



Is Liver Fibrosis Reversible?

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INTRODUCTION

Fibrosis is a process of wound healing where damaged liver tissue is replaced by extra-cellular matrix. Liver fibrosis and cirrhosis is the final common result from the majority of chronic liver insults irrespective of nature of insult. It begins with subendothelial or pericentral fibrosis (hepatic fibrosis) and progresses to panlobular fibrosis with nodule formation (cirrhosis). The development of fibrosis, and particularly cirrhosis, is associated with a significant morbidity and mortality. Thus, there is a considerable imperative to develop antifibrotic strategies that are applicable to liver fibrosis. The general impression is that once established, fibrosis has generally been considered irreversible, but it may not be so. Fibrosis in liver represents a wound healing response that is dynamic and has the potential to resolve without persistent scarring. The point at which cirrhosis or extensive fibrosis becomes irreversible has not been well defined. Interest in cirrhosis has now increased as it can be diagnosed at an early stage by percutaneous liver biopsy (gold standard) or use of non-invasive markers of liver fibrosis. In many cases, cirrhotics are asymptomatic, with normal findings on physical examination, and the disorder is detected initially because of elevated serum liver enzyme levels or a positive serologic test for hepatitis B or C virus on annual health check ups. There is enough evidence to suggest that medical treatment may not only delay the progression of liver fibrosis but may even cause regression. At present, the only curative treatment for end stage cirrhosis is transplantation, and the alternative clinical course is to prevent progression of injury and preventing complications of fibrosis and cirrhosis.

NATURE AND ORIGIN OF FIBROSIS

In hepatic fibrosis, the hepatocytes are replaced by the extra-cellular matrix (ECM) which consists of macromolecules including collagen molecules, glycoproteins, glycoaminoglycans, proteoglycans, etc. The most important content in the ECM is the collagen, which occurs mainly in the capsule, walls of large vessels, portal triads, etc. In a fibrosed liver there is deposition of collagen in the space of Disse, total collagen is increased by 3-10 times, increase in collagen fibril of 1,3,5 types and shift of ECM composition from heparan sulfate to chondroitin and dermatan sulphate containing proteoglycans. The process of hepatic fibrogenesis is dynamic with respect to both cell and extracellular matrix (ECM) turnover and suggest that a capacity for recovery from advanced cirrhosis and fibrosis is possible. In the development of liver fibrosis the primary source of hepatic

ECM are the activated Hepatic Stellate Cells, (HSC, Ito, fat storing cell, or lipocyte) which are the major producers of the fibrotic neomatrix.^{1,2}

Hepatic stellate cells reside in the space of Disse and in normal liver are the major storage sites of vitamin A. Following chronic liver injury, HSC proliferate, lose their vitamin A and undergo a major phenotypical transformation to smooth muscle α -actin positive myofibroblasts (activated HSC) which produce a wide variety of collagenous and non-collagenous ECM proteins. Culture studies have suggested that the neomatrix laid down in the space of Disse may itself contribute to the disease associated alterations in the phenotype of HSC, sinusoidal endothelial cells, and hepatocytes.³ With progressive injury ECM spurs link the vascular structures, ultimately resulting in the architecturally abnormal nodules that characterise cirrhosis. Apart from HSCs, the matrix proteins also influence the formation of fibrosis. This occurs by the alteration in the cellular behaviour by the receptors e.g: Integrins. Integrins constitute a family of homologue membrane proteins that controls gene expression, growth and differentiation. These receptors are present in hepatocytes, fibroblasts and hepatic stellate cells. ECM may also alter cell function by soluble growth factors e.g: PDGF, HGF, TNF, etc. Degradation of ECM is done by matrix metalloproteinases.

ROLE OF HEPATIC STELLATE CELLS (HSCs), MMPs AND TIMPs

It is now clear that the accumulation of extracellular matrix, or scarring, in fibrotic diseases of the liver is not a static or unidirectional event but a dynamic and regulated process that is amenable to intervention⁴. Activation of hepatic stellate cells (formerly known as Ito cells) is a central event in hepatic fibrosis. In liver injury, the accumulation of extracellular matrix by activation of stellate cells is offset by a proportional increase in the degradation of matrix through the action of so-called interstitial collagenases. The cellular sources of these collagenases are still uncertain, but their activity is tightly regulated by the amount of active protein and the concentration of specific inhibitory molecules, called tissue inhibitors of metalloproteinases (TIMPs). Key mediators of the activation of hepatic stellate cells include a host of cytokines and their receptors, reactive oxygen intermediates, and other paracrine and autocrine signals. The process of activation may occur in two phases: The Initiation phase is by early changes in gene expression and phenotype – paracrine method. The paracrine initiation is done by subendothelial cells

(different cytokines), Kupffer cells (cytokines, TGF- α , and ROS) and hepatocytes (fibrotic lipid peroxides). CYP2E1, induced by alcohol generates ROS species and subsequent activation of HSCs. The perpetuation process occurs in discrete changes including proliferation, chemotaxis, fibrogenesis, contractility and matrix degradation.

The matrix metalloproteinases (MMP), a family of zinc dependent endoproteinases, have the capability to degrade these various ECM components and are expressed particularly by HSCs and Kupffer cells.⁵ The first discovered and best characterised interstitial collagenase in humans is MMP-1, which is widely expressed in human tissues including liver, but other human interstitial collagenases with a more limited cell expression include neutrophil collagenase (MMP-8) and collagenase 3 (MMP-13). There is increasing evidence that collagenase inhibition may arise from increased expression in fibrotic liver of endogenous MMP inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). In animal models, as liver injury resolves, activated stellate cells are cleared by apoptosis, and the expression of TIMPs decreases, allowing active enzymes to resorb extracellular matrix.⁶ Expression of both TIMP-1 and -2 is increased in human and rat model fibrotic liver¹⁷ and in human liver the degree of TIMP-1 expression correlates with extent of fibrosis assessed by hydroxyproline content. Studies⁸ indicate that activated HSC may be an important source of these TIMPs in injured liver. In rat models of liver fibrosis, TIMP-1 is expressed early in fibrogenesis before apparent collagen deposition. In contrast to the TIMPs, mRNA for interstitial collagenase (MMP-1 in humans, MMP-13 in rats) remains unaltered in human and rat liver as fibrosis develops. The resulting increase in TIMP: MMP ratio in liver may promote fibrosis by protecting deposited ECM from degradation by MMPs. Activated HSC may however inhibit plasmin synthesis in fibrotic liver through synthesis of plasminogen activator inhibitor-1 (PAI-1).² Plasmin may have an important antifibrotic role, as studies of fibrosis in plasminogen activator knockout mice suggest that an increased PAI-1: urokinase ratio in tissues promotes fibrogenesis. In summary, activated HSC might produce a fibrogenic environment within the liver through a combination of ECM overproduction, diminished MMP activation and inhibition of active MMPs by TIMPs. The removal or inactivation of activated HSC from the liver is therefore likely to be a key process before recovery from fibrosis can occur.

Although these observations in animals need to be validated in humans, they point to the potential for exploiting the factors that regulate collagenase activity in order to develop effective antifibrotic therapies. Key aspects that remain unclear are the cellular sources of interstitial collagenases and the point (in histologic, cellular, or molecular terms) at which fibrosis becomes truly irreversible.

NON-INVASIVE METHODS OF DIAGNOSIS

Gold standard for diagnosing hepatitis fibrosis in liver biopsy, which has limitation of invasiveness, non-representative, risk of complications and inter-observer bias. None of the yet discovered markers are liver specific. Non-invasive may be divided into 3 groups:

A. Markers associated with matrix deposition

- a. Pro-collagen type 3 amino terminal peptidase
- b. Pro-collagen type 1 carboxy terminal peptidase
- c. Type 1 and 4 collagen
- d. Hyaluronic acid
- e. Chondrex

B. Markers associated with matrix degradation

- a. Matrix Metalloproteins MMP2
- b. MMP 3, MM 9
- c. TIMP 1 and 2

Cytokine and chemokines associated with hepatic fibrosis.

- d. *TGF*
- e. *TGF- α*
- f. *PDGF*

RESOLUTION OF FIBROSIS

The concept of reversibility of cirrhosis has undergone great debate. Many experimental pathologists have known for decades that fibrosis from rodent liver may be removed if the injurious agent is removed.^{9,10} Ideal therapy for liver fibrosis should be liver specific, easy to deliver and well tolerable. Treatment of liver fibrosis can be following categories:

- a. Suppression of hepatic inflammation
- b. Modulation of cells and fibrogenic mediators

Possible therapeutic strategies include:

- i. Prevention of stimuli: This is the most effective of preventing fibrosis viz. alcoholic abstinence, anti-viral suppression in viral hepatitis.
- ii. Reduction of inflammation:
 - a. Corticosteroids: Effective in clinical remission and improvement in fibrosis in autoimmune hepatitis
 - b. Colchicine: Initially found to have benefit but later studies have not demonstrated benefit.¹¹
 - c. Ursodeoxycholic Acid: No direct anti-fibrogenetic effect, but benefit shown in cases of Primary Biliary Cirrhosis¹²
 - d. Receptor Antagonists: Neutralise inflammatory cytokines by specific receptor types.¹³
 - e. Immune modulation: In Schistosomiasis induced fibrosis, co-administration of IL-12 and egg antigen resulted in converting a Th2 response to Th1 pattern.¹⁴
- iii. Downregulation of Stellate cell activation
 - a. Interferons: IFN downregulates stellate cells and have an inhibitory effect on m-RNA production of collagen type 1 and 4.¹⁵
 - b. Anti-oxidants
 - c. Sylamarin
 - d. Amiloride: Inhibits hepatic stellate proliferation
 - e. Cytokines: TGF- β antagonists, Endothelin receptor antagonists, Hepatocyte growth factors
- iv. Prevention of matrix deposition and promotion of matrix degradation

There have been many studies have observed that hepatic fibrosis can abate after control of primary disease in both animal

and human studies. This has been most clearly documented in autoimmune disease, in haemochromatotic patients after venesection, liver fibrosis an obstructed biliary system (secondary biliary cirrhosis) after surgical decompression,¹⁶ abstinence from alcohol, surgical reversal of jejunoileal bypass¹⁷ and patients with hepatitis B and C after successful interferon therapy.¹⁸⁻²¹ These suggest that the liver has a ability to remodel scar tissue and offer therapeutic approaches to the treatment of liver fibrosis. In a study for reversibility of fibrosis in experimentally induced cholestasis in rats,⁸ bile duct ligation was done for three weeks, the typical features of bile duct proliferation and periportal fibrosis developed. However, three weeks after reanastomosis of the bile duct to a jejunal loop, there was resorption of periportal fibrosis and liver ECM returned virtually to normal. Spontaneous recovery from liver fibrosis has been also observed in carbon tetrachloride treated rats.²² Rats treated for four weeks with intraperitoneal carbon tetrachloride developed established liver fibrosis with extensive intervascular bridging with collagen fibres. Carbon tetrachloride dosing was then stopped and histological analysis over next 4 weeks showed a return of liver structure to virtual normality.

However, reversal of fibrosis is not true in all cases as there are several patients in whom cirrhosis does not regress. Non-reversal of cirrhosis may be due to multiple factors viz. uncontrollable progression of primary disease as progressive lesions may dominate over regressing lesions; or in a cirrhotic liver a number of physiological changes occur including vascular compromise and cholestasis which may lead to cirrhosis even if the primary disorder has been controlled.

Although liver fibrosis in rats has been shown to be reversible, the implications for recovery from cirrhosis in humans remain to be clarified. Clearly the key question is does liver fibrosis reach a point where it becomes irreversible, and if so what are the qualitative and quantitative differences in the liver structure compared with recoverable fibrosis? Several factors might dictate whether liver fibrosis can recover. Firstly, it is clear that recovery requires degradation of the existing fibrotic matrix, but this matrix may be modified to resist degradation as fibrosis progresses. Newly secreted collagen fibrils can be cross-linked by both tissue transglutaminase and lysyl oxidase pathways; the activity of both pathways is increased during liver fibrogenesis.²³ Such cross-linking during maturation of collagen might reduce its susceptibility to collagenase. Secondly, recovery is unlikely if collagenolytic enzymes remain inactive following cessation of liver injury. However, interstitial collagenase mRNA expression (MMP-1 in humans, MMP-13 in rats) is similar in normal compared with cirrhotic livers, and does not change during recovery in the rat model, even in the face of overt ECM degradation^{1,7}. Continued inhibition of ECM degradation by TIMPs may block the ability to recover from fibrosis, even after removal of the injury. As activated hepatic stellate cells are an important source of both ECM and TIMPs, recovery from fibrosis might require either removal of the activated HSC population, as shown in rat models, or possibly the phenotypical reversal of stellate cell activation, a process yet to be observed in vivo. In non-recovering liver fibrosis activated HSC might persist as a result of a "memory" effect, possibly mediated by collagenous and non-collagenous components of the deposited fibrotic neomatrix,

which either promote HSC activation or protect them from apoptotic stimuli.

In summary, accumulating evidence suggests that liver fibrosis is reversible and that recovery from cirrhosis may be possible. Moreover, the application of cell and molecular techniques to models of reversible fibrosis is helping to establish the events and processes that are critical to recovery. It is anticipated that ultimately these approaches will lead to the development of effective antifibrotics, which harness or mimic the liver's capacity for reversal of fibrosis with resolution to a normal architecture.

CONCLUSIONS

There is a growing body of clinical and scientific evidence that suggests extensive fibrosis in patients with well-preserved liver function should no longer be considered untreatable. Both current and future therapies have the potential for preventing the progression of disease and facilitating endogenous mechanisms that lead to the degradation of extracellular matrix and the regression of fibrosis. Whether, well established cirrhosis *per se* is reversible or not remains to be clearly defined.

Several issues remain to be addressed. Liver fibrosis does not develop at the same rate in all patients, and responses to treatment vary. Therefore, we need to identify host- or disease-specific factors that are associated with both a slower progression of fibrosis and a favorable response to treatment. Furthermore, the possible roles of treatment strategies designed to reverse fibrosis should be analyzed critically.

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