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Reversal of Fibrosis: No Longer a Pipe Dream?

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Fibrosis, or scarring, is a highly conserved evolutionary response to limit tissue damage and serves as a generic response to chronic liver injury, regardless of etiology. Progressive scarring in response to a persisting liver insult eventually leads to "cirrhosis," with disorganization of the normal liver architecture that is characterized by fibrotic bands, parenchymal nodules, and vascular distortion. Alterations in the hepatic vasculature, particularly the development of fibrous vascularized septa that link portal tracts and central veins, result in intrahepatic shunting of blood [1]. Subsequent liver cell dysfunction and portal hypertension result in grave clinical complications, such as variceal hemorrhage, encephalopathy, ascites, and the hepatorenal syndrome. There is the added risk for hepatocellular carcinoma, an aggressive neoplasm that causes death for most patients within 6 months unless it is detected early. Cirrhosis is the seventh leading cause of death by disease; it affects several hundred million people worldwide, and, thus, represents a major global health burden. Overall, chronic viral infection is the dominant cause, but in the developed countries of the West, alcohol is the primary cause. The only curative treatment for end-stage cirrhosis is liver transplantation, but a shortage of available donors and the often poor state of health and nutrition of the potential recipient limit its clinical applicability.

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For years, liver fibrosis was considered irreversible; however, there is accumulating clinical and experimental evidence to suggest that this axiom be rejected. Data from the histologic assessment of biopsy tissue from patients who have chronic liver disease of various etiologies who have been treated successfully, and from animal models of fibrosis, indicate that liver fibrosis is a dynamic, bidirectional process, wherein recovery with remodeling of scar tissue is possible. Furthermore, by understanding the cell and molecular mechanisms that mediate the reversibility of liver fibrosis, we may establish the attributes that are required of an effective antifibrotic therapy. The impact of fibrosis remodelling on clinical parameters, such as portal hypertension or hepatocellular carcinoma, is unknown, but these outcome measures likely will be addressed in future studies.

Evidence for reversibility

The concept of reversibility of liver fibrosis and cirrhosis is not a radical one. Reversal of cirrhosis was reported more than 30 years ago in patients who had hemochromatosis after long-term intensive venesection therapy [2]. Regression of scarring also has been reported in patients who have autoimmune chronic active hepatitis (Fig. 1) [3,4] and primary biliary cirrhosis [5] after successful immunosuppressive therapy. Further support also has been accrued from lamivudine treatment in patients who have chronic hepatitis B [6] and following treatment of chronic delta hepatitis with long-term interferon-α [7]. In addition, a recent study demonstrated regression of liver fibrosis after surgical biliary decompression in patients who had chronic pancreatitis and stenosis of the common bile duct [8]. A justified criticism of many of the earlier reports of the reversibility of fibrosis and cirrhosis was the small numbers of patients that were analyzed. The results from more recent large-scale clinical trials in the treatment of chronic hepatitis C have provided compelling and robust data. Poynard and colleagues [9] analyzed the results of four previous major clinical trials that involved 3010 patients who had chronic hepatitis C and who were randomized to various treatment regimens with interferon or pegylated interferon, with or without the addition of ribavirin. Major beneficial effects of antiviral therapy on liver fibrosis were observed, particularly with combination therapy. The diverse etiologies in which these observations of reversibility have been made suggest that the liver's capacity to remodel scar tissue is a generic phenomenon [10], which, if harnessed and manipulated, may offer innovative therapeutic approaches.

Fibrogenesis: matrix metalloproteinases, tissue inhibitors of metalloproteinases, and the extracellular matrix

Because liver fibrosis is advanced when most patients present, degradation of existing scar will be a critical requirement of effective antifibrotic

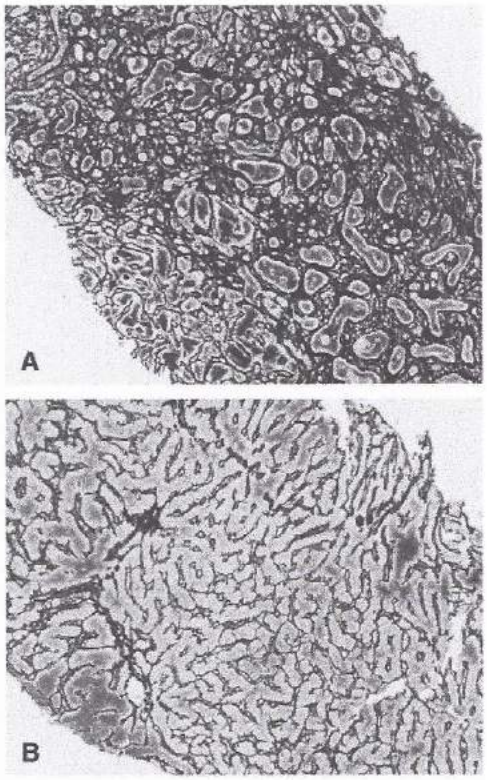


Fig. 1. Photomicrograph of two liver biopsies from a patient with acute-onset autoimmune chronic active hepatitis at the time of presentation before treatment with corticosteroids (*A*) and 18 months later (*B*), demonstrating almost complete resolution of extensive fibrosis (reticulin stain, original magnification □10).

treatment. Liver fibrosis is associated with major alterations in the quantity and composition of extracellular matrix (ECM) [11]. In advanced stages, the liver contains approximately six times more ECM than normal, including collagens (I, III, IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. The hepatic stellate cell (HSC) is the pivotal cell type in the development of liver fibrosis and major source of secreted matrix proteins [12], although other cell types (eg, portal myofibroblasts, hematopoietic stem cells) may have fibrogenic potential [13,14]. In the quiescent state HSCs are perisinusoidal vitamin A–storing cells, but following injury they proliferate and become "activated" to a highly fibrogenic "myofibroblastic" phenotype. In the activated state, HSCs orchestrate an array of changes, including ECM remodelling, vascular contraction, and the release of cytokines. Matrix synthesis is increased markedly by the action of transforming growth factor (TGF)-β1, the major fibrogenic cytokine identified. HSCs are the most important source of TGF-β1, although Kupffer cells

and platelets secrete this cytokine. Recently, connective tissue growth factor also has been implicated in fibrogenic stimulation of HSCs. Accumulation of ECM results from increased synthesis and decreased degradation. Decreased activity of ECM-removing matrix metalloproteinases (MMPs) is due mainly to overexpression of their potent specific inhibitors (tissue inhibitors of metalloproteinases, TIMPs) [15]. Furthermore, the increased expression of TIMPs precedes expression of procollagen-1 mRNA, which suggests that fibrillar collagen is laid down into an extracellular milieu in which matrix degradation is inhibited. In addition, TIMP-1 inhibits apoptosis of some cell types, and, therefore, may be antiapoptotic for activated HSCs [16]. Continued HSC activation is perpetuated by autocrine and paracrine factors and positive feedback loops that involve ECM components and cytokines [17] (also see the article by Rockey elsewhere in this issue).

Mechanisms of reversibility

In bile duct ligation and carbon tetrachloride–induced rat liver fibrosis [18,19] there is spontaneous recovery of the normal liver architecture after reanastomosis of the bile duct [20,21] or cessation of carbon tetrachloride administration [18,22], respectively. During this recovery period, the liver architecture is remodeled, with resorption of fibrotic matrix and reconstitution of the normal architecture. The number of activated HSCs is reduced dramatically during this period. A significant part of this loss of activated HSCs results from stellate cell apoptosis. Apoptosis, or programmed cell death, can be triggered in two ways. A cell will default to the apoptotic sequence if there is a loss of normal constitutive survival signals; alternatively, apoptosis will result if a proapoptotic signal is present. Survival signals can be in the form of cell–cell contact or cell interaction with the local ECM, or it can be provided by soluble factors. Some soluble factors, such as insulin-like growth factor I, prevent cultured activated HSCs from undergoing apoptosis that is triggered by the removal of growth factor–containing serum [21]. Other soluble factors, such as nerve growth factor, are proapoptotic [23]. The importance of myofibroblast apoptosis is shown by experimental evidence that stimulation of rat HSC apoptosis can attenuate liver fibrosis in vivo [24]. Hepatic myofibroblast apoptosis alone may not be sufficient to allow full resolution; there also must be an increase in matrix degradation.

The MMPs are a family of calcium-dependent endopeptidases that specifically degrade collagens and noncollagenous substrates. Matrix degradation occurs predominantly because of the action of these enzymes, which are secreted as proenzymes and activated primarily by way of cell surface–associated cleavage mechanisms. The initiation of matrix degradation represents a pivotal initial step in the process of resolution; this starts a cascade of events that results in loss of fibrillar matrix and apoptosis of HSCs. A key event seems to be the action of the interstitial collagenases (predominantly

MMP-1 in humans and MMP-13 in rodents), which cleave the collagen-1 molecule in a manner that allows it to unwind and become susceptible to degradation by gelatinases and other less selective MMPs. Net collagenase activity reflects the relative amounts of activated MMPs and TIMPs, and progressive fibrosis is characterized by increased expression of TIMP-1. Furthermore, sustained TIMP-1 may represent a major determinant of failure to degrade accumulated scar, and, therefore, its transcriptional regulation is an area of considerable interest [25]. There is a close temporal correlation between HSC apoptosis and matrix degradation, which suggests that the two events may be linked intrinsically [18]. This hypothesis is supported by data that demonstrate that cell–matrix interactions, mediated by integrins, regulate the phenotype, survival, and secretory activity of a variety of cell types, including HSCs [26–29]. Indeed, tissue culture studies showed that type 1 collagen matrix perpetuates the activated phenotype of HSCs. HSC proliferation may be enhanced by pericellular collagen degradation, initiated by collagenase, and mediated by way of αvβ3 engagement. Additionally, contact with type 1 collagen can take hepatocytes out of the cell cycle, which they re-enter after contact with partially degraded collagen-1, an event that also apparently is mediated by the αvβ3 integrin [28]. These interactions may provide a mechanism whereby proteolysis of the fibrillar neomatrix may facilitate resolution and repair processes within the injured liver. Moreover, using mice bearing a mutated collagen-1 gene (r/r mice), which confers complete resistance to collagenase degradation, Issa and colleagues [28] showed that the inability to degrade collagen-1 critically impaired HSC apoptosis with subsequent failure of resolution of fibrosis and blunting of the hepatocyte regenerative response.

It is likely that the same mechanisms that are observed in rodent models are applicable in human liver disease. There is compelling histologic evidence for a reduction in fibrotic matrix after treatment of various human chronic liver diseases. Although HSC apoptosis was not studied specifically in these series, there is histologic evidence of a diminution in HSC numbers. Direct evidence for HSC loss during recovery from injury was provided in a study of acute paracetamol injury, where there was a clear reduction in the numbers of α-sma-positive (activated) HSCs on follow-up biopsy [30].

Factors determining irreversibility of liver fibrosis

Investigating the factors that limit complete reversal of advanced hepatic fibrosis or cirrhosis may reveal important therapeutic insights. Studies have demonstrated that liver fibrosis varies in reversibility according to the nature of matrix components, cellularity of the scar, topography, and duration of fibrosis.

The qualitative and quantitative changes in the ECM that characterize chronic liver disease may determine the extent of reversibility. Tissue

transglutaminase–mediated cross linking of collagen contributes to a variety of irreversible fibrotic diseases and models, including human hepatic fibrosis, scleroderma, and experimental renal fibrosis [31–33]. Cross linking is a feature of matrix maturation and provides matrix proteins, including fibrillar collagens, with a resistance to MMP-mediated degradation; this limits the speed and extent of scar resolution. Moreover, data from animal models and human disease indicate that significant, but incomplete, recovery from advanced cirrhosis is possible and results in remodelling from micronodular cirrhosis to an attenuated macronodular cirrhosis [34].

The topography of fibrotic septa may explain why some areas of scar, such as portal–central septa containing vascular shunts, are more readily reversible than others, such as portal–portal or central–central septa [10]. In addition, the progressive accumulation of collagenous and noncollagenous scar components over time may create a large mass of scar that is inaccessible to degrading enzymes. Furthermore, recent data detailing the kinetics of scar-associated cells also suggest that dense acellular or paucicellular fibrotic tissue may be less reversible than is matrix that is highly cellular and infiltrated by inflammatory cells, because of the relative depletion of collagenolytic MMPs that are derived from the cells present [34,35].

Recent studies have focused on the changes in gene expression and phenotype that are associated with cellular senescence in human HSCs [36]. One intriguing observation is the pronounced proinflammatory response of HSCs at senescence. The potent mixture of cytokines and chemokines might not only promote chemotaxis, adherence, and activation of immune cells or degranulation of leukocytes, but it also might exert paracrine and autocrine effects on HSCs. The significance of the senescent phenotype with regard to the reversibility of fibrosis in vivo remains to be established.

Finally, telomere dysfunction has been proposed to be a factor that leads to end-stage organ failure in chronic diseases of high cellular turnover, such as cirrhosis. Elegant studies have demonstrated that telomere shortening does accelerate the development of cirrhosis in response to chronic liver damage, which affects the ability of hepatocytes to sustain a robust regenerative response [37].

Targeted treatments for fibrosis

Cure of primary disease

Cure of the primary disease to prevent ongoing injury remains the most effective strategy to reverse fibrosis. Clinical efficacy has been shown in venesection in hemachromatosis, copper chelation for Wilson's disease, abstinence in alcoholic liver disease, antivirals for chronic viral hepatitis, immunosuppression for autoimmune liver disease, anthelmintics for parasitic disease, decompressive surgery for secondary biliary obstruction, and drug withdrawal in iatrogenesis [2–4,6–9,38,39]. Successful treatment prevents

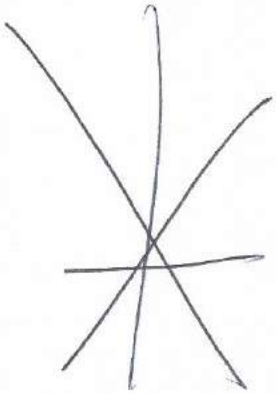
accumulation of further fibrotic neomatrix and accelerates its dissolution as injury resolves. If treatment is instigated early, a near-normal restitution of hepatic histology is possible.

Suppressing hepatic inflammation to prevent hepatic stellate cell activation

Invariably, liver fibrosis is preceded by inflammation. Indeed, persistent inflammation from a sustained hepatic insult perpetuates the fibrogenic HSC phenotype and progression to cirrhosis. Consequently, several agents that attenuate or neutralize upstream inflammatory responses have been studied. Colchicine has anti-inflammatory properties and seems to have an antifibrotic effect in cirrhotics. A recent Cochrane review of randomized controlled trials of colchicine in alcoholic and nonalcoholic cirrhosis demonstrated no clinical, biochemical, or histologic efficacy, however [40]. Malotilate has immune-modulating and anti-inflammatory effects and protected against liver damage and fibrogenesis in dimethylnitrosamine (DMN) and CCl_4-injured rats; however, multi-center trials in primary biliary cirrhosis and alcoholic liver disease failed to demonstrate improved histology or survival [41,42]. Corticosteroids have been used historically to good effect in autoimmune liver disease with responders progressing more slowly to fibrosis and cirrhosis. Patients who have severe alcoholic hepatitis have an improved short-term survival and reduced progression to liver fibrosis with high-dose prednisolone treatment [43]; however, randomized clinical trials of corticosteroids have not shown benefit in patients who have primary biliary cirrhosis or sclerosing cholangitis [44]. Antagonists to proinflammatory cytokines are a credible target and have shown promise in abrogating experimental liver injury. Interleukin (IL)-1 receptor antagonist gene delivery by way of a transgene vector reduced liver damage and proinflammatory cytokines, and increased survival in a rat model of ischemia-reperfusion injury [45]. Tumor necrosis factor (TNF)-α inhibition, for example with soluble TNF-α receptor, has shown some promise in reducing liver injury [46]. The humanized monoclonal antibody infliximab has had an acceptable safety profile in the treatment of rheumatoid arthritis and Crohn's disease. A recent case series of patients who were treated with infliximab in acute alcoholic hepatitis suggested increased survival of historical controls, and a controlled trial was initiated [47]; however, a follow-up trial was stopped after deaths from sepsis in the group that was treated with TNF-α-antibody [48]. Thalidomide also behaves as a TNF-α antagonist and has been shown to be beneficial in experimental liver injury [49]. IL-10 is a potent "suppressor" cytokine and downregulates the immune response. Studies using IL-10 knockout mice highlighted its importance as a major anti-inflammatory effector, by demonstrating a more severe fibrosis phenotype in CCl_4-treated knockout mice compared with wild-type animals [50]. Furthermore, recombinant IL-10 treatment reduced fibrosis in 14 out of 22 patients

who had chronic hepatitis C and were nonresponders to interferon, and it was tolerated well [51].

Antioxidants and other strategies to inhibit hepatic stellate cell activation



Numerous agents have been used experimentally to prevent the transformation of quiescent HSCs to activated myofibroblasts. One approach is to reduce oxidative stress, which is considered a potent stimulus to activation. Vitamin E has been shown to inhibit HSC activation by reducing lipid peroxidation [52]. It suppressed fibrogenesis in some experimental models of liver fibrosis, and in vivo therapy reduced HSC activation in a human pilot study for hepatitis C [53]. Mixed antioxidants also have shown benefits in this disease [54]. Glutathione (GSH) has hepatoprotective and antioxidant properties and attenuates liver fibrosis in alcohol, biliary obstruction, and CCl_4 laboratory models. Furthermore, substrates for GSH have established applications in human liver medicine (eg, *N*-acetyl-L-cysteine for paracetamol overdose). Oral S-adenosyl-L-methionine (SAMe), which promotes GSH synthesis, seemed to improve the outcome of some patients who had alcoholic liver disease significantly, although the same benefits of reduced mortality and need for transplant were not apparent in those who had advanced-stage disease [55]. The cytokines interferon (IFN)-γ and hepatocyte growth factor (HGF) inhibit HSC activation in animal models of liver fibrosis [56]. Mice that lack IFN-γ have an increased tendency to develop hepatic fibrosis after liver injury [57]. IFN-γ has been studied in idiopathic pulmonary fibrosis [58], and a large controlled clinical trial is underway in patients who have established liver fibrosis and hepatitis C. Promising results have been observed using HGF, a hepatocyte mitogen that also modulates HSC functions, as a hepatoprotective agent. Administration of HGF by gene therapy or as a recombinant protein prevents the progression of experimental liver fibrosis [59].

Neutralization of downstream hepatic stellate cell effector functions

Activated HSCs are primed and responsive to many cytokines and growth factors that drive many of the damaging downstream effects that are characteristic of progressive fibrosis. Therefore, it follows that potent mitogens (eg., platelet-derived growth factor [PDGF]) and profibrogenic cytokines (eg, TGF-β), as well as their cognate receptors and intracellular signaling pathways, are potential therapeutic targets. Numerous growth factors, such as PDGF, fibroblast growth factor, epidermal growth factor, and insulin-like growth factor, are mitogenic for HSCs and signal transduce by way of tyrosine kinase receptors. The hepatic expression of these growth factors, in particular PDGF, is increased markedly following acute and chronic liver injury. Furthermore, following cell activation, there is an upregulation of PDGF receptors in HSCs. Pentoxifylline, a phosphodiesterase inhibitor, decreases HSC

proliferation in vitro and in vivo and prevents PDGF-related signaling; its efficacy in liver fibrosis is showing promise [60–63]. The PDGF intracellular signaling pathway is by way of extracellular signal-regulated kinase (ERK) and H-ras, and S-farnesylthiosalicyate (a ras antagonist) reduces HSC proliferation and migration and attenuates thioacetamide-induced liver fibrosis in rats [64]. The semisynthetic analog of fumigillin (TNP-470) was developed as a chemotherapeutic with antiangiogenic properties. It inhibits HSC proliferation by cell cycle arrest and reduced the progression of hepatic fibrosis in experimental models [65]. Peroxisome proliferator activator receptors (PPARs) are a widespread family of receptors that control cell growth and differentiation. PPAR-γ is a receptor transcription factor that decreases during HSC activation [66]. Synthetic PPAR-γ ligands, for example thiazolidinediones, inhibit the profibrotic and proinflammatory effects of HSC as well as promote fibrolysis in experimental models. Furthermore, second- and third-generation "glitazones," which have been used clinically in diabetes for some time, are showing promise in controlled trials in patients who have insulin resistance and non alcoholic steatohepatitis [67–69]. Endothelin-1 (ET-1) is profibrogenic and a potent vasoactive substance. Activated HSCs express contractile intracellular proteins and become sensitive to ET-1, which promotes sinusoidal constriction and increased portal resistance. The dynamic nature of HSC contractility has led to the experimental use of theoretic portal hypotensive agents to manipulate the factors that control this process (ET and nitric oxide [NO]) [70]. Bosentan, a mixed ETA/ETB receptor antagonist, reduced portal pressure when perfused into cirrhotic rat liver [71]. A coexistent reduction in endothelial cell–derived NO also accompanies progressive liver injury; thus, NO augmentation should be capable of countering the procontractile effect of ET-1. Indeed, transduction of cirrhotic rat liver with recombinant adenovirus that contained the neuronal isoform of NO synthetase significantly reduced portal pressure [72].

TGF-β is a key fibrogenic mediator. Following hepatic injury, inflammatory cells and activated Kupffer cells cause release of latent TGF-β from its cognate-binding protein in the local ECM to yield bioactive TGF-β. This binds to the enhanced number of cell surface TGF-β receptors that accompanies HSC activation. Intracellular signaling by way of the SMAD protein pathway leads to increased collagen synthesis, down-regulation of MMPs, and increased TIMP expression. Conversely, TGF-β antagonism stimulates matrix degradation by down-regulating TIMPs, and, thus, boosting net collagenase activity. Several inhibitors of the TGF-β pathway have been effective in experimental models of liver fibrosis. Transgenic inhibition of TGF/SMAD signaling in hepatic myofibroblasts reduced the degree of fibrosis in carbon tetrachloride–induced liver damage [73]. In addition, inhibition of hepatic fibrogenesis has been demonstrated by administration of soluble TGF-β type II receptor and by a dominant negative type II TGF-β receptor in rat models of liver fibrosis [74,75]. Furthermore, the effect of TGF-β1 antisense mRNA on fibrosis was analyzed in a rat bile duct ligation model [76]. Delivery of the

TGF-β1 antisense specifically abolished ongoing TGF-β–driven liver fibrogenesis. More recently, gene transfer of Smad7 (an intracellular antagonist of TGF-β signaling) inhibited experimental fibrogenesis in vivo [77].

Previous studies demonstrated that stimulation of the renin-angiotensin system promotes collagen synthesis by way of stimulation of the mineralocorticoid receptor. Inhibition of this pathway ameliorates human renal and cardiac fibrosis [78,79]. Losartan (an angiotensin-1 receptor antagonist) reduced fibrogenesis in an animal model of biliary fibrosis [80], and enalapril (an angiotensin-converting enzyme [ACE] inhibitor) attenuated liver fibrosis in a rat thioacetamide model of injury [81]. ACE inhibitors also reduce the activation of latent TGF-β, and given their established record of efficacy and safety in other human disease, their potential usefulness in human liver fibrosis is being studied [82]. Finally, the antimicrobial compound halofuginone has antifibrotic activity by blocking collagen expression, and it inhibited cirrhosis in thioacetamide- and DMN-treated rats [83,84].

Activated HSCs play an important role in promoting hepatic inflammation, which is a common response to virtually any significant liver insult, and is an inevitable precursor to hepatic fibrosis. HSCs migrate in response to cytokines that are released by inflammatory cells, and, in addition, secrete a repertoire of chemokines and cytokines themselves. Cytokine secretion by HSCs involves activation of an nuclear factor κB (NF-κB)–dependent pathway, a process that can be inhibited significantly by proteosome inhibitors or an inhibitor κB (IκB) superrepressor [85].

Modulation of degradation of fibrotic neomatrix

Because liver fibrosis is advanced when most patients present, degradation of existing scar will be a critical requirement of effective antifibrotic treatment. This may be facilitated by manipulating the local balance between MMPs and their regulators. One difficulty is that ECM turnover occurs constantly throughout the body and any modulation by targeting MMPs, TIMPs, urokinase plasminogen activator (uPA), or other activators would need to be liver-specific to avoid systemic adverse effects (eg, cataracts and tendon pain). uPA initiates the matrix proteolysis cascade and also induces HGF expression. In a rat cirrhosis model, adenoviral delivery of uPA led to up-regulation of collagenase, reversal of fibrosis, and hepatocyte regeneration [86]. TIMPs bind noncovalently but reversibly to the active site of all of the MMPs that directly inhibit enzymatic activity. Therefore, down-regulating TIMP might be expected to unleash the latent collagenolytic activity in fibrotic liver. Using an antisense TIMP-1–expressing plasmid, collagenase activity was increased and liver fibrosis was abrogated in a pig serum model of liver fibrosis [87]. MMP-1 is the major human interstitial collagenase that is capable of digesting fibrillar collagens. Direct administration of MMP-1 mRNA by way of an adenoviral vector delivery system attenuated established liver fibrosis in thioacetamide-treated rats [88]. Hepatocyte growth

factor decreases hepatic fibrosis in animal models, apparently by inducing MMP-1 expression in HSCs by way of the transcription factor Ets-1 [89]. Moreover, HGF gene transfer into the liver, by infusion of plasmid DNA by way of the portal vein followed by electroporation, prolonged the survival of all DMN-treated rats and attenuated the liver fibrosis [90]. The reproductive hormone relaxin inhibits effective collagen deposition by HSCs in culture, and reduced rat liver fibrosis in vivo by down-regulating TIMPs, and, thus, increasing net collagenase activity [91]. These studies provide proof of concept that disrupting the MMP–TIMP balance effectively is antifibrotic.

Stimulation of hepatic stellate cell apoptosis

Removal of the activated HSC population by apoptosis is a hallmark of spontaneous resolution of experimental liver fibrosis [18]. HSCs are known to express several cell surface death receptors, including Fas, TNF-α, and p75 (low-affinity nerve growth factor receptor). Several agents promote apoptosis of HSCs in vitro by acting as death activators: Fas ligand by way of its receptor Fas [92], nerve growth factor by way of its receptor p75 [93], cyclopentenone prostaglandins [94], and benzodiazepines by way of the peripheral benzodiazepine receptor [95]. Cell–matrix adhesion is recognized as a physiologic determinant of cell growth and survival. Disruption of integrin-mediated cell adhesion using soluble Gly-Arg-Gly-Asp-Ser peptide induced apoptosis of rat HSCs [96]. Activated HSCs express persistently elevated levels of the transcription factor NF-κB, by way of suppression of inhibitory IF-κB by the transcriptional repressor C promoter binding factor-1 [97]. Furthermore, it was shown that NF-κB protects HSCs from apoptosis. Inhibitors of NF-κB activity, such as sulphasalazine, promote apoptosis in rat and human HSCs, and accelerate recovery from CCl_4 and thioacetamide injury in rats [24,98].

It is frustrating that despite all of the experimental approaches that were outlined above, human trial data are limited in number and scope. Furthermore, hardly any have measured the effect of spontaneous resolution or therapeutic intervention on portal hypertension. The same is true of other surrogate end points, which suggests that this area needs to be developed. It is unknown whether a reduction in fibrosis equates to a reduction in portal hypertension, but if portal hypertension has a dynamic component that is due to HSC contractility, then changes in HSC phenotype and apoptosis may impact favorably, even in the absence of significant matrix remodelling. Alternatively, even if matrix does not remodel and portal hypertension remains, if hepatocellular function improves sufficiently, patients may be amenable to transcutaneous intrahepatic portosystemic shunt.

Summary

Reversal of fibrosis is a reality in some cases. Existing treatments, particularly those that treat the primary injury, can allow complete resolution (Fig. 2).

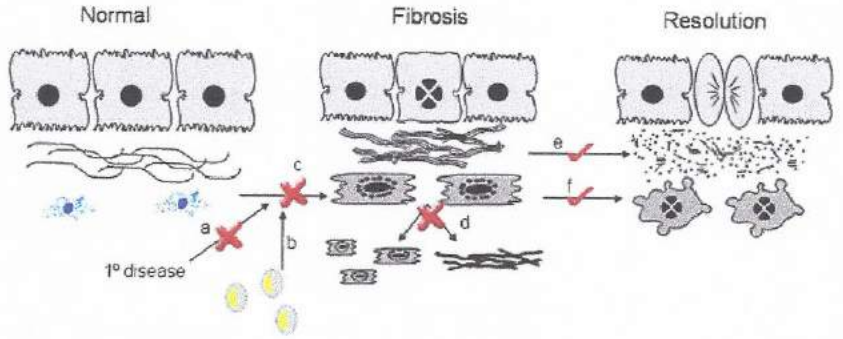


Fig. 2. Strategies to prevent fibrosis or promote resolution. Cure of the primary disease (a) is the most effective strategy; however, other means to prevent stellate cell activation, such as suppression of hepatic inflammation (b) or antioxidants (c), also are effective. The functions of activated myofibroblasts can be inhibited directly (d) and resolution of fibrosis can be enhanced by encouraging dissolution of fibrotic matrix (e) or by promoting myofibroblast apoptosis (f).

New strategies are designed on the foundation of a burgeoning evidence base that documents the reversibility of liver fibrosis, and a detailed understanding of the cell biology of the activated HSC. Many agents were shown to be effective in vitro and in animal models. Translation of this laboratory success into clinical trials is underway, paving the way for use in human liver disease. Increasingly, multiple-agent strategies that work at different mechanistic levels are likely to be assessed. Evidence of the long-term benefits of the reversal of fibrosis on clinical outcome, such as a reduction in portal hypertension or the rate of development of hepatocellular carcinoma, will be sought.

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