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Effect of Chinese herbal compound on liver fibrosis in rabbits with schistosomiasis by B-ultrasound

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ABSTRACT

Objective: To explore the value of B-ultrasound on the evaluation of the effects of traditional Chinese medicine compound of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis*, and TCM + praziquantel on liver fibrosis in rabbits with schistosomiasis. **Methods:** The hepatic fibrosis model in rabbits with schistosomiasis was established. The experimental animals (24 rabbits) were randomly divided into four groups (group A, B, C and D, n=6). Group A (control group) was only treated by praziquantel; Group B was treated by mixture of *Radix astragali* and *Salvia miltiorrhiza* + praziquantel; Group C was treated by mixture of *Radix astragali* and *Angelica sinensis* + praziquantel; Group D was treated by mixture of *Radix astragali, Salvia miltiorrhiza* and *Angelica sinensis* + praziquantel. Then B-ultrasonogram was used to evaluate the effects. **Results:** Each group showed certain curative effect on liver fibrosis in rabbits with schistosomiasis. The efficacy of group B, C and D was better than group A, and that of group D was the best. The differences in long diameter, thickness diameter, transverse diameter and portal vein inner diameter of liver before and after treatment were statistically significant (P<0.05). The liver function indexes and liver fibrosis indexes were significantly improved after treatment (P<0.05). **Conclusions:** The mixture of *Radix astragali, Salvia miltiorrhiza* and *Angelica sinensis* combined with Western medicine treatment can obviously improve the efficacy on liver fibrosis of schistosomiasis.

1. Introduction

Schistosomiasis is a severe endemic parasitic disease caused by schistosomes parasitized in the human body in region with dense water network. It is estimated that there are about 700 million people under the threat of infection by schistosoma all over the world, and about 200 million people are influenced by it in Africa, South America and Asia[1,2]. Liver fibrosis of schistosomiasis, a kind of chronic disease, refers to the granuloma allergy caused by schistosome eggs depositing in the hepatic portal system and also refers to the dysplasia of liver connective tissues including interstitial cells and fibers caused by scar healing in the later period of granuloma allergic reaction[3,4]. Liver fibrosis can further develop into liver cirrhosis, and can also have invertible potency because of the regeneration capacity and gradual absorption of scar of liver itself[5]. Till now, many scholars have believed that liver fibrosis and even the early liver cirrhosis can be reversed[6]. Because of unique clinical efficacy and usability, some traditional Chinese medicines (TCMs) and plant drugs have been used for thousands of years, especially in the treatment of liver diseases[7]. Therefore, exploration of TCM use as the therapeutic drugs

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and methods against liver fibrosis has been a research focus. For example, people have found that through anti-inflammatory response, anti-stress reaction and anti-proliferation and activation of hepatic stellate cells, Chinese herbal medicine compound preparation can reduce the liver fibrosis symptoms of animals, thus protecting liver function, reducing collagen synthesis and promoting extracellular matrix degradation[8-11].

According to the characteristics of invigorating qi, promoting blood circulation and tonifying blood of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis*, this research explored the efficacy of the compound composited by these three TCMs on liver fibrosis in rabbits with schistosomiasis. The pathogen treatment of praziquantel was also observed. The B-ultrasound diagnostic method was used to evaluate the efficacy. This could provide certain experience and reference for Chinese medicine treatment of liver fibrosis of schistosomiasis.

2. Materials and methods

2.1. Preparation of Chinese medicine compound mixture

Angelica sinensis samples (smoke drying) were produced in Min County, Gansu province. *Radix astragali* was purchased from Changsha Jiuzhitang Pharmacy and identified by Institute of Chinese Materia Medica of Hunan Traditional Chinese Medicine Academy. *Salvia miltiorrhiza* was purchased from Beijing Tongrentang Pharmacy, and the Danshen tablet was crushed and then was screened by 40-mesh screen. The compound of *Radix astragali* and *salvia miltiorrhiza* was prepared according to 1:1 compatibility; the compound of *Radix astragali* and *Angelica sinensis* was prepared according to 1:0.2 compatibility; the compound of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* was prepared according to 1:1:0.2 compatibility. These three kinds of Chinese medicines were crushed and screened by 40-mesh screen. Appropriate amount of Chinese medicine powder was prepared according to the previous ratio, extracted by alcohol/water, concentrated, and finally made into liquid medicine with concentration of 5 g/mL (concentration of *Radix astragali*).

2.2. Establishment of animal model[12]

Twenty-four male New Zealand rabbits weighted about 2.5 kg were selected. Fifty positive oncomelanias infected by *Schistosoma japonicum* shed cercarie in 150 mL flask. After full cercarie shedding, each rabbit was infected by 100 *Schistosoma japonicum* cercarie through skin of abdomen. Then the experimental animals were set at the room temperature in different cages, and fed in the experimental conditions of ordinary animals. From the 13th week after schistosoma infection, B-ultrasound examination of the liver was performed every week, the ultrasonography of liver fibrosis of schistosomiasis was obtained, and then liver fibrosis of schistosomiasis was confirmed. This research was approved by Ethics Committee of The Military General Hospital of Beijing PLA.

2.3. Animal grouping and treatment

Eighteen weeks after the infection, 24 experimental rabbits were randomly divided into four groups. Rabbits in group A were given etiological treatment of praziquantel and administered 300 mg/kg praziquantel every day; Group B was treated by mixture of *Radix astragali* and *Salvia miltiorrhiza* + praziquantel, and administered 1.67 mL/kg mixture of *Radix astragali* and *Salvia miltiorrhiza* + 300 mg/kg praziquantel every day; Group C was treated by mixture of *Radix astragali* and *Angelica sinensis* + praziquantel, and administered 1.67 mL/kg mixture of *Radix astragali* and *Angelica sinensis* + 300 mg/kg praziquantel every day; Group D was treated by mixture of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* + praziquantel, and administered by 1.67 mL/kg mixture of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* + 300 mg/kg praziquantel every day.

2.4. Indexes observation

B-ultrasound examination was performed eighteen weeks after schistosoma infection (before treatment) and ten weeks after administration of drugs, and the changes of liver ultrasound was observed, according to the examination method established by TDR Schistosomiasis Ultrasonic Diagnosis Advisory Council hosted by WHO in 1990[13]. Siemens Adam ultrasound diagnostic apparatus was used to show the liver anatomic images. The liver and spleen parenchyma and the coating were mainly observed, and each lead wire diameter of liver and the inner diameter value of PV were measured. Effective criterion: the fish scale or network structure of liver parenchyma disappeared and changed into spot thickening type. Ultrasound diagnosis of schistosomiasis liver cirrhosis: the changes of liver cirrhosis were classified into 0-3 degrees based on literatures.

Serological detection was performed eighteen weeks after schistosoma infection (before treatment) and ten weeks after administration of drugs. A total of 4-5 mL blood was

collected from each rabbit's auricular vein, and the serum was separated immediately for later use. Liver function indexes included alanine aminotransferase (ALT), total bilirubin (TBIL) and albumin (ALB), which were detected by Reitman-Frankel method, biuret method and bromocresol green method, respectively. Liver fibrosis indexes included hyaluronic acid (HA), laminin (LN), type III procollagen (PC Ⅲ) and type Ⅳ collagen (Ⅳ-C), which were detected by radioimmunoassay. All reagent kits were provided by Beijing Zhongshan Golden Bridge Co. Ltd.

2.5 Statistical analysis

SPSS16.0 software was used for data analysis and processing. Measurement data were expressed by mean±SD. Comparison of experimental data in each group before and after treatment used paired *t*-test. Difference was statistically significant when $P<0.05$.

3. Results

3.1. Changes of liver size and PV inner diameter of rabbits before and after treatment

Comparison of B-ultrasound examination results sixteen weeks after administration of drugs and before it indicated that the treatment in group B, C and D had certain efficacy, and the efficacy of group D was the best. The four measurement values in group D were all significant ($P<0.05$, Table 1).

Table 1

Liver size of rabbits before and after treatment of Chinese traditional compound medicines with different formulas (mm).

Group (n=6)		Long diameter	Width	Transverse diameter	PV inner diameter
Group A	before	57.9±5.1	38.2±4.6	70.6±7.3	3.9±0.6
	after	60.7±5.6	51.1±10.8	72.8±1.5	4.1±0.7
	t value	1.2	2.0	1.8	0.6
	P value	>0.05	>0.05	>0.05	>0.05
Group B	before	58.1±5.6	39.8±5.0	70.2±6.8	3.9±0.7
	after	49.7±7.2	36.4±4.3	58.7±8.6	3.1±0.6
	t value	0.9	0.5	2.7	2.7
	P value	>0.05	>0.05	<0.05	<0.05
Group C	before	57.6±4.8	40.0±4.9	69.5±6.9	3.8±0.5
	after	50.8±6.3	33.7±5.2	58.1±5.7	3.0±0.5
	t value	0.7	3.1	3.5	2.8
	P value	>0.05	<0.05	<0.05	<0.05
Group D	before	57.4±5.5	39.6±5.0	69.7±6.7	3.9±0.8
	after	48.2±4.1	30.2±5.7	57.4±6.3	2.8±0.5
	t value	2.6	2.9	3.2	3.7
	P value	<0.05	<0.05	<0.05	<0.05

3.2. Liver parenchyma echo of rabbits before and after treatment

The ultrasonograms of six rabbits in group A before administration of drugs showed small reticulation, which developed into large reticulation for four rabbits after administration of drugs and which was preserved for two rabbits. The liver parenchyma of six rabbits in group B before administration of drugs showed small reticulation, which developed into spot thickening for two rabbits sixteen weeks after administration of drugs and which was preserved for four rabbits. The liver parenchyma echo of six rabbits in group C before administration of drugs showed small reticulation, which developed into spot thickening for four rabbits sixteen weeks after administration of drugs and which was preserved for two rabbits. The liver parenchyma of six rabbits in group D before administration of drugs showed small reticulation, which developed into spot thickening for all rabbits sixteen weeks after administration of drugs.

3.3. Serum liver function indexes in each group before and after treatment

The changes of serum liver function indexes and liver fibrosis indexes in each group before and after treatment were shown in Table 2 and 3. Table 2 showed that concentrations of ALT, TBIL and ALB in group A, B and C decreased, but ALB concentration in these three groups did not change significantly ($P>0.05$). The differences of concentrations of ALT, TBIL and ALB in group D were significant ($P<0.05$). Table 3 showed that the differences of the four indexes in group C and D was significant ($P<0.05$), and the concentration of HA in group B was more significant than that in group A ($P<0.05$).

Table 2

Serum liver function indexes in each group before and after treatment.

Group (n=6)		ALT (U/L)	TBIL (mmol/L)	ALB (g/L)
Group A	before	60.8±11.1	2.5±1.2	35.9±2.1
	after	55.9±10.2	2.0±0.6	38.4±2.7
	t value	2.2	2.8	0.5
	P value	>0.05	<0.05	>0.05
Group B	before	61.2±10.5	2.4±1.0	35.1±1.8
	after	54.2±7.8	1.8±0.8	38.5±3.4
	t value	1.2	2.9	0.6
	P value	>0.05	<0.05	>0.05
Group C	before	60.7±10.6	2.3±0.9	35.4±2.0
	after	52.1±8.3	1.2±0.4	39.7±2.7
	t value	2.9	3.7	2.1
	P value	<0.05	<0.05	>0.05
Group D	before	61.3±11.7	2.4±1.0	35.6±2.2
	after	45.3±8.5	0.8±0.5	41.8±3.0
	t value	4.5	5.7	2.8
	P value	<0.05	<0.05	<0.05

Table 3
Liver fibrosis indexes in each group before and after treatment (n=6).

Group		HA (mg/L)	LN(mg/L)	PC Ⅲ (μg/L)	Ⅳ-C (μg/L)
Group A	before	320.8±55.7	97.3±28.7	41.5±17.4	29.1±8.0
	after	258.7±60.8	80.4±14.6	37.1±7.0	27.5±8.6
	t value	2.4	2.6	0.8	0.9
	P value	>0.05	<0.05	>0.05	>0.05
Group B	before	322.4±60.2	94.7±26.2	40.3±15.9	29.4±8.6
	after	231.3±54.2	73.2±12.3	33.7±6.4	25.3±7.6
	t value	2.7	3.3	2.5	2.4
	P value	<0.05	<0.05	>0.05	>0.05
Group C	before	316.7±56.8	96.5±27.1	42.7±16.1	28.3±7.8
	after	167.5±47.6	65.7±11.6	30.2±6.5	22.4±6.3
	t value	4.1	5.1	3.1	2.9
	P value	<0.05	<0.05	<0.05	<0.05
Group D	before	321.2±58.4	98.1±25.4	39.7±15.4	27.5±6.9
	after	114.6±28.5	55.9±9.4	26.0±6.8	20.4±7.2
	t value	6.7	6.4	4.2	
	P value	<0.05	<0.05	<0.05	<0.05

4. Discussion

Schistosomiasis japonica is caused by eggs which get into liver along with blood and block intrahepatic veins. The growth and death of eggs can cause tissue injuries, form egg granuloma, and then cause hyperplasia of fibrous tissue, which lead to fiber occlusive diseases. The lesions distinctively reflect in the dense echo area along the portal vein, which has been verified by liver biopsy[14-17]. The early diagnosis and treatment of liver fibrosis play an important role in blocking the whole development chain of liver disease, therefore a direct effective diagnostic method is significant for early detection of this disease[18-22]. Ultrasonography is an effective noninvasive ultrasonographic method which can directly check the pathological changes of host caused by schistosoma[6,23-26]. It is generally believed that after appearance of liver fibrosis of rabbit infected with schistosoma, the ultrasonographic features expressed as increase of liver volume, thickening and strengthening of liver parenchyma spots, uneven strength of liver parenchyma echo, and "reticular" structure formed by fine light bands for most cases[27]. Ultrasound images can be used as a good parameter reflecting the degree of infection, so liver ultrasound can be an effective method for understanding and monitoring of liver injury degree caused by *Schistosoma japonicum*[28]. Results of this research showed that after successful modeling of liver fibrosis, the liver ultrasound images of rabbits expressed as small reticulation. After treatment of different medicines, the features of ultrasound images changed a lot, which indicated that ultrasonic diagnosis had certain value and function in early detection and prevention of schistosomiasis.

TCM believes that the pathological features of schistosomiasis are liver meridian obstruction and stagnation of qi and blood, and the treatment should focus on promoting blood circulation by removing blood stasis, combined with reinforcing qi and nourishing blood and liver[29]. Based on the practice and theory of Chinese medicine treatment of schistosomiasis, this research used multiple kinds of compound Chinese medicine preparation cooperated with conventional etiology method to treat this disease. Based on the effect of invigorating qi, promoting blood circulation and enriching blood of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* respectively, researchers prepared three kinds of compound Chinese medicines according to traditional treatment method and compatibility proportion, which was cooperated with praziquantel treatment and had certain efficacy. Results of this study showed that the curative effect of compound Chinese medicines + praziquantel was better than that of praziquantel treatment, and the effect was most obvious when using the prescription of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* + praziquantel. He *et al*[30] affirmed that the compound of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* had curative effect on liver fibrosis of schistosomiasis in rabbits. The changes of serum liver function and liver fibrosis indexes also supported this, which indicated that the compound of *Radix astragali*, *Salvia miltiorrhiza* and *Angelica sinensis* had good function of anti fibrosis. Results of B-ultrasound examination were consistent with it.

The curative effect of treatment of Chinese medicine compound combined with conventional medicine (praziquantel) on liver fibrosis of schistosomiasis is better than single Western medicine liver treatment. It has advantages such as rapid reversal of fibrosis degree, less adverse reaction of drug compatibility and low price, and this is a safe, effective and economic method for treatment of liver fibrosis of schistosomiasis, owning a high clinical application value.

Conflict of interest statement

We declare that we have no conflict of interest.

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