Inhibitory effect of Huangqi Zhechong decoction on liver fibrosis in rat

Shuang-Suo Dang, Xiao-Li Jia, Yan-An Cheng, Yun-Ru Chen, En-Qi Liu, Zong-Fang Li

AIM: To assess the inhibitory effect of Huangqi Zhechong decoction on hepatic fibrosis in rats induced by CCl4 plus alcohol and high fat low protein diet.

METHODS: Male SD rats were randomly divided into hepatic fibrosis model group, control group and 3 treatment groups consisting of 12 rats in each group. Except for the normal control group, all the rats were subcutaneously injected with CCl4 at a dosage of 3 mL/kg. In 3 treated groups, either high-dose group (9 mL/kg), medium-dose group (6 mL/kg), or low-dose group (3 mL/kg) was daily gavaged with Huangqi Zhechong decoction, and saline vehicle was given to model and control rats. Enzyme-linked immunosorbant assay (ELISA) and biochemical examinations were used to determine the changes of alanine aminotransferase (ALT), aspartate aminotransferase (AST), hyaluronic acid (HA), laminin (LN), type-III-procollagen-N-peptide (PIIIP), and type IV collagen content in serum, and hydroxyproline (Hyp) content in liver after sacrificing the rats. Pathologic changes, particularly fibrosis were examined by hematoxylin and eosin (HE) and Van Gieson staining.

RESULTS: Compared with the model control group, serum ALT, AST, HA, LN, PIIIP and type IV collagen levels dropped markedly in Huangqi Zhechong decoction groups, especially in the medium-dose Huangqi Zhechong decoction group (1 954±576 U/L vs 759±380 U/L, 2 735±786 U/L vs 1 259±829 U/L, 42.74±7.04 ng/mL vs 20.68±5.85 ng/mL, 31.62±5.84 ng/mL vs 14.87±1.45 ng/mL, 3.26±0.69 mg/mgprot vs 1.47±0.46 mg/mgprot, 77.68±20.23 ng/mL vs 25.64±4.68 ng/mL, respectively) (P<0.05). The Hyp content in liver tissue was also markedly decreased (26.47±11.24 mg/mgprot vs 9.89±3.74 mg/mgprot) (P<0.01). Moreover, the stage of the rat liver fibrosis in Huangqi Zhechong decoction groups was lower than that in model group, and more dramatic drop was observed in medium-dose Huangqi Zhechong decoction group (P<0.01).

CONCLUSION: Huangqi Zhechong decoction can inhibit hepatic fibrosis resulted from chronic liver injury, retard the development of cirrhosis, and notably ameliorate the liver function. It may be a safe and effective therapeutic drug for patients with fibrosis.

INTRODUCTION
Liver fibrosis is common in most chronic liver diseases regardless of the etiology[1-8]. Although new therapeutic approaches have recently been proposed, there is no established therapy for liver fibrosis[9]. Huangqi Zhechong decoction is a traditional Chinese medicine. The aim of the present study was to investigate the protective effects of Huangqi Zhechong decoction on liver fibrosis in rats of CCl4-induced cirrhosis.

MATERIALS AND METHODS
Reagents
CCl4 (Xi’an Chemical Factory) was diluted into 400 g/L in olive oil (Xi’an Chemical Factory). Huangqi Zhechong decoction was self-made by the Pharmaceutical Department of the Second Hospital, Xi’an Jiaotong University. The kit for Hyp was bought from Nianjing Jiancheng Biological Institute. Kits for HA, LN, PIIIP and type IV collagen were bought from Senxiong Company, Shanghai.

Animals
Sixty adult male SD rats weighing 150-200 g were provided by the Laboratory Animal Center of the College of Medicine, Xi’an Jiaotong University. The rats were randomly divided into 5 groups of 12 each: control group; model group; and 3 treatment groups. Except for the control rats, all rats were subcutaneously injected with 400 g/L CCl4 (CCl4:Olive oil 2:3), 3 mL/kg b.w., at every 3 d for 6 wk, and fed with high fat low protein diet (75% pure maize plus 20% lard and 0.5% cholesterol) and 300 mL/L alcohol as drinks. In the 3 treatment groups, Huangqi Zhechong decoction was administered daily via gastric tube to high-dose, medium-dose and low-dose groups at a dosage of 9 mL/kg, 6 mL/kg and 3 mL/kg for 6 wk, respectively. After 6 wk, except the dead, all the rats were anesthetized with 200 g/L urethane (5 mL/kg, abdominal injection). Blood was taken from abdominal aorta, centrifuged at 4 ℃, and plasma were kept at -20 ℃ for assays.

Pathological observations
Hepatic tissues were fixed in 40 g/L solutions of formaldehyde in 0.1% mol/L phosphate-buffered saline (pH 7.4), and embedded in paraffin. Five-micrometer thick section slides were prepared. All the sections stained with HE and standard van Gieson staining (VG) were coded and scored by blind reading. Van Gieson’s method was used to detect collagen fibers[10]. Liver condition was classified according to the standard formulated by China Medical Association in 1995[11].
and fibrosis was graded from 0 to 4 (0: no fibrosis; 1: portal area fibrosis; 2: fibrotic septa between portal tracts; 3: fibrosis septa and structure disturbance of hepatic lobule; and 4: cirrhosis).

Statistical analysis
Results were expressed as mean±SD. Quantitative data were analyzed by using ANOVA in statistical software SPSS 11.0. A value of \( P<0.05 \) was considered statistically significant. Ridit test was used for statistical analysis of the qualitative data.

RESULTS

Hyp content in liver tissues
Liver Hyp level was significantly lower in rats treated with CCl4 and Huangqi Zhechong decoction compared to the rats treated with CCl4 alone (\( P<0.01 \)). And the liver Hyp level of rat in 3 Huangqi Zhechong decoction treatment groups has no significant difference from control group (Table 1).

Plasma levels of ALT and AST
Plasma levels of ALT and AST in model group were higher than those in the controls (\( P<0.01 \)), while the Huangqi Zhechong decoction treatment groups showed significant lower ALT and AST levels than the model group. Furthermore, among the 3 treatment groups the medium-dose group showed the best effect and the levels of ALT and AST in serum showed no difference compared with the normal group (Table 1).

| Group              | n  | ALT (U/L) | AST (U/L) | Liver Hyp (µg/ mgprot) |
|--------------------|----|-----------|-----------|------------------------|
| Control            | 12 | 86.0±17.7 | 329±40    | 10.02±1.05             |
| Model              | 11 | 1 954±576 | 2 735±786 | 26.47±11.24            |
| High-dose group    | 9  | 989±576   | 1 594±988 | 15.01±7.59             |
| Medium-dose group  | 10 | 759±380   | 1 259±829 | 9.89±3.74              |
| Low-dose group     | 10 | 1 003±530 | 1 650±928 | 10.06±2.58             |

\( P<0.05, \) \( P<0.01 \) vs model group; \( P<0.05, \) \( P<0.01 \) vs control group.

Table 2 serum levels of HA, LN, PIIIP and type IV collagen

| Group                | n  | PIIIP (ng/mL) | Type IV collagen (ng/mL) | LN (ng/mL) | HA (ng/mL) |
|----------------------|----|---------------|--------------------------|------------|------------|
| Control              | 12 | 0.34±0.67     | 18.47±3.43               | 10.07±1.74 | 17.96±5.86 |
| Model                | 11 | 3.26±0.69     | 77.68±20.23              | 31.62±5.64 | 42.74±7.04 |
| High-dose group      | 9  | 2.01±0.40     | 39.14±4.97               | 16.32±2.73 | 21.71±6.69 |
| Medium-dose group    | 10 | 1.47±0.46     | 25.64±4.68               | 14.87±1.45 | 20.68±5.89 |
| Low-dose group       | 10 | 1.84±0.27     | 29.09±2.78               | 17.02±2.74 | 24.18±7.89 |

\( P<0.05, \) \( P<0.01 \) vs model group; \( P<0.05, \) \( P<0.01 \) vs control group.

Table 3 Pathological observation of liver condition

| Group                | n  | 0 | I  | II | III | IV | U        |
|----------------------|----|---|----|----|-----|----|----------|
| Model                | 11 | 0 | 0  | 0  | 3   | 8  |          |
| High-dose group      | 9  | 0 | 0  | 2  | 3   | 3  | 1        |
| Medium-dose group    | 10 | 0 | 4  | 4  | 2   | 0  | 4.01     |
| Low-dose group       | 10 | 0 | 2  | 4  | 3   | 1  | 3.75     |

\( P<0.05, \) \( P<0.01 \) vs model group; The value of \( U \) represents the Ridit value of the two groups, \( U >1.96 \) means \( P<0.05, \) \( U >2.58 \) means \( P<0.01 \).

Figure 1 Liver tissue under light microscope. A: Normal liver tissue in control group (HE staining, original magnification: x400); B: Liver fibrosis tissue in model group, more fibrous tissue was formed in liver. A large amount of inflammatory cells soaked into the intralobular and the interlobular (van Gieson staining, original magnification: x200); C: Liver fibrosis tissue in Huangqi Zhechong decoction group. The pathological change of liver was rather lighter compared with the model (van Gieson staining, original magnification: x200).
**Pathological assay**
At the end of the study, the liver of control rats had no appreciable alterations (Figure 1A). In the model group, the margin of liver was uneven; more fibrous tissues formed and extended into the hepatic lobules to separate them incompletely; a large amount of inflammatory cells infiltrated in the intralobular and the interlobular regions; the liver structure was disordered with some displacement of central veins, and there were more necrotic and degenerated liver cells compared with the control (Figure 1B). While in the 3 treatment groups, especially in the medium-dose group, the pathological changes of liver was rather milder, showing less fibrous tissue proliferation and inflammatory cell infiltration in the interlobular space; the hepatic cell cords arranged radially with less displacement of central veins and less degenerated or necrosis hepatic cells, without any pseudolobule observed (Figure 1C). Compared with the model group, the liver condition of the rats was significantly improved in Huangqi Zhechong decoction groups (Table 3).

**DISCUSSION**
The incidence rate of chronic hepatopathy in China is high, which affects the patients by progressively developing irreversible cirrhosis[12,13]. Hepatic fibrosis is the intermediate and crucial stage of this process, characterized by reversibility. If treated properly in this stage, cirrhosis could be successfully prevented[14]. But it remains a problem to prevent cirrhosis or to control its progression in patients with a chronic liver disease[15]. Great efforts have been made to find safe and effective drugs. Recent clinical and experimental observations have demonstrated that Chinese medicines might be of some preventive and therapeutic values against fibrosis[16-18].

Of Huangqi Zhechong decoction, the Chinese traditional medicine, the Astragalus has the effects of activating blood circulation to relieve stasis, strengthening “spleen”, supplementing and smoothing “qi” to eliminate fullness, reinforcement body’s immunological function. It also could preserve the integrity of hepatocytes, eliminate toxic free radicals, inhibit lipid peroxidation of cytomembrane, relieve necrosis of hepatocytes, and obviously antifibrosis[19-23]. Thoroughfare is mainly used to activate blood circulation, remove stasis, and dredge the liver[24].

Huangqi Zhechong decoction has been used in clinic for many years to prevent liver fibrosis and shown good effect. However, its effect and the associated mechanisms need further experimental evidence. CCl4 is a super-hepatotoxin, with which many years to prevent liver fibrosis and shown good effect. Recent clinical and experimental observations have demonstrated that Chinese medicines might be of some preventive and therapeutic values against fibrosis[16-18].

The decoction may significantly decrease serum levels of ALT and AST in rats with hepatic injury caused by CCl4. It indicates that Huangqi Zhechong decoction may work through protecting the liver cells.

HA, LN, PHII and type IV collagen are good serum markers of hepatic fibrosis. In this study, the serum contents of these 4 markers in the model group were much higher than those of the controls (P<0.01). And the Huangqi Zhechong decoction groups had significantly low HA, LN, PHII and type IV collagen levels in serum than those in the controls, which indicated that Huangqi Zhechong decoction could successfully prevent hepatic fibrosis.

Hyp content in liver is another important index to react the hepatic fibrosis. In fibrotic liver, collagen fibers increase, which induced the rise of Hyp content in liver[28]. So Hyp level could provide the information about the degree and variant process of cirrhosis. In this study, we observed that the liver Hyp level in the model group was much higher than that of the controls and Huangqi Zhechong decoction groups.

In summary, Huangqi Zhechong decoction may play a role in antifibrotic therapy. It can protect the liver cells and inhibit the deposition of collagen fibers in liver. It may provide a safe and effective strategy for inhibition of cirrhosis in clinic use.

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