Abstract

Introduction
Hepatic fibrosis (HF) represents a scarring response to either acute or chronic cellular injury. Following acute liver injury, parenchymal cells regenerate to successfully preserve hepatocellular mass and function. This acute process is associated with an inflammatory and fibrogenic response but limited deposition of extracellular matrix (ECM). In contrast, prolonged liver injury leads to sustained production of growth factors, proteolytic enzymes, angiogenic factors and fibrogenic cytokines. These events culminate in the accumulation of ECM, forming septa that coalesce into broad bands of scar tissue that encircle nodules of hepatocytes and lead to altered microvascular structure. This late stage of fibrosis, termed cirrhosis, ultimately impairs liver function and leads to portal hypertension and its complications. Participation of various cell types, interlinked cellular events and large number of mediator molecules make the fibrotic process enormously complex and dynamic.

For decades, HF was seen as an irreversible disease that progresses to cirrhosis, with a greater risk for developing hepatocellular carcinoma (HCC) and liver failure. This meant the only potential treatment for HF once it had progressed to cirrhosis was transplantation. Research over the past 30 years has yielded increasing insight into the cellular and molecular mechanisms of this disease, uncovering an orchestrated pathophysiology and identifying the hepatic stellate cell (HSC) as the central cell type in fibrogenesis. Most importantly, this revealed the potential reversibility of this disease and the discovery of potential therapeutic targets.

Conclusion
In a prospective study, many traditional Chinese medicines were found to play a chemopreventive role in the development of hepatic fibrosis. Several laboratories, including ours, have clearly demonstrated the preventive and therapeutic effects of some Chinese herbal medicines on experimental hepatic fibrosis, which may provide valuable information on the search for novel antifibrogenic agents.

Antihepatic fibrosis effects of traditional Chinese medicine: A review

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HF research has gained global attention because of its close relationship with disease progression triggered by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, which leads to end-stage liver cirrhosis and ultimately to HCC. Currently, nearly 3 billion and 180 million people have been exposed to HBV and HCV, respectively. Epidemiological studies have shown that in China, HBV infection is the major cause of HF, whereas in the United States, Europe and Japan, HCV infection, alcohol abuse and non-alcoholic steatohepatitis (NASH) are the main causes. In the sub-Saharan African region, Schistosoma mansoni infection is reported to be the major cause of HF, resulting in almost 0.3 million deaths annually. In spite of the high incidence of HF worldwide, no generally accepted antifibrogenic therapy is available. Moreover, the research and development of new drugs with anti-HF activity has been an international puzzle.

In recent years, considerable achievements have been made in China in research of the aetiopathogenesis, diagnosis and especially the treatment of HF. Based on the clinical practice of traditional Chinese medicine (TCM), some effective TCM formulae have been developed and used for the treatment of HF. However, the components in these formulae are complicated. The anti-HF drugs with distinct components, significant effects and definite pharmacological mechanisms but without side or toxic effects are still lacking. Thus, it is a significant trend to find out active anti-HF components in TCM, which may be a consider-

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Molecular mechanisms of hepatic fibrogenesis

HF is an outcome of many chronic liver diseases, such as chronic infection by HBV, HCV and parasite Schistosoma; chronic alcoholism or exposure to certain drugs and toxins; infections; NASH and inherited metabolic diseases like haemochromatosis, Wilson’s disease, α-1 antitrypsin deficiency, autoimmune diseases such as primary biliary cirrhosis, ischaemia and autoimmune hepatitis. Pathogenic mechanism research of HF is now focused on the following respects (Figure 1): mechanisms of HSC activation, inflammatory signalling, peroxidation mechanism, cytokine network and cell apoptosis, as examined briefly in the following sections.

Activation of HSC

Many factors contribute to HSC activation. Hepatocellular damage is the primary and continuing factor leading to HSC activation. Membrane components released from the damaged hepatocytes, such as lipid peroxides from apoptotic cells, intermediate metabolites of drugs or hepatotoxins, infiltrating inflammatory cells and acetaldehyde from alcohol metabolism, as well as ROS (hydrogen peroxide, superoxide radicals), are the strong activators of Kupffer cells. The activated Kupffer cell population is expanded and the cells release a number of soluble agents, including cytokines, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), tumour necrosis factor-α (TNF-α) and other factors, which are crucial for HSC (fat-storing cells or Ito cells) activation. TGF-β not only induces activation of HSC, enhances expression of PDGF receptors on the cells and enhances the autocrine expression of TGF-β by the cells but also prolongs the survival of activated HSC by reducing their apoptosis, which is opposite to its effect on epithelial cells, such as hepatocytes. PDGF is the most potent cytokine that induces proliferation of HSC in vitro, and probably it plays a major role in the activation and proliferation of HSC in vivo. The activated HSCs express more PDGF receptors and release PDGF too. The PDGF and TGF-β secreted by HSCs create an autocrine loop, which is an important mechanism for perpetuating HSC activation. Moreover, liver injury induces cross-talk between hepatocytes, Kupffer cells, inflammatory cells and HSC, which greatly contribute to liver fibrogenesis. In addition, changes in the microenvironment of the HSC also contribute to their activation. In the normal situation, HSC are quiescent and produce small amounts of ECM components, such as laminin and collagen type IV for the formation of basement membrane. When they are exposed to soluble factors from damaged hepatocytes and from activated Kupffer cells, HSCs will lose their lipid content (retinyl palmitate), undergo upregulation of cytoskeletal protein expression such as α-smooth muscle actin (α-SMA) and desmin and undergo morphological transition to myofibroblast-like cells. Furthermore, activated HSCs, in turn, upregulate adhesion molecules and secrete proinflammatory cytokines (IL-6, IL-8 and monocyte chemoattractant protein-1), further accelerating recruitment of inflammatory cells into the injured liver. On the other hand, activated HSCs change their secretion profile of ECM components, releasing more interstitial collagens instead of components of the basement membranes. Accumulated interstitial collagens are regulatory factors for the prolonged production of ECM by HSCs and for other behaviours of the cells. The importance of the ECM as an active, signalling component of fibrogenesis is well accepted now.

Figure 1: Pathogenic mechanism of hepatic fibrogenesis.
Inflammatory signalling
HSCs, which are reviewed above, have emerged as central modulators of hepatic inflammation and immunity, and not just passive targets of inflammatory cytokines. In particular, a growing list of chemokines and their cognate receptors serve the dual function of provoking further fibrogenesis and interacting with inflammatory cells to modulate the immune response during injury. \textsuperscript{12,15,20}

Peroxidation mechanism
Chronic hepatic damage by inflammation, toxins, immunity and malnutrition can activate HSCs. During liver inflammation, ROS, including reactive intermediate metabolites (acetaldehyde) and free radicals, such as $\text{H}_2\text{O}_2$, O$_2^-$ or nitric oxide (NO), can be derived from infiltrating neutrophils, activated Kupffer cells and damaged hepatocytes.\textsuperscript{21} Substantial evidence has demonstrated that these ROS are active inducers of HSC activation \textit{in vitro}\textsuperscript{22} and may also play a pivotal role \textit{in vivo}\textsuperscript{23}. Antioxidants, such as vitamin E, S-adenosyl-l-methionine (SAMe) and polyenylphosphatidylcholine, display protective effects for the liver from injury induced by hepatotoxins, as well as block the progression of the fibrogenesis\textsuperscript{12,14,24}. Recently, the study of De Bleser et al. showed that treatment of HSC with TGF-β induced the production of $\text{H}_2\text{O}_2$ in the cells, whereas treatment of the cells with catalase, a scavenger of $\text{H}_2\text{O}_2$, decreased the production of TGF-β by the cells.\textsuperscript{25} Thus, antioxidants are useful in both the prevention of liver injury and the attenuation of fibrogenesis. Furthermore, many agents such as silymarin, salvianolic acid B (Sal-B), colchicine and Chinese herbal recipes (Xiao Chaihu Tang, Recipe 861) exhibit their antifibrotic effects via this mechanism.

Cytokine network
Activated Kupffer cells release a variety of cytokines closely related to HF such as TGF-α and -β, PDGF and TNF-α. In addition, Kupffer cells, which can be regarded as important ‘coefficients’, help maintain the kinetic equilibrium of HF and mediate the feedback mechanism of some biological messages during the progression of HF. HSCs have both paracrine and autocrine functions, the activation of which can be triggered via a reaction cascade of cytokines and biochemical factors.\textsuperscript{26,27} Two major changes occur after the activation of HSCs, that is, proliferation and phenotypic transition.\textsuperscript{28} As reviewed in the activation of HSCs, PDGF and TGF-β play an important role in the proliferation and transformation of HSCs.\textsuperscript{29} The key role of PDGF, a most effective mitogen during the transformation of HSC DNA, is to convert HSC from G0 to G1 and S phases, whereas TGF-β promotes the synthesis of collagen and tissue inhibitors of matrix metalloproteinases (TIMPs) in activated HSCs.\textsuperscript{31} Moreover, PDGF and TGF-β can interact with each other. PDGF is capable of inducing HSCs to express and secrete PDGF receptors. PDGF and TGF-β can also interact with TNF-α and PDI, leading to the formation of cytokine network with HSCs at the crucial centre.

Activated HSC apoptosis
Recently, activated HSC apoptosis is regarded as a key factor in regression of HF. Activated HSCs are more susceptible to apoptosis and can undergo spontaneous cell death or death receptor-mediated cell death caused by serum deprivation or cytokine signalling. In response to reduced fibrogenic signals or antiviral drug therapy, HSCs increase the expression of Fas receptor or TNF receptor 1 and their ligands and undergo a caspase 8/caspase 3-dependent apoptosis. Alternatively, overexpression of proapoptotic proteins such as p53, Bax and Bcl-2 leads to caspase-9-mediated programmed cell death.\textsuperscript{33} Furthermore, senescence of HSC may be an important mechanism to slow proliferation and favour the regeneration of liver parenchymal cells. Senescence of HSCs depends on the length of telomeres, a non-coding region at the end of chromosomes, which shortens with each division and, once critical length is reached, prevents HSC from further cellular division.\textsuperscript{34}

In addition, activated HSCs produce TIMP, which inhibits collagen-degrading matrix metalloproteinases (MMPs) and shifts the balance between ECM synthesis and degradation towards ECM synthesis, which is considered as the primary mechanism of fibrosis resolution. Therefore, apoptosis of HSC eliminates not only the major source of collagen but also the major source of TIMP-1 and leads to increased activity of MMP interstitial collagenases and subsequent degradation of ECM.\textsuperscript{35,36}

Therapeutic targets of traditional Chinese medicine on HF
TCM has recently attracted the attention of practitioners of western medicine. The principles underlying TCM were established over thousands of years and are based on clinical experience and practice. So far, no satisfactory treatment protocol with western drugs is available for HF because of their severe side effects. However, with the research of the TCM in recent years, we have obtained promising results, and a number of Chinese herbs have been found to reverse the progression of HF without obvious side effects. Chinese herbal recipes display unique features in the treatment of acute and chronic liver injury. Studies have shown that TCM can inhibit the deposition of collagen fibres and promote the reversion of fibrosis, which was confirmed by experimental evidence that Sal-B and salvianolic acid A (Sal-A) extracted from Radix Salviae Miltiorrhizae, matrine (M) and oxytartine (OM) from Seed Sophorae Alopecuroidei or Radix Sophorae Flavescentis, Radix Angelicae Sinensis, Radix Astragalusi seu Hedysari, Radix Paeoniae...
Rbbr, Semen Persicae, Hirudo, Flos Carthami, Radix Notoginseng, Rhizoma Sparganii and Rhizoma Zedoariae could obviously inhibit the formation of collagen fibres. In addition, varieties of recipes or herbal extracts, such as Xiao Chaihu decoction, Recipe 861, Qianggan capsule, Fuzhenghuayu 319, Dahuangzhechong pill, Yigan infusion, Ginkgo biloba extract, glycyrrhizin from Radix Glycyrrhiza, silymarin from Fructus Silybimariani (milk thistle), Desmodium Pulchellum total alkaloid from Desmodium Pulchellum, taurine from Calculus Bovis, cordyceps polysaccharides from Cordyceps or Cordyceps Sinesis Mycelium, tetrandrine from Radix Stephaniae Tetrandrae, gypenosides from Gynostemma pentaphyllum, amygdalin from Prunus persica, ligustrazine from Rhizaome Chuaxiong and Panax notoginsenosides from Radix Notoginseng, 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 HBV infection48, and no serious side effects were observed. Thus, the use of TCM suggests a bright future and a new approach in the treatment of liver disease (Figure 2).

The Chinese herbal medicine Xiao Chaihu decoction (TJ-9) is an officially approved prescription drug in Japan and is presently the most commonly administered drug in Japan to outpatients with chronic liver disease, especially those with chronic hepatitis and liver cirrhosis49. In vivo experiments show that TJ-9 increased liver retinoid levels and decreased the total collagen content and the number of α-SMA positive cells41. 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 baicalein and baicalein41. Other experiments showed similar results in a model of HF induced by a choline-deficient diet53. Extensive clinical use suggested that TJ-9 prevented the development of HCC in patients with HCV infection53.

Recipe 861, which consists of 10 Chinese herbs, has been shown to reduce mRNA expression of pro-collagens I, III, V and TGF-β in the liver of animal models and in cultured HSCs to increase the production of interstitial collagenases (MMP-1) and to suppress TIMP-1 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 stimulation38.

Sal-B, which is one of the water-soluble components of the Salvia miltiorrhiza Bge, has been systematically investigated and used in clinics so far. Previous studies have shown that Sal-B could significantly prevent and treat HF induced by carbon tetrachloride in animal experiments44. The mechanisms of Sal-B on anti-HF were as follows:52,46 anti-lipid peroxidation; inhibiting expressions of TGF-β, and TGF-β receptor protein; inhibiting Smad2 phosphorylation and downregulating TGF-β/Smads signal conduction in HSCs and inhibiting intracellular extracellular signal-regulated kinase signalling conduction induced by TGF-β1. On the other hand, the results of the liver biopsy in 60 patients before and after a 6-month treatment suggested that Sal-B could effectively reverse HF in patients with chronic hepatitis B, with a reverse rate of 36.6% by histological examination, which is superior to interferon-γ.47

OM is one of the most important alkaloids extracted from Sophorae Alopecuroidei L. or Sophorae...
Flaveoncissit ait. Recently, OM has been used in the treatment of chronic liver disease and has a significant effect on the inhibition of HF. The discovered mechanism of actions of OM on anti-HF included inhibiting hepatic expression of Smad4 protein and upregulating Smad7 protein in rats; inhibiting HSC activation and hepatic collagen synthesis and inhibiting Kupffer cell activation and paracrine to restrain HSC proliferation. Furthermore, liver biopsy before and after treatment in 49 patients suggested that OM could reverse HF in chronic viral hepatitis patients, with the histopathological effective rate of 48%.

Glycyrrhizin is extracted from Radix Glycyrrhizae. The study by Wang et al. showed that it has the inhibitory effect on NF-kB-binding activity in CCL_4 plus ethanol-induced liver cirrhosis in rats, as well as on the degree of the liver fibrosis. Therefore, its effects are also thought to be secondary to its antioxidative properties. It has also been used for the treatment of viral hepatitis B because it was found that glycyrrhizin and related compounds could inhibit the release of hepatitis B surface antigen from infected hepatocytes.

In addition to the above description, other recipes have also been shown to be effective in the prevention of HF. Recent experimental studies and clinical trials suggest that Chinese herbal medicines may play a significant role in the therapy of HF. They appear to function at multiple phases of the process of hepatic fibrogenesis. Many groups are presently interested in analysing the effectiveness of these agents. In addition, antifibrotic therapy targets at the inhibition of HSC proliferation, cytokine activity and ECM degradation and also the apoptosis of HSC was another interest of study in recent years. Moreover, we considered that, in view of the complex pathogenesis and the multipathway and multitarget superiority of Chinese herbal medicine, the effective component formula investigations deserve more attention and probably prompt a potential research direction.

Discussion

The author has referenced some of its own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

HF has been investigated actively since HSCs were identified by Dr Scott Friedman in 1985 as the principal collagen-producing cells in the liver. The field of HF is flourishing, thanks to the continued experimental advances complemented by exciting progress in the treatment of chronic liver disease. Recently, there is considerable research going on to develop antifibrotic strategies that are applicable to HF. Such an approach is attractive because it is aimed at the final common pathological pathway of chronic liver disease, regardless of the aetiology. Activation of resident HSCs into proliferative, contractile and fibrogenic cells in liver injury remains a dominant theme driving the field. Based on the complex pathogenesis of HF, the drug treatment of HF focused on a single target seems unfeasible. Thus, approaches that act to inhibit HSC activation and proliferation and matrix production at multiple locations may be more effective than targeting a single step of the fibrogenic cascade. The agents that display multiple actions include antioxidants, such as SAMe, silymarin and TCMs (Xiao Chaihu decoction, Recipe 861).

Moreover, the wholism, the important characteristic and superiority of TCM, may be ignored, while the treatment depends on a single active herbal component. Therefore, with the guidance of the wholism of the TCM, researches of anti-HF components should return to the pharmacological effect with multipathway and multitarget approach. The TCM formulae will be upgraded to the active component formulae after the epistemological circulation of 'herbal formula-effective component-component formula', which makes the TCM formula upgrade to the level of effective component formula. At that time, the novel Chinese medicine formula for anti-HF, based on the theory of TCM and composed of active compounds with significant curative effect, definite material substructure, high stability and quality control, will be available. That is, probably a research trend on anti-HF with integrated Chinese and western medicine, and it deserves more attention.

Problems, however, do exist in TCMs, in the standardisation and purification methodology. Currently, no standardised criteria are available to compensate for the geographical variation in the content of effective components of drugs, and the methods of harvest and refinement are also controversial. In addition, most of the researches on active anti-HF components were pharmacodynamic tests in animals, and there were not many detailed investigations, which is important to find a new active component. However, the further optional mechanism researches on the components available are also valuable. The active components should be further investigated to reveal the effective targets and to be tested in standardisation and strict double-blind, multicentre studies.

Conclusion

From what we reviewed above, it is expected that the favourable prospect of anti-HF Chinese herbal components will be achieved. And the researches on active components provide considerable scientific
References


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Abbreviations list

ECM, extracellular matrix; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HF, hepatic fibrosis; HSC, hepatic stellate cell; MMP, matrix metalloproteinase; NASH, non-alcoholic steatohepatitis; OM, oxymatrine; PDGF, platelet-derived growth factor; Sal-A, salvinolic acid A; Sal-B, salvinolic acid B; TCM, traditional Chinese medicine; TGF-β, transforming growth factor-β; TIMP, tissue inhibitors of matrix metalloproteinase; TNF-α, tumour necrosis factor-α; α-SMA, α-smooth muscle actin