Current and Future Anti-fibrotic Therapies for Chronic Liver Disease

Don C. Rockey, M.D.
From the Division of Digestive and Liver Diseases, The University of Texas, Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390, USA

Abstract

Advances in the understanding of the cellular and molecular basis of hepatic fibrogenesis over the past 2 decades have allowed the emergence of a field dedicated to anti-fibrotic therapy. The liver responds to injury by wound healing and subsequently, fibrosis. This response is after essentially all kinds of injury (whether virus, alcohol, or other) and ultimately leads to cirrhosis in some patients. The observation that any of several types of liver diseases and their injury result in cirrhosis suggests a common pathogenesis. It is now recognized that a population or populations of effector cells play a critical role in the fibrogenic process. A classic effector cell, the hepatic stellate cell, is one of the most important fibrogenic cells in the liver. This cell undergoes a transformation during injury, termed “activation”. The activation process is complex, but one of its most prominent features is the synthesis of large amounts of extracellular matrix, resulting in deposition of scar or fibrous tissue. Thus, the hepatic stellate cell and/or other fibrogenic cell types have been a therapeutic target. It is further noteworthy that the fibrogenic process is dynamic and that even advanced fibrosis is reversible. The best anti-fibrotic therapy is elimination of the underlying disease process. For example, elimination of hepatitis B or C virus can lead to reversal of fibrosis. In situations in which treating the underlying process is not possible, specific anti-fibrotic therapy would be highly desirable. To date, many specific anti-fibrotic treatments have been tried, but none have succeeded yet. Nonetheless, because of the importance of fibrosis, the field of anti-fibrotic compounds is rapidly growing. This review will emphasize mechanisms underlying fibrogenesis as they relate to putative anti-fibrotic therapy, and will review current and potential future anti-fibrotic therapies.

Keywords
fibrosis; cirrhosis; stellate cell; extracellular matrix; myofibroblast; liver biopsy; complication; portal hypertension

Introduction

Chronic injury results in a wound healing response that eventually leads to fibrosis. The response is a generalized one, with features common to multiple organ systems. In the liver, a variety of different types of injury lead to fibrogenesis - implying a common pathogenesis.
Although a number of specific therapies for patients with different liver diseases have been successfully developed, including anti-viral therapies for patients with hepatitis B and hepatitis C virus infection, specific and effective anti-fibrotic therapy remains elusive.

Over the past 2 decades, great advances in the understanding of fibrosis have been made and multiple mechanisms underlying hepatic fibrogenesis have been uncovered. Elucidation of these mechanisms has been of fundamental importance in highlighting novel potential therapies. Indeed, preclinical studies have pointed to a number of putative therapies that might abrogate fibrogenesis. The objective of this review will be to emphasize mechanisms underlying fibrogenesis, and to review the current status of the field with regard to available and future therapeutics.

**Fibrogenesis – Pathophysiology**

**The fibrogenic process**

A fundamental concept is that although the wounding process is complicated, it is characterized by common features that include increased production of extracellular matrix, as a result of a “coordinated” response that includes the action of various events on effector cells that in turn lead to extracellular matrix synthesis. In the liver and in most organs, inflammation often drives the response. Excellent examples include hepatitis B and C infection, autoimmune hepatitis, and alcoholic hepatitis to name a few. The chronicity of inflammation is often important in many types of liver disease, as well as the type of inflammation (i.e., Th2 vs. Th1), and the interplay of inflammation with environmental/metabolic/genetic factors.

The effectors of the fibrogenic response in the liver are diverse and include different cell types including activated stellate cells, peri-portal and peri-central fibroblasts, myofibroblasts (which may be derived from all 3 of the above cell types), bone marrow derived cells, fibroblasts derived from epithelial cells, and even bile duct epithelial and endothelial cells 29,73,81,82, 139,168,169,176. Considerable attention has focused on hepatic stellate cells, which transform from a “quiescent” (normal) to an “activated” (injured liver) state (Figure 1, See the article, ABCD). Although straightforward in concept, the activation process is remarkably complex, and consists of many important cellular changes. Characteristic features of this transition include loss of vitamin A, acquisition of stress fibers, and development of prominent rough endoplasmic reticulum. As intimated above, since the stellate cell has been identified as a key effector of the fibrogenic response, one of the most prominent features of activation is a striking increase in secretion of extracellular matrix (ECM) proteins, including types I, III and IV collagens, fibronectin, laminin and proteoglycans. Some ECM molecules are increased by greater than 50-fold, consistent with the conclusion that stellate cells are the cellular source of the enhanced ECM production at the whole organ level 96. A further critical feature of activation is de novo expression of smooth muscle specific proteins, such as smooth muscle α actin 133. This feature identifies activated stellate cells as liver specific myofibroblasts.

The field of stellate cell biology has exploded over the past 20 years, and a review of this area can be found in the article, ABCD. Importantly, the science has led to multiple therapeutic approaches based on an understanding of this cell’s biology. For example, many pathways lead specifically to stellate cell fibrogenesis, and these have or can be targeted. Theoretical approaches to anti-fibrotic therapy are highlighted in Box 1. A final important concept is that the complexity of the wounding response allows for multiple different “therapeutic” interventions, including those based on stellate cell biology, but also based on other mechanisms active in the wounding milieu. Therefore, it is possible that more than one anti-fibrotic agent may be prescribed, or that an anti-fibrotic agent may be taken along with another agent having a different (anti-inflammatory, anti-oxidant, etc…) mechanism of action.
Pathophysiology of the fibrogenic process and considerations for therapy

When considering anti-fibrotic therapy, it is important to recognize that fibrosis is a dynamic process. While at one time, it had been believed that the fibrotic lesion was static, abundant evidence indicates that this is not the case. Indeed, a prominent feature of liver fibrosis is that of extracellular matrix turnover, including not only its synthesis, but also its degradation. During fibrosis progression, there is increased expression of matrix metalloproteinases (MMPs) as well as their tissue inhibitors (TIMPs). Further, there appears to be an imbalance between MMPs that degrade “good” or normal matrix, and those that degrade “bad” or abnormal matrix. Early in the wounding process, MMPs appear to degrade normal matrix proteins, and this itself may perpetuate the fibrogenic phenotype of effector cells. In advanced fibrosis, overexpression of MMP8 was shown to lead to partial reversal of fibrosis, providing proof of concept for a therapeutic role for overexpressing MMPs.

An enormous body of literature in animal models and a surprisingly robust amount of data in human liver disease emphasizes that fibrosis is reversible. The data come primarily from treatment studies in which the disease has been removed or eliminated. For example, eradication or inhibition of hepatitis B virus (HBV) or hepatitis C virus (HCV) leads to reversion of fibrosis, even in some patients with histological cirrhosis. Additionally, fibrosis (and cirrhosis) in patients with autoimmune hepatitis who respond to medical treatment is reversible. Fibrosis may improve in patients with alcoholic liver disease who respond to anti-inflammatory therapy such as corticosteroids. Fibrosis reverts in patients with hemochromatosis during iron depletion and after relief of bile duct obstruction. Finally, in patients with non-alcoholic steatohepatitis (NASH) treated with the peroxisomal proliferator active receptor (PPAR) gamma agonist, rosiglitazone reduced both steatosis and fibrosis.

Approach to therapy for fibrosis

Monitoring of Hepatic Fibrosis

One of the major challenges in the field of fibrosis therapy currently is now to monitor fibrosis. Emerging evidence suggests that the presence of fibrosis has important prognostic implications. For example, in patients with hepatitis C virus infection after liver transplantation, adverse clinical events appeared to be increased in those patients with the greatest degree of fibrosis. Also, progression of non-alcoholic fatty liver disease, and even liver-related mortality also appeared to be related to initial fibrosis stage.

Additionally, in patients with many different types of liver disease, histological grade and stage may be helpful in identifying those who should receive therapy and those who should not. This is particularly true now for patients with hepatitis C virus infection. Nonetheless, it should be emphasized that use of fibrosis data in treatment algorithms for HCV patients remains controversial. On one hand, patients with advanced fibrosis (e.g., Batts and Ludwig stages 3 and/or 4) may be less likely to respond to antiviral therapy than those with less advanced fibrosis, and moreover it is well appreciated that patients with advanced fibrosis are more likely to experience greater side-effects and often require more aggressive supportive measures (e.g., growth factors) to maintain adequate blood cell counts during treatment. Further, patients with advanced fibrosis may have poorer response rates, and should probably be managed differently (e.g., treated with gradual increments in drug doses and/or for longer periods) than patients with absent or mild fibrosis. Moreover, it has been proposed that patients with absent or minimal fibrosis should simply be observed and perhaps undergo periodic liver biopsy for follow-up staging.
Liver biopsy and histological analysis of the liver has long been considered to be the gold standard for determining the extent of fibrosis and as well to assess fibrosis progression. Qualitative assessment of fibrosis has been made simple by the widespread use of connective tissue stains such as including reticulin, Masson’s trichrome, and picrosirius red (which each readily identify extracellular matrix within tissue). Quantitative measure of collagen content can be performed by colorimetric assay of sirius red in liver tissue or by image analytic quantitation of collagen containing tissue 134. Additionally, scoring systems can quantitate fibrosis 18,113,115 and help standardize the interpretation of biopsies among different centers; such systems are most useful for standardization and comparison of fibrosis in large studies. In single patients, simple inspection of biopsies over time is often the most helpful.

Despite the fact that histological analysis of the liver has been traditionally considered to be the gold standard tool to assess fibrosis, it is not perfect. First, liver biopsy is associated with significant potential morbidity, including a finite risk of death 160. Additionally, it is subject to inter-observer variability, and sampling error may be important, as evidenced by studies examining liver samples from different regions of the liver 127,128. For this reason, noninvasive tools to measure fibrosis would be ideal 132. Noninvasive methods used to assess fibrosis include routine clinical parameters such as physical exam findings, laboratory tests 121,166, radiographic tests 33, combinations of laboratory tests 69,166, and specific serum markers 30,100,132. Serum marker panels, including those that utilize mathematical algorithms 69,166, have recently been emphasized.

Most recently, transient elastography, an ultrasound-based technology, has gained considerable attention 130. This examination involving acquisition of pulse-echo ultrasound signals to measure liver stiffness 141 following the simple placement of an ultrasound transducer probe between two ribs, over the right lobe of the liver. The probe transmits a low amplitude (vibration and frequency) signal to the liver, which induces an elastic shear wave that propagates through the liver. This pulse-echo ultrasound measurement provides a measure of liver stiffness (reported in kilopascals). An advantage of this measurement, beyond its non-invasive nature, is that this technique allows measurement of stiffness across a relatively large area of the liver (1–2 cms), which is at least 100 times greater than for a liver biopsy. Normal liver stiffness is reported to be in the range of 4–6 kilopascals. However, cirrhosis is generally present at levels above 12–14 kilopascals, the higher the level, the more likely that the patient has cirrhosis 31,52,179.

Overview of treatment

Preclinical studies have reported a scientific rationale and experimental evidence supporting the use of many potential therapies for fibrosis. Such therapies have been targeted to any of several different biological targets (e.g., inhibition of collagen synthesis, interruption of matrix deposition, stimulation of matrix degradation, modulation of stellate cell activation, or induction of stellate cell death). In general, these therapies have been highly effective in animal models. A number of these preclinical approaches have been transitioned to clinical trials in humans, which are highlighted below and in Tables 2/3. Therapies have been divided into those that target specifically fibrosis (Table 2) and those that target a more general component of the liver disease process (i.e. oxidative stress) (Table 3). Highlighted below are the major anti-fibrotic agents that have been examined in clinical studies in humans.

Specific anti-fibrotic therapies (studied in human subjects)

Angiotensin II antagonists—The angiotensin II system represents an extremely attractive anti-fibrotic target. Abundant experimental evidence points to overproduction of angiotensin II in the injured liver, and for a role of angiotensin II in stimulation of stellate cell activation and fibrogenesis 16,17. A number of studies have also demonstrated specific anti-fibrotic
Effects of angiotensin II inhibition in a variety of animal models 67,75,107. Angiotensin II may also play a role in the pathogenesis of portal hypertension 131, and thus its inhibition could potentially abrogate not only fibrosis, but also portal hypertension.

Several human studies have examined the effects of angiotensin receptor blockers in humans 37,38,57,144,163, most in the setting of advanced liver disease – and most often in an attempt to reduce portal pressure. A 6 week trial of losartan in 25 patients did not significantly reduce HVPG compared to propranolol in patients with cirrhosis treated after a variceal bleeding episode 57. In a randomized trial of 36 patients with cirrhosis and portal hypertension, irbesartan reduced the hepatic venous pressure gradient by 12.2% +/- 6.6% after 7 days, but it also induced arterial hypotension 144.

In a trial of 39 subjects with cirrhosis who were randomized to losartan (19 patients) or propranolol (20 patients), HVPG was measured at baseline and on day 14 of therapy 37. With losartan, 15 of 19 (79%) patients had a reduction in HVPG >= 20%, while with propranolol, nine of 20 (45%) patients had a reduction in HVPG >= 20% (p < 0.05). Although the hepatic venous pressure gradient reduction (i.e., percentage from baseline) with losartan (27 +/- 22%) was higher than with propranolol (15 +/- 32%), the difference was not significant. In another study, 47 compensated Child A and Child B (8) cirrhotic patients were randomly assigned to receive candesartan (8 mg/d, n = 24) and no treatment (n = 23) for 1 year. The HVPG was decreased significantly in patients treated (-8.4% +/- 2.4mmHg) with candesartan and 25% of patients had a reduction > 20% compared to an increase of +5.6% +/- 2.9 mmHg in the untreated group. Plasma hyaluronic acid levels were also significantly reduced in candesartan treated patients in whom HVPG diminished while they rose in untreated patients in whom HVPG increased 38.

The data in humans are thus mixed, and further have been performed in small numbers of patients. Given the particularly supportive preclinical data, the evidence suggests that there is likely to be some element of anti-fibrotic effect in humans for the angiotensin receptor blockers (or perhaps angiotensin converting enzyme inhibitors). Larger and longer studies appear to be warranted, several of which have recently been closed or are currently underway (http://clinicaltrials.gov/; Clinical Trials.gov Identifier: NCT00298714, NCT00265642).

**Interferon gamma**—The interferons consist of a family of 3 major isoforms including α, β and γ. There are many different interferon α subtypes, while there appear to be only single interferon β and interferon γ species. Interferon α and β bind to the same receptor and therefore share many common properties. Interferon α has much more potent antiviral effects than does interferon γ. Interferon γ has been shown to specifically inhibit extracellular matrix synthesis in fibroblasts 36. Preclinical work with interferon γ in hepatic stellate cells demonstrated that this cytokine inhibited multiple aspects of stellate cell activation 135,136. These data led to an initial pilot study demonstrating that interferon γ 1b was safe and well tolerated in humans with HCV infection and advanced fibrosis 106. In addition, it led to reduction in fibrosis in selected patients 106. A subsequent double-blind, placebo-controlled, multi-center study examined interferon-γ 1b in 488 patients with an Ishak fibrosis score of 4–6 examined 3 treatment groups; interferon-γ 1b 100 micrograms (group 1, n=169), interferon-γ 1b 200 micrograms (group 2, n=157), or placebo (group 3, n=162) 3 times a week for 48 weeks 116. The vast majority of patients (83.6%) had cirrhosis at baseline (Ishak score=5 or 6). Among the 420 patients in whom pre- and post treatment liver biopsies were evaluable, there was no improvement in Ishak score among the 3 groups. Analysis of interferon-γ-inducible biomarkers revealed that interferon-inducible T cell-alpha chemoattractant (ITAC), an interferon-γ-inducible CXCR3 chemokine was an independent predictor of stable or improving Ishak score. Interferon-γ was well tolerated, suggesting that interferon-γ could be effective in certain subgroups of patients.
In a randomized, open-labeled, multicenter trial of interferon-γ in patients with HBV infection and biopsy proven hepatic fibrosis, a total of 99 patients who were not receiving anti-HBV antiviral medications were divided into those receiving diammonium-glycyrrhizinate and potassium-magnesium aspartate alone (n = 33), and those receiving these medications plus 50 micrograms interferon-γ intramuscularly on a daily basis for 3 months, and on alternate days the subsequent 6 months (n = 66). The majority of patients had follow-up biopsies at 9 months. Hepatic fibrosis scores were significantly reduced in 63% of interferon-γ treated patients compared with 24.1% in the control group. Using a semiquantitative scoring system combining the previously described Chevallier 32 and Knodell 83 systems, mean total fibrosis scores decreased from 13.8 +/− 5.8 to 10.1 +/− 5.1 in the interferon-γ group (p = 0.0001), whereas they were unchanged in control subjects (13.2 +/− 6.8 vs 12.6 +/− 4.8, p = 0.937). Using the Scheuer histologic grading system, 12 out of 54 patients improved ·1 stage(s) in the interferon-γ group compared with 1 of 29 in the control group. Interestingly, of 35 patients with compensated cirrhosis, 26 receiving interferon-γ and 9 in the control group, 5 patients in the interferon-γ group were found to have histological reversal of cirrhosis while no patient in the control group had an improvement in fibrosis and 9 month follow-up biopsy.

In summary, the biologic rationale for use of interferon-γ is strong. The data suggest that there are likely to be subgroups of patients for whom interferon-γ may be effective, though whether it would be a cost-effective therapy is not clear.

Peroxisomal proliferator activated receptor (PPAR) gamma ligands—The PPAR family of nuclear hormone receptors consists of 3 subgroups, alpha, gamma, and delta (beta). These receptors heterodimerize with the retinoid X receptor (RXR) and bind to specific regions on target gene DNA. PPAR gamma ligands have received great attention because hepatic stellate cell activation during liver injury is associated with reduced PPAR gamma expression 104, and activation of this receptor by exogenous PPAR gamma ligands 104, or re-expression of PPAR gamma itself in stellate cells reversed the activated phenotype 64. Additionally, expression of PPAR gamma during liver injury led to substantial improvements in fibrosis 174.

In a pilot study, 30 subjects with histologic evidence of NASH, received the PPAR gamma ligand, rosiglitazone, for 48 weeks 110. Twenty-six patients had posttreatment biopsies. Overall, there was significant improvement in hepatocellular ballooning and zone 3 perisinusoidal fibrosis. For the 25 patients completing 48 weeks of treatment, insulin sensitivity and mean serum alanine aminotransferase (ALT) levels (104 initially, 42 U/L at the end of treatment) improved significantly. Weight gain occurred in 67% of patients during treatment. Liver tests returned to baseline after stopping treatment, consistent with data from a subsequent study that demonstrated a return of histologic NASH after cessation of a PPAR gamma ligand used for therapy 95.

In another small study, pioglitazone was examined in subjects with impaired glucose tolerance or type 2 diabetes and liver biopsy-confirmed NASH 19. Subjects were randomized to 6 months of a hypocaloric diet (a reduction of 500 kcal per day in relation to the calculated daily intake required to maintain body weight) plus pioglitazone (45 mg daily) or a hypocaloric diet plus placebo 19. Compared to placebo, diet plus pioglitazone, improved glycemic control and glucose tolerance and led to normalization of aminotransferase levels. Pioglitazone also decreased hepatic fat content, histologic evidence of steatosis (p = 0.003), ballooning necrosis (p = 0.02), and inflammation (p = 0.008), but did not reduce fibrosis significantly compared to placebo (p = 0.08).

The findings in these small studies suggest that treatment of underlying NASH may be associated with an improvement in fibrosis, and warrant larger studies. Particularly given the
preclinical data suggesting a direct effect of PPAR gamma ligands on stellate cell activation, one study examining features of stellate cell biology is currently underway (see http://clinicaltrials.gov; Clinical Trials.gov Identifier: NCT00244751). Preliminary results are expected in 2008.

**Pirfenidone**—Pirfenidone ((5-methyl-1-phenyl-2-(1H)-pyri-) is a small orally bioavailable molecule that appears to inhibit collagen synthesis 40,41, though its molecular mechanism of action is not clearly understood. Pirfenidone has been shown to have anti-fibrotic effects in a variety of fibrogenic animal models, including the lung, kidney and liver 54,90,147,156.

Pirfenidone has been evaluated in small number of patients with fibrosing parenchymal organ diseases. In a double-blind, randomized, placebo-controlled trial of 107 patients with idiopathic pulmonary fibrosis, pirfenidone led to an improvement in vital capacity, and a reduction in the number of episodes of acute exacerbation of IPF compared to placebo (p = 0.0031) 12. Significant adverse events were associated with pirfenidone. In 15 patients with treatment naïve HCV related fibrogenesis, the compound was administered for 12 months (dose 1,200 mg/day) 10 and pre- and post treatment biopsies were compared. Fibrosis was reduced in 5 of 15 patients (30%) by the end of 12 months of treatment.

**Colchicine**—Colchicine is a plant alkaloid that inhibits polymerization of microtubules, a process that is believed to be required for collagen secretion. Thus, this compound is believed to work as an anti-fibrotic compound by preventing collagen secretion and deposition. Colchicine effectively inhibits collagen synthesis and fibrosis in experimental animal models 117,137,138.

Given the rationale for use of colchicine as an anti-fibrotic, as well as its presumed favorable safety profile, colchicine has been studied in a number of clinical trials 77,80,105,124, including in primary biliary cirrhosis, alcoholic cirrhosis, and in various other liver diseases 80. In one primary biliary cirrhosis trial, improvements were noted in a number of biochemical markers, but colchicine failed to reduce fibrosis 77. Interestingly, in a study comparing colchicine and methotrexate for PBC in 42 subjects, there was no change in fibrosis after 24 months of treatment with colchicine (or methotrexate), and in those with stable or improving histologic stage, interleukin-1beta synthesis was elevated in peripheral blood mononuclear cells 103. In a double blind, randomized, controlled trial of colchicine versus placebo in 100 patients with different liver diseases (mostly alcohol or posthepatitic), colchicine led to improved fibrosis as well as a dramatic improvement in survival when followed for up to 14 years 80. However, this study has been questioned because many patients were lost to follow-up, and there was substantial unexplained excess mortality in the control group from causes unrelated to liver disease. A meta-analysis including 1138 subjects found that colchicine had no effect on fibrosis or mortality 124.

In a multicenter study involving 549 patients comparing colchicine (0.6 mg p.o. Bid) to placebo in patients with alcoholic liver disease, there was no effect of active treatment on survival (histologic data were not obtained) 105. In a more recent small trial of colchicine of 38 subjects with miscellaneous liver diseases randomized to receive either colchicine 1 mg per day (n=21) or no agent (n=17) for at least 12 months 111. Liver biopsy was performed prior to beginning colchicine and after 12 months. Interestingly, mean albumin serum levels increased 12 months post-treatment period only in the colchicine group (p < 0.05). Although Knodell fibrosis scores remained unchanged at 12 months, 7 patients were noted to have a reduction in mean serum PIIINP levels during 24-month post-treatment follow-up period.

In summary, the data indicate that colchicine is generally safe (although one report suggested that it may alter the response to interferon alpha bases anti HCV therapy 8) and may lead to
improvement in markers of liver disease and even mortality from liver disease. Overall, however, colchicine does not appear to reduce hepatic fibrosis, and it cannot therefore be recommended as a primary anti-fibrotic treatment. It is also noteworthy that in small randomized studies, colchicine did not appear to be effective for treatment of pulmonary fibrosis 44,145.

Herbal Medicines—A number of herbal medicines have been shown to have anti-fibrotic properties in experimental animal models 98,140,146,177,178. Many of these medications have arisen from China 93. While the mechanism(s) of many of these agents is unknown, these compounds are being used extensively in a wide array of patients with liver diseases 167. Medications containing herbs of the Salvia genus have been popular as anti-fibrotics; salvianolic acid B, a major water-soluble polyphenolic acid appears to be the major active ingredient 93,167. This compound appears to have specific effects on stellate cells. The active ingredients of other agents, including curcumin, glycyrrhizin, celastrol, tetrandrine, berberine, oxymatrine appear to have a wide variety of biologic effects, accounting for their purported activity in human disease.

It is important to emphasize that although some studies have suggested effectiveness of specific herbal medicines 93,167, rigorous evidence is sorely lacking. Since it is well appreciated that such herbal medicines may have significant toxicity, including hepatotoxicity 152, these medications should be used with extreme caution.

Compounds with a potential anti-fibrotic effect occurring due to upstream effects

A number of compounds appear to be capable of affecting fibrogenesis, not through a direct effect on stellate cells or on matrix synthesis per se, but rather by having an effect on other important biologic events such as on lipid peroxidation, on the immune system, or others. These are highlighted briefly below and in Table 3.

Silymarin—The major active component of the milk thistle Silybum marianum, silymarin extract (in turn the major component of which is silybinin), reduces lipid peroxidation and inhibits fibrogenesis in small animals 26,74, as well as in baboons 86. Although fibrosis was not studied as a primary outcome, the compound has been found to be safe. It has been reported to have variable effects 50,114. One study revealed a putative benefit on mortality in patients with alcohol induced liver disease 50. Those with early stages of cirrhosis also appeared to benefit. However, in another study focused solely on alcoholics, no survival benefit could be identified 114. Given the apparent safety of silymarin, and its common use as a complementary and alternative medicine, studies in patients with NASH or in those who have failed conventional antiviral treatment for HCV infection have been initiated (http://clinicaltrials.gov; Clinical Trials.gov Identifier: NCT00680407 and NCT00680342). Although fibrosis is not a primary outcome measure, histological data are planned, and thus information about the effect of silymarin on liver fibrosis is anticipated.

Polyenylphosphatidylcholine—Polyenylphosphatidylcholine has gained considerable interest in the treatment of patients with liver injury. The therapeutic compound is derived from purified soybean extract, consisting of 95–96% polyunsaturated phosphatidylcholines. Polyenylphosphatidylcholine has both has both antioxidant and anti-fibrotic and properties. It is attractive in alcoholic liver injury because this disease is often associated with oxidative stress. Oxidative stress in turn leads to lipid peroxidation, cellular injury, inflammation and subsequently fibrogenesis. It has thus been proposed that because phosphatidylcholine is a prominent component of cell membranes, that supplementation of it should protect cell membranes and might lead to reduced cellular injury and fibrogenesis. Experimental data support this concept 7.
Several large studies have been undertaken in an attempt to determine whether polyenylphosphatidylcholine is beneficial. A multicenter, prospective, randomized, double-blind placebo-controlled VA cooperative clinical trial examined 789 alcoholics (average alcohol intake of 16 drinks/day) 87. Subjects were randomized to either polyenylphosphatidylcholine or placebo for 2 years. Although the majority of subjects substantially reduced their ethanol consumption during the trial (which was felt to result in improvement in fibrosis in the control group), polyenylphosphatidylcholine failed to lead to a comparative improvement in fibrosis. A subsequent study examining the effect of polyenylphosphatidylcholine is currently underway (http://clinicaltrials.gov; Clinical Trials.gov Identifier: NCT00211848).

Ursodeoxycholic acid—Ursodeoxycholic acid, a non-toxic bile acid, binds to hepatocyte membranes and is presumably cytoprotective, thereby reducing inflammation and therefore downstream fibrogenesis 108. It is important to emphasize that neither experimental data nor human studies indicate a primary anti-fibrotic effect of ursodeoxycholic acid in the liver. However, an extensive body of literature, in a variety of liver diseases generally has examined ursodeoxycholic 34,39,59,72,88,89,118,119,154. The aggregate data suggest that ursodeoxycholic acid may impede progression of fibrosis in primary biliary cirrhosis via effects on biliary ductal inflammation, particularly if initiated early in the disease course. Ursodeoxycholic acid is safe, and while it is expensive, in the absence of definitively effective agents, it is this author’s belief that the available data justify its use was an “anti-fibrotic”, primarily in patients with primary biliary cirrhosis.

Interleukin-10—Interleukin-10 is a potent immunomodulatory cytokine which can down regulate production of proinflammatory T cell cytokines, such as tumor necrosis factor-α, interleukin-1, interferon γ, and interleukin-2. Endogenous interleukin-10 appears to attenuate the intrahepatic inflammatory response and reduce fibrosis in several models of liver injury 161. A direct anti-fibrotic effect for interleukin-10 has not been established. Notwithstanding, interleukin-10 was given to 30 subjects with HCV infection and advanced fibrosis who had failed antiviral therapy for 12 months in an effort shift the intrahepatic immunologic balance away from Th1 cytokine predominance (SQ interleukin-10 given daily or thrice weekly) 109. This therapy decreased hepatic inflammatory activity and fibrosis, but led to increased HCV viral levels. Thus, while these results suggest that interleukin-10 might be an attractive anti-fibrotic agent, the adverse effects on HCV viral levels are problematic.

Miscellaneous antioxidants and anti-inflammatory compounds—Oxidative stress has been implicated in a wide variety of biological processes in liver injury (including stellate cell activation and stimulation of extracellular matrix production) 6. Thus, antioxidants have received considerable attention as putative anti-fibrotics 27,63,68,102,153. Compounds, including the vitamin E precursor, d-alpha-tocopherol (1200 IU/day for 8 weeks) 68, vitamin E 102,153, malotilate 9, propylthiouracil 125, penicillamine 24,42,143, and S-adenosylmethionine 92,99 have all been tested in humans; evidence supporting their effectiveness remains lacking. It is noteworthy that for many of these compounds, fibrosis was not typically measured as a specific outcome. Thus, it is not appropriate to consider these agents as primary anti-fibrotics, but rather as compounds that could have secondary effects on fibrogenesis due to other properties.

Metrothrexate is also considered to be an anti-inflammatory compound. However, it has also been believed to be profibrogenic 2,112, although the risk of fibrosis progression when used in patients with skin or rheumatologic disease may be less than commonly believed 2,158. Nonetheless, it has been studied in a substantial number of human trials as a therapeutic agent in patients with primary biliary cirrhosis 13,14,65,103,158. Although improvement in disease and fibrosis have been reported, including reversion of fibrosis 79, the majority of the data on
methotrexate are either negative 13,65 or show that its effects are marginal, either alone 65, or in combination with colchicine 78. If methotrexate is used to treat patients (with primary biliary cirrhosis), an experienced Hepatologist must manage its use.

Experimental evidence suggests that inhibition of tumor necrosis factor alpha (TNF-α) signaling during liver injury may ameliorate fibrosis 15,84. TNF-α is upregulated in alcoholic liver disease, and thus an anti-TNF-α compound would be attractive because it should reduce inflammation and thus fibrosis. Several studies have examined the effect of anti-TNF-α compounds in patients in patients alcohol induced liver disease 4,101,150,162. Available evidence suggests suggest an improvement in inflammation, and acute injury (which presumably precede fibrosis in this disease) 162. In a randomized, double blind, placebo-controlled trial of etanercept in patients with moderate to severe alcoholic hepatitis, there was no improvement in mortality at one month, and patients treated with etanercept had a greater mortality after 6 months; of note, however, this study did not evaluate liver histology, 25.

Why have so many potential therapies been effective in animal models, yet so ineffective in humans?

This key question is a major conundrum for the field of anti-fibrotics. A critical consideration is that experimental models and conditions are dramatically different from real life situations. First, in most animal experiments, anti-fibrotic agents have been tested for their ability to prevent development of fibrosis. This almost never happens in the clinical arena (patients present with advanced fibrosis or cirrhosis, and there is little or no opportunity to treat the patient during fibrosis progression). Second, patients in most of the human trials performed to date have had advanced fibrosis, if not cirrhosis, and since the duration of treatment has been relatively short, it seems unlikely that even if a compound actively inhibited fibrosis, a demonstrable benefit may not be apparent within a short (1 or 2 year) time frame.

It is also possible that there are differences in pharmacokinetics of therapeutic agents among animals and humans. For example, drug levels may be pushed to very high levels in animals, but such levels are not realistically attainable in humans. It is also possible that compounds that truly have anti-fibrotic features in animals are simply not anti-fibrotic in humans; this may be because of differences in basic cell or molecular aspects of the fibrogenic platforms.

Finally, the duration of injury differs markedly between rodent models and human disease, which could lead to significant differences in the cross-linking of ECM, and thus its potential for degradation. Whereas human diseases that lead to fibrosis require decades, in rodents this process is condensed into weeks or months, and thus there is less time for the ECM to ‘mature’, meaning that there is less chemical cross linking and instead the scar remains highly cellular and resorbable.

Future specific targets

A comprehensive discussion of the many different putative pathways that could lead to novel anti-fibrotic therapeutics is beyond the scope of this review. However, there are several systems/areas that are particularly attractive; several are highlighted in Table 4 and below. The most central of fibrogenic pathways involves the cytokine, transforming growth factor beta (TGF-β). Several approaches to inhibit the action of TGF-β can interrupt the TGF-β signaling pathway 56,70,175. The concept is clear, although theoretical concerns include the potential (pro-proliferative) effect of inhibiting TGF-β signaling in vivo.

Recent data implicate the cannabinoid system in fibrogenesis. In the injured liver, the endogenous endocannabinoid receptors, CB1 and CB2 are upregulated and thus facilitate endocannabinoid signaling 148. Additionally, in patients with chronic hepatitis C virus
infection, daily cannabis use is an independent predictor of fibrosis progression. On one hand, upregulation of endogenous hepatic cannabinoid CB2 receptors is associated with progression of experimental liver fibrosis. On the other hand, CB1 receptors were induced in human cirrhotic samples and in liver fibrogenic cells, and in animals undergoing liver injury, a CB1 receptor antagonist inhibited fibrosis, presumably by inhibiting expression of TGF-β1 and by either inhibiting growth hepatic myofibroblasts and/or stimulating apoptosis.

Data is emerging that suggests angiogenesis is important in the fibrogenic response to injury and thus, anti-angiogenic compounds are attractive therapeutic targets. Likewise, as biology uncovering stellate cell signaling pathways continues to emerge, therapy targeted at these pathways will become attractive, with a caveat being that the signaling pathways are extremely complicated, and moreover may vary among models of injury.

An important therapeutic concept is directed or targeted therapy. Since many compounds have adverse affects collateral cells or organs outside the fibrogenic response, it would be most desirable to specifically target fibrogenic cells, particularly hepatic stellate cells. The ability to specifically stimulate stellate cell apoptosis and enhance the resolution of fibrosis is especially attractive. Additionally, the ability to potentially specifically target siRNAs to the liver also makes this approach appealing. MicroRNAs may also be important in fibrogenesis; additional investigation in liver injury models is expected to lead to potential therapies for liver fibrosis. A number of other specific targets are of considerable interest (Table 4).

Farnesoid X receptor (FXR) is a member of the nuclear hormone receptor superfamily or transcription factors that is bile acid-activated. It is not only hepatoprotective in various experimental models of liver injury, but it may also ameliorate fibrosis. FXR activators may be particularly useful in patients with cholestatic injury.

**Summary**

Elucidation of the mechanisms responsible for fibrogenesis, with particular emphasis on stellate cell biology, has generated great hope that novel therapies will evolve; indeed, the field of anti-fibrotic compounds is growing rapidly. A central event in fibrogenesis is the activation of effector cells (hepatic stellate cells are the most prominent). The activation process is characterized by a number of important features, including in particular, enhanced matrix synthesis and transition to a myofibroblast-like (and contractile) phenotype. Factors controlling activation are multifactorial and complex, and thus multiple potential therapeutic interventions are possible. A further critical concept is that even advanced fibrosis is dynamic and may be reversible. Currently, the most effective therapy for hepatic fibrogenesis is to attenuate or clear the underlying disease. The most effective specific anti-fibrotic therapies will most likely be directed at fibrogenic effector cells, either in a targeted fashion, or by using generalized approaches that take in to account biologic differences between fibrogenic cells and their non-fibrogenic neighbors. Additionally, approaches that address matrix remodeling (i.e. by enhancing matrix degradation or inhibiting factors that prevent matrix breakdown) will be pursued. Thus, although there are no specific, effective, safe, and inexpensive anti-fibrotic therapies yet, multiple potential targets have been identified, and it is expected that effective therapies will emerge.

**Acknowledgements**

This work was supported by the NIH (Grants R01 DK 50574 and R01 DK 60338).
References


Clin Liver Dis. Author manuscript; available in PMC 2009 November 1.


Clin Liver Dis. Author manuscript; available in PMC 2009 November 1.


Figure 1. Stellate cell activation
The current consensus is that the key pathogenic feature underlying liver fibrosis and cirrhosis is activation of hepatic stellate cells. This process is complex, both in terms of the events that induce activation and the effects of activation. Multiple and varied stimuli participate in the induction and maintenance of activation, including, but not limited to cytokines, peptides, and the extracellular matrix itself. Key phenotypic features of activation include production of extracellular matrix, loss of retinoids, proliferation, of upregulation of smooth muscle proteins, secretion of peptides and cytokines (which have autocrine effects), and upregulation of various cytokine and peptide receptors (From reference 129). It is likely that other effector cells (fibroblasts, fibrocytes, bone marrow derived-cells), similarly undergo activation and contribute to the fibrogenic response. With permission, Rockey DC: Antifibrotic therapy in chronic liver disease. Clin Gastroenterol Hepatol 3:95, 2005
Table 1
Therapeutic Considerations for Hepatic Fibrosis

- Eliminate the underlying disease process
- Inhibit inflammation (if present)
- Eliminate effector cells
- Inhibit effector cell function (i.e. fibrogenesis, proliferation, cell contraction, motility)
- Other
Table 2
Diseases and Therapies in which Fibrosis can be Reduced by Treating the Underlying Disorder

<table>
<thead>
<tr>
<th>Disease</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Lamivudine, others</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>* Interferon alpha</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Bile duct obstruction</td>
<td>Surgical decompression</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Iron depletion</td>
</tr>
<tr>
<td>Alcohol hepatitis</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>Ursodeoxycholic acid, MTX</td>
</tr>
<tr>
<td>Non-alcoholic steatohepatitis</td>
<td>PPAR gamma ligands</td>
</tr>
</tbody>
</table>

* or PEG-interferon alpha, with or without ribavirin

¶ The effect is minimal if present

§ Evidence is preliminary at this point

References are given in the text

Abbreviations: MTX = methotrexate; PPAR = peroxisomal proliferator activated receptor
### Table 3
Potential anti-fibrotic therapies with “specific effects”

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Efficacy</th>
<th>Safety</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>Misc</td>
<td>+/-</td>
<td>++++</td>
<td>Inhibits collagen synthesis</td>
</tr>
<tr>
<td>Interferon gamma</td>
<td>HCV</td>
<td>+/-</td>
<td>++</td>
<td>Stellate cell specific effects</td>
</tr>
<tr>
<td>ARBs</td>
<td>Misc</td>
<td>+/-</td>
<td>++</td>
<td>Stellate cell specific effects</td>
</tr>
<tr>
<td>PPAR ligands</td>
<td>NASH</td>
<td>++</td>
<td>++</td>
<td>Stellate cell specific effects</td>
</tr>
<tr>
<td>Pirfenidone</td>
<td>Misc</td>
<td>+/-</td>
<td>+++</td>
<td>Mechanism via cytokines?</td>
</tr>
</tbody>
</table>

The scale for efficacy and safety is - to ++++ with - being the lowest rating and ++++ the highest rating. The ratings are empiric based on the aggregate available literature. See text for references and discussion of mechanisms.

Abbreviations: ARBs = angiotensin receptor blockers; PPAR = peroxisomal proliferator activated receptor, HCV = hepatitis C virus, NASH = non alcoholic steatohepatitis, misc = miscellaneous.
### Table 4

Potential anti-fibrotic therapies with “general” effects

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Efficacy</th>
<th>Safety</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-10</td>
<td>HCV</td>
<td>++</td>
<td>+</td>
<td>Increased viral load</td>
</tr>
<tr>
<td>Malotilate</td>
<td>ETOH</td>
<td>−</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>PPC</td>
<td>ETOH</td>
<td>−</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>Propylthiouracil</td>
<td>ETOH</td>
<td>−</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>SAM</td>
<td>ETOH</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Anti-TNFα compounds</td>
<td>ETOH</td>
<td>++</td>
<td>+</td>
<td>Clearly anti-inflammatory</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>Multiple</td>
<td>+</td>
<td>++++</td>
<td>Most studied in PBC</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>HCV/NASH</td>
<td>-</td>
<td>++++</td>
<td></td>
</tr>
</tbody>
</table>

The scale for efficacy and safety is - to ++++ with - being the lowest rating and ++++ the highest rating. The ratings are empiric based on the aggregate available literature. See text for references and discussion of mechanisms.

Abbreviations: PPC = Polyenylphosphatidylcholine; SAM = s-adenosylmethionine, TNF = tumor necrosis factor; ETOH = alcohol, HCV = hepatitis C virus, NASH = non alcoholic steatohepatitis, misc = miscellaneous, PBC = primary biliary cirrhosis.
### Table 5
Experimental Anti-Fibrotic Therapies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoid receptor antagonants</td>
<td>Inhibit stellate cell activation and fibrogenesis</td>
</tr>
<tr>
<td>Rapamycin</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate</td>
<td></td>
</tr>
<tr>
<td>Gliotoxin</td>
<td></td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td></td>
</tr>
<tr>
<td>HOE 77</td>
<td></td>
</tr>
<tr>
<td>Endothelin antagonists</td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II converting enzyme inhibitors</td>
<td></td>
</tr>
<tr>
<td>Sho-saiko-to (TJ-9)</td>
<td></td>
</tr>
<tr>
<td>Retinoic acid</td>
<td></td>
</tr>
<tr>
<td>RGD peptides</td>
<td></td>
</tr>
<tr>
<td>Halofuginone</td>
<td>Inhibit stellate cell fibrogenesis</td>
</tr>
<tr>
<td>Anti-TGF beta</td>
<td></td>
</tr>
<tr>
<td>Follistatin</td>
<td></td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Other/unknown or generalized effect</td>
</tr>
<tr>
<td>Adiponectin</td>
<td></td>
</tr>
<tr>
<td>COX-2 inhibitors</td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td></td>
</tr>
<tr>
<td>Anticoagulation</td>
<td></td>
</tr>
<tr>
<td>Puerarin</td>
<td></td>
</tr>
<tr>
<td>Salvia Milliorrhiza</td>
<td></td>
</tr>
<tr>
<td>IL-1 antagonist</td>
<td></td>
</tr>
<tr>
<td>Octreotide</td>
<td></td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td></td>
</tr>
<tr>
<td>HGF</td>
<td></td>
</tr>
<tr>
<td>*STAP</td>
<td></td>
</tr>
<tr>
<td>PAR antagonists</td>
<td></td>
</tr>
<tr>
<td>PPARs</td>
<td></td>
</tr>
<tr>
<td>C-5 depletion</td>
<td></td>
</tr>
<tr>
<td>5-lipoxygenase inhibitors</td>
<td></td>
</tr>
<tr>
<td>Angiostatin</td>
<td></td>
</tr>
<tr>
<td>Anti-TNF alpha compounds</td>
<td></td>
</tr>
<tr>
<td>Hepatocyte growth factor (inhibition)</td>
<td></td>
</tr>
<tr>
<td>Connective tissue growth factor (inhibition)</td>
<td></td>
</tr>
<tr>
<td>Relaxin</td>
<td></td>
</tr>
</tbody>
</table>

*STAP = stellate cell activation-associated protein

---

Clin Liver Dis. Author manuscript; available in PMC 2009 November 1.