Cirrhosis: new research provides a basis for rational and targeted treatments

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Clinical review

Science, medicine, and the future
Cirrhosis: new research provides a basis for rational and targeted treatments
John P Iredale

Liver transplantation and antiviral treatments for hepatitis have improved the outlook for many patients with liver disease. For patients with cirrhosis, new developments herald targeted treatments.

It is an exciting time to be working in hepatology. The success of liver transplantation and the advances in the radiological and endoscopic management of portal hypertension have improved the longevity and quality of life of patients with liver cirrhosis. Additionally, the development of effective antiviral treatments means that disease can be cured in many patients infected with hepatitis B and hepatitis C. However, these interventions also serve to highlight our current impotence in altering the underlying fibrotic process in many patients with liver disease. This rather depressing perspective may soon change: data from clinical and laboratory based research are showing that cirrhosis may be reversible. By highlighting the attributes required of an effective antifibrotic, a new dynamic model is likely to lead to the development of targeted treatment for liver cirrhosis.

Methods
This article is based on knowledge accrued over 13 years of work investigating the mechanisms of fibrosis and aspects of hepatic stellate cell biology and on regular Medline searches of the peer reviewed scientific literature during that time. The field has benefited from the recognition that certain mechanisms are common to hepatic, renal, and pulmonary fibrosis, and I have reviewed models of these processes while preparing this article. My examples highlight how laboratory based studies of the biology of hepatic fibrosis may inform the design of future treatments.

Clinical impact of cirrhosis
Liver fibrosis and its end stage, cirrhosis, represent enormous worldwide healthcare problems. In the United Kingdom, more than two thirds of the 4000 people who died of cirrhosis in 1999 were under 65, and the incidence of cirrhosis related death is increasing. Worldwide, the common causes of liver fibrosis and cirrhosis include hepatitis B and hepatitis C and alcohol. Other causes include immune mediated damage, genetic abnormalities, and non-alcoholic steato-hepatitis, which is associated with diabetes and the metabolic syndrome. Changing patterns of alcohol consumption in the West and the increasing rates of obesity and diabetes mean that advances in preventing and treating viral hepatitis may be offset by an increasing burden of fibrosis and cirrhosis related to alcohol and non-alcoholic steatohepatitis.1–3

Current treatments for cirrhosis are limited to removing the underlying injurious stimulus (where possible); eradicating viruses using interferon, ribavirin, and lamivudine in viral hepatitis; and liver transplantation. Transplantation is a highly successful treatment for end stage cirrhosis, with a 75% five year survival rate. But limited availability of organs, growing lists of patients needing a transplant, issues of compatibility, and comorbid factors mean that not everyone is eligible for transplantation. As a result, effective antifibrotic treatments are needed urgently.

Summary points

||
| Fibrosis, the liver's wound healing response, is bi-directional and potentially reversible. |
| Antiviral treatments provide increasing evidence for reversibility of fibrosis and cirrhosis. |
| Excess fibrillar (scar) matrix can be degraded even in advanced cirrhosis but is held in check by protease inhibitors termed TIMPs. |
| Antifibrotic treatments are likely to be developed in the next decade, on the basis of a better understanding of the pathogenesis of fibrosis. |
| Hepatic stellate cells have been shown to contribute to portal hypertension by dynamic contractile activity; this could lead to the design of specific agents to reduce portal hypertension. |

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Pathogenesis of fibrosis

High quality experimental evidence supports the hypothesis that the final common pathway of fibrosis is mediated by the hepatic stellate cells. Hepatic stellate cells in normal liver store retinoids and reside in the spaces of Disse (Fig 2). In injured areas of the liver, hepatic stellate cells undergo a remarkable transformation: they resemble myofibroblasts and express contractile proteins. In this "activated" phenotype, hepatic stellate cells proliferate and are known to be the major source of the fibrillar collagens that characterise fibrosis and cirrhosis (Fig 2). The mechanisms mediating activation of hepatic stellate cells are a major subject of research.

In injured areas, soluble factors (cytokines) are released by the incoming inflammatory cells, the damaged and regenerating hepatocytes, and other liver cells that target the hepatic stellate cells, activating them so they become the central mediators of wound healing. Because of the key role of inflammation, removing the causative agent and treating the patient with immunosuppressive drugs are effective interventions for some diseases (box). Greater understanding of the specific cytokine and chemokine messengers that mediate the inflammatory process in liver disease is informing the design of future treatments. This is exemplified by the identification of interleukin-10 as a downregulator of the inflammatory response and tumour necrosis factor α as a pro-inflammatory mediator. Studies using interleukin-10 knockout mice have identified this cytokine as a major anti-inflammatory effector in fibrotic liver injury. A pilot study suggested that interleukin-10 may be valuable clinically in the context of hepatitis C virus infection, but definitive evidence of efficacy has yet to be produced in a large scale clinical trial. Antagonising tumour necrosis factor α would also be expected to downregulate hepatic inflammatory. Reagents to neutralise tumour necrosis factor α are available for clinical use, and this approach is likely to be investigated further in the clinic.

Another approach to chronic liver fibrosis is to block the signals which promote transition of hepatic stellate cells from a quiescent to an activated phenotype and promote collagen secretion. Foremost among the soluble mediators promoting the fibrogenic response from hepatic stellate cells is transforming growth factor β-1 (box). This cytokine also has a role in the development of fibrosis in other organs, including the lung and kidney. The activated hepatic stellate cells respond to it by increasing production of collagen and decreasing its breakdown (see below). Models in other internal organs suggest that modifying the secretion or activity of transforming growth factor β-1 can attenuate fibrosis, which indicates that this is a possible anti-fibrotic target in the liver. Recent studies of experimental liver fibrosis have shown the potential of this approach.

Matrix synthesis and turnover in fibrosis and cirrhosis

Activated stellate cells proliferate, with the result that increases in numbers of hepatic stellate cells, in addition to increases in secretion of the fibrillar (or

Inflammation and repair

Liver fibrosis and cirrhosis represent a continuous disease spectrum characterised by an increase in total liver collagen and other matrix proteins which disrupt the architecture of the liver and impair liver function. Fibrosis results from sustained wound healing in the liver in response to chronic or iterative injury. The wound healing response is an integral part of the overall process of inflammation and repair: it is dynamic and has the potential to resolve without scarring (Fig 1).

Fig 1 Liver cirrhosis as wound healing: damage to the normal liver (a) results in inflammation (b) and activation of hepatic stellate cells (brown) to secrete collagen, culminating in the development of fibrosis (c) and ultimately in cirrhosis (d). Withdrawal of the injurious agent may allow remodelling of the fibrillar matrix, leaving an attenuated cirrhosis (e). Spontaneous resolution of fibrosis after removal of injury results in a return to near normal architecture (f). Whether "complete" resolution of cirrhosis can occur is currently unknown.

Fig 2 Normal liver (top) and liver injury (bottom). After liver injury, hepatic stellate cells become activated and secrete a collagen rich matrix. Through associated changes in cell-matrix interactions, hepatocytes lose their microvilli and sinusoidal endothelial cells lose their fenestrations. Reproduced with permission.
scarring) collagens, result in the deposition of excess fibrotic matrix. Collagen synthesis is therefore clearly a target for therapeutic intervention. Because fibrosis is advanced when most patients present, understanding the processes regulating matrix degradation is likely to be pivotal to the development of effective anti-fibrotic treatments. Effective treatment will require breakthrough of the pre-existing matrix.

Stellate cells and other cells involved in the fibrotic process, including macrophages and Kupffer cells, secrete a repertoire of matrix degrading metalloproteinase enzymes (MMPs). These enzymes degrade collagen and other matrix molecules, and their presence in the fibrotic liver highlights the potential dynamic nature of scarring within the liver. Molecular studies of the expression of mRNA for these enzymes (including those with collagenolytic activity) have shown that they are expressed in the liver even in cirrhosis, but their activity is held in check by powerful inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). The potential for matrix degradation is present, even in advanced cirrhosis—but it is held in check by concurrently secreted TIMPs (fig 3). It should be possible to unharness the latent matrix degrading capacity of a fibrotic or cirrhotic liver and to facilitate matrix degradation, resulting in a return to normal or near normal architecture.

**Models of resolution of liver fibrosis**

Studies that used pathological specimens and paired biopsies from trials of antiviral treatments in chronic hepatitis have shown that matrix degradation occurs in advanced human cirrhosis. In parallel, rodent models in which spontaneous recovery from liver fibrosis and cirrhosis occurs have allowed the frequent sampling that is needed to identify the critical features of the process. In recovery, expression of TIMPs 1 and 2 decreases rapidly while matrix degrading metalloproteinases continue to be expressed, resulting in increased collagenase activity and consequent matrix degradation within the liver (see fig 1).

Together with these changes, apoptosis of the hepatic stellate cells occurs. Apoptosis, in effect the suicide of a cell, fulfils a function in mammalian tissue, removing unwanted cells when they become too numerous or redundant. During progressive liver injury, when stellate cells are activated in the normal wound healing response, stellate cell apoptosis is forestalled, probably through signals from soluble factors and changes in the matrix. When the injurious stimulus is withdrawn and remodelling of matrix is required, the loss of these survival factors causes the activated stellate cell to default into apoptosis, which facilitates the remodelling process by removing the major cellular source of collagen and TIMPs. Logically, therefore, manipulating matrix degradation or enhancing hepatic stellate cell apoptosis might be expected to reduce fibrosis and promote a return to normal liver architecture. Studies in this area are currently limited to experimental models but show promise that liver fibrosis can be attenuated by manipulating the TIMP-MMP balance or enhancing hepatic stellate cell apoptosis.

**Possible therapeutic interventions in liver fibrosis**

**In progressive or established fibrosis**

- **Inflammation**
  - Removal of injurious agent
  - Interleukin-10—anti-inflammatory effect
  - Tumour necrosis factor α inhibitors—anti-inflammatory effect
  - Antioxidants—suppress fibrotic response to oxidative damage

- **Stellate cell activation**
  - Interferon gamma (or interferon alfa)—inhibits activation of hepatic stellate cells
  - Hepatocyte growth factor—inhibits activation of hepatic stellate cells
  - Peroxisome proliferator-activated receptor ligand—reduces activation of hepatic stellate cells

- **Perpetuation of stellate cell activation**
  - Transforming growth factor β-1 antagonists—reduce matrix synthesis and enhance matrix degradation
  - Platelet derived growth factor antagonists—reduce proliferation of hepatic stellate cells
  - Nitric oxide—inhibits proliferation of hepatic stellate cells
  - Angiotensin-converting-enzyme inhibitors—reduce proliferation of hepatic stellate cells

- **Stellate cell secretion of collagen rich matrix**
  - Angiotensin converting enzyme inhibitors—reduce fibrosis
  - Polyhydroxylase inhibitors—reduce experimental fibrosis
  - Interferon gamma—reduces fibrosis
  - Endothelin receptor antagonists—reduce fibrosis and portal hypertension

**To enhance or initiate resolution of fibrosis**

- **Stellate cell apoptosis**
  - Gilotoxin—causes apoptosis of hepatic stellate cells
  - Nerve growth factor—causes apoptosis of hepatic stellate cells
  - Peripapillary cell secretion of collagen rich matrix
  - Polyhydroxylase inhibitors—reduce experimental fibrosis
  - Interferon gamma reduces fibrosis

- **Metalloproteinases**
  - Metalloproteinases—enhance activity of metalloproteinases
  - Tissue inhibitor of matrix (TIMP) antagonists—enhance activity of metalloproteinases
  - Transforming growth factor β-1 antagonists—downregulate TIMPs and increases activity of metalloproteinases
  - Relaxin—downregulates TIMPs and increases activity of metalloproteinases

**Stellate cells as mediators of portal hypertension**

A major and life threatening consequence of cirrhosis is the development of portal hypertension. Studies of isolated hepatic stellate cells have revolutionised our understanding of these cells as mediators of portal hypertension.
Clinical review

Educational resources

The August 2001 edition of Seminars in Liver Diseases is devoted to the hepatic stellate cell and reviews of hepatic stellate cell biology. Chapters of particular interest are:

Rockey DC. Hepatic blood flow regulation by stellate cells in normal and injured liver. (pp 337-50)
Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. (pp 351-72)
Benyon RC. Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. (pp 373-84)
Pinzani M, Marra, F. Cytokine receptors and signalling in hepatic stellate cells. (pp 397-416)
Maher J. Interactions between hepatic stellate cells and the immune system. (pp 417-26)
Iredale JP. Hepatic stellate cell behaviour during resolution of liver injury. (pp 427-37)

Design of anti-fibrotic treatments:

For information on the incidence and epidemiology of liver disease, the addresses of patient support groups, and information for patients:
British Liver Trust (www.britishlivertrust.org.uk)
Children’s Liver Disease Foundation (http://childliverdisease.org)

view of the mechanisms underlying portal hypertension and point to a role for these cells. Activation of hepatic stellate cells is associated with the expression of contractile intracellular proteins such as smooth muscle actin, and activated cells become sensitive to the potent vasoactive substance endothelin. Endothelin concentrations increase after fibrotic liver injury, promoting contraction of hepatic stellate cells. In parallel, injury results in a reduction in nitric oxide derived from hepatic endothelial cells, which antagonises the effect of endothelin (fig 4). The net result of this imbalance is that stellate cell contraction is stimulated, and the consequent increases in intrahepatic sinusoidal resistance contribute to portal hypertension. The observation that this process is dynamic and might be manipulated has led to the exciting concept that effective endothelin antagonism might reduce portal hypertension in cirrhosis.1

Serum markers of fibrosis

At present, the clinical assessment of antifibrotic interventions relies on serial liver biopsies. Liver biopsy remains associated with a (small) morbidity and mortality, and even though effective fibrosis scoring systems have been introduced, liver biopsy is prone to sampling error. It may not be an appropriate way of monitoring in a dynamic situation as a clinical trial of an antifibrotic agent. A further likely development is the identification of a panel of serum fibrosis markers which can be used to predict the stage of fibrosis and monitor disease progression or resolution without recourse to repeated liver biopsies.2

The future

In future, patients with cirrhosis are likely to be treated simultaneously with a targeted anti-inflammatory agent, an agent to lower portal pressure, and an anti-fibrotic or fibrolytic agent, and the effectiveness of the treatment may well be monitored by using a panel of serum markers. The development of effective targeted treatments and the tools to monitor their effectiveness non-invasively will change the way we view and treat cirrhosis.

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2 Day CP. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? Gut 2002;50:585-8.
15 George J, Rosoli D, Zeteliansky VC, Bissell DM. In vivo inhibition of rat stellate cell activation by soluble transforming growth factor beta type II.
Lesson of the week

Interaction of spironolactone with ACE inhibitors or angiotensin receptor blockers: analysis of 44 cases

Eike Wrenger, Regina Müller, Michael Moesenthin, Tobias Welte, Jürgen C Frölich, Klaus H Neumann

The randomised aldactone evaluation study (RALES) proved a substantial (30%) reduction in risk of mortality in patients with severe congestive heart failure by treatment with low dose spironolactone (25–50 mg a day) in addition to standard treatment. 

Exclusion criteria for treatment in the study were a plasma potassium concentration > 5.0 mmol/l and serum creatinine concentration > 221 μmol/l. A pilot study had previously shown that the higher the dosage of spironolactone (up to 24%) with 75 mg a day) the higher the risk of hyperkalaemia. 

Standard treatment for patients with heart failure categorised as New York Heart Association class II to IV includes angiotensin converting enzyme (ACE) inhibitors or angiotensin II AT1 receptor antagonists (AT1 receptor blockers). Both spironolactone and ACE inhibitors or AT1 receptor blockers reduce the renal elimination of potassium. 

In RALES, the increase in potassium was judged not to be important as serious hyperkalaemia (>6 mmol/l) occurred in only 10 (1%) of 822 patients taking spironolactone, with no significant difference between the groups. Discontinuation of the treatment was necessary in only one patient taking placebo and three patients taking spironolactone.

We present a larger case series of life threatening hyperkalaemia in patients who were receiving spironolactone plus ACE inhibitors or AT1 receptor blockers. We identify clinical circumstances associated with this medical emergency and suggest recommendations for prevention.

Case series

From January 1999 until December 2002 we observed 44 patients (17 men) with congestive heart failure who were taking spironolactone and ACE inhibitors or AT1 receptor blockers and were admitted to our nephrology unit (serving a population of about 250 000) for treatment of life threatening hyperkalaemia. Their mean age was 76 (standard deviation 11) years. The mean dosage of spironolactone was 88 (SD 45, range 25–200) mg daily. All patients also received ACE inhibitors or AT1 receptor blockers.

Thirty five of the 44 patients had diabetes mellitus type 2. Symptoms on admission were vomiting (19), diarrhoea (8), bradyarrhythmia (14), muscle weakness and paralysis (27), and severe dehydration (28). Mean plasma potassium concentration on admission was 7.7 (SD 0.9, range 6.0–9.6) mmol/l and mean serum creatinine concentration was 294 (SD 175, range 88–940 μmol/l). Nineteen of the 44 patients had a serum creatinine concentration < 221 μmol/l. Creatinine clearance estimated by the formula of Cockroft and Gault was 0.38 (SD 0.22) ml/min and below the lower limit of normal in all patients. Five patients had to be resuscitated on admission. The figure shows electrocardiograms for patient 39, who on admission had a serum potassium concentration of 9.65 mmol/l and, after dialysis, had a lowered value of 4.65 mmol/l.

Haemodialysis was immediately started in 37 patients. Seven patients received conventional potassium lowering treatment consisting of intravenous sodium bicarbonate, intravenous furosemide (frusemide), and intravenous glucose and insulin. The mean number of dialysis treatments per patient was 2.5 (SD 1.2). However, in six patients renal function did not recover, and they had to be kept on chronic dialysis treatment. Two patients developed fatal complications during their stay in the intensive care unit: a 75 year old man died of gastrointestinal bleeding and an 88 year old woman died of sepsis. Renal function recovered in 34 patients after closely monitored volume administration as dehydration was a common problem. At discharge, creatinine clearance in the patients who were not receiving dialysis was 0.65 (0.27) ml/min, with serum creatinine values between 79 (SD 0.9) μmol/l and 821 μmol/l.