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• BASIC RESEARCH •

Effects of pentoxifylline on the hepatic content of TGF-b1 and collagen in Schistosomiasis japonica mice with liver fibrosis

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Abstract

AIM: To study the effects of pentoxifylline (PTX) on the content of hepatic TGF-β1, type I and type III collagen in schistosomiasis japonica mice with liver fibrosis and its mechanism of anti-fibrosis.

METHODS: Forty mice with schistosomiasis were divided into four 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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INTRODUCTION

Liver fibrosis is the main reason for portal hypertension and hemorrhagic of upper digestive tract in schistosomiasis and there fore 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[1,2]. 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[6,7]. 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 (provided as SHUANLIN tablet by Shijiazhuang Pharmaciticus CO.) 360 mg/(kg· d) for 8 wk, and low dose PTX 180 mg/(kg· d) for 8 wk respectively. The mice were then deceased and hepatic tissue sections were prepared for examination. Immunohistochemical technique and multimedia color pathographic analysis system were applied to observe the content of hepatic TGF-β1, type I and type III collagen before and after treatment.

Assay of TGFb1, collagen I and collagen III

Rabbit anti-mouse TGFβ1 was purchased from Santa Cruz. Rabbit anti-mouse collagen I and III, and SABC kit were provided by Boster Biological Technology Co., Ltd. The immunohistochemical studies were performed by the avidin-biotin- peroxidase method, briefly described as following. The tissue sections were blocked in 3 % hydrogen peroxide, washed in buffer solution, and then incubated in mixed digestive solution for 5 min in room temperature. The sections were washed in PBS and then incubated in goat serum blocking solution for 10 min. The sections were then incubated with the primary antibodies at 37 ℃ for 30 min, washed and then incubated with biotin conjugated secondary antibodies at 37℃ for 20 min, washed with PBS, and then labeled with peroxidase-conjugated streptavidin for 20 min at 37 ℃. The washed

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 $\bar{x}\pm s$. Results were then analyzed with student *t* test.

RESULTS

Effects of PTX on TGF-b1 expression

The contents of TGFβ1 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 praziquentel group and control group is very significant ($P<0.01$). The effect of PTX on TGFβ1 content is dose related and there is significant difference on TGFβ1 contents between high and low dose groups. The TGFβ1 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 dose and low dose PTX groups have significant difference on TGFβ1 contents between praziquentel group and themselves. The results are shown in Table 1.

Table 1 Content of TGF-β1, collagen I and III in liver of each treated group and control group ($\bar{x}\pm s$, ILD, $n=10$)

Group	TGF-β1	Collagen I	Collagen III
Control	0.883±0.140	0.771±0.156	0.822±0.129
Praziquantel	0.489±0.105[a]	0.596±0.103[a]	0.613±0.116[a]
High dose PTX	0.709±0.111[bd]	0.644±0.108[bd]	0.654±0.152[be]
Low dose PTX	0.752±0.152[cdf]	0.733±0.117[cdf]	0.788±0.147[cdg]

[a]$P<0.01$, *vs* control group; [b]$P<0.05$, *vs* control group; [c]$P>0.05$, *vs* control group; [d]$P<0.01$, *vs* praziquantel group; [e]$P>0.05$, *vs* praziquantel group; [f]$P<0.05$, *vs* high dose PTX group; [g]$P<0.01$, *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 praziquentel 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 praziquentel 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 praziquentel 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 praziquentel 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 alleviated 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 TGFβ1 by autocrine[27,28] and TGFβ1 has been proved to be a strong mitogen to HSC. This autocrine effect is upgraded when HSC has been activated. TGFβ1 depresses the regeneration of hepatic cells, activates and promotes HSC to synthesize extracellular matrix such as collagen, fibronetin proteinopolysarrcride, 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 TGFβ1, type I and III collagens shows that, high dose of PTX can reduce the content of TGFβ1, 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 praziquentel ($P>0.05$) and its effects on TGFβ1 and type I collagen are weaker than praziquentel. Compared with the control group, low dose of PTX can also reduce the contents of TGFβ1, 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 TGFβ1, type I and III collagens.

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