# Fibrosis of liver, pancreas and intestine : common mechanisms and clear targets ?

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## Abstract

**Chronic diseases of the liver, pancreas, intestine, kidneys, skin and lungs are usually accompanied by scarring. Loss of organ function is often progressive despite the use of immunosuppressive, antiviral or antiinflammatory agents. Therefore, well tolerated antifibrotic therapies are urgently needed. The targets for such therapies are activated mesenchymal cells that synthesize an excess of matrix proteins and resemble the myofibroblasts of healing wounds. These cells derive from normally quiescent fibroblasts or smooth muscle cells and from stellate cells of liver and pancreas. Their activation is triggered and maintained by mechanical stress and several fibrogenic modulators and cytokines. Some agents inhibit myofibroblast proliferation and collagen synthesis *in vitro*, but only few of them are effective in vivo. Potential antifibrotic drugs have been tested mainly in models of liver fibrosis. In the suitable rat model of biliary fibrosis, an antifibrotic effect was demonstrated for silymarin, a defined mixture of flavonoids, and to a lesser degree for pentoxifylline. A spin-off of the large multicenter trials for hepatitis C is the finding that interferon-α given for 6-12 months may halt or reverse fibrosis, even in virological non-responders. This has to be proven in prospective randomized trials. Specific inhibitors of the endothelin-A-receptor which are orally available can suppress liver collagen accumulation by 40-60%. Other strategies aim at inhibition of the profibrogenic cytokines TGF-β or connective tissue growth factor. Effective drug targeting to the fibrogenic liver cells is now possible by use of cyclic peptides that bind to receptors which are specifically upregulated on activated stellate cells. Blockade of such activation receptors can induce stress-relaxation which reverts the fibrogenic cells to a fibrolytic, collagen degrading phenotype. Fibrosis has been discovered as a novel target for the pharmaceutical industry. This implies the use of combinatorial chemistry and an automatized screening machinery, greatly speeding up the design and selection of specific antifibrotic agents. Combined with the rapidly evolving validation of serological markers of fibrogenesis and fibrolysis unforeseen progress in the treatment of organ fibrosis can be expected.** (Acta gastroenterol. belg., **2000**, 63, **366-370**).

**Key words** : fibrosis, fibrogenesis, collagen, antifibrotic, silymarin, pentoxifyllin, endothelin, TGF-β, liver, pancreas.

## Abbreviations

CTGF, connective tissue growth factor ; ECM, extracellular matrix ; ET, endothelin ; $ET_AR$, endothelin A receptor ; MMP, matrix metalloproteinase ; MF, myofibroblast ; PDGF, platelet derived growth factor ; SC, stellate cell ; TGF, transforming growth factor ; TIMP, tissue inhibitor of metalloproteinases.

## The development of fibrosis

Fibrosis results from excessive accumulation of extracellular matrix (ECM). ECM describes the connective tissue molecules found in all multicellular organisms. These molecules are grouped into major molecular classes, mainly the collagens, noncollagenous glycoproteins, glycosaminoglycans, proteoglycans and elastin. In most organs, collagens, especially the fibril forming collagen types I and III, but also basement membrane collagen type IV, are the most abundant ECM components (1). In liver cirrhosis their relative tissue content may increase up to tenfold. This increase explains most of the complications of advanced fibrosis. In the liver, this leads to an impaired exchange of metabolites between the sinusoidal blood and the hepatocytes by sinusoidal sclerosis (capillarization) and the formation of porto-venous shunts that prevent sinusoidal perfusion. The latter is also the basis for the increase in portal pressure that leads to esophageal or gastric varices and the development of ascites. Lastly, the continuous stimulus for epithelial proliferation in an abnormal ECM environment (regenerative nodules) predisposes for the development of carcinoma.

As demonstrated in Fig. 1 a variety of adverse stimuli such as toxins, viruses, hypoxia, immune reactions, metabolic diseases, excretory stasis or simply mechanical stress can trigger *fibrogenesis*, i.e., the excess synthesis and deposition of ECM. In acute diseases, such as self-limited viral hepatitis, infectious enteritis or ERCP-induced pancreatitis, fibrogenesis is balanced by *fibrolysis*, i.e., the removal of excess ECM by proteolytic enzymes, the most important of which are the matrix metalloproteinases (MMPs). With repeated injury of sufficient severity, as occurs in most chronic diseases, fibrogenesis prevails, finally resulting in morphologically apparent fibrosis. Fibrogenesis is accompanied by an upregulation of collagen synthesis, a downregulation of MMP secretion and activity, and by an increase of the physiological inhibitors of the MMPs, the tissue inhibitors of MMPs (TIMPs), of which the universal MMP-inhibitor TIMP-1 is the most important (1,2). Collagens, MMPs and TIMPs are mainly produced by activated hepatic or pancreatic stellate cells (SC) and by activated fibroblast and myofibroblasts (MF) (1-6).

Activated macrophages (Kupffer cells in the liver) or proliferating ductular epithelia are major sources of potentially fibrogenic cytokines and growth factors that

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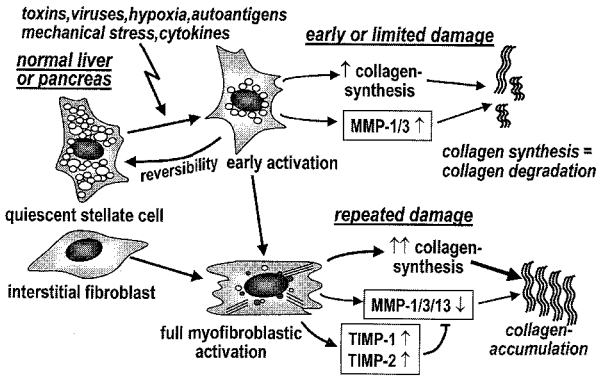


Fig. 1. — Initiation and maintenance of fibrogenesis.
The normally quiescent mesenchymal cells may undergo intermediate activation to a transitional cell type that synthesizes balanced amounts of collagens and collagen dissolving metalloproteinases (MMPs). With continuous activation as occurs in chronic inflammation these cells transdifferentiate to myofibroblasts which are characterized by excessive collagen synthesis, decreased release of MMPs and an enhanced expression of the physiological inhibitors of MMPs (TIMP-1 and -2).

Table 1. — **Related fibrogenic cell types**
In most organs normally quiescent mesenchymal cell types that resemble the hepatic stellate cell or the interstitial (portal) fibroblast can be activated to fibrogenic myofibroblast-like cells which are the main target for antifibrotic therapies.

| Liver | Pancreas | Kidney | Lung |
|---|---|---|---|
| portal fibroblast | interstitial fibroblast | interstitial fibroblast | interstitial fibroblast |
| stellate cell | stellate cell | alveolar-fibroblast | mesangial-cell |

| Gut | Artery | Skin |
|---|---|---|
| interstitial fibroblast | intima fibroblast | dermal fibroblast |
| subepithelial fibroblast | media-myo-fibroblast | subepithelial fibroblast |

stimulate SC and fibroblasts to become MF (1-6). Similar cell types are found in most organs prone to fibrosis such as the kidney, lung, skin and arteries (1,7,8) (Table 1). Usually, activation to MF is the key step of a protective program aimed at rapid closure of a potentially lethal wound (1,8). This program is self-limiting if the offending agent is present for a short period of time but leads to fibrosis when continuously activated. It follows that activated SC and MF are the prominent target for antifibrotic therapies in chronic diseases.

## Potential antifibrotic agents

Since SC and MF were identified as the major fibrogenic cell types and since they undergo spontaneous activation in cell culture, the stage has been set for the development of specific antifibrotic agents. Such agents are currently identified and tested in several laboratories worldwide. Once active in the *in vitro* culture, all substances have to undergo "proof of principle" in a suitable animal model, such as experimental hepatic fibrosis and cirrhosis of the rat. Models that evolve chronically and reproducibly, especially biliary cirrhosis due to bile duct occlusion or seruminduced fibrosis, are preferable over those characterized by major hepatocyte necrosis, like those induced by carbon tetrachloride, dimethylnitrosamine or galactosamine, because the former more closely resemble human chronic liver disease and allow to identify a "true" antifibrotic instead of an anti-inflammatory, anti-necrotic or radical scavenging effect.

Table 2 lists substances which have been tested in suitable animal models of liver fibrosis (8). Some of these drugs are currently undergoing phase 2 or 3 clinical testing with pre- and post-treatment biopsy for exact morphometrical determination of the area of connective tissue and with a spectrum of surrogate markers of liver fibrogenesis (see below). Promising drugs are oral endothelin A receptor ($ET_AR$) antagonists (9), silymarin (10), interferon alpha (11,12), and derivatives of pentoxifyllin (13). $ET_AR$ antagonists are of particular interest, since the $ET_AR$ is highly upregulated on most mesenchymal cells, including hepatic SC, in their intermediate state of activation (9,14,15), allowing for a targeted approach to the fibrotic lesion. Antagonizing the $ET_AR$ also offers the additional advantage to lower an elevated portal pressure which is in part mediated by endothelin-1 induced contraction of activated hepatic SC (Fig. 2).

Transforming growth factor beta (TGF-β) is considered to be the most potent fibrogenic cytokine and its inhibition therefore appears attractive. Though peptidic antagonists to TGF-β are available (16,17), only a targeted approach is feasible (see below), since its recep-

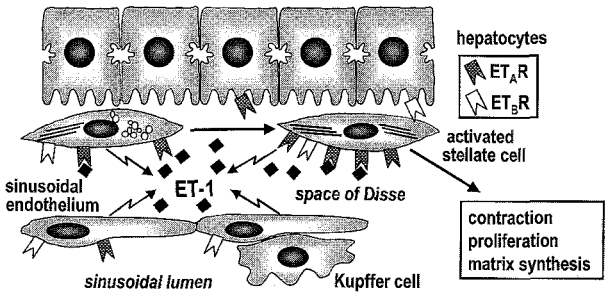


Fig. 2. — Hepatic endothelin/endothelin receptor system in hepatic stellate cell activation.
Upon early activation endothelin A receptors ($ET_AR$) are upregulated on the perisinusoidal stellate cells (SC). An increased release of endothelin-1 by the injured sinusoidal endothelium and the SC themselves then leads to para- and autocrine activation. However, with complete myofibroblastic transformation SC decrease expression of the profibrogenic $ET_AR$ and lower expression of the anti-fibrogenic $ET_BR$ (ref.14) which may be a counter-regulatory mechanism. Similar mechanisms are operative in other organs.

Table 2. — **Potential antifibrotic drugs**

Examples of drugs for which antifibrotic activity has been shown in suitable animal models of liver fibrosis. Most of these agents are also expected to be effective in fibrogenesis of other organs. $ET_AR$, endothelin A receptor ; CTGF, connective tissue growth factor.

| Drug | Antifibrotic Effect Animal | Man | Mechanism |
|---|---|---|---|
| $ET_AR$-antagonists | yes | (studies) | stellate cell activation ↓ |
| pentoxifyllin | yes | ? | proliferation/collagen ↓ |
| silymarin | yes | studies | free radicals/collagen ↓ |
| interferon α,β,γ | (yes) | studies | proliferation ↓, MMPs ↑ |
| anti-TGF-β/-CTGF | (yes) | ? | collagen ↓, MMPs ↑ |
| hepatocyte growth factor | (no) | ? | hepatocyte-/ bile duct-proliferation ↑ |

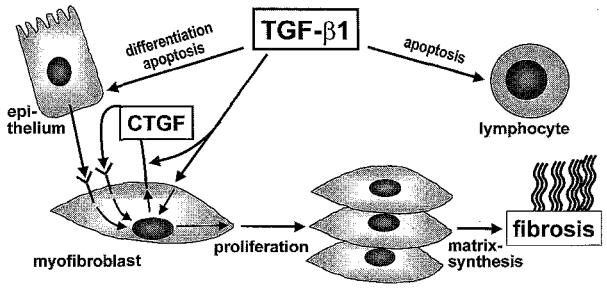


Fig. 3. — CTGF as mesenchymal mediator of TGF-β action. In conjunction with transforming growth factor (TGF)-β, connective tissue growth factor (CTGF) induces myofibroblast proliferation and collagen synthesis. CTGF is produced by proliferating biliary epithelia and induced in mesenchymal cells by TGF-β. Due to its specific action on mesenchymal cells alone it is a better target for antifibrotic therapies than TGF-β which has a regulatory role in numerous other cell types.

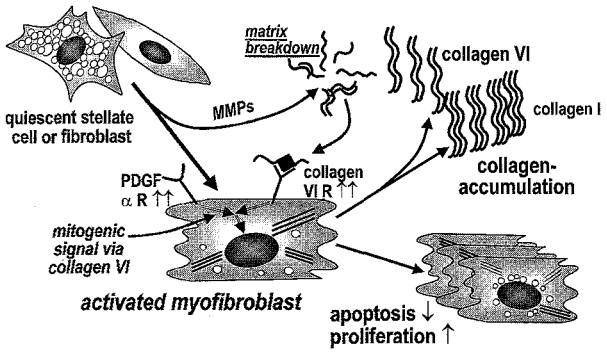


Fig. 4. — Collagen VI as auto- and paracrine fibrogenic factor. Activated myofibroblasts are characterized by an increased surface expression of receptors for platelet-derived growth factor (PDGF) and collagen VI. PDGF and collagen VI serve as para and autocrine growth factors for these cells. Their blockade by receptor antagonists may result in an antifibrotic effect which is limited to fibrogenic fibroblasts and devoid of unwanted sideeffects in uninvolved tissues.

tors are expressed on most cell types and its systemic inhibition is expected to trigger autoimmune disease and cellular dedifferentiation. Antagonizing connective tissue growth factor (CTGF), the auto- and paracrine synthesis of which is triggered by TGF-β primarily in mesenchymal cells may render a more specific antifibrotic strategy (18) (Fig. 3).

Reports on the antifibrotic activity of hepatocyte growth factor (HGF) (19) have to be interpreted with care, since this cytokine rather causes hypertrophy and hyperplasia of epithelia, thus reducing the *relative* and not the *absolute* collagen content in the organ, with the additional danger of promoting epithelial malignancy.

A promising target is the induction of stress relaxation of fibrogenic cells, a matrix (integrin) receptor-mediated process that is associated with a decrease in collagen synthesis and an increase in collagenase activity. This stress relaxation occurs once mesenchymal cells are placed from a "stressed", two-dimensional environment (mimicking a situation of wounding) into a "relaxed", three-dimensional environment (8,20). Stress relaxation mitigates or even abrogates signals transferred via certain mitogenic growth factors and can revert the same cell that caused fibrogenesis into a fibrolytic cell that preferably releases MMPs instead of collagens. Thus, the receptors for platelet-derived growth factor and endothelin-1 (via the $ET_AR$) transmit potent stress signals that trigger proliferation and ECM synthesis in activated SC and in MF. Interestingly, also soluble proteolytic fragments of collagen VI which are released from the liver matrix during remodeling serve as a potent growth stimulator and anti-apoptotic factor for fibrogenic cells, an effect that is mediated via a non-integrin collagen VI receptor (21-23) (Fig. 4). Apart from direct inhibition of these receptors by peptides or peptide analogues, coupling specific receptor recognizing cyclic peptides or other ligands to a drug carrier allows highly specific targeting to the activated fibrogenic cells in the liver and most problably also in other organs subjected to active fibrogenesis (Fig. 5). This has been shown both *in vitro* and *in vivo* with cyclic peptides recognizing the receptors for PDGF, collagen VI and mannose-6-phosphate (24-26). With these ligands specific uptake of the targeted carrier in activated SC of fibrotic rat livers can reach up to 50% *in vivo*.

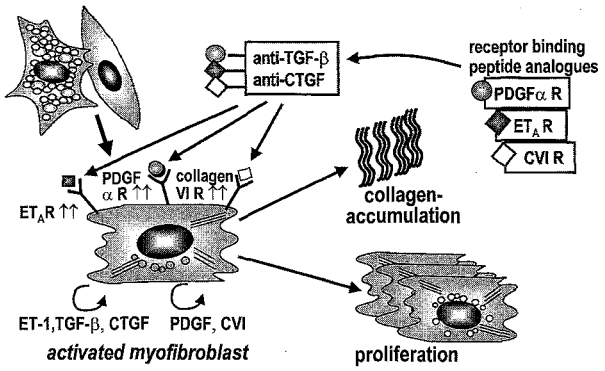


Fig. 5. — Receptor-targeted antifibrotic therapy.
The myofibroblastic activation receptors can serve as ideal targets to deliver potential antifibrotic agents exclusively to the fibrogenic focus. These agents can be coupled to cyclic receptor-recognizing peptides that allow highly efficient delivery to activated myofibroblasts and their rapid internalization with release of the antifibrogenic priniciple.

Table 3. — **Serum assays to assess fibrosis**
The shown serum makers are currently being validated in patients with chronic liver diseases, but some of the parameters may also prove useful for assessment of matrix turnover in renal and pancreatic fibrosis. Most of them appear to reflect fibrogenesis rather than fibrolysis.

| | Fibro-genesis | Fibro-lysis | Liver specificity |
|---|---|---|---|
| PIIINP | + | (+) | + |
| collagen IV | + | – | + |
| collagen VI | + | (+) | + |
| collagen XIV | + (portal) | – | + |
| laminin | + | (+) | (+) |
| tenascin | + (lobular) | – | (+) |
| hyaluronan | (+) | (+) | (+) |
| TIMP-1 | + | – | + |
| MMP-2 | – | + | (+) |
| MMP 2 | + | (+) | + |
| MMP-9 | (+) | (+) | (+) |

## Serum markers of liver fibrosis

Serum fibrosis markers show the highest levels in chronic liver diseases (3,8,27), although they may also be useful to monitor renal and pancreatic matrix turnover (28,29). These parameters mainly appear to reflect fibrogenesis rather than fibrolysis (Fig. 6). They open the possibility to assess the future evolution of fibrosis and the effect of potential antifibrotic treatment in an individual patient on a frequent basis. However, these markers still await validation in large prospective follow-up studies of patients with liver, pancreatic or renal diseases. Several such studies are currently underway for chronic liver diseases. They involve more than 1000 patients with sequential liver biopsies 18-24 months apart. From these biopsies the increase of the connective tissue area and volume will be determined by densitometry. In addition, quantitative

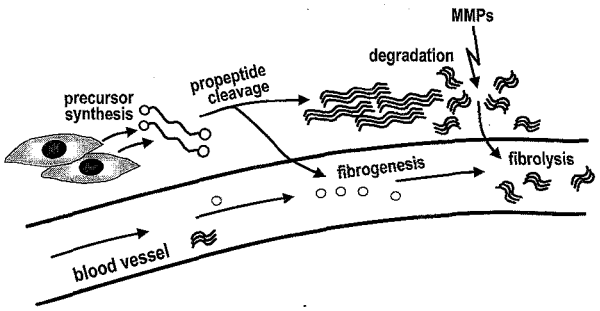


Fig. 6. — Circulating matrix proteins related to fibrogenesis and fibrolysis.
Procollagen precursors released by fibrogenic cells are processed by procollagen peptidases. Only removal of the bulky propeptides allows the formation of collagen fibrils in the extracellular space. Thus circulating propeptide levels should reflect de novo synthesis and deposition of collagen, i.e. fibrogenesis. On the other hand, action of matrix metalloproteinases (MMPs) is expected to generate fragments of already deposited matrix proteins the levels of which should reflect matrix dissolution, i.e. fibrolysis.

RT-PCR, to quantitate hepatic expression of several collagens, of MMPs and TIMP-1 will be performed from fractions of diagnostic biopsies, allowing a direct comparison with the serum fibrosis markers. Table 3 shows serum fibrosis markers that may prove to be useful in future studies of antifibrotic drug effects.

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