Liver fibrosis and altered matrix synthesis

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Liver fibrosis represents the uniform response of liver to toxic, infectious or metabolic agents. The process leading to liver fibrosis resembles the process of wound healing, including the three phases following tissue injury: inflammation, synthesis of collagenous and noncollagenous extracellular matrix components, and tissue remodelling (scar formation). While a single liver tissue injury can be followed by an almost complete restitution ad integrum, the persistence of the original damaging noxa results in tissue damage. During the establishment of liver fibrosis, the basement membrane components collagen type IV, entactin and laminin increase and form a basement membrane-like structure within the space of Disse. The number of endothelial fenestrae of the sinusoids decreases. These changes of the sinusoids are called 'capillarization' because the altered structure of the sinusoids resembles that of capillaries. At the cellular level, origin of liver fibrogenesis is initiated by the damage of hepatocytes, resulting in the recruitment of inflammatory cells and platelets, and activation of Kupffer cells, with subsequent release of cytokines and growth factors. The hepatic stellate cells seem to be the primary target cells for these inflammatory stimuli, because during fibrogenesis, they undergo an activation process to a myofibroblast-like cell, which represents the major matrix-producing cell. Based on this pathophysiological mechanism, therapeutic methods are developed to inhibit matrix synthesis or stimulate matrix degradation. A number of substances are currently being tested that either neutralize fibrogenic stimuli and prevent the activation of hepatic stellate cells, or directly modulate the matrix metabolism. However, until now, the elimination of the hepatotoxins has been the sole therapeutic concept available for the treatment of liver fibrogenesis in humans.

Key Words: *Liver fibrosis; Matrix*

Fibrose du foie et altération de la synthèse matricielle

RÉSUMÉ : La fibrose hépatique est une réaction uniforme du foie à des agents toxiques, infectieux ou métaboliques. Le processus aboutissant à la fibrose du foie ressemble au processus de cicatrisation en trois étapes à la suite d'une lésion tissulaire : l'inflammation, la synthèse de composants matriciels extracellulaires collagènes et non collagènes, et le remodelage du tissu (formation de la cicatrice). Même s'il ya reconstitution quasi complète du tissu hépatique à la suite d'une seule lésion du foie, la présence continue du facteur agressant initial entraîne des lésions tissulaires. Durant le processus de fibrose du foie, les composants collagènes de type IV de la membrane basale, l'entactine et la laminine, se développent et forment une structure semblable à celle de la membrane basale dans l'espace de Disse, tandis que le nombre de fenêtres endothéliales des sinusoïdes diminue. Ce dernier phénomène s'appelle « capillarisation » en raison de la transformation de la structure des sinusoïdes en une structure semblable à celle des capillaires. Au niveau cellulaire, la fibrogenèse du foie commence par l'atteinte des hépatocytes, qui se traduit par l'arrivée de cellules inflammatoires, de plaquettes et par l'activation des cellules de Kupffer, suivies de la libération de cytokines et de facteurs de croissance. Les cellules stellaires du foie semblent la principale cible de la réaction inflammatoire parce que, durant la fibrogenèse, elles subissent un processus de transformation en myofibroblastes, qui sont les plus grands producteurs de tissu matriciel. Aussi cherche-t-on à mettre au point des moyens thérapeutiques visant à inhiber la synthèse du tissu matriciel ou à en stimuler la dégradation. Certaines substances sont actuellement à l'essai : ou bien elles neutralisent l'activité fibrogène et empêchent l'activation des cellules stellaires du foie, ou bien elles modulent directement le métabolisme du tissu matriciel. Toutefois, le seul moyen dont on dispose actuellement pour traiter la fibrogenèse du foie est l'élimination des hépatotoxines.

Liver cirrhosis is the result of a progressive increase in connective tissue and a progressive reduction in functional active parenchyma; in other words, liver cirrhosis is a continuous wound healing process due to a continuous loss of vital parenchyma caused by different noxa (alcohol, viruses, metabolic disorders). This process leading to liver fibrosis includes the three phases following tissue injury: inflammation, synthesis and deposition of collagenous and noncollagenous extracellular matrix (ECM) components, and tissue remodelling (scar formation). Data from experimental animal models and observations of humans indicate that liver cirrhosis may be reversible when the damaging agent is withdrawn (eg, eradication of viruses).

MECHANISMS OF ACUTE AND CHRONIC LIVER INJURY

Inflammation: Inflammatory processes are characterized by migration of inflammatory cells into areas affected by various noxious agents. Migration induced by chemokines released at sites of injury is mediated by the interaction of adhesion molecules on migrating inflammatory cells and adhesion molecules on resident cells (mostly endothelial cells first and hepatic stellate cells [HSCs] afterwards) (1-4). Because noxious agents may only induce a 'disturbance' of hepatocytes and not necessarily induce cell death, hepatocellular necrosis may be a result of the interaction of 'disturbed' hepatocytes with recruited inflammatory cells (Figure 1). Following hepatocellular necrosis, there is an activation of repair mechanisms that either lead to a restitution ad integrum or, in the case of progressive disease, to the replacement of functional parenchyma by matrix (fibrogenesis). In different animal models of acute liver injury, it has been shown that the accumulation of inflammatory cells and the hepatic necrosis precede the proliferation and activation of HSCs as well as the formation of a 'provisional clot', which, therefore, are considered to constitute a part of the expected healing response to hepatocyte necrosis. On the other hand, HSCs may also be capable of contributing to the recruitment of inflammatory cells because it has been demonstrated that HSCs express chemokines (5-9). Furthermore, HSCs may be important for the transmigration of inflammatory cells by the synthesis of adhesion molecules as intercellular cell adhesion molecule-l, vascular cell adhesion molecules and neural cell adhesion molecules under conditions of inflammation (2,10,11). Nevertheless, it is not definitively clear whether necroinflammation is always needed for the development of liver fibrosis. In fact, cirrhosis may develop in patients with hemochromatosis without inflammation. However, in most cases, following the persistence of noxious agents, necrosis and inflammation, changes occur that include the so called 'capillarization' of the sinusoids, the progressive deposition of ECM and the reduced matrix degradation, which finally leads to a scar formation.

'Capillarization' of the sinusoids: Changes of the hepatic sinusoids referred to as 'capillarization' are characterized by
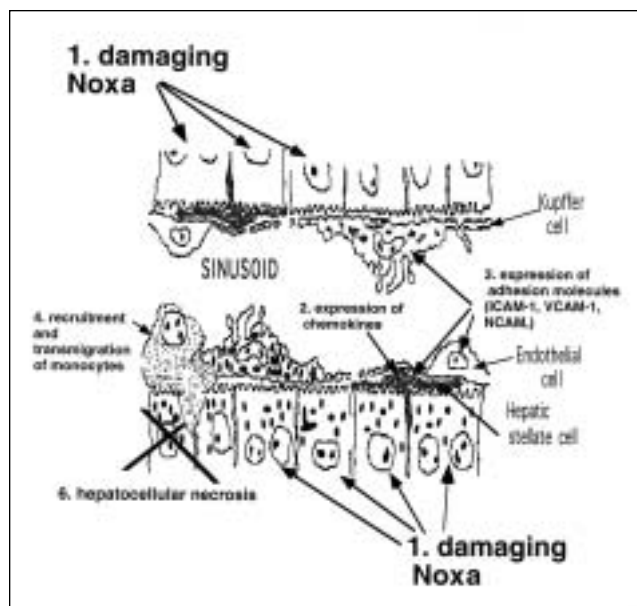

Figure 1) *Pathogenesis of liver fibrogenesis. Recruitment of inflammatory cells induced by stressed hepatocytes followed by hepatocellular death. ICAM-I Intercellular adhesion molecule-1; NCAM Neural cell adhesion molecule; VCAM-1 Vascular cell adhesion molecule-1*

a decrease in the size and number of endothelial cell fenestrae, and the development of a basement membrane within the space of Disse (12,13). Endothelial cell defenestration precedes sinusoidal fibrosis. It has been suggested that defenestration may contribute to fibrogenesis by causing a reduction in endothelial cell sieving of lipids and vitamin A in the perisinusoidal cell, leading to a depletion of vitamin A (14). The initial defenestration of endothelial cells before the development of fibrosis appears to be reversible with the removal of the hepatotoxin. However, in the case of persistent damage, matrix proteins, including the basement membrane components collagen type IV, laminin, entactin and fibronectin, increase with the formation of an organized basement membrane-like structure immediately beneath the endothelial cells. There is also accumulation of collagen types I, II, V and VI, tenascin and undulin in the space of Disse (15,16). The increase of interstitial collagens and of laminin is thought to contribute to further endothelial cell defenestration and phenotypic change. 'Capillarization' of the sinusoids hinders the normal exchange between plasma and hepatocytes and is, therefore, a major contributor to the deterioration of liver function that occurs with liver disease. Capillarization takes place in the sinusoids lining the bridge between the portal and the central vein, or between two central veins or two portal tracts (Figure 2).

Progressive deposition of ECM – Scar formation: The persistence of noxious agents leads to a progressive replacement of hepatocytes by connective tissue. Whereas the components of the ECM of the fibrotic liver qualitatively resemble that of normal liver, all components are increased
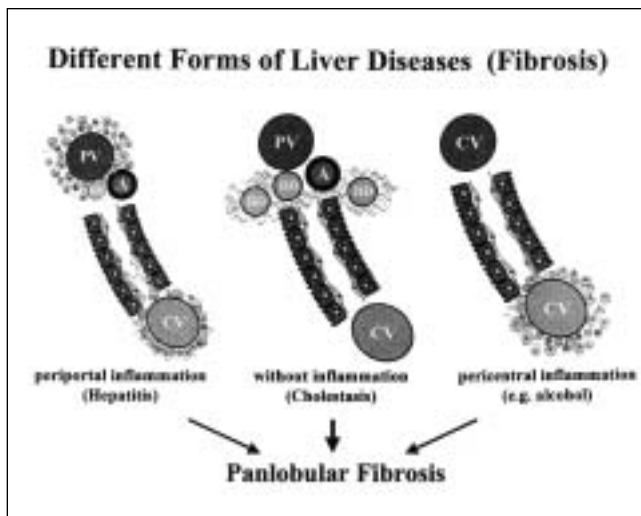
Figure 2) *Scar formation*

Figure 3) *Mechanisms of transforming growth factor-beta-induced accumulation of extracellular matrix through interaction with activated hepatic stellate cells. TIMP Tissue inhibitor of metalloproteinase; α2-M Alpha₂-macroglobulin*

in fibrotic liver with an over-proportional increment of single ECM proteins.

During early liver fibrogenesis, fibronectin and tenascin increase within necroinflammatory areas, early fibrous septa and the outer regions of larger septa. The deposition of fibrinogen/fibrin suggests the involvement of a 'clotting-like process during short term liver injury and liver fibrosis, indicating that fibrin/fibronectin constitutes a 'provisional matrix', which influences attraction and proliferation of inflammatory and matrix-producing cells (17,18). The glycoprotein laminin was found in early septa and the outer regions of larger septa but is of less or no importance in necroinflammatory areas (19,20). In fibrous septa, the presence of entactin, undulin and von Willebrand factor was demonstrated, whereby the von Willebrand factor is closely related to newly formed vessels (16,21,22). SPARC (secreted protein acidic and rich in cystein; osteonectin, BM-40) expression also increases during the course of liver fibrogenesis (23).

In accordance with the scheme of wound healing and scar formation, collagen type III is first increased after liver damage and is finally substituted with collagen type I, the latter constituting 60% to 70% of the total collagen in the cirrhotic liver (sixfold increment compared with normal liver). In the case of alcoholic liver disease, however, collagen type III is the major component of the fibrotic septa (19,24,25). Furthermore, collagen type IV, V and VI are also detected in the fibrous septa (19).

Although the amount of matrix in the liver is similar in different forms of liver damage, the distribution of the ECM varies with the site and origin of the injury. For example, in the case of viral hepatitis, there is a periportal inflammation, which is then followed by fibrosis in the periportal zones. On the other hand, ECM deposition during the development of cholestatic fibrosis occurs without inflammation (26). Finally, during alcoholic liver disease, early injury and fibrosis are mainly pericentral. However, during
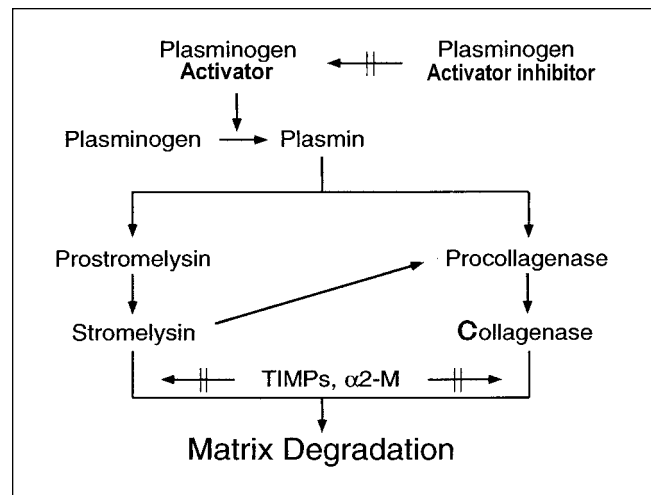
progressive disease, both periportal and pericentral injury lead to panlobular fibrosis (Figure 2).

Matrix degradation: Accumulation of connective tissue reflects an alteration in the balance between synthesis and degradation of ECM. Metalloproteinases (matrix metalloproteinase [MMP]) and their specific inhibitors, tissue inhibitor of metalloproteinase (TIMP)-1 and -2, are believed to play a crucial role in ECM remodelling. In addition to HSCs (27-30), TIMP-1 is expressed in rat hepatocytes in primary culture and regulated by inflammatory cytokines (in particular interleukin-6), illustrating that hepatocytes are involved in ECM protein degradation (31).

Levels and cellular sites of gene expression of two major collagen-degrading enzymes for MMP-1 and MMP-2 in normal and fibrotic human livers as well as in human HSCs were analyzed by Milani et al (32). HSCs expressed both MMP-1 and MMP-2 mRNA in vitro. The expression of MMPs and TIMPs in all rat liver cell types was recently demonstrated, although the cellular expression levels were markedly different. Gelatinase-B was predominantly expressed by Kupffer cells (KCs), gelatinase A, stromelysins 1 and 2, and collagenase in HSCs (33). TIMP-1 was mainly present in HSCs and TIMP-2 in KCs, while TIMP-3 expression was detectable only in hepatocytes. Furthermore, a cytokine-specific regulation of TIMP/MMP expression was demonstrated, suggesting that the initial matrix breakdown following liver injury might be enhanced by tumour necrosis factor (TNF)-α, while the diminished matrix degradation during chronic tissue injury might be due to the activation of transforming growth factor (TGF)-β1 through TIMP induction (Figure 3) (33).

CELLS INVOLVED IN LIVER FIBROGENESIS

At the cellular level, origin of liver fibrogenesis is initiated by the damage of hepatocytes, resulting in a recruitment of inflammatory cells and platelets, and activation of KCs,
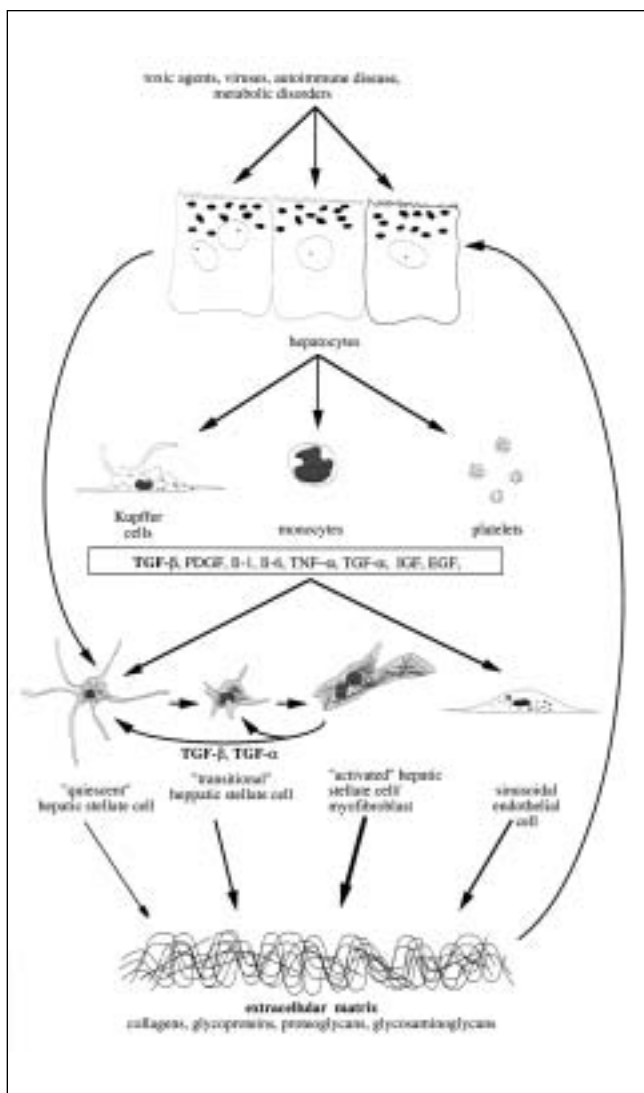
Figure 4) *Pathogenesis of liver fibrogenesis. Activation of matrix-producing cells (hepatic stellate cells, myofibroblasts and sinusoidal endothelial cells). EGF Epithelial growth factor; IGF Insulin growth factor; Il Interleukin; PDGF Platelet-derived growth factor; TGF Transforming growth factor; TNF Tumour necrosis factor*

with subsequent release of cytokines and growth factors. Whereas it could be shown that sinusoidal endothelial cells (SECs), bile duct epithelial cells, hepatocytes, KCs, fibroblasts and myofibroblasts are involved in fibrogenesis through the formation of ECM, HSCs seem to be the primary target cell for these inflammatory stimuli.

HSCs and myofibroblasts: Interstitial fibroblasts, as well as myofibroblasts, have been shown to be of particular fibrogenic importance in early stages of cholestatic and serum-induced hepatic fibrosis models (26). In this context, however, it must also be mentioned that myofibroblasts are only insufficiently delimited from activated HSCs (34).

The 'activation' of quiescent HSCs is recognized to be one of the most important steps in the development of liver fibrosis. Activation describes the proliferation (?) of HSCs as well as the morphological transformation from vitamin

A-storing 'quiescent' cells, through an intermediate state ('transitional' HSCs), to myofibroblast-like 'activated' HSCs, which represent the major matrix-producing cells during liver fibrogenesis. In vivo initiation of activation of HSCs is caused by the synthesis of cytokines and non-peptide mediators produced by damaged hepatocytes, endothelial cells, fibroblasts, platelets and activated macrophages/KCs. Regarding matrix synthesis in activated HSCs, the growth factors TGF-α, TGF-β and TNF-α are of particular importance because all of these, especially TGF-β, upregulate matrix gene expression in these cells (Figure 4) (35-38).

For a long time, the mechanisms that result in the termination of HSC proliferation when tissue repair is concluded were poorly understood. However, it was recently shown that, in vitro and in vivo after acute liver damage, HSCs express CD95 and CD95L in increasing amounts in parallel with activation; furthermore, spontaneous apoptosis has been shown to occur (39). This suggests that HSCs induce a self-activated cell death when their work is no longer needed. However, the fate of activated HSCs during liver injury is still unclear. It has been suggested that HSCs continue to stay alive by a mechanism of self perpetuation. It was recently demonstrated that TGF-β, the main fibrogenic mediator, as well as TNF-α, not only reduces proliferation of activated HSCs, but also inhibits spontaneous and induced apoptosis in vitro (40). This may be a mechanism that allows HSCs to survive and continue matrix production. However, when the damaging insult is withdrawn, the increased number of stellate cells is reduced by apoptosis.

During activation of HSCs, the synthesis of ECM proteins by HSCs increases, and the pattern of the synthesized proteins changes. The ECM proteins produced by quiescent HSCs are typically deposited in basement membranes (laminin, collagen type IV, entactin), whereas activated HSCs synthesize more proteins located in the interstitium, eg, collagen type 1 (15,41-43).

SECs: Evidence has been published that, in addition to HSCs, SECs are capable of synthesizing ECM proteins, since it has been demonstrated that SECs synthesize collagen types I, II and IV, fibronectin, tenascin, undulin, laminin, entactin, thrombospondin and von Willebrand factor in vivo and in vitro (13,22,44-48). Whereas perlecan is expressed by both SECs and portal endothelial cells, syndecan 1 is produced by SECs and syndecan 3 by portal endothelial cells (49,50). On the mRNA level, differences in matrix proteins expressed were found between SECs isolated from normal liver and SECs isolated from injured liver because SECs from injured liver produce elevated mRNA levels for collagen types I, III, IV, fibronectin and laminin (13,44). Furthermore, it could be demonstrated that SECs respond upon stimulation with TGF-β1 with an enhanced synthesis of ECM proteins (Figure 4) (13), which is in accordance with the fact that TGF-β1 receptors are expressed by SECs (35). The data suggest that, in addition to HSCs, SECs are also a source of matrix proteins present in the space of Disse. Although HSCs seem to be responsible for

the synthesis of matrix proteins at later stages of disease, endothelial cells play a role under basal conditions as well as during 'capillarization' of the sinusoids at the beginning of fibrosis.

KCs: Little matrix production has been ascribed to KCs. Macrophage cultures contain small amounts of fibronectin and proteoglycans, and mRNA for collagen types I, III and IV (47,51,52). Furthermore, KCs have been shown to express aggrecan in an in vivo study (53). In mononuclear phagocytes from damaged livers, increased fibronectin synthesis was demonstrated (54). Nevertheless, KCs and inflammatory mononuclear phagocytes are of critical importance for repairing processes as well as for liver fibrogenesis due to their capacity to synthesize a high amount of cytokines, which influence matrix synthesis and degradation by myofibroblast-like cells and other liver cells (Figures 1 and 4) (55,56).

Hepatocytes: Previous reports have claimed that hepatocytes contain collagen types I, III and IV, as well as plasma and cellular fibronectin; laminin; hyaluronic acid; sulphated glycoproteins, especially heparan sulphate, dermatan sulphate, chondroitin sulphate, syndecans and perlecan; and recently also collagen type XVIII (47,51,52,57-62). Caution is required before interpreting matrix synthesis by cultured hepatocytes following the recognition that contaminating HSCs may be responsible for ECM production in hepatocyte cultures (63). Studies of the in vivo incorporation of radiolabelled collagen precursors suggest that hepatocytes play only a minor role in total collagen production in normal liver and during early fibrosis (64).

Bile duct epithelial cells: Bile duct epithelial cells produce procollagen type I, prolylhydroxylase, syndecan 1, syndecan 4, glycipan and heparan sulphate. Beyond this, mRNA for collagen type IV and laminin has been shown in bile duct epithelium (65,66). This could be of great relevance during the development of fibrosis in cholestatic liver diseases.

Therapeutic approaches: The identification of ECM- or MMP- and TIMP-producing cells as well as the characterization of regulatory mechanisms are the basis for therapeutic approaches, which either inhibit fibrogenesis or activate matrix degradation. Such approaches should be taken into consideration if there is no way of eliminating the hepatic noxa (67-69).

According to the pathophysiological concepts, reduced fibrogenesis can be induced by inhibition of fibrogenic mediators using neutralizing antibodies or cytokine-binding proteins; on the other side, neutralization of lipid peroxidation products may also be important. It has been demonstrated that the inhibition of the fibrogenic mediator TGF-β1 by neutralizing antibodies or the administration of hepatocyte growth factor decreased fibrogenesis (70,71).

Intending to bind free oxygen radicals, recent studies have successfully employed antioxidants such as vitamin E and polyenylphosphatidylcholine for therapy of fibrogenesis (72,73). In in vitro studies, the activation of HSCs in culture was inhibited by vitamin A treatment. Because vitamin A, however, is potentially toxic, its therapeutic impact may be not relevant.

During later stages of disease, pharmaceuticals should be employed that inhibit the ECM synthesis, change the macromolecule organization of the ECM and stimulate the degradation. So far, substances have been identified that inhibit collagen synthesis, as for example the prolylhydroxylase inhibitor HOE 077, the collagen type I synthase inhibitor halofuginone or pentoxifyllin, which also inhibits proliferation of HSCs (74,75).
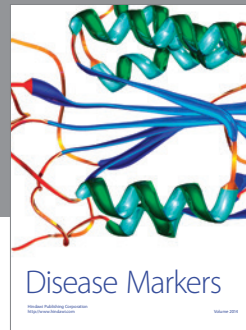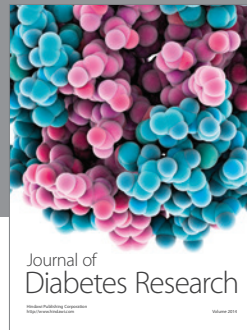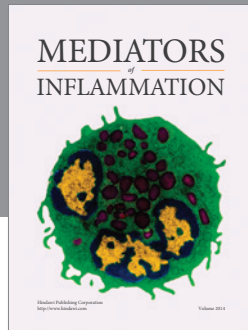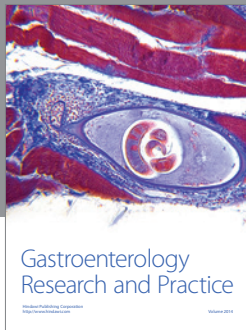
Taken together, only results from animal experiments are available, and the published data are often conflicting. Therefore the elimination of the hepatotoxins is so far the sole therapeutic concept available for the treatment of liver fibrogenesis in humans.

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