

BASIC RESEARCH

## Therapeutic effect of interleukin-10 on CCl<sub>4</sub>-induced hepatic fibrosis in rats

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inflammation, inhibiting expression of MMP-2 and TIMP-1 and promoting resolution of collagen types I and III.

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**Key words:** Rat; Hepatic fibrosis; Interleukin-10; Tumor necrosis factor; Matrix metalloproteinase; Collagen

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### Abstract

**AIM:** To study the therapeutic effect of exogenous interleukin-10 on CCl<sub>4</sub>-induced hepatic fibrosis in rats and its possible mechanisms.

**METHODS:** Forty-seven SD rats were randomly divided into control group (group N) and CCl<sub>4</sub>-induced hepatic fibrosis model group (group C). After CCl<sub>4</sub> was given for 9 wk, the model group was divided into three groups. Rats in group M were put to death immediately, rats in group T were treated with IL-10 for another three wk and then put to death, rats in group R recovered after three weeks and were then killed. The degree of hepatic fibrosis was measured by HE staining and histological activity index (HAI). Histological activity index (HAI), change of collagen types I and III were measured by Picosirius staining. The expression of TNF- $\alpha$ , MMP-2 and TIMP-1 in liver tissue was measured by S-P immunohistochemistry.

**RESULTS:** CCl<sub>4</sub>-induced experimental rat hepatic fibrosis model was established successfully. The degree of hepatic fibrosis was markedly lower in group T than in groups M and R, and there was no difference between the two groups. The expression of collagen types I and III was significantly suppressed in group T and was slightly suppressed in groups M and R. The positive levels of TNF- $\alpha$ , MMP-2 and TIMP-1 in group M increased significantly compared to those in group N ( $P < 0.01$ ). The positive signals decreased significantly in groups T and R ( $P < 0.01$ ), but positive score was significantly lower in group T than in group R ( $P < 0.01$ ).

**CONCLUSION:** Exogenous IL-10 can reverse CCl<sub>4</sub>-induced hepatic fibrosis in rats. IL-10 may exert its reversible effects on hepatic fibrosis by blocking CCl<sub>4</sub>-induced

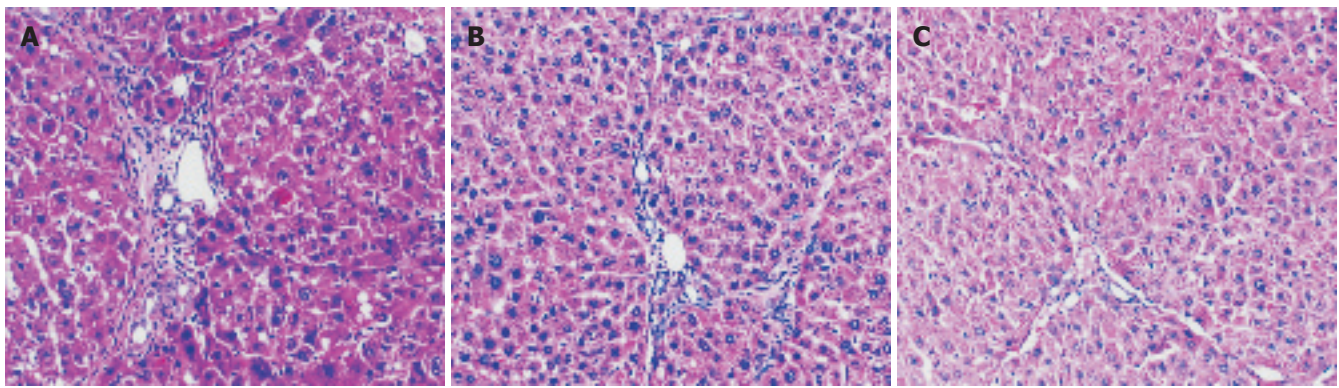
### INTRODUCTION

Hepatic fibrosis represents the final common pathological outcome for the majority of chronic liver insults. Its final stage is cirrhosis. Liver cirrhosis, the irreversible terminal stage of chronic liver disease, characterized by widespread fibrous scarring, is a major cause of morbidity and mortality worldwide, with no effective therapy. Regardless of causes, hepatic fibrosis involves abnormal accumulation of extracellular matrix (ECM) components, particularly collagens. Present data indicate that a specific potentially safe orally bioavailable and inexpensive antifibrotic agent is not yet available<sup>[1]</sup>. Interleukin-10 (IL-10) has anti-inflammatory and immunomodulatory effects and can down-regulate production of proinflammation cytokines, such as interleukin-1, interferon- $\gamma$  and interleukin-2 from T cells. Previous reports indicate that IL-10 might have antifibrogenic properties by downregulating profibrogenic cytokines, such as TGF- $\beta$ 1. Many studies indicate that IL-10 may become a new therapeutic target<sup>[2]</sup>. Therefore, the aim of this study was to evaluate its therapeutic effect on reversing well-established hepatic fibrosis after 9 wk of CCl<sub>4</sub> administration through different markers.

### MATERIALS AND METHODS

#### Materials

Forty-seven clean male Sprague-Dawley rats weighing 180-280 g were provided by Shanghai Experimental Animal Center. All the rats were bred under routine condition (room temperature of  $22 \pm 2^\circ\text{C}$ , humidity of  $55 \pm 5\%$ ) in a 12 h light/dark cycle with free access to water and food. The food was provided by BK Company (Shanghai,



**Figure 1** Effects of IL-10 on histology of CCl<sub>4</sub>-induced fibrotic rat liver after treated with CCl<sub>4</sub> for 9 wk (A), IL-10 for 3 wk (B) and spontaneous recovery for 3 wk (C).

China). IL-10 was purchased from Jingmei Biotechnology Company of Shenzhen. MMP-2 monoclonal antibody was purchased from NeoMarkers Company. TNF- $\alpha$ , TIMP-1 polyclonal antibodies and S-P immunohistochemical kit were obtained from Zhongshan Company of Beijing.

#### Preparation of rats

Forty-seven clean SD rats were randomly divided into control group (group N,  $n=6$ ) and CCl<sub>4</sub>-induced hepatic fibrosis model group (group C,  $n=41$ ). The rats of group N were injected intraperitoneally with saline (2 mL/kg) twice a week. After 9 and 12 wk, 3 rats of groups N were sacrificed and their livers were taken out. The rats of group C were injected intraperitoneally with 50 % CCl<sub>4</sub> dissolved in castor oil (2 mL/kg) twice a week for nine weeks. After injection group C was divided into three groups. Rats in group M were put to death immediately by the end of week 9, rats in group T were treated with IL-10 (4  $\mu$ g/kg) three times a week for three weeks and then put to death, rats in group R recovered after three weeks and were then killed.

#### Histopathological examination

Rats of groups N, M, T and R were sacrificed and their livers were taken out. The specimens were fixed in 10% formalin and embedded with paraffin. The sections stained with hematoxylin and eosin were evaluated by two pathologists. Histological activity index (HAI) was evaluated using a numerical system proposed by Knodell *et al*<sup>[3]</sup>.

#### Picrosirius staining and collagen measurement

The sections were deparaffinized with xylene and rehydrated with graded ethanol. After rinsing, the sections were washed 3 times with distilled water, stained in 0.1% Sirius red in saturated picric acid solution for 30 min, and put into ethanol for differentiation for 2 min. The sections were then rinsed once in phosphate-buffered saline and twice in water for 30 s each to remove any unbound dye. After drying for two hours, slides were mounted. Quantitative analysis of collagen types I and III was made with the Olympus-BX41 image analyzing system in 5 microscopic fields (40x magnification) of per section. The average of the 5 fields was calculated for assessment of the

degree of fibrosis in each case. All sections were examined by the same person. The liver tissue was distinguished from the background according to a difference in light density, which allowed the measurement of the total liver tissue area. Then the amount of connective tissue stained red was measured. Finally, the percentage of collagen on the section was calculated.

#### Immunohistochemistry

Rat liver tissues were sectioned at the thickness of 4  $\mu$ m. After deparaffinization with xylene and rehydration in graded ethanol, the sections were incubated in PBS containing 30 mL/L H<sub>2</sub>O<sub>2</sub> to remove endogenous peroxidase and in PBS containing 0.1 mol/L citrate to retrieve microwave antigens and then with normal goat serums to block the nonspecific binding sites. After incubation with rabbit anti-rat MMP-2(monoclonal antibody) as well as TNF- $\alpha$  and TIMP-1 polyclonal antibodies respectively, the sections were treated with instant S-P immunohistochemical reagents and then incubated in a buffer solution containing 3, 3-diaminobenzidine tetrahydrochloride (DAB) and H<sub>2</sub>O<sub>2</sub> to produce a brown reaction product, dehydrated and coverslipped. Microscopic examination of the sections was then performed as previously described<sup>[4]</sup>.

#### Statistical analysis

All data were expressed as mean  $\pm$  SE. The difference between groups was studied with SPSS11.0 by one-way ANOVA.  $P<0.05$  was considered statistically significant.

## RESULTS

#### Histological examination

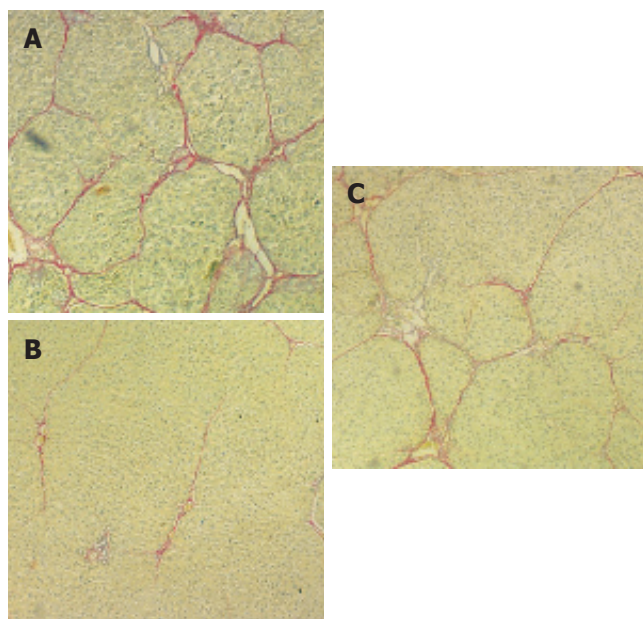
CCl<sub>4</sub>-induced experimental rat hepatic fibrosis model was established successfully. After treatment with CCl<sub>4</sub> for 9 wk (group M), the liver had severe pathological damages, such as hepatocyte degeneration and necrosis, mononuclear cells and neutrophil infiltration and collagen deposition. The collagen fibers began to extend with hepatic plate and formed intact fibrous septum and distorted tissue architecture, abnormal hepatic lobules could be observed occasionally (Figure 1A). The liver tissue in group N showed normal lobular architecture with central veins and radiating hepatic cords. The liver of group T showed that



Table 1 Grading and staging of rat liver histopathology

Group	Number	Grading of inflammation					Staging of fibrosis				
		0	1	2	3	4	0	1	2	3	4
N	6	6	0	0	0	0	6	0	0	0	0
M <sup>bf</sup>	9	0	0	9	0	0	0	0	0	0	9
R <sup>bdf</sup>	9	0	7	2	0	0	0	0	0	0	9
T <sup>bdfn</sup>	9	0	7	2	0	0	0	0	3	6	0

Grading of inflammation: <sup>b</sup> $P < 0.01$  vs group N; <sup>d</sup> $P < 0.01$  vs group M; staging of fibrosis: <sup>f</sup> $P < 0.01$  vs group N; <sup>h</sup> $P < 0.01$  vs group M; <sup>i</sup> $P < 0.01$  vs group R.

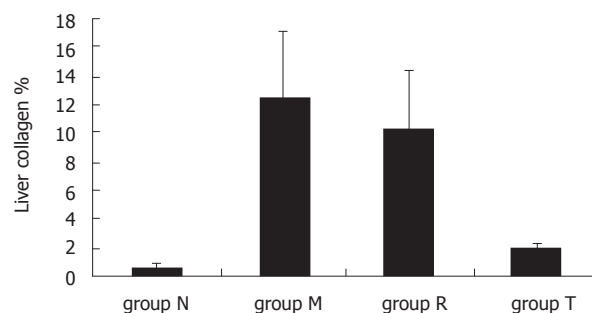


**Figure 2** Photomicrographs of liver tissue from rats after treatment with with CCL<sub>4</sub> (A), IL-10 for 3 wk (B) and spontaneous recovery for 3 wk (C) by Picrosirius staining (40x).

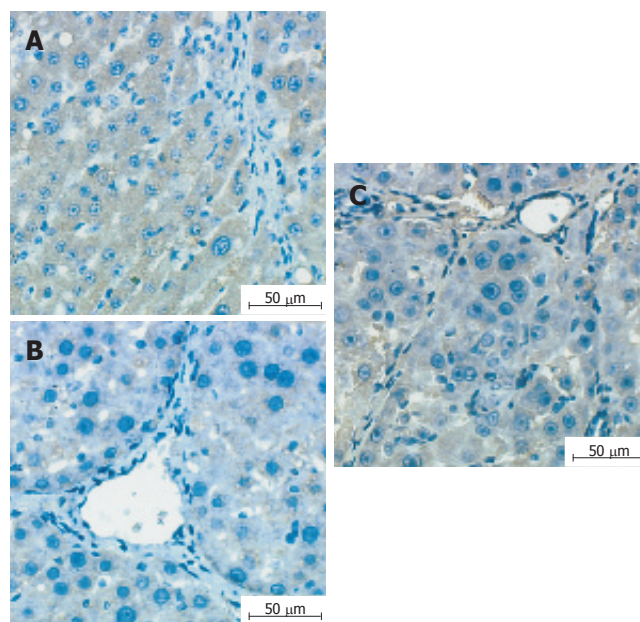
the degree of hepatocyte necrosis and degeneration was decreased markedly, and there were a few inflammatory cell infiltrates around central lobular veins, deposition of collagen fibers was relieved (Figure 1B). In addition, collagen fibers were changed slightly in group R (Figure 1C). According to HAI score, the degree of inflammation of liver decreased markedly in groups T and R compared to group M. The degree of hepatic fibrosis decreased markedly in group T compared to group M ( $P < 0.01$ ), but there was no difference between groups R and M ( $P > 0.05$ , Table 1).

### Change of collagen types I and III in liver

Collagen types I and III were stained intensely red with the Picrosirius procedure, while the non-collagen tissue was stained yellow. CCL<sub>4</sub> treatment for 9 wk induced a significant deposition of collagen types I and III, leading to severe fibrosis (Figure 2A). Little collagen was deposited around central vein in group N. After IL-10 treatment for 3 wk, the degree of hepatic fibrosis was markedly reduced, the area of collagen types I and III was significantly decreased (Figure 2B). After spontaneous recovery for 3 wk, obvious deposition of collagen types I and III was observed (Figure 2C).



**Figure 3** Percentage of collagen types I and III in fibrotic rats.



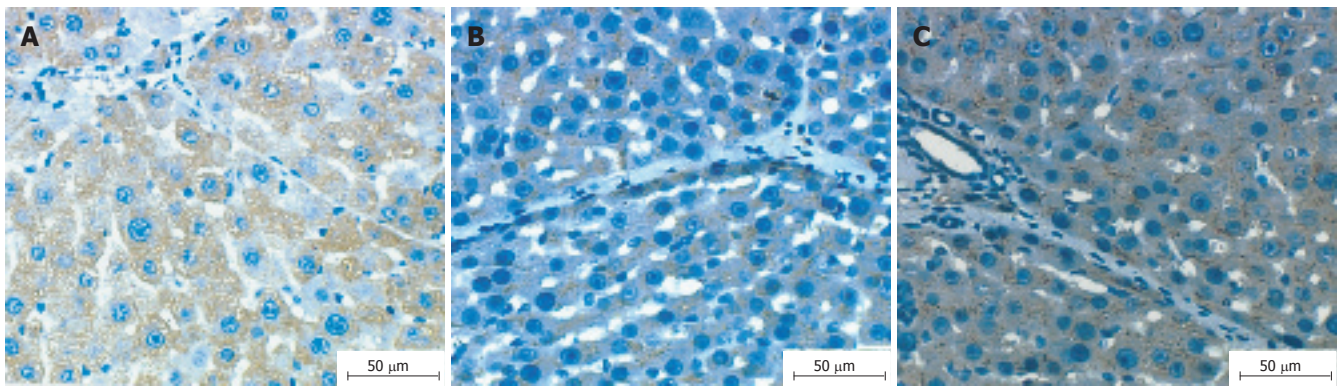
**Figure 4** Expression of MMP-2 protein in liver tissue of rats treated with CCL<sub>4</sub> for 9 wk (A), IL-10 for 3 wk (B) and spontaneous recovery for 3 wk (C).

The area of collagen types I and III increased from  $0.64 \pm 0.11\%$  of field area in group N to  $12.41 \pm 4.62\%$  of field area in group M ( $P < 0.01$ ), while the areas of collagen dramatically decreased to  $2.00 \pm 0.31\%$  in group T ( $P < 0.01$ , Figure 3). Although there was a trend toward lower values of collagen types I and III in group R ( $10.24 \pm 4.12\%$ ) compared to group M, the difference failed to reach any statistical significance ( $P > 0.05$ ).

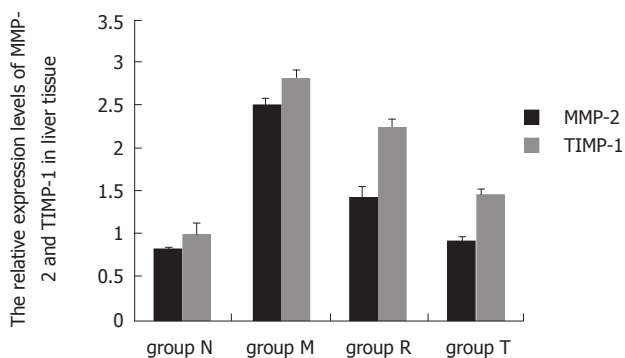
### Relative quantities of MMP-2 and TIMP-1 in the liver

Positive expression of MMP-2 was localized in endothelial and hepatic cells. Positive expression of TIMP-1 was localized in cytoplasm of hepatocytes and biliary epithelial cells, but not in nuclei. After treatment with IL-10, the distribution area of MMP-2 and TIMP-1 was smaller and the color was lighter (Figures 4 and 5).

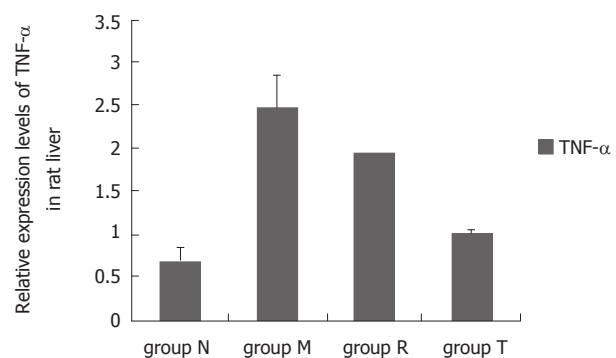
The expression of MMP-2 and TIMP-1 was markedly higher in group M than in group N ( $P < 0.05$ ). The expression of these two cytokines in group T and R was significantly reduced compared to those in group M ( $P < 0.05$ ). However, the levels of MMP-2 and TIMP-1 decreased markedly in group T compared to those in group R ( $P < 0.05$ ). Tissue concentrations of MMP-2 and



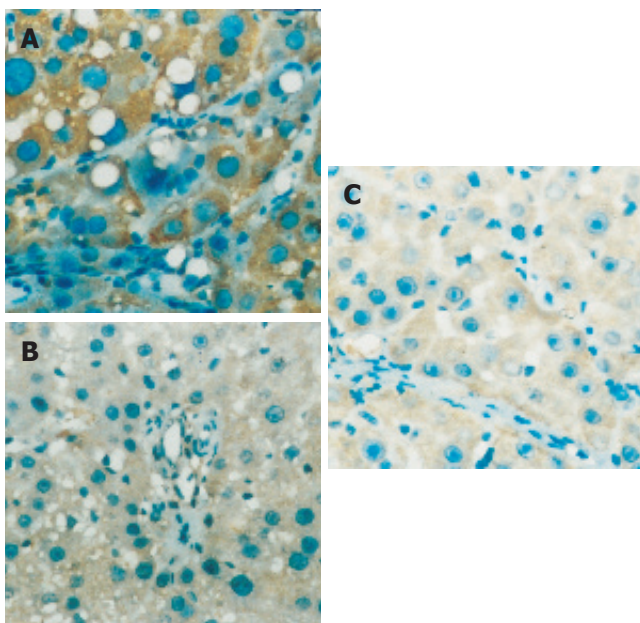
**Figure 5** Expression of TIMP-1 protein in liver tissue of rats treated with CCL<sub>4</sub> for 9 wk (A), IL-10 for 3 wk (B) and spontaneous recovery for 3 wk (C).



**Figure 6** Relative expression levels of MMP-2 and TIMP-1 in liver of different groups.



**Figure 8** Relative expression of TNF-α in rat liver of different groups.



**Figure 7** Expression of TNF-α protein in liver tissue of rats treated with CCL<sub>4</sub> for 9 wk (A), IL-10 for 3 wk (B) and spontaneous recovery for 3 wk (C).

TIMP-1 were decreased after IL-10 treatment for 3 wk (Figure 6).

#### Relative quantity of TNF-α in liver

Positive expression of TNF-α was localized in most he-

patic cells. After treatment with IL-10, the distribution area was smaller and the color was lighter (Figure 7).

The expression of TNF-α was markedly higher in group M than in group N ( $P < 0.05$ ). The expression of this cytokine in group T was significantly reduced compared to group M ( $P < 0.01$ ), but the level of TNF-α in group R was lower than that in group M but higher than that in group T ( $P < 0.01$ , Figure 8).

## DISCUSSION

Hepatic fibrosis is a progressive pathological process involving multi-cellular and molecular events that ultimately lead to deposition of excess matrix proteins in the extracellular space. It is generally accepted that hepatic stellate cells (HSCs) are central to the process of fibrosis as the major source of ECM components<sup>[5-10]</sup>. Advanced fibrosis and cirrhosis are generally considered to be irreversible conditions even after removal of the injurious agent. However, data from the histological assessment of biopsy tissue from patients with liver fibrosis complicating chronic viral infection after successful treatment and from animal models of fibrosis indicate that recovery with remodeling of the excess collagens is possible<sup>[11-15]</sup>, but current therapies targeting at arresting or reversing hepatic fibrosis are largely ineffective and have unacceptable side effects in long-term therapy.

IL-10, initially discovered in 1989, is a cytokine synthesis inhibitory factor for T lymphocytes<sup>[16]</sup>. IL-10 is produced by other cells of the immune system including



the liver. Within the liver, production of IL-10 has been documented within hepatocytes, sinusoidal cells, kupffer cells, stellate cells and liver-associated lymphocytes<sup>[17]</sup>. It was reported that endogenous IL-10 can decrease intrahepatic inflammatory response and fibrosis in several models of liver injury<sup>[18, 19]</sup>. It is well known that HSCs are the principal cells involved in hepatic fibrosis. Our previous studies indicate that exogenous IL-10 down regulates collagen type I in cultured HSCs and up regulates metalloproteinase gene expression *in vitro*<sup>[20, 21]</sup>. IL-10 may promote apoptosis of HSCs by up-regulating the expression of FasL and Bax and down-regulating the expression of Bcl-2 *in vitro*<sup>[22]</sup>. It also exerts antifibrogenic effect by down regulating profibrogenic cytokines such as TGF- $\beta$ 1 and TNF- $\alpha$ <sup>[23, 24]</sup>. All these studies indicate that IL-10 might become a new therapeutic target. In this study, exogenous IL-10 was used to treat well-established hepatic fibrosis after 9 wk administration of CCL<sub>4</sub>. Liver biopsy, the gold-standard method for detecting changes in liver fibrosis, showed that advanced fibrosis or cirrhosis was established after 9 wk administration of CCL<sub>4</sub>, most of the fibrotic septa were resolved, and only small fibrotic fragments could be found after 3 wk of IL-10 treatment. The results indicated that exogenous IL-10 had therapeutic effect on advanced fibrosis. Predominant collagens in fibrotic liver were collagen types I and III. There were massive depositions of collagen types I and III in the peak of fibrosis, while the areas of collagen types I and III were significantly decreased 3 wk after IL-10 treatment. Matrix degradation may occur predominantly as a consequence of the action of a family of enzymes called matrix metalloproteinases (MMPs), and the expression of these enzymes is inhibited by a family of TIMPs<sup>[25-27]</sup>. To explore the reason why IL-10 results in a significant reduction of hepatic fibrosis, we investigated the effect of IL-10 on expression of MMP-2 and TIMP-1 by immunohistochemistry. The data showed that in the peak of fibrosis, the expression levels of MMP-2 and TIMP-1 were significantly increased. After 3 wk IL-10 treatment, the expression levels of MMP-2 and TIMP-1 were significantly decreased, indicating that collagen types I and III are associated with the decrease of MMP-2 and TIMP-1 levels.

IL-10 is a pleiotropic cytokine, which has anti-inflammatory inhibitory action on the immune response under various stimuli. TNF- $\alpha$  is a pro-inflammation cytokine and a major endogenous mediator of hepatotoxicity<sup>[28]</sup>. TNF- $\alpha$  is expressed in chronic liver injuries by both infiltrating inflammatory cells and hepatocytes and plays an important role in tissue damage<sup>[29]</sup>. In this study, the results of HE staining showed that IL-10 could suppress inflammation induced by CCL<sub>4</sub>. TNF- $\alpha$  was significantly increased 9 wk after initial CCL<sub>4</sub> treatment, but 3 wk treatment with exogenous IL-10 markedly suppressed the expression of TNF- $\alpha$  in liver, suggesting that IL-10 exerts its inhibitory effect on hepatic fibrosis by blocking the release of inflammatory mediators such as TNF- $\alpha$ , which may consequently suppress HSC activation leading to hepatic fibrosis. A similar result has been reported by Nakamuta *et al*<sup>[30]</sup>.

Issa *et al*<sup>[31]</sup> studied a model of cirrhosis to determine

the mechanisms mediating and limiting spontaneous recovery, and found that micronodular cirrhosis undergoes remodeling to macronodular cirrhosis. Lee *et al*<sup>[32]</sup> reported that hepatic fibrosis can reverse gradually<sup>[32]</sup>. In this study, livers were harvested from rats for spontaneous recovery from hepatic fibrosis induced by CCL<sub>4</sub>. Histology of liver sections indicated that advanced septal fibrosis observed at peak fibrosis was not remodeled. On the other hand, the degree of inflammation of liver decreased markedly after spontaneous recovery and the expression levels of TNF- $\alpha$ , MMP-2 and TIMP-1 decreased, suggesting that inflammation is decreased, but fibrosis is not significantly changed after removal of CCL<sub>4</sub>. The mechanisms need to be elucidated.

In conclusion, the therapeutic effect of IL-10 on hepatic fibrosis is not only related with removal of deposited collagen and expression levels of MMP-2 and TIMP-1, but also related with the degree of inflammation.

## REFERENCES

- 1 **Rockey DC**. Antifibrotic therapy in chronic liver disease. *Clin Gastroenterol Hepatol* 2005; **3**: 95-107
- 2 **Asadullah K**, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. *Pharmacol Rev* 2003; **55**: 241-269
- 3 **Knodell RG**, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435
- 4 **Huang YH**, Zhang LJ, Zheng WD, Shi MN, Chen ZX, Wang XZ. Effects of Interleukin-10 on Expression of Transforming Growth factor- $\beta$ 1 and its Receptors in Fibrotic Rat Liver. *Fujian Yikedaxue Xuebao* 2005; **39**: 277-280
- 5 **Albanis E**, Friedman SL. Hepatic fibrosis. Pathogenesis and principles of therapy. *Clin Liver Dis* 2001; **5**: 315-334, v-vi
- 6 **Li D**, Friedman SL. Liver fibrogenesis and the role of hepatic stellate cells: new insights and prospects for therapy. *J Gastroenterol Hepatol* 1999; **14**: 618-633
- 7 **Benyon RC**, Iredale JP. Is liver fibrosis reversible? *Gut* 2000; **46**: 443-446
- 8 **Murphy FR**, Issa R, Zhou X, Ratnarajah S, Nagase H, Arthur MJ, Benyon C, Iredale JP. Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition: implications for reversibility of liver fibrosis. *J Biol Chem* 2002; **277**: 11069-11076
- 9 **Rockey DC**. The cell and molecular biology of hepatic fibrogenesis. Clinical and therapeutic implications. *Clin Liver Dis* 2000; **4**: 319-355
- 10 **Du WD**, Zhang YE, Zhai WR, Zhou XM. Dynamic changes of type I,III and IV collagen synthesis and distribution of collagen-producing cells in carbon tetrachloride-induced rat liver fibrosis. *World J Gastroenterol* 1999; **5**: 397-403
- 11 **Arif A**, Levine RA, Sanderson SO, Bank L, Velu RP, Shah A, Mahl TC, Gregory DH. Regression of fibrosis in chronic hepatitis C after therapy with interferon and ribavirin. *Dig Dis Sci* 2003; **48**: 1425-1430
- 12 **Pol S**, Carnot F, Nalpas B, Lagneau JL, Fontaine H, Serpaggi J, Serfaty L, Bedossa P, Brechot C. Reversibility of hepatitis C virus-related cirrhosis. *Hum Pathol* 2004; **35**: 107-112
- 13 **Malekzadeh R**, Mohamadnejad M, Rakhshani N, Nasseri-Moghaddam S, Merat S, Tavangar SM, Sohrabpour AA. Reversibility of cirrhosis in chronic hepatitis B. *Clin Gastroenterol Hepatol* 2004; **2**: 344-347
- 14 **Luo YJ**, Yu JP, Shi ZH, Wang L. Ginkgo biloba extract reverses CCl<sub>4</sub>-induced liver fibrosis in rats. *World J Gastroenterol* 2004; **10**: 1037-1042
- 15 **Xu JW**, Gong J, Chang XM, Luo JY, Dong L, Hao ZM, Jia A,

- Xu GP. Estrogen reduces CCL4- induced liver fibrosis in rats. *World J Gastroenterol* 2002; **8**: 883-887
- 16 **Fiorentino DF**, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989; **170**: 2081-2095
  - 17 **Le Moine O**, Louis H, Sermon F, Goldman M, Deviere J. Interleukin-10 and liver diseases. *Acta Gastroenterol Belg* 1999; **62**: 1-8
  - 18 **Thompson K**, Maltby J, Fallowfield J, McAulay M, Millward-Sadler H, Sheron N. Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. *Hepatology* 1998; **28**: 1597-1606
  - 19 **Louis H**, Van Laethem JL, Wu W, Quertinmont E, Degraef C, Van den Berg K, Demols A, Goldman M, Le Moine O, Geerts A, Deviere J. Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. *Hepatology* 1998; **28**: 1607-1615
  - 20 **Zheng WD**, Zhang LJ, Shi MN, Chen ZX, Chen YX, Huang YH, Wang XZ. Expression of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 in hepatic stellate cells during rat hepatic fibrosis and its intervention by IL-10. *World J Gastroenterol* 2005; **11**: 1753-1758
  - 21 **Zhang LJ**, Chen YX, Chen ZX, Huang YH, Yu JP, Wang XZ. Effect of interleukin-10 and platelet-derived growth factor on expressions of matrix metalloproteinases-2 and tissue inhibitor of metalloproteinases-1 in rat fibrotic liver and cultured hepatic stellate cells. *World J Gastroenterol* 2004; **10**: 2574-2579
  - 22 **Wang XZ**, Zhang SJ, Chen YX, Chen ZX, Huang YH, Zhang LJ. Effects of platelet-derived growth factor and interleukin-10 on Fas/Fas-ligand and Bcl-2/Bax mRNA expression in rat hepatic stellate cells in vitro. *World J Gastroenterol* 2004; **10**: 2706-2710
  - 23 **Zhang LJ**, Yu JP, Li D, Huang YH, Chen ZX, Wang XZ. Effects of cytokines on carbon tetrachloride-induced hepatic fibrogenesis in rats. *World J Gastroenterol* 2004; **10**: 77-81
  - 24 **Kovalovich K**, DeAngelis RA, Li W, Furth EE, Ciliberto G, Taub R. Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. *Hepatology* 2000; **31**: 149-159
  - 25 **Okazaki I**, Watanabe T, Hozawa S, Arai M, Maruyama K. Molecular mechanism of the reversibility of hepatic fibrosis: with special reference to the role of matrix metalloproteinases. *J Gastroenterol Hepatol* 2000; **15 Suppl**: D26-D32
  - 26 **Wang JY**, Guo JS, Yang CQ. Expression of exogenous rat collagenase in vitro and in a rat model of liver fibrosis. *World J Gastroenterol* 2002; **8**: 901-907
  - 27 **Wang LT**, Zhang B, Chen JJ. Effect of anti-fibrosis compound on collagen expression of hepatic cells in experimental liver fibrosis of rats. *World J Gastroenterol* 2000; **6**: 877-880
  - 28 **Louis H**, Le Moine O, Goldman M, Deviere J. Modulation of liver injury by interleukin-10. *Acta Gastroenterol Belg* 2003; **66**: 7-14
  - 29 **Hernandez-Munoz I**, de la Torre P, Sanchez-Alcazar JA, Garcia I, Santiago E, Munoz-Yague MT, Solis-Herruzo JA. Tumor necrosis factor alpha inhibits collagen alpha 1(I) gene expression in rat hepatic stellate cells through a G protein. *Gastroenterology* 1997; **113**: 625-640
  - 30 **Nakamuta M**, Ohta S, Tada S, Tsuruta S, Sugimoto R, Kotoh K, Kato M, Nakashima Y, Enjoji M, Nawata H. Dimethyl sulfoxide inhibits dimethylnitrosamine-induced hepatic fibrosis in rats. *Int J Mol Med* 2001; **8**: 553-560
  - 31 **Issa R**, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, Sands E, Suliman I, Trim N, Knorr A, Arthur MJ, Benyon RC, Iredale JP. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* 2004; **126**: 1795-1808
  - 32 **Lee HS**, Huang GT, Chen CH, Chiou LL, Lee CC, Yang PM, Chen DS, Sheu JC. Less reversal of liver fibrosis after prolonged carbon tetrachloride injection. *Hepatogastroenterology* 2001; **48**: 1312-1315

S- Editor Wang J L- Editor Wang XL E- Editor Ma WH