Hepatic stellate cells targeting – a specific approach to liver cirrhosis

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ABSTRACT

Cirrhosis is one of the chronic generalised disease and has a variety of clinical manifestations and complications some of which can be a life threatening. This results in decrease in hepatocellular mass and thus functions. It is 12th leading cause of death in United States. Hepatic stellate cells (HSC) plays a crucial role in the development of liver fibrosis because of their prominent role in extracellular matrix production, regulation of vascular tone, and production of inflammatory mediators such as transforming growth factor-b (TGF-b) and platelet-derived growth factor (PDGF). Therefore, these cells are major target for the treatment of Cirrhosis. Cell-specific delivery can provide a solution to these problems, but a specific drug carrier for HSC has not been described until now. The mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor, which is expressed in particular during fibrosis, may serve as a target-receptor for a potent carrier. Carrier molecules are designed for their selective cellular uptake, taking advantage of specific receptors or binding sites present on the surface membrane of the target cell.

Introduction

Liver Cirrhosis is the end stage of all the diseases like viral hepatitis, alcohol abuse, non-alcoholic steatohepatitis and other diseases [1]. In Liver Cirrhosis acute liver damage leads to chronic inflammation and fibrosis. Unfortunately there is no treatment currently available for liver fibrosis apart from organ transplantation but in that also donor organ shortage and high costs remain a serious problem. Hepatic failure after transplantation is still burdened by a high mortality rate. So finding a proper pharmacotherapeutic treatment for the liver fibrosis is very challenging.

Selective targeting of anti-fibrotic drugs to hepatic stellate cells (HSC) has recently been proposed which are identified as the key fibrogenic cell type in the progression of Cirrhosis[2, 3, 4].

With the help of targeted drug delivery system potent antifibrotic drug can be delivered intracellular within the diseased liver and even within the desired cell type. For this mannose-6-phosphate modified albumin (M6PHSA) has been proposed which binds with high affinity to the insulin-like growth factor II/mannose-6-phosphate receptor on activated HSC [5].

The current status of cirrhosis disease

Cirrhosis is one of the major disease affecting millions of people world-wide. Cirrhosis is a chronic liver disorder caused by a variety of diseases, with the most common being hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, and alcoholic liver disease. These disease attack the liver, leading to progressive liver damage and, ultimately, liver failure and death. For example, 1–46% of patients with chronic HCV infection will likely develop cirrhosis during a 30-year period. Cirrhosis, the twelfth leading cause of death in
the United States in 2007, represents a large economic burden, with the national cost for treatment in 2008 ranging from $14 million to $2 billion, depending on disease etiology. This has been estimated that the burden is expected to rise over the next 20 years, given that the percentage of patients with HCV-related cirrhosis is predicted to almost double. The overall cost of cirrhosis includes direct costs (drug and hospitalization costs) and indirect costs (due to loss of work productivity and reduction in health-related quality of life [HRQOL]).

In 2004, the direct costs of cirrhosis and chronic liver disease in the United States (excluding patients with HCV infection) were estimated to be $2.5 billion, whereas indirect costs were estimated to be $10.6 billion. Because cirrhosis is a progressive disorder, preventing or arresting its causes may substantially reduce the monetary burden of the disease. Given that liver transplantation entails a large economic outlay for relatively few individuals, the cost-effectiveness of the procedure, particularly in terms of the allocation of available livers and patient’s HRQOL post-transplantation, may be questionable [6].

**Functional classification of cirrhosis**

On the basis of their anatomy and pathogenesis Cirrhosis can be divided into following categories:

- Alcohol Cirrhosis.
- Cirrhosis following viral hepatitis.
- Chemically (drug) induced Cirrhosis.
- Obstructive biliary Cirrhosis.
- Congestive Cirrhosis.
- Neural hepatic Cirrhosis.
- Cirrhosis from iron overload (Hemochromatosis).
- Cirrhosis on hereditary basis.
- Multifactorial Cirrhosis.
- Cirrhosis on unknown etiology.

**Hepatic stellate cells**

**Ultrastructure and retinoid storage**

Hepatic stellate cells are located in the sub endothelial space, between the basolateral surface of hepatocytes and the antiluminal side of sinusoidal endothelial cells. They comprise approximately one-third of the nonparenchymal cell population and 15% of the total number of resident cells in normal liver. Stellate cells in normal liver have spindle-shaped cell bodies with oval or elongated nuclei. Ultra structurally, there have moderately developed rough endoplasmic reticulum (rER), juxtanuclear small Golgi complex, and prominent dendritic cytoplasmic processes. The sub endothelial processes wrap around sinusoids between endothelial cells and hepatocytes. On each of these processes, there are numerous thorny micro projections (spines).

The function of these projections had been obscure until a recent, elegant study has demonstrated that these protrusions serve a vital role as the cell’s leading edge in “sensing” chemotactic signals, and then transmitting them to the cell’s mechanical apparatus to generate a contractile force. A single stellate cell usually surrounds more than two nearby sinusoids. On the other side of the cell (i.e. the anti-luminal surface), multiple processes extend across the space of Disse to make contact with hepatocytes. This intimate contact between stellate cells and their neighbouring cell types may facilitate intercellular transport of soluble mediators and cytokines. In addition, stellate cells are directly adjacent to nerve endings which are consistent with reports identifying neurotrophin receptors and with functional studies confirming neurohumoral responsiveness of stellate cells.

**Retinoid Storage**

The most characteristic feature of stellate cells in normal liver is their cytoplasmic storage of vitamin (retinoid) droplets. The number of droplets varies with the species and the abundance of vitamin A stores of the organism. During liver injury, the fine structure of stellate cells changes considerably. They lose their characteristic droplets and become “activated”. The rER becomes enlarged, accompanied with a well-developed Golgi apparatus, suggesting active protein synthesis. Bundles of numerous microfilaments appear beneath the cell membrane. The activated stellate cells then evolve into myofibroblast-like cells with newly formed collagen fibrils surrounding them.

**Fibrogenic cells of the liver**

Activated hepatic stellate cells show de novo fibrogenic properties, including proliferation and accumulation in areas of parenchymal cell necrosis, secretion of proinflammatory cytokines and chemokines, and synthesis of a large panel of matrix proteins and of inhibitors of matrix degradation, leading to progressive scar formation (Figure 1).
Hepatic myofibroblasts are another source of fibrogenic cells that derive from fibroblasts of the portal connective tissue, perivascular fibroblasts of portal and central veins, and periductular fibroblasts in close contact with bile duct epithelial cells. Contribution of these cells to fibrogenesis was initially demonstrated in experimental biliary cirrhosis by showing that myofibroblastic transformation of portal and periductular fibroblasts precedes activation of hepatic stellate cells in the lobule. Phenotypic and functional properties of hepatic myofibroblasts are grossly similar overall to those of activated hepatic stellate cells. However, culture studies have clearly established that several phenotypic markers distinguish both cell types, including selective expression of fibulin-2 and interleukin-6 by hepatic myofibroblasts and protease P100 and reelin by activated hepatic stellate cells. Cell-specific expression of these markers has also been described in experimental models and suggests that hepatic myofibroblasts derived from portal (myo) fibroblasts are present within fibrotic septa, whereas activated hepatic stellate cells are found in the subendothelial sinusoidal space close to portal tracts.

Regarding biological functions, activated hepatic stellate cells show minor functional differences with hepatic myofibroblasts, such as a short life span owing to rapid apoptosis and low proliferative capacity. Further work is needed to fully delineate the precise contribution of each cell type to the fibrogenic process, and characterization of the fibrogenic cell lineage may provide useful information. In this respect, recent studies indicate that as yet undefined bone marrow cells constitute a significant source of hepatic stellate cells. In addition, bone marrow myofibroblasts represent a significant proportion of hepatic myofibroblasts incirrhosis of diverse etiologies [30].

**Functions of hepatic stellate cells in normal liver**

- Role in liver development and regeneration
- Retinoid metabolism
- Immunoregulation
- Secretion of lipoproteins, growth factors, and cytokines
- Biology of membrane and nuclear receptors
- Adipogenic features
- Detoxifying and antioxidant enzymes, pH regulation, and generation of oxidant stress
- Transcriptome and proteome analyses [7].
M6PHSA as a soluble carrier protein

Albumin is the most abundant plasma protein, and has a biological half-life of 19 days. It consists of a single chain of 585 amino acids organized in a three-dimensional structure in a helical conformation. The helices are bound by 17 disulfide bridges, leaving only one free thiol (Cys34) [8]. Albumin is biodegradable and therefore biocompatible and contains many different functional groups, i.e. -NH₂ of the lysine residues or methionine, which can be used for conjugation of the homing device, the linker, or the drug. In addition, due to its size and charge, it is not cleared from the blood by renal filtration.

In our strategy, albumin was modified with sugar mannose-6-phosphate groups on its surface resulting in M6PHSA. M6PHSA has been shown to specifically interact with mannose 6-phosphate/insulin-like growth factor II (M6P/IGFII) receptors expressed on the surface of hepatic stellate cells. Due to stellate cell proliferation during liver fibrosis and a concomitant increase in M6P/IGF II receptor expression on this cell type [9], the disease process itself may selectively direct the carriers to the diseased tissue.

This targeting strategy may largely contribute to the increased therapeutic concentration of drug in the target tissue.

HSC targeting materials

In HSC-selective targeting strategies, the receptor expression for some growth factors on the cell surface is drastically upregulated. Examples of receptors that are upregulated on activated cells are the platelet-derived growth factor (PDGF) receptors, the mannose-6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor and many receptors that allow HSCs to interact with the surrounding ECM. Thus, HSCs form an attractive cellular target for the treatment of hepatic fibrogenesis. In several recent studies, some modified albumins have been used to target drugs to HSCs: losartan, mycophenolic acid, DOX, 15d-prostaglandin J₂, giotioxin, the viral vector HVJ, pentoxifylline, IL-10 and a kinase inhibitor. Most of these constructs displayed antifibrotic effects in vitro. Losartan-M6PHSA was synthesized by a method in which M6PHSA was prepared as follows: HSA was modified in vivo with mannose-6-phosphate groups. Briefly, p-nitro phenyl-a-Dmannopyranoside was phosphorylated and after reduction of the nitro group it was coupled with HAS [10]. In other method prepared M6P-modified albumin, which was purified using an Amicon Stirred Cell (Amicon, Danvers, MA, USA) followed by Sephadex G-25 gel chromatography (Pharmacia, Uppsala, Sweden) [11].

The results demonstrated that animals receiving losartan-M6PHSA showed losartan levels that corresponded to 62% of the last injected dose, which was at least 20% of the cumulative dose, while oral losartan yielded liver tissue levels corresponding to only 4% of the cumulative dose (15% of the last dose administered). These results illustrate the preferential hepatic accumulation of losartan-M6PHSA [13].

Isolation of hepatic stellate cells

Here firstly the livers of male Wistar rats (450–550 g) were perfused with Gey’s balanced salt solution (GBSS) containing collagenase P (Roche Molecular Biochemicals, Mannheim, Germany), Pronase (Merck, Darmstadt, Germany), and DNase (Roche Molecular Biochemicals). The HSC were separated from the other hepatic cells by density gradient centrifugation and collected at the top of an 11% Nycodenz solution (Nyegaard, Oslo, Norway). The cells were then cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc., Paisley, Scotland) containing 10% foetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. After 2 days, cell debris and nonadherent cells were removed by washing and the medium was changed every 2 or 3 days thereafter. Cells cultured for 2 days after isolation represented quiescent HSC, whereas those cultured for 10 days after isolation represented activated HSC [13].

Drug targeting to hepatic stellate cells (hsc)

HSC, which comprise about 5–10% of the total amount of liver cells, play a pivotal role in cirrhosis. Factors generated by the damaged hepatocytes and activated nonparenchymal cells subsequently induce activation, transformation and proliferation of fibroblast-like cells (hepatic stellate cells and portal fibroblasts) and these cells in turn produce excessive amounts of scar tissue (extracellular matrix proteins like collagen types I and III) forming a fibrous scar. Eventually this leads to portal hypertension due to the increased intrahepatic resistance. This increased portal pressure represents one of the major clinical problems because it leads to variceal bleeding, one of the most life threatening complications in cirrhotic patients. A complicating factor in the management of high portal blood pressure is the reduced mean arterial blood pressure in these patients. Treatment of the high pressure in the portal vein without affecting the systemic blood pressure represents a major challenge and requires cell-selective approaches. Selective delivery of Nitric oxide donor prodrugs to the liver and intrahepatic delivery of genes encoding for NO synthesizing enzymes have been explored but so far without clinical success. Although liver-specific, these delivery systems were not specifically directed at the cells responsible for the increased portal pressure. The use of cell-specific drug carriers to HSC, whose contractile characteristics significantly contribute to the increased intrahepatic resistance and hence portal pressure, may open new therapeutic options in this area. However, this cell type is not easily accessible for drugs, and for this reason many drugs that showed antifibrotic activities in vitro were ineffective in vivo. To optimize the therapeutic success of potential antifibrotic drugs, targeting to hepatic stellate cells has been explored [14] (Figure 2).
Binding sites expressed on (activated) HSC were considered for their ability to serve as potential targets for carrier molecules. One of these is the mannose 6-phosphate/insulin like growth factor II (M6P/IGF-II) receptor, whose expression is increased on activated rat HSC, particularly during fibrosis. About 10-20% of the receptor is expressed at the cell surface, and the receptor has binding sites for IGF-II and M6P-containing ligands such as latent transforming growth factor β (L-TGFβ), proliferin, and lysosomal enzymes. This cation-independent type of M6P receptor plays a role in the lysosomal enzyme targeting and the regulation of cell growth. In addition, a soluble form of the M6P/IGF-II receptor is found in rat and human serum. The main source of this soluble receptor in adult rats appears to be activated HSC [15].

**Modified albumin-based carriers**

The first carrier that accumulated in HSC was reported in 1999 which was recognized as a fundamental new approach and since then several other drug carriers to this cell type have been developed. Cell-specificity for activated HSC in fibrotic livers was obtained by using albumin-based carriers that bind to receptors which are highly up regulated on activated HSC:

1. mannose-6-phosphate/insulin-like growth factor II receptor
2. collagen type VI receptor
3. platelet derived growth factor β (PDGF-β) receptor.

To reach these receptors, albumin molecules were substituted with mannose-6-phosphate (M6P) or with peptides that recognized either the PDGF-receptor or the collagen type VI receptor. For all these carriers, extensive hepatic accumulation was found in experimental models of fibrosis in animals (approx. 70% of the dose accumulated in fibrotic livers) and the main target cells responsible for this accumulation were identified as activated HSC, although some uptake in other liver cells was also found depending on the carrier used. This was confirmed in human tissue. Since for at least two of the carriers receptor-mediated endocytosis was demonstrated, these carriers open the opportunity to deliver anti-fibrotic compounds to the key players in this disease. In the past years, various antifibrotic compounds have been targeted to HSC ranging from drugs that interfere either with proliferation, apoptosis or activation of HSC or with matrix deposition or oligonucleotides. An angiotensin II inhibitor and several kinase inhibitors, affecting key signalling pathways in HSC, like PDGF-, and Rho-kinase activated pathways were successfully delivered to these cells. In all of these studies, a significant reduction of crucial profibrotic parameters was seen in experimental animal models. The albumin-based HSC-specific drug carrier M6P-HSA has been used to deliver losartan and the Rho-kinase inhibitor Y27632 to HSC in several animal models of fibrosis. Mannose6-phosphate residues have also been applied for the delivery of siRNA to HSC, although this has only been demonstrated in vitro. In vivo delivery of siRNA to HSC has been demonstrated in a study of using liposomes substituted with vitamin A.

In this study significant antifibrotic effects were found in an animal model of fibrosis in rats using siRNA blocking heat shock protein 47 expression. In normal livers, quiescent HSC store vitamin A and these authors used vitamin A as a homing device to deliver liposomes to activated HSC in fibrotic tissue. This is the first study showing effective delivery of siRNA in vivo and it may represent an important step in siRNA-based therapies although further confirmation of this study is still awaited [17].

**Specific delivery of therapeutic cytokines to HSC**

The targeting to HSC via the M6P homing device was also used to deliver a cytokine to HSC. Interleukin-10, a cytokine endowed with anti-inflammatory and anti-fibrotic activities was successfully delivered to HSC by coupling M6P-residues to it. This re-direction of cytokines opens many new opportunities for the clinical application of these powerful mediators. The most recent and important illustration is the
use of Interferon γ (IFNγ) for antifibrotic purposes. IFNγ is a cytokine with potent antifibrotic activities but its clinical use is limited due to its many adverse effects induced by the uptake of this cytokine in nearly all cells of the body. Efficacy is also limited by the short half-life of IFNγ which could not be overcome by dose increments because of the adverse effects. By coupling of PDGF-β receptor binding peptides to this cytokine, accumulation of IFNγ in HSC was achieved and its antifibrotic effects were strongly enhanced while adverse effects were completely abolished.

Other approaches to target HSC

In this approach M6P is combined with a RGD homing device. This RGD sequence is also present in the collagen type VI receptor recognizing peptide. Studies with HSC in cultures proved that M6PRGD constructs were more effective in inhibiting fibrotic activities in HSC than RGD or M6P alone. Further studies in vivo must prove whether this RGD-M6P can be applied as a cell-selective drug, targeting selectively the HSC [18]. In addition to these protein-based carriers substituted with sugars or peptides, targeting to HSC was also achieved using antibodies, liposomes and viruses as carrier. In another study a human monoclonal antibody fragment is developed with affinity for synaptophysin, a protein expressed on the surface of HSC [19]. This was conjugated with a toxin to achieve cell-selective killing in HSC. This is the first report on the use of antibodies as a cell-specific drug carrier to resident hepatic cells. The liposomal research area also benefits from the new drug carriers to HSC. To ensure specific accumulation of liposomes within HSC in the fibrotic liver, in an experiment M6P-albumin is attached to liposomes [20-23], and in one liposomes substituted with the collagen type VI receptor recognizing peptide were used [24]. Efficacy studies in animal models showed an enhanced effect of hepatocyte growth factor on liver fibrogenesis when this growth factor was delivered into HSC using RGD-based liposomes. Another lipid that was reported to accumulate in HSC was cholesterol. Oligonucleotides conjugated with cholesterol induced increased hepatic accumulation after systemic administration and HSC were found to be the major site of uptake (35% of the dose). Although all HSC-selective carriers display some uptake in other non-parenchymal cells (extends and cell types varies with the carrier), much progress has been made in the last decade and this field is rapidly progressing to a situation where cell-selective drug targeting in vivo is a feasible option for therapy.

Gene delivery to HSC

Not only drugs but also genes are targeted to HSC. In most of the studies on gene delivery to HSC, adeno viral vectors are used. However, adenoviruses predominantly transduce hepatocytes due to their CAR expression. Internalization of adeno viral particles is additionally promoted by integrin present on cell membranes. HSC and myofibroblasts express high levels of various types of integrin, which may account for the adeno viral-mediated transfections of HSC. [25, 26]. Other examples of adeno viral gene delivery as an experimental approach to treat liver fibrosis are the hepatic delivery of telomerase RNA by of PPAR-gamma, interference with TGFβ activities, and MMP gene delivery [27]. However, to achieve specific expression of genes into HSC using adeno viral vectors, virus re-targeting strategies need to be developed.

Retargeting can be achieved at three levels:

1. adenoviruses can be conjugated with re-targeting moieties to induce binding to another receptor and avoid binding to the CAR receptor
2. expression of adeno viral transgenes can be limited to target tissue by the introduction of cell-specific promoters, or
3. the transgene product can be secreted locally and thereby delivered to neighbouring cells in the diseased area [28].

An example of the first is reported in which adenovirus is re-directed to PDGF-β receptor using PDGF-receptor binding peptides. An example of the second retargeting strategy was there in which specificity of adeno viral transfection is improved by using a promoter in the viral construct which is only present in activated HSC? The promoters tested were CSRP2 (a gene encoding the LIM domain protein CRP2), SM22alpha (smooth muscle-specific gene encoding a 22-kDa protein), and TIMP-1 (tissue inhibitor of metalloproteinase-1) [29]. It was demonstrated that all of these promoters were effective in producing a strong or partially selective expression in HSC in vivo. However, none of these promoters was able to create a specific or inducible expression of transgenes in these cells HSC in vivo. To date, mostly adeno viral vectors are applied to achieve transfection in HSC, and in these cases the HSC is just one of the cells that take up the vector. In general, the field of HSC targeting has seen much progress in recent years and most progress has been made with new protein-based drug carriers [12].

Therapeutic approaches to inhibit HSC activation

Antioxidants

Acetaldehyde generated from alcohol metabolism is responsible for both hepatocellular injury and HSC activation in alcoholic liver disease. Other ROS generated in patients with alcoholic liver disease (ALD) include H2O2 and hydroxyl ethyl radicals. These radicals are responsible for the apoptosis of hepatocytes exposed to ethanol in vitro. Adducts of these reactive metabolites and free radicals are immunogenic and may be the auto antigens that participate in the pathogenesis of chronic ALD. Therefore, antioxidative agents appear to be a good choice for preventing the hepatocellular injury and for attenuating the fibro genesis in ALD. Other conditions in which antioxidative agents are useful include drug-induced chronic liver injury and injury induced by hepatotoxins, such as acetaminophen.
Silymarin has been shown to be beneficial in inhibiting liver injury and in lessening the accumulation of hepatic collagen in advanced biliary fibrosis. However, a double-blind clinical trial showed that treatment of alcoholics with silymarin at a daily dose of 450 mg for 2 years did not improve the survival of the patients with advanced cirrhosis when compared with a placebo, and the treatment did not change the course of the disease. Nonetheless, it is argued that, whereas improvement of liver functions and reduction of ECM deposition in an early stage of cirrhosis or fibrosis may not necessarily change the survival rate, the evaluation of other parameters may indicate the clinical efficacy of the treatment in alcoholics.

*S-adenosyl-L-methionine (SAMe)* is a substrate of glutathione (GSH) synthesis and a donor of methyl groups which are involved in the metabolism of several amino acids in the body. It is thought that SAMe is crucial for the maintenance of normal biochemical functions in the nervous system and in the liver. It has been seen that SAMe protected isolated rat hepatocytes from the toxicity of bromobenzene and d-galactosamine and replenished glutathione levels in liver. It has been seen that SAMe protected isolate d rat normal biochemical functions in the nervous system and in the It is thought that SAMe is crucial for the maintena nce of involved in the metabolism of several amino acids i n the body.

SAMe attenuated hepatic fibrosis induced in vivo by CCl₄ toxicity or bile duct ligation, and that SAMe was also useful in alcohol-induced fibrosis in baboons. Although the role of glutathione in HSC activation is not well defined, SAMe treatment will increase GSH levels in hepatocytes, which are often depleted in patients with ALD. The treatment probably will affect GSH levels in HSC too. Clinical trials have demonstrated that SAMe may significantly improve liver function test results (serum bilirubin levels, alkaline phosphatase, γ-glutamyltransferase) and alleviate symptoms (pruritus, fatigue, etc.) in patients with cholestasis, as primary biliary cirrhosis, drug-induced liver injury with cholestasis, or recurrent intrahepatic cholestasis of pregnancy. A large randomized, placebo-controlled, double-blind clinical trial demonstrated that SAMe treatment in alcoholic cirrhosis increased the patient survival rate and delayed liver transplantation, and that no significant adverse effects occurred during long-term treatment.

**Pentoxifylline** an analogue of the methyl xanthine theobromine, is clinically useful for the treatment of conditions involving defective regional microcirculation. It was employed to inhibit the transition of HSC to myofibroblast-like cells (activated HSC) and to inhibit the proliferation of the cells *in vitro*. A study showed that pentoxifylline inhibited LPS-stimulated TNF-α production in monocytes, nuclear factor-kB (NF-kB) activation in HSC *in vitro*, and activated HSC *in vivo* (isolated from CCl₄-treated rats).

It also reduced the acute liver toxicity of CCl₄. Because NF-kB activity is induced by ROS, the inhibitory effect of pentoxifylline on HSC isolated from CCl₄-treated rats is the inhibition of phosphodiesterase. It was recently reported that pentoxifylline abrogated HSC proliferation, measured by proliferating cell nuclear antigen (PCNA) staining, and activation (expression of smooth muscle α1-actin) in animal models of hepatic fibrosis induced by CCl₄ intoxication and bile duct ligation. Pentoxifylline also inhibited HSC proliferation and activation *in vitro*, which implies a direct effect on the cells.

**Polyenylphosphatidylcholine (PPC)** is a mixture of polyunsaturated phosphatidylcholine (PC) rich in dilinoleoyl PC. PPC not only corrected ethanol-induced phosphatidylcholine depletion but also prevented septal fibrosis and cirrhosis in baboons fed adequate diets. PPC treatment also led to a reduced number of HSC in patients with alcoholic disease. *In-vitro* experiments showed that PPC attenuated the transformation of stellate cells to myofibroblast-like cells, and inhibited PDGF-induced proliferation in rat HSC. PPC also acts on Kupffer cells by reducing LPS-stimulated TNF-α release and by enhancing interleukin-1β (IL-1β) secretion, which exerts an opposing action on TNF-α effect. It has been shown recently that PPC is an antioxidant that abrogated arachidonate-induced lipid peroxidation in a tumour cell line by 50%. Thus, PPC seems to be a potent antifibrotic agent, who appears to be beneficial for patients with alcoholic liver disease; a large clinical trial is in progress TGF-α and its antagonists. The critical roles of TGF-α in hepatic fibrosis have been established by many *in vivo* and *in vitro* investigations. Any approaches that reduce TGF-α secretion, neutralize its activity by specific antibodies or an antagonist (relaxin), block its conversion from the latent to active form, interrupt its affinity or reaction with the receptor, or inhibit its signal transduction, will minimize its biological effects.

**Blocking cell-matrix interaction**

Integrins are a group of protein molecules on the cell surface that play a significant role in the mediation of ECM and HSC interaction and in the contraction of connective tissue (α1β1 integrin). Integrins serve as a bridge or “receptor” for cell interaction with the ECM through the adhesion activation of focal adhesion kinase (FAK), by increasing the levels of tyrosine phosphorylation in the cells. A recent study showed that P21 Ras, a member of a family of proteins that induce cell proliferation, cytoskeleton organization, and cell motility, operates as a protein-linking PDGF receptor to FAK in human HSC. Using soluble Arg-Gly-Asp peptides diminished the adhesion-induced tyrosine phosphorylation of FAK and inhibited HSC activation in vitro, as well as in a rat model of hepatic fibrosis induced by thioacetamide.

**Herbal medicine**

Chinese herbal recipes display unique features in the treatment of acute and chronic liver injury. Varieties of recipes or herbal extracts, such as Xiao Caihu Tang Sho-aiko-to in Japanese), Recipe 861, glycyrrhizin, or silymarin (milk thistle), have been shown to be effective in the prevention and treatment of
Liver injury and fibrosis. Some of them were even found to be able to “reverse” the fibrotic liver to a nearly normal histology in patients with hepatitis B viral infection. Thus, the use of herbal medicines is providing a new approach in the treatment of liver disease. Recipe 861, which consists of ten Chinese herbs, has been shown to reduce mRNA expression of procollagens I, III, V, and TGF-β in the liver of animal models and in cultured HSC, to increase the production of interstitial collagenases (MMP1), and to suppress TIMP1 synthesis in HSC. Liver biopsies showed that the fibrosis was reduced or had completely disappeared in three-quarters of patients with hepatitis B after 6 months of treatment with Recipe 861. In a separate study; it was shown that Recipe 861 inhibited HSC proliferation induced by PDGF stimulation. Dietary supplementation with Xiao Caihu Tang (TJ-9) prevented dimethyl nitrosamine- or pig-serum induced hepatic fibrosis in rats. TJ-9 supplementation increased liver retinoid levels, and supplementation with Xiao Caihu Tang (TJ-9) prevented proliferation induced by PDGF stimulation. Dietary supplementation with Xiao Caihu Tang (TJ-9) prevented dimethyl nitrosamine- or pig-serum induced hepatic fibrosis in rats. TJ-9 supplementation increased liver retinoid levels, and decreased total collagen content and the number of smooth muscle α-actin-positive cells. In vitro experiments show that TJ-9 is a strong antioxidative agent that inhibited both lipid peroxidation in hepatocytes and oxidative stress induced HSC activation. Furthermore, TJ-9 also displays radical scavenger activity. The main components in TJ-9 that exert antioxidative effects are baicalin and baicalein. Extensive clinical use suggested that TJ-9 prevented the development of hepatocellular carcinoma in patients with hepatitis C virus infection. We have previously reported that glycyrrhizin, an extract of liquorice, abrogated ethanol plus CCl₄-induced hepatic fibrogenesis in rats. It reduced liver injury and NF-κB binding activity, as well as the degree of the liver fibrosis.

Conflict of interest statement
We declare that we have no conflict of interest.

References


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