Effects of pentoxifylline on the hepatic content of TGF-β1 and collagen in Schistosomiasis japonica mice with liver fibrosis

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INTRODUCTION
Liver fibrosis is the main reason for portal hypertension and hemorrhagic of upper digestive tract in schistosomiasis and therefor the main reason for the mortality of schistosomiasis. The basic pathological changes of liver fibrosis are the disturbance and degredation of extracellular matrix (ECM), which causes accumulation of ECM in the liver[12]. Within the major components of ECM, type I and type III collagen constitute more than 95 % of the total content of increased collagen in liver fibrosis[3-5]. It is well known that fibrosis is reversible whereas cirrhosis is irreversible, so it is important to prevent fibrosis progressing to cirrhosis[6]. However, there is no ideal antifibrosis drug to date. Recent researches found that PTX has antifibrosis function[8,9], while its effects on hepatic fibrosis of schistosomiasis japonica are still unknown. Since the main pathological characteristic of schistosomiasis japonica is the deposition of type I and III collagen and TGFβ1 has very important influence on the fibrosis development, it is considered the key cytokine to accelerate cirrhotic procession[10-15]. We studied the effects of PTX on the expression of collagen I and III and TGFβ1 in mice with schistosomiasis japonica and intended to evaluate the roles of PTX in hepatic fibrosis.

MATERIALS AND METHODS

Materials
Forty female Qunming mice, weighted 16-20 g and aged 4-6 w, provided by Experimental Animal Center of Tongji Medical College, were infected with 25 cercaria of schistosome japonica (provided by Wuhan Institute of Schistosomiasis Prophylactic and Therapy) and fed for 2 weeks and then divided randomly and equally into 4 groups: one group as control without any treatment, other three were treated with Praziquantel 500 mg/(kg·d) for 2 d, high dose PTX 360 mg/(kg·d) for 8 wk, and low dose PTX 180 mg/(kg·d) for 8 wk respectively. Immunohistochemical technique and multimedia color pathographic analysis system were applied to observe the content change of hepatic TGF-β1, type I and type III collagen in schistosomiasis japonica mice with liver fibrosis before and after PTX treatment.

RESULTS: Effects of PTX on the content change of hepatic TGF-β1, type I and type III collagen in schistosomiasis japonica mice with liver fibrosis were related to the dosage of PTX, high dose PTX treated group could significantly reduce the content of TGF-β1 (0.709±0.111), type I (0.644±0.108) and type III (0.654±0.152) collagen compared with those of control group (0.883±0.140, 0.771±0.156, 0.822±0.129) with statistical significance (P<0.05). Low dose PTX could also reduce the hepatic content of TGF-β1 (0.752±0.152), type I (0.733±0.117) and type III (0.788±0.147) collagen, but without statistical significance (P>0.05). Both high dose and low dose PTX groups have significant differences on the content of TGF-β1, type I and type III collagen (P<0.05, P<0.05, P<0.01, respectively).

CONCLUSION: High dose of PTX treatment could reduce the content of hepatic TGF-β1, type I and type III collagen significantly in schistosomiasis japonica mice with liver fibrosis, and thus plays its role of antifibrosis.

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sections were then incubated with Diaminobenzidine (DAB), counterstained and prepared for microscopic examination.

**Results analysis**

The sections were analyzed with MPZAS-500 multimedia color pathological graph analyzing system. The average integral light density (ILD) of positive staining in each section was obtained and presented as \( \pm x \). Results were then analyzed with student \( t \) test.

**RESULTS**

**Effects of PTX on TGF-\( \beta \)1 expression**

The contents of TGF\( \beta \)1 in praziqunatel group, high dose PTX group and low dose PTX group decrease by 44.62 %, 19.71 %, 14.84 % respectively compared with control group. The difference between praziqunatel group and control group is very significant (\( P<0.01 \)). The effect of PTX on TGF\( \beta \)1 content is dose related and there is significant difference on TGF\( \beta \)1 contents between high and low dose groups. The TGF\( \beta \)1 content in high dose PTX group is significantly (\( P<0.05 \)) different from that of control group while no significant difference between low dose PTX group and control group. Both high and low dose PTX groups have significant difference on TGF\( \beta \)1 contents between praziqunatel group and themselves. The results are shown in Table 1.

**Table 1** Content of TGF-\( \beta \)1, collagen I and III in liver of each treated group and control group (x\( \pm x \), ILD, n=10)

| Group   | TGF-\( \beta \)1 | Collagen I | Collagen III |
|---------|-----------------|------------|--------------|
| Control | 0.883±0.140     | 0.771±0.156 | 0.822±0.129  |
| Praziquentel | 0.489±0.105  | 0.996±0.103 | 0.613±0.116  |
| High dose PTX | 0.709±0.111** | 0.644±0.108** | 0.654±0.152** |
| Low dose PTX | 0.752±0.152** | 0.733±0.117** | 0.788±0.147** |

\( * P<0.01 \), vs control group; \( * P<0.05 \), vs control group; \( P>0.05 \), vs control group; \( P<0.01 \), vs praziqunatel group; \( P<0.05 \), vs praziqunatel group; \( P<0.01 \), vs high dose PTX group.

**Effects of PTX on collagen I expression**

The contents of collagen I in praziqunatel group, high dose PTX group and low dose PTX group decrease by 22.70 %, 16.47 %, 4.93 % respectively compared with control group. The difference between praziqunatel group and control group is very significant (\( P<0.01 \)). The effect of PTX on collagen I content is dose related and there is significant difference on collagen I contents between high and low dose groups. The collagen I content in high dose PTX group is significantly (\( P<0.05 \)) different from that of control group while no significant difference between low dose PTX group and control group. Both high and low dose PTX groups have significant difference on collagen I contents between praziqunatel group and themselves. The results are shown in Table 1.

**Effects of PTX on collagen III expression**

The contents of collagen III in praziqunatel group, high dose PTX group and low dose PTX group decrease by 25.43 %, 20.44 %, 4.14 % respectively compared with control group. The difference between praziqunatel group and control group is very significant (\( P<0.01 \)). The effect of PTX on collagen III content is dose related and there is significant difference on collagen III contents between high and low dose groups (\( P<0.01 \)). The collagen III content in high dose PTX group is significantly (\( P<0.05 \)) different from that of control group while no significant difference between low dose PTX group and control group (\( P>0.05 \)). Compared with praziqunatel group, high dose PTX group has no difference on collagen III contents (\( P>0.05 \)), whereas low dose PTX group has significant difference (\( P<0.01 \)). The results are shown in Table 1.

**DISCUSSION**

PTX is a trimethylated xanthine derivative product. As an inhibitor of phosphodiesterase, it can induce the increase of intracellular cAMP, dilation of the blood vessels and smooth muscles, ameliorating the microcirculation. It has been used to improve the peripheral blood vessel disease for many years\(^{[16,17]}\). Recently, PTX has been found to have antifibrosis effect. In vitro studies show that PTX can inhibit the proliferation of myofibroblast from hepatitis patients and depress the synthesis of collagen. Treatment with PTX in early stage can alleviate the hepatic lesion and inflammatory reaction\(^{[18]}\). In animal hepatic fibrosis models, PTX also has anti-fibrosis effect. It has been reported that treated with PTX prior to the inducing of hepatic fibrosis with CCL\(_{4}\)-acetone can alleviate the proliferation of hepatic stellate cell (HSC), and previous treatment with PTX decelerate the differentiation of HSC in mouse with hepatic fibrosis induce by bile duct ligation\(^{[19]}\). It was reported that previous treatment with PTX could improve the regeneration and function of liver after partial hepatectomy in mice with hepatic fibrosis and alleviate the hepatic fibrosis.

But there is no report on the effects of PTX on schistosomatic hepatic fibrosis\(^{[20]}\). The fibrosis in schistosomatic has its special characteristics against those caused by hepatic cell lesion or bile duct obstruction. Therefore, the effects of PTX in the schistosomatic hepatic fibrosis should be explored.

Hepatic stellate cell (HSC) plays a pivotal role in the fiber synthesis and degradation. The activation of HSC is mediated by various cytokines and reactive oxygen species released from the damaged hepatocytes and activated Kupffer cells\(^{[21-26]}\). HSC can release TGF\( \beta \)1 by autocrine\(^{[27,28]}\) and TGF\( \beta \)1 has been proved to be a strong mitogen to HSC. This autocrine effect is upgraded when HSC has been activated. TGF\( \beta \)1 depresses the regeneration of hepatic cells, activates and promotes HSC to synthesize extracellular matrix such as collagen, fibrinogen proteinopolysarccide, promotes the synthesis of TIMP and inhibits the synthesis of MMPs\(^{[29-37]}\).

We established a mouse hepatic fibrosis model induced by cercaria of schistosomiasis japonica infection and studied the effect of PTX on the fibrosis development in the early stage. We found that PTX could inhibit the development of fibrosis in this model significantly. The quantitative immunohistochemical evaluation of TGF\( \beta \)1, type I and III collagens shows that, high dose of PTX can reduce the content of TGF\( \beta \)1, type I and III collagens in hepatic tissue of mice with schistosomatic hepatic fibrosis. Its capability to reduce the hepatic content of type III collagen is similar to praziqunatel (\( P<0.05 \)) and its effects on TGF\( \beta \)1 and type I collagen are weaker than praziqunatel. Compared with the control group, low dose of PTX can also reduce the contents of TGF\( \beta \)1, type I and III collagens but the effects have no statistical significance.

The results indicate that PTX treatment in the early stage inhibits the development of schistosomatic hepatic fibrosis by reducing the content of TGF\( \beta \)1, type I and III collagens.

**REFERENCES**

1. Qing JP, Jiand MD. Phenotype and regulation of hepatic stellate cell and liver fibrosis. Shiji Meihuan Xiuhaou Zazhi 2001; 9: 801-904
2. Dai WJ, Jiang HC. Advances in gene therapy of liver cirrhosis: a review. World J Gastroenterol 2003; 7: 1-8
Wang GQ, Lu HQ, Wang H, Kong XT, Zhong RQ, Huang C, Gao F. Effects of Decorin on collagen of hepatic stellate cells. Xin Xiaohuabingxue Zazhi 2001; 9: 1395-1398

Wang JY, Guo JS, Yang CQ. Expression of exogenous rat collagen gene in vitro and in a rat model of liver fibrosis. World J Gastroenterol 2002; 8: 903-907

Zhang YT, Chang XM, Li X, Li HL. Effects of spironolactone on expression of type I/III collagen proteins in rat hepatic fibrosis. Xin Xiaohuabingxue Zazhi 2001; 9: 1120-1124

Jiang SL, Yao XX, Sun YF. Therapy of liver fibrosis. Shijie Huaren Xiaohua Zazhi 2000; 8: 684-686

Okazaki I, Kanzler S, Raetsch C, Keio J Med 2000; 30: 181-196

Wang GQ, Jiang SL, Wang JY, Gao F. Effects of Decorin on collagen of hepatic stellate cells. Okazaki I, Kanzler S, Raetsch C, Keio J Med 2000; 30: 181-196

Tsuakamoto H. Cytokine regulation of hepatic stellate cells in liver fibrosis. Alcohol Clin Exp Res 1999; 23: 911-916

Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. Gastroenterology 2000; 35: 665-672

Reeves HL, Friedman SL. Activation of hepatic stellate cells—a key issue in liver fibrosis. Front Biosci 2002; 7: D330-D366

Devereux D, Friedman SL. Activation of hepatic stellate cells—a key issue in liver fibrosis. Front Biosci 2002; 7: D330-D366

Wu J, Zern MA. Hepatic stellate cells: a target for the treatment of liver fibrosis. Gastroenterology 2000; 35: 665-672

Beljaars L, Meijer JK, Poelstra K. Targeting hepatic stellate cells for cell-specific treatment of liver fibrosis. Front Biosci 2002; 7: e214-e222

Liu T, Hu JH, Cai Q, Ji YP. The signal transducing molecular in HSC. Shijie Huaren Xiaohua Zazhi 2001; 9: 805-807

Wang GC, Zhang JS. Activated in vivo signal transduction of HSC. Xin Xiaohuabingxue Zazhi 2001; 9: 1056-1060

Dooley S, Delvoux B, Streckert M, Bonzel L, Stopa M, Ten Dijke P, Gresner AM. Transforming growth factor beta signal transduction in hepatic stellate cells via Smad2/3 phosphorylation, a pathway that is abrogated during in vitro progression to myofibroblasts. TGFbeta signal transduction during transdifferentiation of hepatic stellate cells. FEBS Lett 2003; 502: 1-3

Yata Y, Gobals P, Koteliansky V, Rockey DC. Dose-dependent inhibition of hepatic fibrosis in mice by a TGF-beta soluble receptor: implications for antifibrotic therapy. Hepatology 2002; 35: 1022-1030

Tahashi Y, Matsuzaki K, Date M, Yoshida K, Furukawa F, Sugano Y, Matsuishi M, Himeno Y, Inagaki Y, Inoue K. Differential regulation of TGF-beta signal in hepatic stellate cells between acute and chronic liver injury. Hepatology 2002; 35: 49-61

Okuno M, Akita K, Moriaki H, Kawada N, Ikeda K, Kameda K, Suzuki Y, Kojima S. Prevention of rat hepatic fibrosis by the protease inhibitor, camostat mesilate, via reduced generation of matrix metalloproteinase-2 activation in human hepatic stellate cells. J Gastroenterol Hepatology 2000; 15: 875-881

Rees LF, Souza SO, Arana-Pino A, Pelajo-Machado M, Pereira MJ, Lenzi HL, Conceicao MJ, Takiya CM. Quantitative analysis of transforming growth factor beta1 mRNA in patients with alcoholic liver disease. World J Gastroenterol 2002; 8: 379-381

Moser M, Pereira MJ, Lenzi HL, Conceicao MJ, Takiya CM. Quantitative analysis of transforming growth factor beta1 mRNA in patients with alcoholic liver disease. Adv Anat Embryol Cell Biol 2001; 161: 1-151

Du WD, Zhu W, 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

Biselli DM. Chronic liver injury, TGF-beta, and cancer. Exp Mol Med 2001; 33: 179-190

Schuppan D, Koda M, Bauer M, Hahn EG. Fibrosis of liver, pancreas and intestine common mechanisms and clear targets? A cta Gastroenterol Bieg 2000; 63: 366-370

Windecker C, Gressner AM. Pharmacological aspects of pentoxifylline with emphasis on its inhibitory actions on hepatic fibrogenesis. Gen Pharmacol 1997; 29: 183-196

Preaux AM, Mallat A, Rosenbaum J, Zafrani ES, Mavier P. Pentoxifylline inhibits growth and collagen synthesis of cultured human hepatic myofibroblast-like cells. Hepatology 1997; 26: 315-322

Desmouliere A, Xu G, Costa AM, Yousef LM, Gabbiani G, Tuchweber B. Effects of pentoxifylline on early proliferation and phenotypic modulation of fibrogenic cells in two rat models of liver fibrosis and on cultured hepatic stellate cells. J Hepatol 1999; 30: 621-631

Moser M, Zhang M, Gong Y, Johnson J, Kneteman N, Minuk GY. Effects of preoperative interventions on outcome following liver resection in a rat model of cirrhosis. J Hepatol 2000; 32: 287-292

Wu J, Zern MA. Hepatic stellate cells: a target for the treatment of liver fibrosis. Gastroenterology 2000; 35: 665-672

Reeves HL, Friedman SL. Activation of hepatic stellate cells—a key issue in liver fibrosis. Front Biosci 2002; 7: D330-D366

Preaux AM, Mallat A, Nhieu JT, D’orthe MP, Hembry RM, Mavier P. Matrix metalloproteinase-2 activation in human hepatic fibrosis regulation by cell-matrix interactions. Hepatology 1999; 30: 944-950

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