Minireview

The Pathogenesis of Liver Fibrosis:
A Role for Altered Matrix Degradation

M. J. P. ARTHUR, R. C. BENYON, D. A. MANN and J. P. IREDALE

University of Southampton, UK

Received, 12 June, 1998; accepted, 25 June, 1998

Abstract: Hepatic stellate cells (HSC) are involved in regulating matrix degradation in liver. In early liver injury or in early HSC culture there is increased expression of metalloproteinases that are capable of degrading normal liver matrix including gelatinase A, stromelysin-1 and MT1-MMP. There is increasing evidence to suggest that the action of gelatinase A is profibrogenic, possibly mediated via disruption of cell matrix interactions or release of matrix bound growth factors.

In advanced liver disease and in the later phases of HSC culture there is net inhibition of degradation of fibrillar liver matrix. This is mediated by increased expression of TIMP-1 and TIMP-2 relative to interstitial collagenase by fully activated HSC. Recent evidence indicates that decreased expression of TIMPs relative to interstitial collagenase is the mechanism by which fibrillar matrix is degraded as liver fibrosis resolves after cessation of liver injury. These studies indicate that alterations in matrix degradation play a significant role in the development, progression and resolution of liver fibrosis.

Key words: cirrhosis, matrix, metalloproteinases, TIMPs, collagenase.

Introduction

The space of Disse in normal liver contains a highly differentiated mesenchymal cell called the hepatic stellate cell (HSC; also called the Ito cell, fat-storing cell or hepatic lipocyte), which is involved in storage and metabolism of vitamin A. After liver injury HSC proliferate and undergo a major phenotypic change to become myofibroblast-like cells, sometimes termed activated HSC. There is now a large body of evidence demonstrating that activated HSC are the major source of the matrix proteins (type I and III collagen and others) which are laid down in liver fibrosis.

The net deposition of matrix in any tissue is dependent on the balance between matrix synthesis and matrix degradation. This article concentrates on the role of altered matrix degradation in the pathogenesis of liver fibrosis, with particular emphasis on the role of activated HSC. Recent evidence shows that these cells not only synthesize several metalloproteinases, but also regulate their extracellular activity. This is achieved through control of cleavage of pro-metalloproteinases to their active enzymatic forms and through simultaneous expression of a class of specific inhibitors, called the tissue inhibitors of metalloproteinases or TIMPs.

Liver Fibrosis - Cellular and Molecular Events

The key early event in the pathogenesis of liver fibrosis is the proliferation and activation of HSC. This occurs in close relationship to the site of liver injury; in rat CCI, liver injury, HSC proliferation occurs around the central vein in association with areas of hepatocellular necrosis whereas HSC proliferation occurs in the peripoortal zones following bile duct ligation.

A key phenotypic feature of the activated HSC is the expression of α-smooth muscle actin, which defines the myofibroblastic nature of these cells.

There are three well defined pathways which lead to HSC proliferation and activation (fig 1). The most important mechanism is the release of soluble factors from activated Kupffer cells, particularly when isolated from models of liver injury.

These factors include platelet derived growth factor, transforming growth factor β1 and novel low molecular weight factors which have yet to be characterised. Hepatocytes which are damaged and necrotic or undergoing apoptosis can also release factors (insulin-like growth factor-1, fibroblast growth factor and transforming growth factor-α) that are mitogenic for hepatic stellate cells. Finally, there is increasing evidence that HSC subjected to oxidant stress can react by becoming activated in response to NF-κB and c-myb transcription factor pathways.

Once activated to a myofibroblastic phenotype, these cells participate in a number of autocrine and paracrine pathways that maintain their activation. The factors released include a wide array of pro-fibrogenic and pro-inflammatory cytokines, growth factors and chemokines. Of major importance is the autocrine expression of TGF-β1, which is accompanied temporally by expression of TGF-β receptors. This combination is profibrogenic as the action of TGF-β1 on hepatic stellate cells is to increase synthesis of matrix proteins, particularly collagen I, whilst inhibiting matrix degradation (collagenase is decreased, TIMP-1 is increased - see later). Activated HSC have also been

Reprints requests to: MJP Arthur
University Medicine Level D
South Block (811)
Southampton General Hospital
Tremont Road
Southampton
Hampshire SO16 6YD
United Kingdom
Tel: +44
Fax: +44
E-mail: bct@soton.ac.uk
Matrix degradation in liver fibrosis

Over the past decade, increasing evidence indicates that liver fibrosis is a dynamic process involving alterations in matrix degradation as well as matrix synthesis. In the extracellular space the most important enzymes involved in matrix degradation are the metalloproteinases (or MMPs). At least 20 members of this family of enzymes have now been identified by a combination of biochemical and genetic techniques (for recent review see Cawston24). Those relevant to matrix degradation in liver are summarised in Table 1.

(i) Studies in cultured cells

Of the different cell types in liver, HSC and Kupffer cells have a prominent role in degradation of extracellular matrix. HSC studied in primary culture have been described to synthesise several members of the matrix metalloproteinase family. In early primary culture (days 1-3) there is transient expression of interstitial collagenase (MMP-1 in humans, MMP-13 in rats) and stromelysin-1 (MMP-3) both of which are then spontaneously down-regulated (days 3-5) and are not detectable in

<table>
<thead>
<tr>
<th>Group/Name</th>
<th>MMP Number</th>
<th>Cellular origin in liver</th>
<th>Matrix degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Collagenases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial Collagenase</td>
<td>MMP 1</td>
<td>HSC, KC + (BDEC ?)</td>
<td>Fibrillar collagens</td>
</tr>
<tr>
<td>Collagenase 3</td>
<td>MMP 13</td>
<td>HSC (in rat)</td>
<td>I and III</td>
</tr>
<tr>
<td>The Gelatinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatinase A</td>
<td>MMP 2</td>
<td>HSC</td>
<td>Basement membrane proteins; collagen IV, proteoglycans, laminin</td>
</tr>
<tr>
<td>Gelatinase B</td>
<td>MMP 9</td>
<td>KC</td>
<td></td>
</tr>
<tr>
<td>The Stromelysinis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stromelysin 1</td>
<td>MMP 3</td>
<td>HSC</td>
<td>Basement membrane proteins; collagen IV, proteoglycans, laminin</td>
</tr>
<tr>
<td>The Membrane-type MMPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>MMP 14</td>
<td>HSC, Hepatocytes (?)</td>
<td>Activation of progelatinase A</td>
</tr>
</tbody>
</table>

Key to abbreviations:
- HSC = Hepatic stellate cell
- KC = Kupffer cell
- BDEC = Bile duct epithelial cells
- MT1-MMP = Membrane-type 1 metalloproteinase
fully activated HSC (days 7-21). In contrast, gelatinase A (MMP-2) expression is not detectable in early HSC culture but increases from days 3-5 and is then a prominent feature of fully culture-activated HSC. This is accompanied by a similar pattern of expression of membrane-type 1-MMP (MT1-MMP), which mediates conversion of progelatinase A to active gelatinase A at the cell surface. Recent preliminary data from our group indicates that treatment of cultured HSC with concanavalin A converts latent MT1-MMP to the active form and that this markedly enhances activation of progelatinase A by these cultures. These data indicate that HSC can express the relevant MMPs and their activation systems, providing them with the ability to degrade extracellular matrix proteins in liver, particularly during the early phase of HSC activation. As these MMPs (gelatinase A and stromelysin) are able to degrade basement membrane matrix proteins (type IV collagen, laminin, proteoglycans) they are able to destroy the normal liver matrix and thus disrupt normal cell-matrix interaction. There is evidence to suggest that this per se might contribute to activation of HSC and be profibrogenic.

Kupffer cells also participate in matrix degradation in liver, through their synthesis of gelatinase B, small amounts of gelatinase A and interstitial collagenase (MMP-1). There is no conclusive evidence that hepatocytes release matrix metalloproteinases into the extracellular space, although they are able to activate progelatinase A, via mechanisms which may include expression of other members of the newly described MMP family of enzymes.

(ii) Studies in whole liver

In vivo changes in MMP expression in diseased compared to normal liver have been studied by several groups using a variety of techniques in either human tissue or animal models. By in situ hybridisation, Herbst and Milani and colleagues found that expression of gelatinase A and stromelysin-1 mRNA transcripts were low in normal liver but increased dramatically in liver disease or after experimental liver injury. In contrast interstitial collagenase mRNA transcripts were minimal in normal liver, with little or no change after liver injury. Using gelatin zymography, Takahara and colleagues described a progressive increase in detection of the active form of gelatinase A as liver fibrosis progresses. Moreover, using in situ hybridisation this group has also recently shown that mRNA transcripts for progelatinase A and MT1-MMP are increased in fibrotic and cirrhotic human liver and are co-localised to α-smooth muscle actin positive activated HSC. As active gelatinase A can degrade type IV collagen, a paradox is apparent as fibrotic liver contains increased, not decreased, amounts of type IV collagen. The explanation for this is unclear but, recent data from our laboratory suggest that gelatinase A might have functions other than matrix degradation; for example the active form of the enzyme promotes proliferation of HSC by a mechanism which has yet to be defined. The body of evidence therefore points towards active gelatinase A having profibrogenic effects despite the fact that it can degrade matrix. As suggested earlier this may be mediated via disruption of normal cell-matrix interactions or possibly via localised release of matrix-bound pro-fibrogenic growth factors. Understanding the mechanisms by which proteinases may be pro-fibrogenic is an area of active research interest.

With regard to degradation of fibrillar matrix proteins, previous studies have described interstitial collagenase activity in whole liver homogenates. In general, these studies demonstrate an increase in collagenase activity in the early stages of liver injury/disease, but this is followed by a progressive decrease as the liver becomes more fibrotic or cirrhotic. A reproducible theme in all of these studies is early expression of metalloproteinases with liver injury, but then as fibrosis progresses, metalloproteinase activity is either absent or ineffective. These observations raised the possibility of altered matrix degradation mediated by simultaneous expression of metalloproteinase inhibitors (TIMPs) in progressive liver disease.

Matrix degradation is inhibited in progressive liver fibrosis

Matrix metalloproteinases are inhibited by a family of specific inhibitors called tissue inhibitors of metalloproteinases or TIMPs, of which there are now four distinct members described. The properties of TIMPs 1-4 have recently been reviewed elsewhere. Although there is a great deal known about TIMPs 1 and 2, TIMP-3 and particularly TIMP-4 are relatively new members of this family, which have yet to be characterised in more detail.

We have investigated the role of TIMPs in liver fibrosis, concentrating mainly on TIMP-1 and TIMP-2. Our initial studies concentrated on TIMP-1 expression and release by HSC in primary culture. In early culture (day 3) HSC do not express TIMPs but do transiently express interstitial collagenase. With culture activation of HSC (towards a profibrogenic myofibroblastic phenotype the reverse pattern is observed; there is a striking increase in TIMP-1 expression, synthesis and extracellular release while interstitial collagenase expression becomes undetectable. TIMP-2 expression by activated HSC and in models of liver injury parallels that observed for TIMP-1. In early primary HSC culture, TIMP-2 is not detectable, but TIMP-2 mRNA and TIMP-2 activity are evident in activated HSC. By use of affinity chromatography to separate TIMPs from metalloproteinases in HSC culture supernatants we found that total detectable metalloproteinase activity increased by more than 20-fold, indicating that TIMPs play a major regulatory role in this system. We have therefore suggested that inhibition of matrix degradation, mediated by increased HSC expression of TIMPs 1 and 2 relative to interstitial collagenase may play an important role in the pathogenesis of liver fibrosis.

This hypothesis was examined further both in human liver disease and in animal models of liver fibrosis. In fibrotic human liver we demonstrated a 5-fold increase in mRNA levels for TIMP-1 and TIMP-1 protein with no significant change in interstitial collagenase (MMP-1) expression. TIMP-2 mRNA expression is also increased in human liver disease as determined by RNAase protection analysis of total liver RNA obtained from liver explants in primary biliary cirrhosis, sclerosing cholangitis and biliary atresia. We have also studied sequential changes in expression of TIMP 1 and 2, interstitial collagenase and procollagen I after rat liver injury induced by CCl4 or bile duct ligation. In both these models, TIMP-1 mRNA was upregulated within 6 hours, peaked at 72 hours post injury and remained elevated throughout the time course of the study (up to 8 weeks in the CCl4 model). TIMP-2 gene expression was also increased, but there was no change in rat interstitial collagenase (MMP-13) gene expression. More-
over increased TIMP gene expression occurred before up-regulation of procollagen I. By in situ hybridisation, Herbst and colleagues have also found an increase in TIMP-1 and TIMP-2 transcripts as early as 1-3 hours after CCI4 injury with markedly increased expression by 72 hrs\(^{40}\). Similarly, they also found increased TIMP-1 and -2 transcripts following bile duct ligation in rat and in a variety of human liver diseases.

TIMP-1 expression has also been described for HEP-G2 cells and hepatocytes in response to acute phase stimuli such as IL-6\(^45\)-\(^47\). Although this may be relevant to some disease states, in models of liver injury that lead to fibrosis, we\(^26\),\(^41\) and others \(^48\) have found the majority of TIMP-1 and TIMP-2 expression is confined to non-parenchymal cells and in particular the activated HSC. By in situ hybridisation there are, however, some transcripts detectable over hepatocytes\(^44\) and this source of TIMP-1 may also be relevant to progression of liver fibrosis.

In recent studies, we have examined expression of TIMPs relative to interstitial collagenase and procollagen I in a rat model of reversible liver fibrosis\(^49\). In this model CCI4 is given twice a week for 4 weeks which results in development of significant liver fibrosis. The injury is then stopped and natural resolution of liver fibrosis occurs over the next four weeks. Early in the recovery period (day 3-10) TIMP-1, TIMP-2 and procollagen I mRNA levels return to control values with no change in rat interstitial collagenase (MMP-13) expression. These changes in mRNA are accompanied by a net increase in detectable collagenase activity in whole liver homogenates. These observations imply that the changes in TIMP-1 and TIMP-2 relative to rat interstitial collagenase is the mechanism by which detectable collagenase activity increases and that this is responsible for the observed degradation of fibrillar liver matrix that occurs in this model. These exciting findings are potentially relevant to human liver disease, as reversibility of advanced fibrosis and cirrhosis has been described in patients with autoimmune chronic active hepatitis in whom effective immunosuppression has been achieved\(^49\).

**Regulation of TIMP-1 gene expression in hepatic stellate cell**

Because of the overall importance of TIMP-1 in controlling matrix metabolism in liver we have studied regulation of TIMP-1 gene transcription in quiescent and activated HSCs. The strategy employed was to use transient transfection of primary HSC cultures with a series of TIMP-1 promoter-CAT constructs\(^50\). These studies have demonstrated that TIMP-1 promoter activity in HSC involves novel regulatory mechanisms. Initial studies using a series of truncated constructs described a minimal promoter that contained AP-1, Pea 3 and Sip1 transcription factor binding sites. Further studies concentrated on AP-1 binding factors in nuclear extracts derived from HSC in various stages of activation. Electromobility shift assays demonstrated that AP-1 binding due to c-fos and c-jun was a feature of the early phase of HSC activation (20hrs) at a time when TIMP-1 was not expressed\(^51\). Lack of TIMP-1 promoter activity was found to be related to the absence of a transcription factor called Ets which binds to the PEA-3 site and is necessary for AP-1 mediated transcriptional regulation of TIMP-1 gene expression. Conversely in activated HSC, TIMP-1 gene expression and TIMP-1 promoter activity was increased in the absence of the classical c-fos and c-jun mediated AP-1 binding activity. We have recently shown that this prolonged late expression of TIMP-1 in activated HSC is mediated by Fra-2 and Jun-D transcription factors. Further analysis of TIMP-1 gene regulation in HSC is the subject of current research in our laboratories to determine if there are other novel transcriptional regulatory mechanisms.

**Conclusion**

Studies of the mechanisms of extracellular matrix degradation in liver have improved our understanding of the role this plays in the pathogenesis of liver fibrosis. In the early phase of liver injury, several metalloproteinases are expressed and there may be localised extracellular matrix degradation. Current evidence indicates that this is profibrogenic, perhaps through disruption of cell matrix interactions or possibly via release of matrix-bound growth factors. Both these potential mechanisms act via altering the phenotype of HSC, promoting their activation. In contrast, in more advanced disease, degradation of fibrillar matrix is inhibited by TIMP-1 and TIMP-2 derived from activated HSC. The balance between TIMPs and interstitial collagenase also mediates the degradation of fibrillar liver matrix during the natural resolution of liver fibrosis. These observations provide new horizons in considering strategies for the development of antifibrotic therapies.

**Acknowledgements:** Studies described in this manuscript were supported by the Wellcome Trust (grants G038712/Z/93 and G050443/Z/97), the Wessex Medical Trust, the British Liver Trust and the Medical Research Council (UK).

**References**

23) Thompson, K.C., Millward-Sadler, G.H., Gentry, J., and Sheron, N. (1997) Transgenic IL-10 deleted mice develop increased fibrosis in a carbon tetrachloride-induced model of hepatic cirrhosis. Hepatology 26, 335A
38) Benyon, R.C., Hovell, C.J. and Arthur, M.J.P. (1997) Gelatinase A (72kDa type IV collagenase) is an autocrine proliferation factor for rat hepatic stellate cells. Hepatology 26, 186A
45) Kordula, T., Guttgemann, I., Rosejohn, S., et al. (1992) Synthesis of tissue inhibitor of metalloproteinase-1 (TIMP-1) in human
Matrix degradation in liver fibrosis

hepatoma cells (HepG2) - Up-regulation by interleukin-6 and transforming growth factor-beta. FEBS Lett. 313, 143-147


47) Roeb, E., Graeve, L., Mullberg, J., Matern, S., and Rosejohn, S. (1994) TIMP-1 protein expression is stimulated by IL-1 beta and IL-6 in primary rat hepatocytes. FEBS Lett. 349, 45-49


