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Relationship between anti-fibrotic effect of Panax notoginseng saponins and serum cytokines in rat hepatic fibrosis

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ABSTRACT

The aim of this study was to investigate the relationship between anti-fibrotic effect of Panax notoginseng saponins (PNS) and serum cytokines in rat hepatic fibrosis. Hepatic fibrosis induced by carbon tetrachloride (CCl_4) was studied in animal models using SD rats. Liver index, serum alanine amino transferase (ALT), aspartate amino transferase (AST), transforming growth factor-β1 (TGF-β1), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured, respectively. Liver index and the degree of liver fibrosis were also determined. Our results showed that the levels of ALT, AST and liver index in PNS-treated group were markedly lower than those in model group. PNS therapy also significantly attenuated the degree of hepatic fibrosis, collagen area and collagen area percent in liver tissue. Furthermore, the levels of serum TGF-β1, TNF-α and IL-6 were strikingly reduced in PNS-treated group compared with model group while the production of IL-10 was up-regulated. These findings demonstrate that PNS has certain therapeutic effects on hepatic fibrosis probably by immunoregulating the imbalance between pro-fibrotic and anti-fibrotic cytokines.

Introduction

Hepatic fibrosis is a common pathological process of chronic liver injuries, regardless of etiology, and its progression leads to cirrhosis and liver cancer [1]. Despite extensive efforts, its etiology and pathogenesis remain unclear and effective therapies with limited side effects are still deficient [2]. PNS is the major active constituent of the isolated root of Panax notoginseng, a well known traditional Chinese medicine. We have reported that PNS has significant scavenging effects on oxygen free radicals and protective effects on liver injuries induced by CCl_4 [3,4]; however, its exact mechanisms remain unclear. Therefore, it is necessary to be further elucidated.

CCl_4-induced hepatic fibrosis is a well-established animal model to study the pathogenesis and therapy of chronic liver injury diseases. Zhang et al. have reported that several pro-fibrotic cytokines including TGF-β1, TNF-α and IL-6 play an important role in the initiation and perpetuation of CCl_4-induced liver fibrosis while IL-10 play an antifibrogenic role by counterbalancing their effects [5]. This study aimed to further investigate the effect of PNS on hepatic fibrosis induced by CCl_4 and its relationship with the expression of TGF-β1, TNF-α, IL-6 and IL-10.

Materials and methods

Reagents and animals. PNS was provided by Kunming Zhongxing Pharmaceutical Co., Ltd (purity > 98%, Yunnan, China). Colchicine was purchased from Xiamen Sanland Chemical Co., Ltd (purity > 99%, Fujian, China). CCl_4 was obtained from Chongqing Chemical Reagent Co., Ltd (Chongqing, China). Male SD rats weighing 150–180 g were purchased from the Experimental Animal Center of Third Military Medical University. All studies involving animals were approved by the Institutional Animal Care and Use Committee.

Induction of liver fibrosis and PNS treatment. Forty male SD rats were randomly divided into four groups: normal control group ($n = 9$), model group ($n = 11$), PNS-treated group ($n = 10$), colchicine-treated group ($n = 10$). Except normal control group, all rats were treated with subcutaneous injection of 40% CCl_4 (0.3 ml/kg, but 0.5 ml/kg for the first injection), mixed with vegetable oil, twice a week for 8 weeks. For the last two groups, PNS (130 mg/kg, dissolved in sterile normal saline, intraperitoneal injection, once daily) or colchicine treatment (50 μg/kg, dissolved in sterile normal saline, intraperitoneal injection, once daily as positive group) was initiated at the same day as CCl_4 administration and

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Table 1
Levels of liver index and serum AST, ALT in different treatment groups (mean ± SD).

Group	*n*	Liver index	ALT (U/L)	AST (U/L)
Normal	9	0.026 ± 0.004	114.50 ± 8.16	183.09 ± 26.70
Model	7	0.049 ± 0.009[b]	193.58 ± 24.35[b]	404.37 ± 68.29[b]
PNS	8	0.038 ± 0.005[b,d]	163.36 ± 19.91[b,c]	321.70 ± 50.94[b,c]
Colchicine	9	0.031 ± 0.004[a,d]	167.60 ± 21.66[b,c]	325.61 ± 52.83[b,c]

Significantly different: [a]$P < 0.05$, [b]$P < 0.01$ vs. normal control group; [c]$P < 0.05$, [d]$P < 0.01$ vs. model group.

continued till the end. The two drug doses were selected based on the previous study [4]. Simultaneously, normal control and model groups were intraperitoneally administered with the same volume of vehicle (sterile saline water) once daily. At the end of the 8-week experimental period, all animals were anesthetized with 3% chloral hydrate and dissected. Blood and liver were obtained for further analysis.

Measurement of serum AST and ALT. Serum AST and ALT levels were measured using on an automated analyzer of biochemistry (Hitachi 7170, Tokyo, Japan) according to the manufacturer's instructions.

Liver index calculation. Liver index was measured according to the formula: (rat liver weight/rat weight) × 100% [6].

Histopathology. Samples were obtained from the same liver lobe in all animals and fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin (HE) or Van Gieson (VG) solutions.

The degree of liver fibrosis was evaluated on HE stained sections according to the previous report [7]. The collagen content of the sections was also determined on VG stained sections by a computer image analysis system (CM2000B, Beijing University of Aeronautics & Astronautics, china). Five random fields were chosen in each section and the amount of total collagen was detected in the area stained by VG and expressed as percentage relative to the total area [8].

ELISA of TGF-β_1, TNF-α, IL-6 and IL-10. Cytokine levels in the serum samples were measured by a commercially available ELISA kit (Biosources, San Jose, CA, USA) according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed with the SPSS software system (SPSS for Windows, version 13.0; SPSS Inc, Chicago, IL). Parametric data were statistically analyzed by one-way ANOVA followed by post hoc tests when appropriate. Degree of hepatic fibrosis was analyzed by Kruskal–Wallis nonparametric test. Data were expressed as the means plus or minus SD. A significant difference was defined as $p < 0.05$.

Results

Animals

Irritability, aggression, and weight loss was present predominantly in rats of the model group. At the end of 8-week experimental period, No death was found in normal control group. All rats' death in other groups was as follows: 4 rats died in model group, 1 in colchicine-treated group, 2 in PNS-treated group.

Liver index and serum aminotransferases

Liver index in the normal control group was 0.026 ± 0.004. However, 8 weeks after the CCl_4 injection, the level of Liver index increased markedly. The increase was significantly attenuated by PNS or colchicine treatment ($P < 0.01$; Table 1).

We then measured serum aminotransferase activities in different experimental groups. The levels of serum AST, ALT were significantly increased in model group compared with those in normal control group. In contrast, PNS or colchicine treatment significantly suppressed up-regulations of these parameters induced by CCl_4 ($P < 0.05$; Table 1).

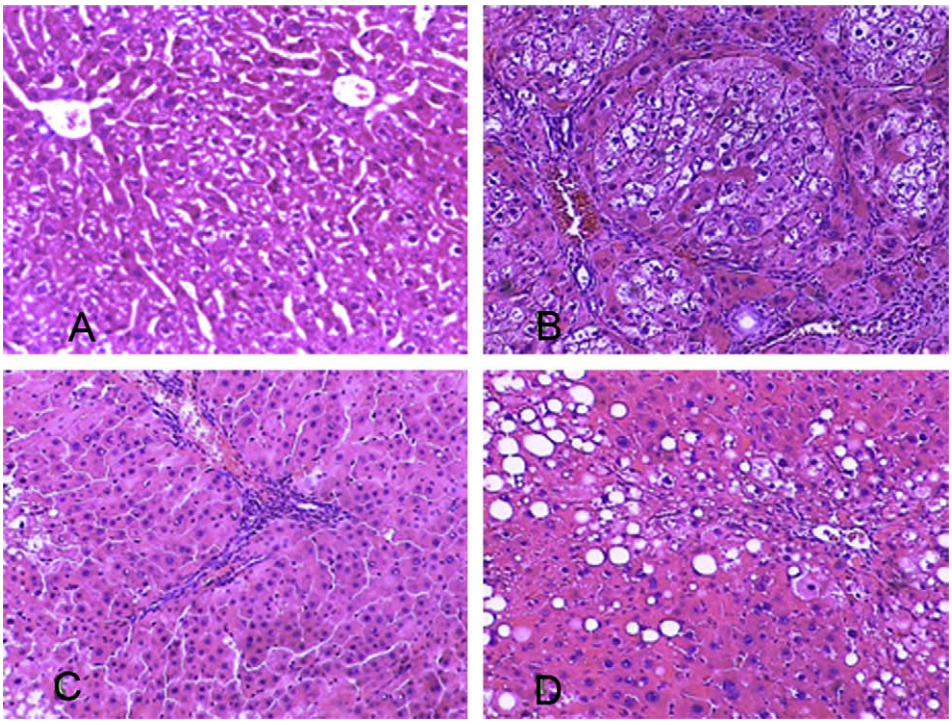

Fig. 1. The representative pathological changes of liver section taken from four experimental groups (A: normal; B: model; C: PNS; D: colchicine), HE stain, original magnification, ×100.