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TCM Clinical Research

Progress of Research on Organic Fibrosis with Traditional Chinese Medicine

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ABSTRACT

Fibrosis is the remodeling and repair processes of chronic injuries. There are few effective therapies. Chinese medicine formula, the main pattern of traditional Chinese medicine (TCM) in clinic, applies a multi-component, multi-target and complicated approach in the treatment of diseases, and certainly shows good comprehensive therapeutic effects on fibrosis. In this review, the clinical study, effects and mechanism of action of Fuzheng Huayu recipe in the treatment of liver, pulmonary, and renal fibrosis was analyzed and evaluated. Meanwhile, based on the understanding of TCM pathogenesis for liver cirrhosis, this review will also briefly introduce the research of different classical Chinese medicine formulae with various functions in the treatment of experimental liver fibrosis induced by different methods, including comparing the efficacy, analyzing the action characteristics and mechanism of effective formulae, exploring pathological and biological basis of TCM diagnostic and therapeutic pattern, which will contribute to the research of TCM in the treatment of organ fibrosis.

Key words: Organic fibrosis, traditional Chinese medicine (TCM), clinical efficacy, Fuzheng Huayu recipe

Abbreviations: Alb: albumin; EMT: epithelial-to-mesenchymal transition; TGF-β: transforming growth factor β; PI: phosphatidylinositol; TCM: traditional Chinese medicine; FZHY: Fuzheng Huayu; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; COPD: chronic obstructive pulmonary diseases; IL: interleukin; α-SMA: α-smooth muscle actin; TNF-α: tumor necrosis factor α; $HgCl_2$: mercuric chloride; RIF: renal interstitial fibrosis; UUO: unilateral ureteral obstruction; SCr: serum creatinine; BUN: blood urea nitrogen; Hyp: hydroxyproline; HA: hyaluronic acid; BDL: bile duct ligation; HGF: hepatocyte growth factor; Prdx6: peroxiredoxin 6; Hsp70: heat shock protein 70; SOD: superoxide dismutase; TIMP: tissue inhibitor of metalloproteinase; CD31: endothelial cell adhesion molecule-1; vWF: von Willebrand factor; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor-2; DAF: decay-accelerating factor; KCs: kupffer cells; MDA: malondialdehyde; HSP: heat shock protein; HO: heme oxygenase; GSH: glutathione; HIF-1α: hypoxia inducible factor-1α; EGFP: enhanced green fluoresenct protein

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Fibrosis is a common pathological consequence of injury, inflammatory, regeneration, and repair processes, namely the remodeling and repair processes of chronic injuries. The biology of fibrogenesis is dynamic, although the degree of plasticity appears to vary from organ to organ. The fact that diverse diseases in different organ systems are associated with fibrotic changes suggests common pathogenic pathways. Disease-related injury in any organ triggers a complex cascade of cellular and molecular responses. When it progresses over a prolonged period of time, parenchymal scarring and ultimately cellular dysfunction and organ failure ensue[1].

In the developed world, nearly 45% of all deaths are attributed to some type of chronic fibroproliferative diseases[2]. This "wounding response" of organ is orchestrated by complex activities within different cells in which specific molecular pathways have emerged. Cellular constituents include inflammatory cells (e.g., macrophages and T cells), epithelial cells, fibrogenic effector cells, endothelial cells, and others. Many different effector cells, including fibroblasts, myofibroblasts, cells derived from bone marrow, fibrocytes, and possibly cells derived from epithelial tissues (epithelial-to-mesenchymal transition, EMT) have been identified[1].

As fibrosis progresses, myofibroblasts proliferate and contractile mediators trigger pathological tissue contraction. This chain of events, in turn, causes physical organ deformation, which impairs organ function. Although we understand many of the cellular and molecular processes underlying fibrosis, there are few effective therapies and fewer that target fibrogenesis specifically[1].

So far, some antifibrotic drugs (including kidney, skin, liver and pulmonary fibrosis) are undergoing clinical trial evaluation. The targets of these drugs are mainly the molecules of fibrotic-related signaling pathway, including transforming growth factor β (TGF-β), phosphatidylinositol (PI), chemokines and microRNAs. Chinese medicine formula, the main pattern of traditional Chinese medicine (TCM) in clinic, applies a multi-component, multi-target and complicated approach in the treatment of diseases, which is a perfect match with the complicated pathological characteristics of fibrosis, and certainly shows good comprehensive therapeutic effects on fibrosis. Furthermore, one same

formula has effects on multiple organ fibrosis, on the other hand, different formulae have effects on the specific stage of one organ fibrosis. Chinese medicine formula has gradually showed the promising development prospect in the treatment of organ fibrosis.

Chinese Medicine Formula for Tonifying Deficiency and Dissolving Stasis Can Effectively Treat Multiple Organ Fibrosis.

1. Fuzheng Huayu (FZHY) recipe can effectively reverse liver fibrosis

FZHY recipe is a SFDA-approved anti-fibrotic medicine in China, which consists of 6 Chinese herbs, namely Danshen (*Radix Salvia Miltiorrhizae*), Chongcaojunsi (*Cordycepic hyphae*), Taoren (*Semen Persicae*), Jiaogulan (*Gynostemma Pentaphyllammak*), Songhuafen (*Pollen Pini*) and Wuweizi (*Fructus schisandrae Chinensis*). A series of clinical trials have been carried out to evaluate the efficacy and safety of FZHY recipe. A multi-center, randomized, double blinded and paralled-controlled clinical study was conducted in 216 eligible patients with liver fibrosis due to chronic hepatitis B in order to evaluate the efficacy and safety of FZHY recipe[3]. All the patients received histological diagnosis before treatment, among them 50 cases in FZHY and 46 cases in control group received liver biopsy again after treatment. The results showed there was no significant difference in fibrotic stage between two groups before treatment, the fibrotic stage score was 2.33 in FZHY group, and 2.11 in control group. The score of fibrosis stage in FZHY group after treatment (1.80) decreased significantly as compared to that before treatment (2.33, $P<0.05$), but there was no significant difference before (2.11) and after (2.14) treatment in the control group. There was a significant difference in reverse rate between FZHY group and control group, with the reverse rate of 52% in FZHY group. There was no significant difference in serum liver function between two groups before treatment. The serum albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) was improved in two groups after treatment, FZHY recipe was superior to the control drug. Both FZHY recipe and control drug also had good effects on the score of TCM syndrome. No adverse reaction was observed. Thereafter, a phase III clinical trial with more cases further confirmed the good effects of FZHY recipe on liver fibrosis.

In Xie's study[4], 82 patients with chronic Hepatitis B were randomly divided into treatment group and control group. Patients in the treatment group were treated with FZHY capsule plus adefovir dipivoxil, while patients in the control group were treated with adefovir dipivoxil only. The treatment duration was 48 weeks. After treatment, serum parameters of liver fibrosis and liver histopathologic examination were evaluated. The results showed that the degree of liver inflammation grading and the stage of liver fibrosis in the treatment group were significantly better than those in control group.

Wang[5] et al compared the therapeutic efficacy between FZHY capsule and AnLuo HuaXian pill in the treatment of liver fibrosis. In this study, 99 patients with liver fibrosis were randomly divided into two groups, namely FZHY group and ALHX group, and received FZHY capsule or AnLuo HuaXian pill respectively for 12 months. The results showed that FZHY capsule had better effects on reducing fibroscan value, serum liver fibrosis paramaters, the inside diameter of the portal vein and the thickness of the spleen, with good effects on improving the stage of liver fibrosis.

The effects of FZHY capsule on the prevention of esophageal variceal bleeding in cirrhotic patients were further evaluated[6]. A multi-center randomized and placebo-controlled trial was carried out. One hundred and forty-six cirrhotic patients with esophageal varices were enrolled to compare the probability of esophageal variceal bleeding between FZHY capsule group and controlled group for the duration of 2 years. The results demonstrated that FZHY capsule could effectively reduce the risk of variceal bleeding of cirrhotic patients with varices. Among these 146 cases, 56 patients who met the criteria for inclusion of mild varices level were divided into FZHY group (29 cases) and placebo group (27 cases). Esophageal variceal bleeding occurred in 1 patients in FZHY group, with the acturial probability of bleeding of 3.7%, and 5 patients in the placebo group, with the acturial probability of bleeding of 23%. The acturial probability of bleeding in FZHY group was much lower than that of the placebo group. Ninety patients were recruited in the level of moderate and severe varices, and divided into 3 groups: FZHY group, propranolol group, and combination group (FZHY plus propranolol) (30 patients for each group). Five patients in FZHY group, 8 in propranolol group, and 3 in bombination group had esophageal variceal bleeding, with the actuarial probability of bleeding of 23.9%, 43%, and 12.4% respectively. The actuarial probability of bleeding in combination group was much lower than that in FZHY group and propranolol group. Thirty five patients with the level of moderate or severe varices, and the history of bleeding, were randomly divided into propranolol group (17 cases), and combination group (FZHY plus propranolol) (18 cases). The results showed that 10 patients in propranolol group had variceal rebleeding, with the actuarial probability of bleeding of 76.5%, median time of non-bleeding of 8±2.56 months; 7 patients in combination group had variceal rebleeding, with the actuarial probability of bleeding of 55.2%, median time of non-bleeding of 22±1.38 months. This study demonastrated that FZHY could effectively reduce the risk of variceal bleeding, confirmed the advantage of chinese medicine formula in improving the pathologic histology of liver cirrhosis.

Furthermore, a systematic analysis was used to assess the the curative effects of FZHY on liver fibrosis and cirrhosis[7]. Twelve articles with 1392 subjects were finally selected (714 cases in treatment group and 678 cases in control group) for the meta-analysis. The results suggested that FZHY recipe

could effectively improve liver function and life quality by relieving symptoms, alleviating liver fibrosis and decreasing Child-Pugh score.

2. FZHY recipe has good effects on chronic obstructive pulmonary diseases (COPD)

A randomized, paralled-controlled clinical study was conducted to evaluate the clinical efficacy of FZHY recipe in the treatment of COPD patients[8]. 62 eligible patients were divided into FZHY group (30 patients) and control group (30 patients). Patients in FZHY group received basic treatment of Western medicine plus FZHY capsule, while patients in control group only received basic treatment of Western medicine. The treatment duration was 8 weeks. The results showed that FZHY could improve the lung functions and symptoms, decrease serum interleukin (IL)-8 and tumor necrosis factor α (TNF-α) expression, ameliorate inflammatory reaction and pulmonary fibrosis.

The effects of FZHY recipe on pulmonary fibrosis were also observed in a rat model[9]. Rat pulmonary fibrosis model was induced by bleomycin, and treated by FZHY recipe, methylprednisolone was used as a control drug. The results showed that FZHY recipe could significantly improve the lung coefficient and attenuated infiltration of inflammatory cells, with good effects on inhibiting pulmonary alveolitis; FZHY recipe could also decrease pulmonary collagen deposition, especially the deposition of elastin, down-regulate protein expressions of α-smooth muscle actin (α-SMA) and collagen I/IV. These results demonstrate that FZHY has good effects on rat pulmonary fibrosis.

3. FZHY recipe has good effects on rat renal interstitial fibrosis (RIF)

The effect of FZHY recipe on renal interstitial fibrosis was observed using mercuric chloride ($HgCl_2$) induced rat RIF model. The results showed that FZHY recipe could improve renal function, alleviate inflammation and collagen deposition in kidney, and reduce the expression of α-SMA. These results demonstrated that FZHY recipe could ameliorate $HgCl_2$ induced rat RIF[10].

In a RIF model induced by unilateral ureteral obstruction (UUO), FZHY recipe also showed good effects on reversing fibrosis. The results showed that FZHY down-regulated serum creatinine (SCr) and blood urea nitrogen (BUN) level, improved renal function; alleviated collagen content[11].

The Compatibility Principle and Mechanism of Action of FZHY Recipe in the Treatment of Organ Fibrosis

The compatibility principle of FZHY recipe was revealed by orthogonal experiment. In this formula, *Semen Persicae* and *Radix Salvia miltiorrhiza* are the herbs with the function of resolving tasis, *Cordycepic hyphae* is the herb with the function of tonifying deficiency. For the treatment of liver fibrosis, *Semen Persicae* could increase interstitial collagenase activity, thus promote collagen degradation; *Radix Salvia miltiorrhiza* could protect hepatocytes from injury; *Cordycepic hyphae* could inhibit HSC activation and generation of collagen, and interact with *Radix Salvia miltiorrhiza*. *Cordycepic hyphae, Radix Salvia miltiorrhiza* and *Cordycepic hyphae* are the main drugs in FZHY recipe in the treatment of liver fibrosis[12]. The compatibility principle of FZHY recipe in the treatment of pulmonary fibrosis was studied using a rat model[13]. The results showed the most effective herb in decreasing hydroxyproline (Hyp) content is *Gynostemma Pentaphyllammak*, while *Radix Salvia Miltiorrhizae* and *Semen Persicae* take the second place; *Cordycepic hyphae* shows strong effects on anti-oxidative damage; *Cordycepic hyphae*, *Fructus Schisandrae Chinensis* and *Radix Salvia Miltiorrhizae* have combination effect on inhibiting collagen generation. The main effective herbs of FZHY recipe in the treatment of pulmonary fibrosis are *Gynostemma Pentaphy llammak* , *Radix Salvia Miltiorrhiza,* and *Cordycepic hyphae*. For the treatment of renal interstitial fibrosis, the main effective herbs are *Radix Salvia Miltiorrhiza*, *Cordycepic hyphae, Semen Persicae* and *Pollen Pini*[14]. *Cordycepic hyphae* has good effects on decreasing Hyp content, improving renal function, alleviating urine protein. *Radix Salvia Miltiorrhiza* and *Semen Persicae* show prominent effects on decreasing inflammation; *Radix Salvia Miltiorrhiza*, *Cordycepic hyphae, Semen Persicae* and *Pollen Pini* have combination effects on anti-fibrosis. Taken together, "*Radix Salvia Miltiorrhiza*, *Cordycepic hyphae, Semen Persicae* and *Gynostemma Pentaphyllammak"* are the common main effective herbs in FZHY recipe for the treatment of liver, pulmonary and renal fibrosis, the combination of herbs for tonifying deficiency and herbs for resolving stasis have the synergistic effects on inhibiting fibrogenesis. Whereas, each organ has a specific characteristic, *Cordycepic hyphae* and *Radix Salvia Miltiorrhizae* have prominent role against liver and renal interstitial fibrosis, *Cordycepic hyphae* and *Gynostemma Pentaphyllammak* show a significant effect on pulmonary fibrosis. These results show the comprehensive advantage of Chinese medicine formula, simultaneously reveal the different pathological feature and TCM pathogenesis of different organ fibrosis.

The mechanism of action of FZHY recipe against organ fibrosis was investigated. The mechanism includes: 1) Affecting fibrotic-related cells and substance metabolism: in liver fibrosis, proteomic analysis results showed that FZHY recipe could regulate cell proliferation, metabolism, stress response and cytoskeleton[15]; it could also inhibit hepatic sinusoid capillarization and angiogenesis[16]; In renal interstitial fibrosis, FZHY recipe could regulate substance and energy metabolism, stress response and inflammation reaction, inhibit EMT of tubular epithelial cells[17]. Regulating substance metabolism, oxidative stress and cytoskeleton were the common mechanism of FZHY recipe against renal and liver fibrosis, and may be the important substance basis of "same treatment for different diseases" of FZHY recipe. 2) Inhibiting the peroxide damage, protecting tissue microenvironment and inhibiting apoptosis of parenchymal cells[15,18]. FZHY recipe could alleviate lipid peroxidation

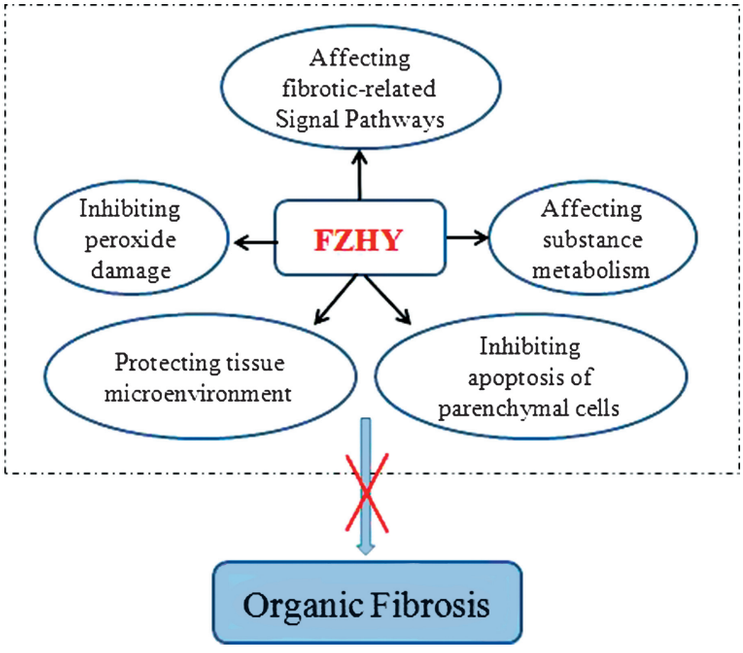

Figure 1. The action mechanism of FZHY recipe against Organic Fibrosis.

reaction, inhibit metalloproteinases-2/9 (MMP-2/9) activation, protect basement membrane. 3) Affecting the signaling pathway of pro-fibrotic factors: In liver fibrosis, FZHY recipe could down-regulate fibronectin/integrin signaling pathway[19], TGF-β/Smads signaling pathway[20,21], thus inhibit HSC activation; In pulmonary fibrosis, FZHY recipe could regulate hyaluronic acid (HA)/ NF-κB signaling pathway, inhibit inflammation response; In renal interstitial fibrosis, FZHY recipe could down-regulate TGF-β/Smads signaling pathway, inhibit EMT of tubular epithelial cells[22]. [Figure 1.]

Based on The Pathogenesis and Syndrome of Liver Cirrhosis, Exploring the Biological Basis of Different Classical Chinese Medicine Formula in the Treatment of Liver Cirrhosis

Using factors analysis, C mean clustering and fuzzy comprehensive assessment, the clinical symptoms and signs collected from 900 patients with posthepatitic cirrhosis were analyzed. The analysis results reveal that the basic pathogenesis of cirrhosis is Qi deficiency and blood stasis, the basic TCM syndromes include Gan-Shen yin-deficiency, dampness-heat accumulation[23]. Based on these analysis results, combining with the rule of medication and ancient TCM books, the following representative classical Chinese medicine formulae were selected: Huangqi Decoction for tonifying Qi, Yiguanjian Decoction for nourishing Yin, Xiayuxue Decoction for resolving stasis, Yinchenhao Decoction for clearing heat and promoting dieresis. The effects of these formulae on different liver cirrhosis models were observed and compared dynamically, and the pathological and biological basis of these formulae in the treatment of liver cirrhosis are explored through analyzing the different action effects and mechanism of these formulae.

1. Different Chinese medicine formulae with various functions have an effect on the same disease model induced by different methods. What is the pathological and biological basis?

1) The effective Chinese medicine formulae for liver fibrosis induced by dimethylnitrosamine (DMN). Huangqi Decoction and Yinchenhao Decoction are the effective formulae for liver fibrosis/cirrhosis induced by DMN. DMN-induced liver fibrosis model was intervened by the four classical formulae from the second week of DMN intoxication for 2 weeks. The results showed that just Yinchenhao Decoction showed good effect on this model, manifested by decreasing Hyp content, improving liver inflammation and pathological changes. When the treatment duration extended to 4 weeks, from the second week to the 6th week, both Yinchenhao Decoction and Huangqi Decoction significantly improved liver pathological changes, reducing collagen content, showed good effects on DMN-induced liver fibrosis[22,24]. These results showed that dampness-heat accumulation with Qi deficiency may be the main TCM syndrome of DMN-induced liver fibrosis.

2) The effective Chinese medicine formulae for liver cirrhosis induced by tetrachloride carbon (CCl_4). Yiguanjian Decoction and Xiayuxue Decoction are the effective formulae for liver fibrosis/cirrhosis induced by CCl_4. The effects of the four classical Chinese medicine formulae on CCl_4 induced rat liver cirrhosis formation were investigated[25–26]. The model was established by subcutaneous injecting of CCl_4 for 12 weeks. The treatment was from the 9th week to 12th week. The results showed that Yiguanjian

Decoction and Xiaxuxue Decoction could improve liver function and pathological changes, reduce Hyp content, reverse liver fibrosis. Xiayuxue Decoction showed prominent effect on reducing Hyp content, while Yiguanjian Decoction was perfect at increasing serum Alb content. These results showed that the main pathological changes during CCl_4 induced liver cirrhosis formation is the rapid hyperplasia of hepatic fibrous connective tissue and obstruction of collaterals by blood stasis, thus induced reconstruction of the tissue structure, which could be treated with Xiayuxue Decoction effectively, while the severe injury of liver parenchyma in this phase is another pathological change of Gan-Shen yin deficiency syndrome, which could be effectively treated with Yiguanjian Decoction.

3) The effective Chinese medicine formula for immunological liver cirrhosis induced by pig serum. Xiayuxue Decoction is the effective formulae for liver fibrosis/cirrhosis induced by pig serum. Rat liver fibrosis was induced by intraperitoneal injection of pig serum for 12 weeks. The treatment of different classical Chinese medicine formulae was from the 9th week to the end of 12th week. Only Xiayuxue Decoction could effectively reverse liver fibrosis in this model. The results indicated that blood stasis congesting vessels may be the TCM syndrome of liver fibrosis induced by pig serum[27].

4) The effective Chinese medicine formulae for liver cirrhosis induced by common bile duct ligation (BDL). Huangqi Decoction and Yinchenhao Decoction are the effective formulae for liver fibrosis/cirrhosis induced by BDL[28]. Rat liver fibrosis model was induced by BDL. The Chinese medicine formulae were administrated for 4 weeks from the end of the first week after operation. The results showed that only Huangqi Decoction and Yinchenhao Decoction could significantly improve liver function and liver pathological changes, reduced Hyp content, reverse liver fibrosis. Huangqi Decoction shows the best effect on this model.

2. The mechanism of action of different Chinese medicine formula against liver fibrosis

1) The mechanism of action of Huangqi Decoction. The main mechanism of Huangqi Decoction is increasing hepatocyte growth factor (HGF) expression, inhibiting TGF-β1 expression and TGF-β1/Smads signaling pathway, thus inhibiting activation and transdifferentiation of liver cells, including the transdifferentiation of hepatic stellate cells (HSCs) to myofibroblast-like cells, hepatocytes to cholangiocytes, and liver sinusoidal endothelial cells to vascular endothelial cells[29–32]; it can also increase the expression of peroxiredoxin 6 (Prdx6), heat shock protein 70 (Hsp70), catalase and superoxide dismutase (SOD), which are important antioxidants, indicates that anti-oxidative stress is another important mechanism of Huagnqi Decoction anti-fibrosis [33–34]

2) The mechanism of action of Xiayuxue Decoction. The main mechanism of Xiayuxue Decoction is increasing interstitial collagenase activity, accelerating the degradation of abnormal collagen, promoting the apoptosis of HSCs, and inhibiting sinusoid capillarization and angiogenesis. In a rat immunological liver fibrosis model induced by pig serum, Xiayuxue Decoction could regulate the balance between matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMP), increase the activity of MMP-9[35].

In CCl_4 induced liver fibrosis, Xiayuxue Decoction could effectively decrease endothelial cell adhesion molecule-1 (CD31), von Willebrand factor (vWF), vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor-2 (VEGFR2), complement decay-accelerating factor (DAF) and α-SMA expression, down-regulate the activities of MMP-2 and MMP-9, up-regulate the expression of MMP-13, thus inhibit the liver pathological angiogenesis [36]. The extracts from Xiayuxue Decoction could promote the apoptosis of HSCs and the proliferation of hepatocytes, inhibit the dedifferentiation of liver sinusoidal endothelial cells, improve sinusoid capillarization[37].

3) The mechanism of action of Yinchenhao Decoction. In DMN induced fibrotic liver, during the fibrosis progression, there are a massive increase of CD68 positive Kupffer cells (KCs), and Yinchenhao Decoction could decrease CD68 and TNF-α expression, reduce the activation of KCs, inhibitthe inflammation response. During the stage of DMN-induced liver cirrhosis, KCs could increase the secrete of MMP-13, and Yinchenhao Decoction could increase the expression of CD68 and MMP-13, degrade the abnormal extracellular matrix (ECM), and it could also protect hepatocytes from apoptosis[32]. These results indicate regulating the function of KCs, protecting hepatocytes from apoptosis is the mechanism of Yinchenhao Decoction for clearing heat and promoting dieresis.

4) The mechanism of action of Yiguanjian Decoction.

①Increasing the biotransformation ability of liver. Gene microarray results showed that Yiguanjian Decoction could up-regulate CYP3A13, arginine vasopressin receptor 1A, Beta-glo, down-regulate lymphotoxin A, MMP-23, RNA binding motif protein 3, thrombospondin 2, AP1 gamma subunit binding protein 1, growth hormone releasing hormone receptor, amiloride binding protein 1 expression in CCl_4 induced rat liver cirrhosis, which are related to hepatic biotransformation ability[38].

②Protecting liver tissue from oxidative stress injury. Differential proteomics analysis showed that Yiguanjian Decoction could up-regulate the expression of Cu/Zn SOD, DJ-1, glutathione synthetase, glutathione S-transferase Yb-1 subunit and aldo-keto reductase family 7, A2 in CCl_4 induced rat liver cirrhosis[39]. Yiguanjian Decoction could also decrease malondialdehyde (MDA), heat shock protein (HSP)70 and heme oxygenase (HO)-1, but significantly increase SOD, L-glutathione (GSH), Prdx6 and transferring[40]. These results indicate that Yiguanjian could reduce lipid peroxidation damage by preventing generation of oxidizing substances.

③Improving hepatic oxygen-deficiency, protecting liver sinusoidal endothelial cells, inhibiting liver angiogenesis.

Yiguanjian Decoction could decrease α-SMA, CD31, VEGF, VEGFR, and hypoxia-inducible factor-1α (HIF-1α) expression in CCl_4 induced liver fibrosis, indicates that Yiguanjian Decoction could inhibit liver angiogenesis by improving the hepatic hypoxic microenvironment[41].

④Inhibiting the migration of bone marrow cells into the liver as well as inhibiting their differentiation and suppressing the proliferation of both progenitors and hepatocytes in fibrotic liver[42]. Liver cirrhosis in mice received transplantation of enhanced green fluorescent protein (EGFP)-positive bone marrow were induced by CCl_4 and treated by Yiguanjian Decoction. The results showed EGFP-positive marrow cells migrated into the liver and were mainly distributed along the fibrous septa, and co-expression with α-SMA. Yiguanjian Decoction abrogated the increases in the number of bone marrow-derived fibrogenic cells in the liver, inhibited expression of both progenitor and mature hepatocyte markers, and reduced fibrogenesis.

3. The overall effect of Chinese medicine formula can reveal the key pathological feature of diseases

Huangqi Decoction can inhibit TGF-β1 and α-SMA expression, up-regulate HGF-α expression in DMN-induced liver cirrhosis, indicating its effects on the key factors during liver fibrogenesis. However, in this model it did not suppress the expression of proinflammatory factors secreted by Kuffer cells, which strongly indicates that during the severe inflammation stage of fibrogenesis, the therapy may has no significant effect if it is only aiming at the effector cells or the key pro-fibrotic factors[30]. On the other hand, Yinchenhao Decoction significantly inhibited liver inflammation, which indicates that liver inflammation (the early stage of liver fibrosis) may be the pathological and biological basis of Yinchenghao Decoction in the treatment of liver cirrhosis.

The main mechanism of action of Huangqi Decoction against liver fibrosis is inhibiting the generation of fibrosis at the source, namely inhibiting TGF-β1 (a key cytokines during fibrogenesis) and its signaling transduction pathway, preventing the activation and transdifferentiation of its effector cells; while the main mechanism of Xiayuxue Decoction is aiming at the key factors of fibrous tissue proliferation, namely down-regulating the activity of MMP-2, protecting normal liver matrix from disturbing and promoting the apoptosis of activated HSCs. These results further verify that the basic syndrome pathogenesis of liver cirrhosis is Qi deficiency and blood stasis. Meanwhile, to some extent, it also interprets the pathological and biological basis of the syndrome pathogenesis, and provides a promising idea for the progress of therapy and drug development.

Based on the thinking method of TCM, focusing on the problem of "syndrome pathogenesis, and its correlation with diseases and Chinese medicine formula", which is the important scientific problem of syndrome research. Based on clinical analysis results of TCM syndrome pathogenesis, combining with disease-syndrome specific formulae and animal model, comparing the overall effects of Chinese medicine formula on the same animal model induced by different methods, analyzing the correction between diseases, syndrome, formula and efficacy, investigating the pathological basis of the effective formulae, exploring the scientific basis of disease-syndrome relationship; these studies will provide biology basis for developing TCM theory, and contribute to developing effective Chinese medicine and method for the treatment of organ fibrosis.

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