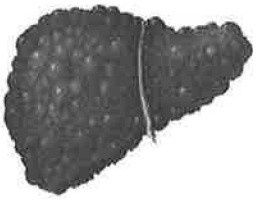
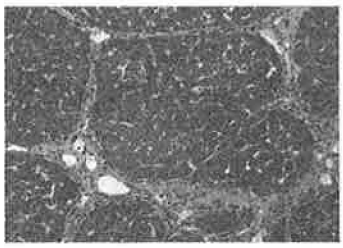
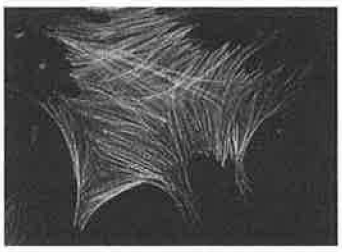
The Cell and Molecular Basis for Cirrhosis - A Bench to Bedside Approach

Medical Grand Rounds
August 17, 2006

Don C. Rockey, M.D.
Department of Internal Medicine
University of Texas Southwestern Medical Center

This is to acknowledge that Dr. Rockey has disclosed any financial interests or other relationships with commercial entities related directly or indirectly to this program. Dr. Rockey will not be discussing off-label uses in this presentation.

Don C. Rockey
Professor of Medicine, UT Southwestern
Division of Digestive and Liver Diseases
Internal Medicine Grand Rounds
August 17, 2006

Abstract

Fibrotic liver disease occurs after any of various forms of injury to the liver. Moreover, fibrosis appears to be a critical factor leading to hepatic dysfunction and portal hypertension and its complications. The fibrogenic cascade is complex, but leads to accumulation of extracellular matrix proteins, followed by nodular fibrosis, tissue contraction, and alteration in blood flow. A critical concept emerging over the past 2 decades is that activation of effector cells – which produce extracellular matrix – underlies the fibrogenic process. In the liver, these effectors are primarily hepatic stellate cells, although some data suggests that other mesenchymal cell types contribute to fibrogenesis, particularly after certain kinds of liver injury. Stellate cell activation is characterized by many important features, including enhanced extracellular matrix synthesis. The aggregate data has not only helped lead to an understanding of the pathophysiologic basis of hepatic fibrogenesis, but it has also provided an important conceptual framework with which to base novel anti-fibrotic therapy.

Introduction and Background

Hepatic fibrogenesis is prominent after injury to the liver. Further, fibrosis is believed to lead to hepatic dysfunction and portal hypertension. The fibrogenic process represents a complex biologic set of events ultimately leading to extensive accumulation of extracellular matrix proteins, tissue contraction and portal hypertension. A fundamental concept in this field is that new (and presumably effective) therapies for hepatic fibrogenesis will be predicated on understanding of laboratory based scientific advances, rather than on empiric observations and trials. This aim of this review is to provide a pathophysiologic framework for understanding important clinical and therapeutic issues in hepatic fibrogenesis and to highlight potential anti-fibrotic therapies.

Fibrogenesis leading to cirrhosis develops in response to many multiple different types of liver injury. While chronic hepatitis C infection is a prominent cause of fibrogenesis, and perhaps at the time of this writing, the leading cause of hepatic fibrosis, a multitude of other liver diseases (i.e., non-alcoholic fatty liver disease, sustained alcohol ingestion, iron overload due to genetic hemochromatosis, recurrent injury to the bile ducts - as in primary biliary cirrhosis and primary sclerosing cholangitis, autoimmune injury, alpha-1-anti-trypsin disease, copper overload and perhaps congenital lesions) also cause fibrogenesis. Importantly, regardless of the etiologic basis for fibrosis and cirrhosis, the clinical outcome, fibrosis, is similar (Figure 1 and see (1) for review).

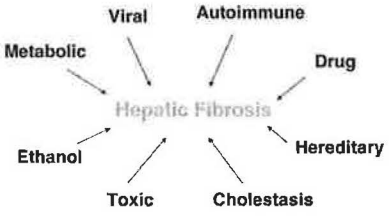


Figure 1. *Injury leads to fibrosis.* Many types of injury lead to the same common result -- fibrosis.

Cirrhosis is currently a leading cause of morbidity and mortality, both in the U.S. and worldwide. The burden of liver disease in the United States appears to be expanding (2). We are currently in the middle of virtual epidemics – in hepatitis C virus (HCV) infection and non-alcoholic steatohepatitis (NASH). Notably, hepatocellular cancer, which usually arises in cirrhotic livers, is increasing in incidence (3). Importantly, although current data suggest that despite an apparent decrease in the prevalence of HCV infection, hepatic fibrosis and cirrhosis due to HCV is and will continue to rise for many years to come (4). In addition, it is predicted that hepatocellular carcinoma due to HCV will dramatically increase as well (4). Further evidence of the impending burden of liver disease comes from data indicating that hepatocellular carcinoma is the most rapidly increasing neoplasm in the U.S. and Western Europe (5).

Risk factors for development of advanced fibrosis in patients with HCV include the following: increasing age at infection, male gender, ethanol consumption, co-infection with HIV, and immunosuppression (i.e. after orthotopic liver transplantation) (6).

Pathogenesis of Hepatic Fibrosis

Table 1

Matrix Proteins in Cirrhosis

Prominent	Minor
Collagen type I	collagen type V
Collagen type III	SPARC
Collagen type IV	vitronectin
Fibronectin	Nidogen (entactin)
*Proteoglycans	elastin
Laminin	
Tenascin	
Undulin	

Essentially all matrix proteins known are upregulated during hepatic fibrogenesis; the designation betwe "prominent" and "minor" is arbitrary, those categorized as prominent matrix proteins are most abundant and have received the greatest attention

* multiple species based on glycosaminoglycan content including heparin sulfate, dermatan sulfate, chondroitin sulfate

Abbreviations: SPARC = secreted protein, acidic and rich in cysteine

Increased production of extracellular matrix constituents is a central component of all forms of hepatic fibrogenesis (Table 1). The most prominent (and abundant) extracellular matrix types include the interstitial collagens, types I and III (7, 8). Quantitative and qualitative changes in many other matrix components have been described, including proteoglycans (9, 10) and matrix glycoproteins including laminin (11, 12), fibronectin, including its EDA (or "cellular fibronectin") isoforms (13) and tenascin (14). Specific changes in matrix composition are similar in chronic injury of all forms of liver injury and hepatic fibrogenesis, suggesting that the general mechanisms of fibrosis are similar. This fact also underscores the importance of identifying central regulatory components of the fibrotic response - since such components may be selectively targeted without respect to etiology of disease.

Hepatic fibrosis is the body's wound healing response to injury and is similar to the response of other organs to recurrent injury (15, 16). Many forms of wound healing are characterized by a relatively typical cascade of events. This cascade of events (highlighted in Figure 2, below) includes components of epithelial injury (in liver – hepatocytes) (17-19), followed by activation and mobilization of a variety of inflammatory cells which release cytokines. Cytokines not only lead to amplification of the overall response, but also contribute directly (and indirectly) to "activation" of effector cells (20-22). In the liver, effector cells are hepatic stellate cells (23-25), which once activated, produce cytokines

(and biologically active peptides) that amplify the response in an autocrine fashion. It is also noteworthy that the process includes release of matrix degrading proteases and their regulation by specific inhibitors and plasma proteins, which provide for dynamic turnover of the matrix.

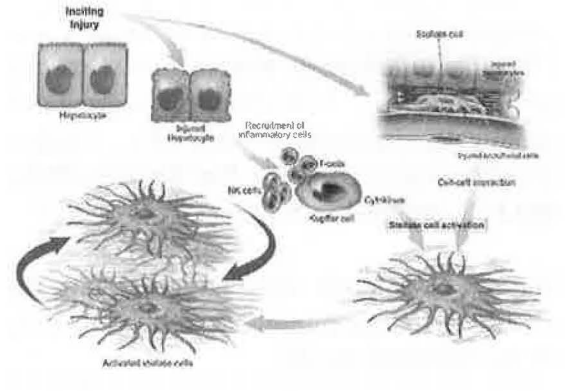


Figure 2. *Fibrogenesis.* A simplified version of the wounding response in the liver is depicted. Most forms of liver injury result in hepatocyte injury, followed by inflammation, leading to recruitment of inflammatory effectors including T cells, NK and NKT cells as well as Kupffer cells. These cells produce growth factors, cytokines, and chemokines that play an important role in stellate cell activation. Additionally, injury leads to disruption of the normal cellular environment, and also to stellate cell activation (right upper panel). Once activated, stellate cells themselves produce a variety of compounds, including growth factors, cytokines, chemokines, and vasoactive peptides, which have pleotrophic effects in the local environment, including many of which have autocrine effects on stellate cells themselves. One of the major results of stellate cell activation is extracellular matrix synthesis, as well as production of matrix degrading enzymes.

Stellate Cell Biology

Abundant evidence now indicates that the hepatic stellate cell (also known as a lipocyte or Ito cell) is a critical effector in hepatic fibrogenesis. Under normal circumstances, these perisinusoidal cells are found throughout the hepatic lobule, and are the principal storage site for retinoids (vitamin A metabolites) in the body (26). A number of investigators have now developed methods to isolate this cell type, representing a major advance in the field. The current working model is one in which after injury, hepatic stellate cells undergo "activation" (Figure 3). Stellate cell activation is prominent after infection of the liver with HCV. Activated stellate cells are proliferative, fibrogenic and contractile - representing liver specific myofibroblasts. Activation is characterized by many important events including fibrogenesis (27), proliferation (28), contractility (29, 30), release of proinflammatory cytokines (31-34) and release of matrix degrading enzymes and their inhibitors (35-37), each of which contribute to activation (and fibrogenesis), and each of which represent a potential target for novel therapy.

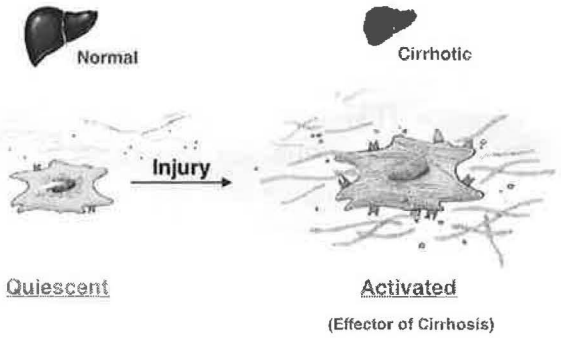


Figure 3. *Stellate Cell Activation.* Stellate cell activation is a key pathogenic feature underlying liver fibrosis and cirrhosis. Multiple and varied stimuli contribute to the induction and maintenance of activation, including, but not limited to cytokines, peptides, and the extracellular matrix itself. Shown in the diagram are key phenotypic features of activation, which 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 (1).

Many different factors lead stellate cell activation (See (1). For example, cytokines, such as TGFβ are prominent in stimulating fibrogenesis. Other cytokines stimulate proliferation, and thus fuel the incipient fibrotic lesion. While a number of typical factors prominent in the injured liver are important in stimulation activation (i.e., cytokines, chemokines, extracellular matrix, etc...), it is notable that many factors may contribute to activation. For example, the extracellular matrix itself is important in stimulation of stellate cell

activation (1). In addition, apoptotic fragments derived from hepatocytes appear to stimulate stellate cell fibrogenesis (38). Further, HCV core and non-structural (NS3-NS5) proteins directly interact with stellate cells and may stimulate stellate cell activation (39, 40).

Germaine to this discussion, perhaps the most prominent feature of stellate cell activation is enhanced extracellular matrix production (12). Investigation has turned toward understanding the mechanisms underlying this process. It appears that a number of events, typically acting in concert, play a role in stimulating stellate cell fibrogenesis. For example, TGFβ directly stimulates fibrogenesis, while PDGF stimulates cellular proliferation, thus contributing to the accumulation of extracellular matrix. A further prominent feature of activation, and one that may be important in the pathophysiology of portal hypertension, is the upregulation of many proteins that are characteristic of contractile cells such as smooth muscle α actin and smooth muscle myosins (41). Available data suggest that stellate cells control sinusoidal blood flow by perisinusoidal constriction, analogous to the way that tissue pericytes control blood flow in systemic capillary structures (42, 43). Further, since stellate cell contractility is greatest after stellate cell activation and since endothelin-1 is overproduced in the injured liver, enhanced contractility after activation appears to contribute to elevated intrahepatic resistance to blood flow (44, 45). Second, contraction of stellate cells residing within bands of extracellular matrix are likely to lead to the whole organ contraction characteristic of end-stage liver disease (46).

Stellate cells produce a number cytokines and biologically active peptides that have autocrine effects on themselves and that further have a broad range of effects in the wounding milieu. Prominent examples include TGF-β1 (47), PDGF (48), and CTGF (49), to name a few; these and other cytokines typically bind to specific endogenous receptors on stellate cells. Stellate cells also produce a variety of peptides important in the wounding response, and in addition, several vasoactive peptides that may be important in portal hypertension (43). Among the most notable of these peptides is endothelin-1 (50). The role of endothelin-1 in stellate cell activation, fibrogenesis, and in the injury milieu is notable, and is likely to be serve as an model for its role in other diseases. Another vasoactive peptide important in fibrogenesis is angiotensin II, which appears to be produced by stellate cells themselves and has autocrine profibrogenic effects (51).

A further important aspect of stellate cell biology is their apoptosis or programmed cell death, which appears to be prominent during spontaneous recovery of liver fibrosis (52). When stellate cells undergo apoptosis, they obviously loose their fibrogenic potential; the role of apoptotic stellate cell bodies in activation of stellate cells remains open. The data suggest that stellate cell apoptosis may play a role in resolution of fibrosis, and imply that a balance between stellate cell proliferation and death is important in determining the dynamics of the total overall stellate cell population in the liver.

Extracellular matrix biology in fibrogenesis

Extracellular matrix remodeling is an integral part of the fibrogenic process. Extracellular matrix proteins are typically degraded by the action of a family of enzymes known as the matrix metalloproteinases (MMPs). Many different matrix degrading enzymes are produced by stellate cells during fibrogenesis (53, 54). For example, stellate cells express MMP-

2 after activation (35), where it appears to disrupt the normal subendothelial basement membrane matrix. Enhanced production of abnormal interstitial collagens (i.e. types I and III) by stellate cells leads to an abnormal basement membrane which subsequently disrupts hepatocellular dysfunction (13). Additionally, the MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs); also synthesized by stellate cells (36, 55, 56) and which appear to be important in the fibrogenic response (57). Finally, HCV envelope E2 glycoprotein binds to stellate cells, inducing increased expression of MMP-2, which leads to increased degradation of the normal hepatic extracellular matrix, which in turn facilitates stellate cell activation and fibrogenesis (40).

A number of studies have emphasized that changes in basement membrane extracellular matrix play an important role in fibrogenesis. For example, the EDA (or cellular) fibronectin isoform is expressed early after liver injury, and once synthesized, it leads directly to stellate cell activation (13). A further example of the importance of the extracellular matrix comes from work demonstrating that stellate cells exposed to an abnormal basement membrane (type I collagen), exhibited marked activation of MMP-2, which in turn would be predicted to further degrade normal basement membrane extracellular matrix (58). Additionally, type I collagen promoted activation of hepatic stellate cells through discoidin domain tyrosine kinase receptor 2 (DDR2) signaling, which increased expression of active matrix metalloproteinase 2 (MMP-2), leading to enhanced proliferation and invasion (59). Finally, stimulation with IL-1 alpha caused robust induction of pro-MMP-9 (the precursor of matrix metalloproteinase-9) in stellate cells and induced conversion of pro-MMP-9 to the active form when the cells were exposed to type I collagen (60). Thus, a multitude of factors appear to be important in modulating MMP expression and activity.

The family of integrins are important mediators cell-matrix interactions. These heterodimeric molecules (made up of an alpha chain and a beta chain) recognize a number of motifs on extracellular proteins, perhaps the most prominent of which include the amino acids Arg-Gly-Asp (RGD), which mediate a number of important cellular functions (61). Hepatic stellate cells express the integrin $\alpha 1\beta 1$, which mediates not only stellate cell adhesion to type I collagen, but also stellate cell contraction (62). Stellate cells express a multitude of other integrins, which are important in a variety of responses in the wounding milieu (63).

The immune system, stellate cells, and fibrogenesis

More and more evidence now points to a role for the immune system in regulation of stellate cell fibrogenesis. For example, interferon γ has direct and potent anti-fibrogenic activity on stellate cells and in the whole liver (64, 65). Other immunomodulatory cytokines by lymphocytes, natural killer (NK) cells and macrophages also appear to be important. These can typically be divided into pro-inflammatory Th1 cytokines (interferon γ, interleukins 2, 3, and 12, and TNF-α) and anti-inflammatory, pro-fibrogenic Th2 cytokines (interleukins 4, 5, 9, 10, and 13). A number of studies suggest a role for lymphocyte subsets in fibrogenesis, including those that produce immunomodulatory cytokines (21, 66, 67) as well as those that do not, such as B cell subsets (22).

Summary – Pathophysiology of hepatic fibrosis

Abundant evidence suggests that activation of stellate cells is a key feature in hepatic fibrosis. During liver injury, the following model is evolving; liver injury leads to stellate cell activation which is associated with prominent phenotypic alterations in stellate cells, among which, fibrogenesis is prominent. Fibrogenesis is driven also by autocrine signaling in stellate cells, in particular in response to various cytokines and peptides. A key concept is that understanding the biology of stellate cell activation is likely to highlight potential anti-fibrotic therapies, and emphasizes the point that multiple pathways could be targeted.

Measuring Fibrosis

Implicit in the attempt to more actively treat hepatic fibrosis is the need to develop robust and non-invasive markers of liver fibrosis. This is particularly true since with specific therapy, fibrosis is dynamically reversible. The gold standard for assessment of fibrosis has historically been liver biopsy. However, liver biopsy is invasive, makes both patients and physicians anxious, and can be associated with substantial sampling-error (Table 3). This latter feature is in particular problematic. For example, in a recent study in which 124 patients with chronic HCV infection underwent laparoscopy guided biopsy of the right and left hepatic lobes, 33.1% had a difference of at least one histologic stage (modified Scheuer system) between the right and left lobes (68). Non-invasive tests such as serum markers of fibrosis have been advanced as potential alternatives to liver biopsy (Table 4). Additionally, besides serum markers of fibrosis, a number of models for predicting liver fibrosis have been developed. These include combinations of clinical signs, routine laboratory tests, radiologic imaging modalities, and/or quantitative assays of liver function. Discussion of these methods to non-invasively assess fibrosis can be found in recent reviews (see (69)). Unfortunately, at the current time, while required to non-invasively quantitate the response to therapy, neither single tests nor a combination of tests appears to be highly accurate or reliable.

Table 3.

Feature	Liver Biopsy	Serum Markers
Accuracy	Considered to be the gold standard	Correlates 70-90% with biopsy
Sampling	Samples 0.001% of liver	Samples (in theory) the entire liver
Cost	Expensive	Variable, typically expensive
Risk	Invasive	Safe
Experience	Great	Limited
Availability	Widely available	Limited

Table 4.

"Name"	Components	Sensitivity / Specificity for Advanced fibrosis	PPV / NPV for Advanced fibrosis
AST/ALT ratio	AST/ALT	53%/100%	100%/81%
"Forns" test	[5]platelets, GGT, cholesterol	94%/51%	40%/96%
APRI	AST, platelets	41%/95%	88%/64%
[1]PGA index	platelets, GGT, apolipoprotein A	91%/81%	85%/89%
Fibrotest	GGT, haptoglobin bilirubin, apolipoprotein A alpha-2-macroglobulin	87%/59%	63%/85%
Fibrospect	Hyaluronic acid TIMP-1 alpha-2-macroglobulin	83%/66%	72%/78%
[6]FPI	AST, cholesterol, HOMA-IR	85%/48%	70%/69%
ELF	[1]Numerous ECM proteins	90%/41%	35%/92%

[a]The tests vary substantially with regard to types of liver disease and numbers of patients studied, although most panels have been studied in patients with HCV. The definition of fibrosis has varied for histologic grading systems, as well as in the degree of fibrosis considered to be advanced (for example some studies consider Metavir stage 2-4 to be advanced, while other studies consider Scheuer stage 3-4 to be advanced). Importantly, use of different cutoffs leads to variation in sensitivity/specificity/PPV/NPV (cutoffs recommended by authors was used when possible).
[b]Also includes age in the panel
[c]The PGAA added - alpha-2-macroglobulin
[d]This panel additionally includes age and history of alcohol intake
[e]Components tested include: collagen IV, collagen VI, amino terminal propeptide of type III collagen (PIIINP), matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), tenascin, laminin, and hyaluronic acid (HA).
Abbreviations: AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; APRI, AST to platelet ratio index; TIMP-1, tissue inhibitor of metalloproteinase 1; ECM, extracellular matrix; HOMA-IR = homeostasis model assessment (for insulin resistance)

Therapy for hepatic fibrosis

Advances in understanding of the pathophysiologic basis of fibrogenesis are now leading to novel therapeutic approaches. Several important points should be kept in mind. First, fibrosis results from chronic, not acute liver injury. Thus, it is likely that therapy will need to also be chronic. Although it is likely that newly synthesized collagen may be more susceptible to degradation than old collagen, there is abundant evidence in animal models that even advanced cirrhosis is reversible, and in humans, the data suggest that fibrosis is reversible in hepatitis C (70, 71), hepatitis B (72), autoimmune hepatitis (73), alcoholic liver disease (74), hemochromatosis (75), and in secondary biliary cirrhosis (76). Diseases for which treatment appears to effectively inhibit, reduce, or reverse fibrosis are highlighted in Table 5.

Table 5.

Disease	Therapy
Hepatitis B	Lamivudine
Hepatitis C	*Interferon alpha
Bile duct obstruction	Surgical decompression
Hemochromatosis	Iron depletion
Autoimmune hepatitis	Corticosteroids
Alcoholic hepatitis	Corticosteroids
§Primary biliary cirrhosis	Ursodeoxycholic acid, MTX
‡Non-alcoholic steatohepatitis	PPAR ligands

*or PEG-interferon alpha, with or without ribavirin
§The effect is minimal if present
‡Evidence is weak at this point
Abbreviations: MTX = methotrexate; PPAR = peroxisomal proliferator activated receptor

The most convincing evidence for therapy, as implied above, is for treatment of the underlying disease process. However, there is hope that fibrosis resulting from liver disease not amenable to treatment of the underlying disease process can be treated with agents specifically targeted at fibrosis. It is this authors' belief that the earlier the incipient fibrotic lesion is detected and treated, the more likely it will be amenable to therapy. Implicit in this conclusion is the belief that better mechanisms to detect fibrosis must be developed, preferably methods that simply utilize serum (see above and (69)).

Preclinical studies have highlighted multiple different targets and compounds that could specifically abrogate fibrogenesis. Such therapies have been targeted at many different areas in the fibrogenic cascade, including inhibition of matrix deposition, collagen synthesis, modulation of stellate cell activation, enhancing matrix degradation or stimulation of stellate cell death. Although a number of compounds with specific "antifibrotic activity" have been studied in human trials (in a variety of diseases), at this point, none have been proven to be clearly effective (Table 6). Indeed, a specific anti-fibrotic that fits the profile of an ideal agent - one that is potent, safe, orally bioavailable, and inexpensive - is not yet available.

Table 6.

Therapy	Disease
Colchicine	Multiple
Silymarin	Multiple
Methotrexate	PBC
Ursodeoxycholic acid	PBC, multiple others
Vitamin E	HCV, others
PPC	Alcohol
Malotilate	Alcohol
Penicillamine	PBC
S-Adenosylmethionine	Alcohol
Propylthiouracil	Alcohol

Therapies specifically targeting fibrosis

Colchicine

Colchicine is a plant alkaloid that inhibits polymerization of microtubules, a process that in turn is believed to be required for collagen secretion. Preclinical work indicates that

colchicine has anti-fibrotic properties (77) and colchicine has been examined in a number of human clinical trials (78-81). Numerous different types of liver disease have been studied. In a double blind, randomized, controlled trial examining colchicine in primary biliary cirrhosis, improvements were noted in a number of biochemical markers, but colchicine failed to reduce fibrosis (78). In an often cited, double blind, randomized, controlled trial of colchicine versus placebo in patients with various liver diseases, colchicine led to improved fibrosis as well as a dramatic improvement in survival (79). However, this study was thought to suffer from a variety of methodological concerns and its findings have not been applied in clinical practice. In a recent large VA cooperative multicenter study involving 549 patients comparing colchicine (0.6 mg p.o. Bid) to placebo in patients with alcoholic liver disease, there was no apparent effect of active treatment on survival (histologic data were not obtained) (80). Finally, a meta-analysis including 1138 subjects found that colchicine had no effect on fibrosis or mortality (81). In summary, the data surrounding colchicine suggest that this compound is safe but probably ineffective.

Polyenylphosphatidylcholine

Polyenylphosphatidylcholine has antioxidant properties and since oxidant stress is likely involved in the inflammatory and fibrogenic response to injury, it is an attractive candidate therapy in patients with alcoholic liver disease. In a large, carefully performed randomized, double-blind placebo-controlled trial in 789 alcoholic VA patients with alcoholic hepatitis (82) with a daily average alcohol intake of 16 drinks/day, polyenylphosphatidylcholine failed to lead to significant improvement in fibrosis. Subjects were randomized to either polyenylphosphatidylcholine or placebo for 2 years (the long period of treatment is noteworthy as it is likely that long periods of treatment will be required to effect changes in the liver inflammation/injury/fibrosis axis). Notably, many subjects substantially reduced their ethanol consumption during the trial, making it difficult to show differences between treatment and therapeutic groups.

Interleukin-10

Interleukin-10 is an anti-inflammatory and immune-suppressive cytokine and appears to reduce production of proinflammatory cytokines, such as tumor necrosis factor-α, interleukin-1, interferon γ, and interleukin-2 from T cells (TH1 cytokine family members). Endogenous interleukin-10 appears to reduce the intrahepatic inflammatory response, shift the cytokine milieu toward a TH2 predominance. In addition, it has been shown to reduce fibrosis in several *in vivo* models of liver injury (83). However, a direct anti-fibrotic effect for interleukin-10 on stellate cells has not been established.

In patients with chronic HCV infection mediated fibrosis who had failed antiviral therapy, treatment with IL-10 (84) for 12 months led to reduced intrahepatic inflammation and fibrosis (mean change from 5.0 ± 0.2 to 4.5 ± 0.3, $p < 0.05$). However, serum HCV RNA levels increased during therapy (mean HCV RNA at day 0: 12.3 ± 3.0 meq/mL; and at 12 months: 38 meq/mL; $p < 0.05$). There was also an apparent shift in lymphocyte response toward a Th2 predominant phenotype. Thus, while longer-term therapy with interleukin-10 decreased hepatic inflammatory activity and appeared to have an inhibitory overall effect on fibrosis it also led to increased HCV viral levels, causing concern about long-term effects of this treatment in patients

with HCV. Indeed, while potentially anti-fibrotic, interleukin-10 is unlikely to emerge as a viable anti-fibrotic compound because of its virological effects.

Interferon gamma (γ)

The interferons consist of a family of 3 major isoforms. The 3 isoforms, α, β and γ are unique structurally and also in their biologic actions. Interferon α and β bind to the same receptor and therefore share common functional and signaling characteristics. A number of interferon α subtypes exist, while there appear to be only single interferon β and interferon γ species. Interferon α has more potent antiviral effects than does interferon γ, while interferon γ has been shown to inhibit extracellular matrix synthesis in hepatic stellate cells (64, 85).

The preclinical data have generated considerable enthusiasm about the use of interferon γ in patients with hepatic fibrogenesis, although there is theoretical concern about its use because it is proinflammatory and moreover, its overexpression in the liver leads to chronic hepatitis (86). Nonetheless, in a pilot study, it appeared to be safe in patients with chronic HCV infection and in a subgroup of patients may have had anti-fibrotic effects (87). While this pilot study provides a firm foundation underscoring the potential use of interferon γ in patients, larger randomized studies are needed to prove a therapeutic benefit.

Silymarin

Silymarin is the active ingredient from the milk thistle, Silybum marianum. Preclinical data indicate that silymarin reduced lipid peroxidation and fibrogenesis in rodents (88, 89) and in baboons (90). It has been tested in human clinical trials, although fibrosis was not used as an outcome. The compound appears to be safe, but has been reported to have mixed effects (91, 92). In one study examining silymarin in alcoholics (91), mortality was reduced; in addition, patients with early stages of cirrhosis also appeared to benefit. However, in another study in alcoholics, no survival benefit was found (92). Nonetheless, because silymarin appeared to be safe, and may be effective an NIH sponsored clinical trial is planned, hopefully including patients with chronic HCV infection.

Ursodeoxycholic acid

Ursodeoxycholic acid presumably stabilizes cell membranes and is thus cytoprotective. This cytoprotective action in turn theoretically reduces inflammation and thus may have a beneficial effect on fibrogenesis (93). While neither experimental nor human data indicate a primary anti-fibrotic effect, the compound has been examined extensively in humans in many different diseases (94-102). The greatest experience has been in primary biliary cirrhosis, a disease in which results with have been mixed. Both symptomatic and biochemical improvements have been observed in patients with primary biliary cirrhosis, but data on histological improvement and survival have not been consistent. For example, in a randomized controlled trial examining patients with primary biliary cirrhosis, ursodeoxycholic acid led to reduced fibrosis in those with mild disease, but had no effect on those with severe disease (95). Longer term follow-up revealed no effect on orthotopic liver transplantation or mortality (103). In another study, survival was improved in patients treated with ursodeoxycholic acid, but

fibrogenesis was not improved (99). Further, in a histopathological study of 54 patients with primary biliary cirrhosis and paired liver biopsies, 4 years of ursodeoxycholic acid therapy was associated with a significant decrease in the prevalence of florid interlobular bile duct lesions, lobular inflammation, and necrosis. Worsening of fibrosis was observed in 14 patients (the majority had only a one grade progression in fibrosis score) whereas stabilization was noted in the 40 remaining patients (100). Results of meta-analyses have also been mixed, and have largely reported that ursodeoxycholic acid is not effective in primary biliary cirrhosis (98). A combined analysis of the histologic effect of ursodeoxycholic acid on paired liver biopsies including a total of 367 patients suggested that ursodeoxycholic acid delayed histologic progression of disease patients with early disease (101). The data suggest that ursodeoxycholic acid may impede progression of fibrosis in primary biliary cirrhosis, presumably via effects on (bile duct) inflammation, particularly if given early in the disease course.

Ursodeoxycholic acid has been studies in several other liver diseases, including familial intrahepatic cholestasis (96), cystic fibrosis (97), and non-alcoholic steatohepatitis (102). In children with progressive familial intrahepatic cholestasis, it appeared to improve fibrogenesis (96). Additionally, a small series indicated that 7 of 10 patients with cystic fibrosis treated with ursodeoxycholic acid had a reduction in liver fibrosis (97). Although these effects are promising, it should be emphasized that the numbers of patients studied has been small and studies have not been randomized. Finally, in a large randomized controlled trial of ursodeoxycholic acid in patients with non-alcoholic steatohepatitis over 2-years, including 107 subjects who had paired biopsy data, there was no improvement in fibrosis (102). Thus, although ursodeoxycholic acid is expensive, in the absence of more effective therapy, the available data probably justify the use of ursodeoxycholic acid in patients with primary biliary cirrhosis as an anti-fibrotic, but do not support its use in other liver diseases including chronic HCV.

Miscellaneous

A variety of compounds have been examined in patients with various types of liver disease. A preliminary report in patients with non-alcoholic steatohepatitis (NASH) treated with the peroxisome proliferator-activated receptor (PPAR) gamma agonist, rosiglitazone reduced both steatosis and fibrosis (104). Antioxidants such as vitamin E have been examined in animal models (105) as well as in humans (106-109). d-alpha-tocopherol, a vitamin E precursor (1200 IU/day for 8 weeks), was studied in 6 patients with HCV infection who failed to respond to interferon α therapy (106), and was found to inhibit stellate cell activation, but did not affect fibrosis. A randomized controlled trial examined vitamin E in patients with mild to moderate alcoholic hepatitis found that vitamin E reduced serum hyaluronic acid, but did not lead to a change in type III collagen (108). Combined antioxidant therapy, including vitamin E, had no effect on outcome in patients with severe alcoholic hepatitis, although fibrosis was not specifically addressed (109). In patients with primary biliary cirrhosis, malotilate, a presumed cytoprotective agent, was found to diminish plasma cell and lymphocytic infiltrate and piece-meal necrosis, but had no significant effect on fibrogenesis (110). Penicillamine, a heavy metal chelating compound with presumed anti-inflammatory and thus antifibrogenic effects (111) had no effect on fibrogenesis in patients with primary biliary cirrhosis (112, 113). Metrothrexate, typically considered to be profibrogenic (114) has received great attention in patients with primary biliary cirrhosis. Some investigators have reported highly favorable effects of

methotrexate in this disease, including improvement the disease and reversion of fibrosis (115), but a large randomized trial revealed no added benefit over ursodeoxycholic acid (116) (if methotrexate is used to treat patients with primary biliary cirrhosis, this must be overseen by an experienced Hepatologist). S-adenosylmethionine, important in the synthesis of the anti-oxidant, glutathione has been found to be present in reduced amounts in the injured liver (117) and thus, it has been hypothesized that if S-adenosylmethionine were replaced, then injury and fibrogenesis might be reduced. S-adenosylmethionine led to an improvement in overall mortality/need for liver transplantation in the treatment arm, especially in patients with Child's A/B cirrhosis, although information fibrosis was limited (118). Propylthiouracil, another presumed antioxidant is an anti-thyroid drug that reacts with some of the oxidizing species derived from the respiratory burst and thus may be protective in alcoholic liver disease, a disease in which an increase in hepatic oxygen consumption may predispose the liver to ischemic injury. Thus, propylthiouracil has been tested in randomized clinical trials in patients with alcoholic liver disease. Unfortunately, a systematic review and meta-analysis found that propylthiouracil led to no benefit in fibrosis (or other outcome variables) (119). Anabolic-androgenic steroids such as oxandrolone have been examined in randomized trials including patients with alcoholic liver disease, but have not been found to have significant effects on fibrosis (or other outcomes) (120). Several recent small studies have examined the effect of anti-tumor necrosis factor alpha (TNF-α) compounds in patients with alcohol induced liver disease (121-124), and have reported improved histology. Concerns about potential toxicity however must be addressed, especially if this compound were to be considered in patients with HCV.

Future Anti-fibrotics

With the vast number of advances in understanding the biology of hepatic fibrogenesis, it is not surprising that multiple pathways have been targeted as having therapeutic potential. Many compounds have been studied in experimental models and have been shown to have anti-fibrotic properties (see (125) for review). Several of the canonical pathways are attractive as therapeutic targets (Table 7). An important example is the TGF-β pathway, since it plays a central role in the fibrogenic cascade. Several approaches to inhibit the action of TGF-β have been proposed and include use of molecules such as decorin, the protein core component of proteoglycan, which binds and inactivates TGF-β (126), antibodies directed against TGF-β1, and soluble receptors which typically encode for sequences that bind active TGF-β and prevent it from binding to cognate receptors. The concept has been well established experimentally; indeed, the effect of inhibition of TGF-β in animal models of liver injury and fibrogenesis has been striking (127, 128). Additionally, stellate cells express angiotensin and endothelin receptors and stimulation of stellate cells with their respective ligands leads to stellate cell activation (43). The angiotensin II pathway is particularly attractive because there are already numerous safe and potent compounds in use in

Table 7

Compound	Mechanism
Rapamycin Mycophenolate Gliotoxin Pentoxifylline HOE 77 Endothelin antagonists Prazosin *Angiotensin II receptor antagonists Sho-saiko-to (TJ-9) Retinoic acid RGD peptides	Inhibit stellate cell activation and fibrogenesis
Halofuginone Anti-TGF beta Follistatin	Inhibit stellate cell fibrogenesis
Prostaglandins Adiponectin Estradiol Salvia Miltiorhiza IL-1 antagonist Octreotide Glycyrrhizin HGF **STAP PAR antagonists PPARs C-5 depletion 5-lipoxygenase inhibitors Angiostatin	Other/unknown

*Also angiotensin converting enzyme inhibitors
**STAP = stellate cell activation-associated protein

humans. Others compounds such as pirfenidone (129), peroxisomal proliferator activated receptor (PPAR) gamma ligands (130-132), and halofuginone (133) appear to have direct effects on stellate cells and thus could evolve into effective anti-fibrotic compounds.

Summary and Future

New therapies and effective for treatment of fibrosis are soon to emerge. A critical concept is that the fibrotic lesion, in particular, the extracellular matrix component, is dynamic and that the accumulation of fibrosis may be inhibited. Further, it is possible that fibrosis, including even advanced fibrosis may be reversible under the appropriate conditions. A key element is that anti-fibrotic approaches should be mechanism based (e.g. focused on the activation of hepatic stellate cells). It should be noted that factors controlling activation are multifactorial, and thus multiple different potential therapeutic interventions are likely to be possible. Indeed, it is this author's opinion that multi-drug treatments will be more effective than single agents.

Currently, effective therapy for hepatic fibrogenesis exists in the form of removal of the underlying disease process in certain diseases. In contrast, specific therapy directed only at the fibrotic lesion is not currently available; the most effective therapies will most likely be directed at the stellate cell. It is predicted that in the future, patients with chronic HCV infection will be treated with combination approaches that target both viral eradication, as well as the fibrotic lesion, particularly in patients with advanced (e.g., stage 3 fibrosis). At the time of writing of this review, several different compounds are being explored in clinical trials and it is expected that specific, effective, safe, and inexpensive compounds will soon be identified.

Acknowledgment

This work was supported by the National Institutes of Health (Grants R01 DK50574, DK57830, and DK63308).

References

1. Rockey DC, Friedman SL. Hepatic Fibrosis. In Hepatology: A Textbook of Liver Disease; 5th Edition, 2006.
2. Kim WR, Brown RS, Jr., Terrault NA, El-Serag H. Burden of liver disease in the United States: summary of a workshop. Hepatology 2002;36:227-242.
3. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. Hepatology 2002;36:S74-83.
4. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. Liver Transpl 2003;9:331-338.
5. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular cancer. Cancer Cell 2004;5:215-219.
6. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. J Hepatol 2001;34:730-739.
7. Rojkind M, Martinez-Palomo A. Increase in type I and type III collagens in human alcoholic liver cirrhosis. Proc.Natl.Acad.Sci.U.S.A. 1976;73:539-543.
8. Schuppan D. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. Semin.Liver.Dis. 1990;10:1-10.
9. Meyer DH, Krull N, Dreher KL, Gressner AM. Biglycan and decorin gene expression in normal and fibrotic rat liver: cellular localization and regulatory factors. Hepatology 1992;16:204-216.
10. Gallai M, Kovalszky I, Knittel T, Neubauer K, Armbrust T, Ramadori G. Expression of extracellular matrix proteoglycans perlecan and decorin in carbon-tetrachloride-injured rat liver and in isolated liver cells. Am J Pathol 1996;148:1463-1471.
11. Jezequel AM, Ballardini G, Mancini R, Paolucci F, Bianchi FB, Orlandi F. Modulation of extracellular matrix components during dimethylnitrosamine-induced cirrhosis. J.Hepatol. 1990;11:206-214.
12. Maher JJ, McGuire RF. Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. J.Clin.Invest. 1990;86:1641-1648.
13. Jarnagin WR, Rockey DC, Koteliansky VE, Wang SS, Bissell DM. Expression of variant fibronectins in wound healing: cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis. J.Cell Biol. 1994;127:2037-2048.
14. Van Eyken P, Geerts A, De Bleser P, Lazou JM, Vrijsen R, Sciot R, Wisse E, et al. Localization and cellular source of the extracellular matrix protein tenascin in normal and fibrotic rat liver. Hepatology 1992;15:909-916.
15. Schaffer CJ, Nanney LB. Cell biology of wound healing. Int Rev Cytol 1996;169:151-181.
16. Martin P. Wound healing--aiming for perfect skin regeneration. Science 1997;276:75-81.
17. Holstege A, Bedossa P, Poynard T, Kollinger M, Chaput JC, Houglum K, Chojkier M. Acetaldehyde-modified epitopes in liver biopsy specimens of alcoholic and nonalcoholic patients: localization and association with progression of liver fibrosis [published erratum appears in Hepatology 1994 Dec;20(6):1664]. Hepatology 1994;19:367-374.

18. Lee KS, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. J Clin Invest 1995;96:2461-2468.
19. Svegliati Baroni G, D'Ambrosio L, Ferretti G, Casini A, Di Sario A, Salzano R, Ridolfi F, et al. Fibrogenic effect of oxidative stress on rat hepatic stellate cells. Hepatology 1998;27:720-726.
20. Choy C, Sempkowski G, Rockey DC. 2002.
21. Safadi R, Ohta M, Alvarez CE, Fiel MI, Bansal M, Mehal WZ, Friedman SL. Immune stimulation of hepatic fibrogenesis by CD8 cells and attenuation by transgenic interleukin-10 from hepatocytes. Gastroenterology 2004;127:870-882.
22. Novobrantseva TI, Majeau GR, Amatucci A, Kogan S, Brenner I, Casola S, Shlomchik MJ, et al. Attenuated liver fibrosis in the absence of B cells. J Clin Invest 2005;115:3072-3082.
23. Kent G, Gay S, Inouye T, Bahu R, Minick OT, Popper H. Vitamin A-containing lipocytes and formation of type III collagen in liver injury. Proc.Natl.Acad.Sci.U.S.A. 1976;73:3719-3722.
24. Friedman SL, Roll FJ, Boyles J, Bissell DM. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. Proc.Natl.Acad.Sci.U.S.A. 1985;82:8681-8685.
25. Johnson RJ, Iida H, Alpers CE, Majesky MW, Schwartz SM, Pritzi P, Gordon K, et al. Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. Alpha-smooth muscle actin is a marker of mesangial cell proliferation. J Clin Invest 1991;87:847-858.
26. Wake K. Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs. Int.Rev.Cytol. 1980;66:303-353.
27. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. Roles of tgf-Beta in hepatic fibrosis. Front Biosci 2002;7:D793-807.
28. Friedman SL, Arthur MJ. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J.Clin.Invest. 1989;84:1780-1785.
29. Rockey DC, Housset CN, Friedman SL. Activation-dependent contractility of rat hepatic lipocytes in culture and in vivo. J.Clin.Invest. 1993;92:1795-1804.
30. Rockey D. The cellular pathogenesis of portal hypertension: stellate cell contractility, endothelin, and nitric oxide. Hepatology 1997;25:2-5.
31. Pinzani M, Abboud HE, Gesualdo L, Abboud SL. Regulation of macrophage colony-stimulating factor in liver fat-storing cells by peptide growth factors. Am.J.Physiol. 1992;262:C876-C881.
32. Marra F, Valente AJ, Pinzani M, Abboud HE. Cultured human liver fat-storing cells produce monocyte chemotactic protein-1. Regulation by proinflammatory cytokines. J.Clin.Invest. 1993;92:1674-1680.
33. Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. Semin Liver Dis 2001;21:397-416.
34. Marra F. Chemokines in liver inflammation and fibrosis. Front Biosci 2002;7:d1899-1914.
35. Arthur MJ, Friedman SL, Roll FJ, Bissell DM. Lipocytes from normal rat liver release a neutral metalloproteinase that degrades basement membrane (type IV) collagen. J.Clin.Invest. 1989;84:1076-1085.

36. Iredale JP, Murphy G, Hembry RM, Friedman SL, Arthur MJ. Human hepatic lipocytes synthesize tissue inhibitor of metalloproteinases-1. Implications for regulation of matrix degradation in liver. J.Clin.Invest. 1992;90:282-287.
37. Iredale JP. Stellate cell behavior during resolution of liver injury. Seminars in Liver Disease 2001;21:427-436.
38. Canbay A, Taimr P, Torok N, Higuchi H, Friedman S, Gores GJ. Apoptotic body engulfment by a human stellate cell line is profibrogenic. Lab Invest 2003;83:655-663.
39. Bataller R, Paik YH, Lindquist JN, Lemasters JJ, Brenner DA. Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. Gastroenterology 2004;126:529-540.
40. Mazzocca A, Sciammetta SC, Carloni V, Cosmi L, Annunziato F, Harada T, Abrignani S, et al. Binding of hepatitis C virus envelope protein E2 to CD81 up-regulates matrix metalloproteinase-2 in human hepatic stellate cells. J Biol Chem 2005;280:11329-11339.
41. Rockey DC, Boyles JK, Gabbiani G, Friedman SL. Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. J.Submicrosc.Cytol.Pathol. 1992;24:193-203.
42. Sims DE. Recent advances in pericyte biology--implications for health and disease. Can.J.Cardiol. 1991;7:431-443.
43. Rockey DC. Vascular mediators in the injured liver. Hepatology 2003;37:4-12.
44. Rockey DC, Weisiger RA. Endothelin induced contractility of stellate cells from normal and cirrhotic rat liver: implications for regulation of portal pressure and resistance. Hepatology 1996;24:233-240.
45. Bauer M, Paquette NC, Zhang JX, Bauer I, Pannen BH, Kleeberger SR, Clemens MG. Chronic ethanol consumption increases hepatic sinusoidal contractile response to endothelin-1 in the rat. Hepatology 1995;22:1565-1576.
46. Irle C, Kocher O, Gabbiani G. Contractility of myofibroblasts during experimental liver cirrhosis. Journal of Submicroscopic Cytology 1980;12:209-217.
47. Bissell DM, Wang SS, Jarnagin WR, Roll FJ. Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J Clin Invest 1995;96:447-455.
48. Marra F, Choudhury GG, Pinzani M, Abboud HE. Regulation of platelet-derived growth factor secretion and gene expression in human liver fat-storing cells. Gastroenterology 1994;107:1110-1117.
49. Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, et al. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. Hepatology 1999;30:968-976.
50. Shao R, Yan W, Rockey DC. Regulation of endothelin-1 synthesis by endothelin-converting enzyme-1 during wound healing. J Biol Chem 1999;274:3228-3234.
51. Bataller R, Gines P, Nicolas JM, Gorbig MN, Garcia-Ramallo E, Gasull X, Bosch J, et al. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. Gastroenterology 2000;118:1149-1156.
52. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998;102:538-549.

53. Arthur MJ, Stanley A, Iredale JP, Rafferty JA, Hembry RM, Friedman SL. Secretion of 72 kDa type IV collagenase/gelatinase by cultured human lipocytes. Analysis of gene expression, protein synthesis and proteinase activity. Biochem.J. 1992;287:701-707.
54. Takahara T, Furui K, Funaki J, Nakayama Y, Itoh H, Miyabayashi C, Sato H, et al. Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats. Hepatology 1995;21:787-795.
55. Herbst H, Wege T, Milani S, Pellegrini G, Orzechowski HD, Bechstein WO, Neuhaus P, et al. Tissue inhibitor of metalloproteinase-1 and -2 RNA expression in rat and human liver fibrosis. Am J Pathol 1997;150:1647-1659.
56. Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2000;279:G245-249.
57. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, et al. Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. Hepatology 2002;36:850-860.
58. Preaux AM, Mallat A, Van Nhieu JT, D'Ortho MP, Hembry RM, Mavier P. Matrix metalloproteinase-2 activation in human hepatic fibrosis regulation by cell-matrix interactions. Hepatology 1999;30:944-950.
59. Olaso E, Ikeda K, Eng FJ, Xu L, Wang LH, Lin HC, Friedman SL. DDR2 receptor promotes MMP-2-mediated proliferation and invasion by hepatic stellate cells. J Clin Invest 2001;108:1369-1378.
60. Han YP, Zhou L, Wang J, Xiong S, Garner WL, French SW, Tsukamoto H. Essential role of matrix metalloproteinases in interleukin-1-induced myofibroblastic activation of hepatic stellate cell in collagen. J Biol Chem 2004;279:4820-4828.
61. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Ann Rev Cell Dev Biol 1996;12:463-518.
62. Racine-Samson L, Rockey DC, Bissell DM. The role of alpha1beta1 integrin in wound contraction. A quantitative analysis of liver myofibroblasts in vivo and in primary culture. J Biol Chem 1997;272:30911-30917.
63. Zhou X, Murphy FR, Gehdu N, Zhang J, Iredale JP, Benyon RC. Engagement of alphavbeta3 integrin regulates proliferation and apoptosis of hepatic stellate cells. J Biol Chem 2004;279:23996-24006.
64. Rockey DC, Chung JJ. Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis. J Investig Med 1994;42:660-670.
65. Baroni GS, D'Ambrosio L, Curto P, Casini A, Mancini R, Jezequel AM, Benedetti A. Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. Hepatology 1996;23:1189-1199.
66. Shi Z, Wakil AE, Rockey DC. Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. Proc Natl Acad Sci U S A 1997;94:10663-10668.
67. Radaeva S, Sun R, Jaruga B, Nguyen VT, Tian Z, Gao B. Natural Killer Cells Ameliorate Liver Fibrosis by Killing Activated Stellate Cells in NKG2D-Dependent and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Dependent Manners. Gastroenterology 2006;130:435-452.

68. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614-2618.
69. Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. Hepatology 2006;43:S113-120.
70. Dufour JF, DeLellis R, Kaplan MM. Regression of hepatic fibrosis in hepatitis C with long-term interferon treatment. Dig Dis Sci 1998;43:2573-2576.
71. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122:1303-1313.
72. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.
73. Dufour JF, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. Ann Intern Med 1997;127:981-985.
74. Ramond MJ, Poynard T, Rueff B, Mathurin P, Theodore C, Chaput JC, Benhamou JP. A randomized trial of prednisolone in patients with severe alcoholic hepatitis. N Engl J Med 1992;326:507-512.
75. Perez-Tamayo R. Cirrhosis of the liver: a reversible disease? Pathol Annu 1979;14:183-213.
76. Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Flejou JF, Degott C, et al. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med 2001;344:418-423.
77. Rodriguez L, Cerbon-Ambriz J, Munoz ML. Effects of colchicine and colchiceine in a biochemical model of liver injury and fibrosis. Arch Med Res 1998;29:109-116.
78. Kaplan MM, Alling DW, Zimmerman HJ, Wolfe HJ, Sepersky RA, Hirsch GS, Elta GH, et al. A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 1986;315:1448-1454.
79. Kershenobich D, Vargas F, Garcia-Tsao G, Perez Tamayo R, Gent M, Rojkind M. Colchicine in the treatment of cirrhosis of the liver. N Engl J Med 1988;318:1709-1713.
80. Morgan TR, Nemchausky B, Schiff ER, Anand BS, Lodap J, Bennett C, Mendenhall C, et al. Colchicine does not prolong life in patients with advanced alcoholic cirrhosis: results of a prospective, randomized, placebo-controlled trial. Gastroenterology 2002:641A.
81. Rambaldi A, Gluud C. Colchicine for alcoholic and non-alcoholic liver fibrosis or cirrhosis. Liver 2001;21:129-136.
82. Lieber CS, Weiss DG, Groszmann R, Paronetto F, Schenker S. II. Veterans Affairs Cooperative Study of Polyenylphosphatidylcholine in Alcoholic Liver Disease. Alcohol Clin Exp Res 2003;27:1765-1772.
83. Thompson K, Maltby J, Fallowfield J, McAulay M, Millward-Sadler H, Sheron N. Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis [see comments]. Hepatology 1998;28:1597-1606.
84. Nelson DR, Tu Z, Soldevila-Pico C, Abdelmalek M, Zhu H, Xu YL, Cabrera R, et al. Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology 2003;38:859-868.
85. Rockey DC, Maher JJ, Jarnagin WR, Gabbiani G, Friedman SL. Inhibition of rat hepatic lipocyte activation in culture by interferon-gamma. Hepatology 1992;16:776-784.

86. Toyonaga T, Hino O, Sugai S, Wakasugi S, Abe K, Shichiri M, Yamamura K. Chronic active hepatitis in transgenic mice expressing interferon-gamma in the liver. Proc.Natl.Acad.Sci.U.S.A. 1994;91:614-618.
87. Muir AJ, Sylvestre PB, Rockey DC. Interferon gamma-1b for the treatment of fibrosis in chronic hepatitis C infection. J Viral Hepat 2006;13:322-328.
88. Boigk G, Stroedter L, Herbst H, Waldschmidt J, Riecken EO, Schuppan D. Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. Hepatology 1997;26:643-649.
89. Jia JD, Bauer M, Cho JJ, Ruehl M, Milani S, Boigk G, Riecken EO, et al. Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagen alpha1(I) and TIMP-1. J Hepatol 2001;35:392-398.
90. Lieber CS, Leo MA, Cao Q, Ren C, DeCarli LM. Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons. J Clin Gastroenterol 2003;37:336-339.
91. Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, Meryn S, et al. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. J Hepatol 1989;9:105-113.
92. Pares A, Planas R, Torres M, Caballeria J, Viver JM, Acero D, Panes J, et al. Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial [see comments]. J Hepatol 1998;28:615-621.
93. Nava-Ocampo AA, Suster S, Muriel P. Effect of colchiceine and ursodeoxycholic acid on hepatocyte and erythrocyte membranes and liver histology in experimentally induced carbon tetrachloride cirrhosis in rats. Eur J Clin Invest 1997;27:77-84.
94. Stiehl A. Ursodeoxycholic acid in the treatment of primary sclerosing cholangitis. Ann Med 1994;26:345-349.
95. Combes B, Carithers RL, Jr., Maddrey WC, Lin D, McDonald MF, Wheeler DE, Eigenbrodt EH, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1995;22:759-766.
96. Jacquemin E, Hermans D, Myara A, Habes D, Debray D, Hadchouel M, Sokal EM, et al. Ursodeoxycholic acid therapy in pediatric patients with progressive familial intrahepatic cholestasis. Hepatology 1997;25:519-523.
97. Lindblad A, Glaumann H, Strandvik B. A two-year prospective study of the effect of ursodeoxycholic acid on urinary bile acid excretion and liver morphology in cystic fibrosis-associated liver disease. Hepatology 1998;27:166-174.
98. Goulis J, Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic-acid therapy for primary biliary cirrhosis: a meta-analysis. Lancet 1999;354:1053-1060.
99. Poupon RE, Bonnand AM, Chretien Y, Poupon R. Ten-year survival in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. The UDCA-PBC Study Group. Hepatology 1999;29:1668-1671.
100. Degott C, Zafrani ES, Callard P, Balkau B, Poupon RE, Poupon R. Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression. Hepatology 1999;29:1007-1012.
101. Poupon RE, Lindor KD, Pares A, Chazouilleres O, Poupon R, Heathcote EJ. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. J Hepatol 2003;39:12-16.

102. Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology 2004;39:770-778.
103. Combes B, Luketic VA, Peters MG, Zetterman RK, Garcia-Tsao G, Munoz SJ, Lin D, et al. Prolonged follow-up of patients in the U.S. multicenter trial of ursodeoxycholic acid for primary biliary cirrhosis. Am J Gastroenterol 2004;99:264-268.
104. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology 2003;38:1008-1017.
105. Brown KE, Poulos JE, Li L, Soweid AM, Ramm GA, O'Neill R, Britton RS, et al. Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload. Am J Physiol 1997;272:G116-123.
106. Houglum K, Venkataramani A, Lyche K, Chojkier M. A pilot study of the effects of d-alpha-tocopherol on hepatic stellate cell activation in chronic hepatitis C. Gastroenterology 1997;113:1069-1073.
107. Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. Aliment Pharmacol Ther 2001;15:1667-1672.
108. Mezey E, Potter J, Rennie-Tankersley L, Caballeria J, Pares A. A randomized placebo controlled trial of vitamin E in alcoholic hepatitis. Hepatology 2003;38:264A.
109. Stewart S, Prince M, Bassendine M, Hudson M, James O, Jone D, Record C, et al. A trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. Journal of Hepatology 2002;36 (S):16.
110. Anonymous. The results of a randomized double blind controlled trial evaluating malotilate in primary biliary cirrhosis. A European multicentre study group. J Hepatol 1993;17:227-235.
111. Schaff Z, Lapis K, Szende B, Jeney A, Gergely P, Simon K, Divald A, et al. The effect of D-penicillamine on CCl4-induced experimental liver cirrhosis. Exp Pathol 1991;43:111-120.
112. Bodenheimer HC, Jr., Schaffner F, Sternlieb I, Klion FM, Vernace S, Pezzullo J. A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 1985;5:1139-1142.
113. Dickson ER, Fleming TR, Wiesner RH, Baldus WP, Fleming CR, Ludwig J, McCall JT. Trial of penicillamine in advanced primary biliary cirrhosis. N Engl J Med 1985;312:1011-1015.
114. Aithal GP, Haugk B, Das S, Card T, Burt AD, Record CO. Monitoring methotrexate-induced hepatic fibrosis in patients with psoriasis: are serial liver biopsies justified? Aliment Pharmacol Ther 2004;19:391-399.
115. Kaplan MM, DeLellis RA, Wolfe HJ. Sustained biochemical and histologic remission of primary biliary cirrhosis in response to medical treatment. Ann Intern Med 1997;126:682-688.
116. Combes B, Emerson SS, Flye NL, Munoz SJ, Luketic VA, Mayo MJ, McCashland TM, et al. Methotrexate (MTX) plus ursodeoxycholic acid (UDCA) in the treatment of primary biliary cirrhosis. Hepatology 2005;42:1184-1193.
117. Lu SC, Tsukamoto H, Mato JM. Role of abnormal methionine metabolism in alcoholic liver injury. Alcohol 2002;27:155-162.
118. Mato JM, Camara J, Fernandez de Paz J, Caballeria L, Coll S, Caballero A, Garcia-Buey L, et al. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. J Hepatol 1999;30:1081-1089.

119. Rambaldi A, Gluud C. Meta-analysis of propylthiouracil for alcoholic liver disease--a Cochrane Hepato-Biliary Group Review. Liver 2001;21:398-404.
120. Rambaldi A, Iaquinto G, Gluud C. Anabolic-androgenic steroids for alcoholic liver disease: a Cochrane review. Am J Gastroenterol 2002;97:1674-1681.
121. Akriviadis E, Botla R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. Gastroenterology 2000;119:1637-1648.
122. Spahr L, Rubbia-Brandt L, Frossard JL, Giostra E, Rougemont AL, Pugin J, Fischer M, et al. Combination of steroids with infliximab or placebo in severe alcoholic hepatitis: a randomized controlled pilot study. J Hepatol 2002;37:448-455.
123. Tilg H, Jalan R, Kaser A, Davies NA, Offner FA, Hodges SJ, Ludwiczek O, et al. Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. J Hepatol 2003;38:419-425.
124. Menon KV, Stadheim L, Kamath PS, Wiesner RH, Gores GJ, Peine CJ, Shah V. A pilot study of the safety and tolerability of etanercept in patients with alcoholic hepatitis. Am J Gastroenterol 2004;99:255-260.
125. Rockey DC. Antifibrotic therapy in chronic liver disease. Clin Gastroenterol Hepatol 2005;3:95-107.
126. Isaka Y, Brees DK, Ikegaya K, Kaneda Y, Imai E, Noble NA, Border WA. Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney. Nature Medicine 1996;2:418-423.
127. George J, Roulot D, Koteliansky VE, Bissell DM. In vivo inhibition of rat stellate cell activation by soluble TGF beta type II receptor: a potential new therapy for hepatic fibrosis. Proceedings of the National Academy of Sciences:USA 1999;96:12719-12724.
128. Yata Y, Gotwals P, Koteliansky V, Rockey DC. Dose-dependent inhibition of hepatic fibrosis in mice by a TGF-beta soluble receptor: implications for antifibrotic therapy. Hepatology 2002;35:1022-1030.
129. Di Sario A, Bendia E, Svegliati Baroni G, Ridolfi F, Casini A, Ceni E, Saccomanno S, et al. Effect of pirfenidone on rat hepatic stellate cell proliferation and collagen production. J Hepatol 2002;37:584-591.
130. Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF, Jr., Motomura K, Anania FA, et al. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000;275:35715-35722.
131. Marra F, Efsen E, Romanelli RG, Caligiuri A, Pastacaldi S, Batignani G, Bonacchi A, et al. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. Gastroenterology 2000;119:466-478.
132. Yang L, Chan CC, Kwon OS, Liu S, McGhee J, Stimpson S, Chen L, et al. Regulation of peroxisome proliferator-activated receptor gamma (PPAR γ) in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2006 (In Press).
133. Bruck R, Genina O, Aeed H, Alexiev R, Nagler A, Avni Y, Pines M. Halofuginone to prevent and treat thioacetamide-induced liver fibrosis in rats. Hepatology 2001;33:379-386.