–57]. A complementary approach is to supplement zinc with the aim of decreasing intestinal permeability along with inhibition of inflammatory pathways with pentoxifylline and an IL-1 receptor antagonist [58].

Increased oxidant stress is present in all forms of inflammatory liver diseases, and anti-oxidant approaches are an attractive strategy, and have been added to the standard prednisone therapy. *N*-acetylcysteine, when added to a full 28-day prednisone course, showed improvement in survival at the 1-month time point [59]. Metadoxine, another antioxidant, was beneficial in combination with prednisolone or with pentoxyfyilline, and improved survival at 3 and 6 months [60]. Metadoxine is being studied in further studies which are ongoing [61, 62].

Challenges in the study of fibrosis

In order to study the pathogenesis of fibrosis and potential target for therapies an adequate model of fibrosis has to be used. Unfortunately there is no perfect model as in vivo models with mice cannot simulate all the different etiologies of liver disease. Fibrosis is usually a disease of older age and the simulated liver fibrosis mouse models are usually in young mice [1]. Other models that have been used include precision cut tissue slices [1, 63]. The optimal model should be a liver that has intermediate level of fibrosis with the possibility of reversal so as to evaluate anti-fibrolytic activity [1]. In addition the model should account for the heterogeneity of the individuals affected [1]. Evaluating fibrosis in an individual with active alcoholism is also challenging due to poor compliance with follow-up or confounding factors such as patients decreasing their alcohol intake after being enrolled into a trial [32].

Potential target therapies for liver fibrosis

Fortunately with the rise of the recent anti-viral therapies for hepatitis C and suppressive viral therapies for hepatitis B, the burden of fibrosis from viral hepatitis will hopefully be decreasing. Currently, there are multiple target therapies that are being studied for diseases with fibrosis-mostly in NASH and viral hepatitis. Potential targets for anti-fibrotic therapies in NASH have included FXR agonist Obetacholic acid, PPAR agonists—GFT-505, bile acid conjugate-aramchol, caspase inhibitors emricacsan, and GLP agonists—liraglutide, thiazolididinediones. Other new anti-fibrotic medications recently approved by the FDA for idiopathic pulmonary fibrosis-simtuzumab, nintedanib and pirfenidone are exciting therapies that can be considered for patients with liver fibrosis [1]. As there are many potential therapies for fibrosis that act on different targets for fibrosis, combination therapies may be of benefit. Unfortunately there are few trials evaluating the pathogenesis of fibrosis in alcoholic liver disease due to issues of non-compliance with medications, visits and recidivism [32]. Potential targets to evaluate for liver fibrosis from alcohol injury include restoration of the NK cell, increase IL-22 expression and potentially target the gut bacteria and gut epithelial integrity [32]. Nonetheless there is still much to be studied before a potential targeted therapy can be found.

Treatment of alcohol abuse

Alcohol addiction remains a substantial portion of treatment for patients with alcoholic hepatitis and cirrhosis [64]. Unfortunately a recent health care audit showed that approximately only 15–25 % received the recommended care for alcohol addiction [64]. Enrollment in a substance abuse program has shown to improve alcohol cessation and maintain abstinence, but recidivism can be as high as 70 %. There is no single agreed upon approach, but in general a team and multifaceted strategy seems to work the best. Current medications that are approved for alcohol addiction include naltrexone, disulfram, and acamprosate [65]. Off label use of gabapentin, baclofen and topiramate has also been used to treat alcohol addiction [65].

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