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Effect of total glucosides of paeony on nuclear factor-κB and transforming growth factor β1 in liver tissue of rats with immune hepatic fibrosis.

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Abstract

Objective: To investigate effect from total glucosides of paeony on protein expression of nuclear factor-κB and transforming growth factor β1 in liver tissue of rats with hepatic fibrosis.
Methods: Healthy SD rats were randomly chosen and divided into normal group (group N), model group (group M) and TGP high, medium, low dose treatment group (T1-T3). Except the normal group, all the groups received intraperitoneal injection of porcine serum for modelling, and after 6 w, the treatment group were given different doses of TGP. Rats' liver tissue received pathological examination to determine the degree of liver fibrosis hyperplasia after 15 w, and Nuclear Factor-κB (NF-κB) as well as Transforming Growth Factor β1 (TGF-β1) protein expression were detected.
Results: HE staining and VG staining showed that the degree of liver fibrous tissue hyperplasia in group M was higher, and the state of fibrosis was obviously improved after treatment by TGP. The protein expression of NF-κB and TGF-β1 in M group was significantly increased compared with the N group (19.65 ± 2.53 *vs.* 1.58 ± 0.73, 24.43 ± 2.32 *vs.* 2.01 ± 0.56, all P<0.05). The protein expression of NF-κB and TGF-β1 in treatment group were significantly decreased compared with the M group (P<0.05), the difference of which was statistically significant.
Conclusion: TGP can significantly improve the liver tissue of rats with immune hepatic fibrosis, reduce the symptoms of liver fibrosis, and can inhibit the protein expression of NF-κB and TGF-β1.

Keywords: TGP, NF-κB, TGF-β1, Protein expression, Immune hepatic fibrosis.

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Introduction

Immune Hepatic Fibrosis (IHF) refers to the liver injury caused by various reasons, making the disorder of body's immune system adjustment, characterized by generation and decomposition imbalance of Extracellular Matrix (ECM), resulting in a series of pathological changes and hepatic fibrosis, cirrhosis and may even causing hepatic encephalopathy, liver cancer and other complications [1,2]. At present, liver disease model are believed to perform in the order of 'acute- chronic-liver fibrosis-liver cirrhosis/liver cancer' sequence. Thus, it is particularly important to block the occurrence and development of liver fibrosis symptoms for prevention and treatment of liver fibrosis, cirrhosis and liver cancer [3].

The Total Glucosides of Paeony (TGP) is the effective component extracted from Radix Paeoniae Alba in traditional Chinese medicine. TGP mainly includes paeoniflorin, albiflorin, hydroxyl-paeoniflorin, etc. [4], and it has a strong inflammatory immune regulating function, which has important influence on the treatment of immune diseases [5,6]. Nuclear factor-κB (NF-κB) is an important nuclear transcription factor existing commonly in cells, and it is involved in the body's inflammatory response, immune response, apoptosis and other stress responses, existing in the cells of a variety of tissues. Transforming Growth Factor beta 1 (TGF-β1) [7] plays an important role in the pathogenesis of liver fibrosis. Thus it can be seen that it has important significance to study the changes of NF-κB and TGF-β1 by TGP.

Materials and Methods

Materials

Clean level male SD rats with the weight of (170 ± 20 g) were supplied by the animal center of the Nanjing Medical University. Dry powder formulation for TGP was offered by the Ning Po Lihua Pharmaceutical Co., Ltd, and before using, it would be diluted with saline and prepared into suspension. Porcine serum, taken from Nanjing meat processing factory, was preserved after centrifugal separation, filtration and sterilization. Nuclear Factor-κB (BNF-κB) antibody was provided by Kamaishu (Shanghai) ELISA biotechnology research (batch number 20161008). Transforming Growth Factor β1 (TGF-β1) antibody was provided by Shanghai Juli

Biological Technology Co., Ltd (Batch number 20162006). IgG was provided by Shanghai Jixing Biotechnology Co., Ltd (Batch number 20163009). TGL18M desktop high speed refrigerated centrifuge (Product from Yancheng Kate Experimental Equipment Co., Ltd), BM-37XB inverted biological microscope (Shanghai biaimu Optical Instrument Manufacturing Co., Ltd).

Establishment and treatment of animal models

The experiment was approved by the hospital ethics committee. 50 clean grade healthy male SD rats were chosen for 1 w of adjustment in the laboratory, 10 of which were randomly selected as normal control group (N group), and the rest received intraperitoneal injection of porcine serum 0.5 ml, 2 times a week for constant 15 w. The control group was injected with normal saline. The rats after modeling were randomly divided into the animal model type group (M group), low dose of TGP group (T1 group), medium dose group (T2 group), and high dose group (T3 group) with 10 rats in each group. After modeling for 6 w, T1-T3 groups were conducted with gavage by TGP. T1 group was given by low dose of TGP (50 mg/(kg•d)), T2 group was given by medium dose of TGP (100 mg/(kg•d)), and T3 group received low dose of TGP (150 mg/(kg•d)), N and M group were fed with the same amount of normal saline. All the rats were sacrificed after modeling for 15 w, and the liver tissue was fixed by 10% formalin to make light microscopy samples.

Tissue sections and light microscopy specimens making

The same position to the rat liver lobe was cut as specimens and placed in liquid PBS for full wash, and after fixed with 100 g/L neutral formalin, paraffin was used to embed before 4 μm serial sections. Then HE staining and VG staining were conducted, with semi quantitative standard to judge the changes of collagen fibers.

The detection of the expression of NF-κB and TGF-β1 through immunohistochemical staining method

Paraffin sections were dewaxed to water, 3% methanol hydrogen peroxide was adopted to block endogenous peroxidase with 0.01 mol•L^{-1} (PH 7.2) phosphate buffer washing for 5 min, and 0.01 mol•L^{-1} sodium citrate buffer for microwave repair, blocking non-specific peroxidase. NF-κB, TGF-β1 were diluted in 1:100 for incubation at 37°C for 1 h. Biotinylated second antibody (IgG) was incubated at 37°C for 30 min with DAB/H_2O_2 for coloring. PBS was used to replace the primary antibody as negative control.

Result judgment

According to the HAI scoring system, the degree of hyperplasia for fibrous tissue is divided into 0~4 scores [8]. 0 score means no fibrosis; 1 score refers to fibrous connective tissue gradually expanding in the sink area; 2 scores mean fibrosis appearing around the sink area, and the fibrous septa forming lobular structure; 3 scores refer to structure disorder of fibrous septa combined with lobular; 4 scores mean the fibrous connective tissues presents multiple diffuse hyperplasia in total lobule, and hepatic cirrhosis symptoms come out. 10 better visions were randomly taken in each slice and pathological image analysis system was adapted to measure collagen size in vision, and at the same time, the percentage of collagen area in the total area of the field was calculated.

Under light microscope, HE staining and VG staining results were observed. The positive staining state of NF-κB was the yellow or brown particles appeared on the cytoplasm and (or) the nucleus, and TGF-β1 positive staining referred to yellow or brown yellow particles come out on cytoplasm and (or) the cell membrane. The photographic field of microscope vision were analysed by the image analysis system.

Data analysis

All the data in the experiment were input into the Excel table for statistics and analysed by SPSS11.5 software package. $P<0.05$ meant the difference was statistically significant.

Result

Pathological staining state of liver tissue

According to the observation under microscope and analysis of the degree of hyperplasia of fibrous tissue in the normal group, model group, TGP treatment group through HAI scoring system, it was found that the hepatic lobules structure of liver tissue was normal in N group, and no fibrosis appeared in hepatocyte (0 score). In M group, inflammatory cells and necrotic cells increased in sink area, and the structure of liver interlobular presented permutation disorder, fibrous septal was thickened, pseudolobuli was formed, and there were continuous or interrupted annular collagen depositing in hepatic sinus (3~4 scores). After different doses of TGP functioning in model group, the structure of hepatic lobule was significantly improved, hepatic cell necrosis was improved significantly, the hyperplasia of fibrous tissue was reduced especially in T3, whose structure of hepatic lobule was basically intact without obvious hyperplasia of fibrous tissue, and there were few intervals in collagen fibers (1~2 scores). Table 1 summarized the percentage of each collagen field area in the total vision area, and results showed that fibrosis in the model group was significantly increased, and compared with model group, fibrosis state in TGP treatment group was significantly improved.

Table 1. *The percentage of collagen area in total field area (n=10).*

Group	Dosage mg/(kg·d)	Percentage
N group	-	1.98 ± 0.92
M group	-	12.32 ± 2.31*
T1 group	50	6.48 ± 1.76**
T2 group	100	4.92 ± 1.89**

T3 group	150	3.78 ± 2.14**

Note: M group compared with N group-*P<0.05; T1-T3 groups compared with M group-**P<0.05

Effects of TGP on expression of NF-κB protein in liver tissue

Only a little NF-κB protein was expressed in liver tissue of rats in normal group, and there was expression of NF-κB protein showing in the cytoplasm and on the nucleus in M group. Positive staining mainly distributed in the area of fibrosis and bile duct area, mostly coloring as yellow or brown. Compared with N group, the protein expression of M group increased significantly, and statistical analysis showed that the difference was statistically significant. NF-κB protein expression in T1-T3 groups was lower than that of M group, which was obvious in T3 group (P<0.05). It indicated that TGP could significantly inhibit protein expression of NF-κB in liver tissue of rats with immune hepatic fibrosis.

Effects of TGP on protein expression of TGF-β1 in liver tissue

TGF-β1 protein showed weak expression in normal liver tissues with very shallow color. Compared with N group, positive signal of TGF-β1 was enhanced in M group, and the positive staining distributed in the denatured liver cells, fibrosis area, hepatic area, sinusoid area and sink area with coloring mostly yellow or dark brown yellow. The protein expression of TGF-β1 in T1-T3 groups was lower than that of M group, especially in T3 group. It indicated that TGP could significantly inhibit protein expression of TGF-β1 in liver tissue of rats with immune hepatic fibrosis. The results are shown in Table 2.

Table 2. *Effect of TGP on protein expression of NF-κB and TGF-β1 in hepatic tissue of rats with hepatic fibrosis (n=10).*

Group	Dosage mg/ (kg·d)	NF-κB/OD	TGF-β1/OD
N group	-	1.58 ± 0.73	2.01 ± 0.56
M group	-	19.65 ± 2.53*	24.43 ± 2.32*
T1 group	50	12.68 ± 1.88**	15.35 ± 2.11**
T2 group	100	10.92 ± 2.66**	13.91 ± 2.27**
T3 group	150	7.36 ± 2.08**	10.55 ± 1.75**

NoteM group compared with N group-*P <0.05; T1-T3 groups compared with M group-**P<0.05.

Conclusion

Hepatic fibrosis is common in various liver diseases, which is a repair process after liver injury, as well as pathological features of chronic liver disease, and the pathological changes are abnormal hyperplasia in liver's connective tissue. At present in our country, the incidence of liver fibrosis is about 2% or so with an increasing trend [9]. Thus, it is particular important to study the pathogenesis of disease prevention and control method.

In the course of hepatic fibrosis, Hepatic Stellate Cells (HSC) and Transforming Growth Factor beta (TGF-β) accounts for an important position [7]. Various liver injury leads to the activation of HSC, making it continuously dissolve and proliferate, and migrate to the injured region, generating a large number of ECM protein (including collagen, elastic matrix proteins, glycoproteins and proteoglycans) [10]. With the ECM formation and decomposition ratio disorder, increasing formation and decreasing decomposition can also promote the further development of hepatic fibrosis [11]. And the activation of HSC can make the synthesis of TGF-β1, etc. factors contributing to liver fibrosis [12].

In this study, we used porcine serum for modeling [13], which lead to little overall injury to rats, showed obvious liver fibrosis through the pathological examination, lasted long model duration, and presented low rate of self-healing. NF-κB is a protein which has transcriptional activation function, widely exists in various tissues, and many molecules involved in the immune response and inflammation reaction will be subject to regulation of NF-κB protein. The study found that [14,15] NF-κB took part in the regulation of Hepatic Stellate Cells (HSC) activated. TGF-β1 is promoting fiber factor, which plays an important role in hepatic fibrosis.

The experiment was divided into normal group, model group and TGP treatment group. The results showed that comparing the degree of hyperplasia of liver fibrosis in the treatment group and the model group, with the dose increased, the degree of fiber decreased in treatment group. The protein expression of NF-κB and TGF-β1 in the model group was higher than the normal group, while the protein expression of NF-κB and TGF-β1 were lower than the model group, and the difference was statistically significant. The study found that there was a positive correlation relationship between the protein expression of NF-κB and TGF-β1, indicating the two has some relevance in the process of hepatic fibrosis. In addition, TGP can significantly improve the symptoms of liver fiber, reduce the fiber tissue hyperplasia, decrease protein expression of NF-κB and TGF-β1, explaining that the inhibition of NF-κB and TGF-β1 protein expression is most likely to be the action mechanism of TGP anti-fibrosis.

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