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Original article

Effect of medicated serum of *Curcumae Radix* extract on mRNA expression of TIMP-1, MMPs-13 and aI-collagen of HSC-T6 cell

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ABSTRACT

To study the effect of medicated serum of *Curcumae Radix* (Yujin) on the mRNA expression of Tissue inhibitor of metalloproteinase-1 (TIMP-1), Matrix metalloproteinases-13 (MMPs-13) and aI-collagen of Hepatic stellate cell-T6 (HSC-T6) cell. Twenty SD rats were randomly divided into 4 groups: high dose of Yujin group (16.2 g kg^{-1}), medium dose of Yujin group (8.1 g kg^{-1}), low dose of Yujin group (4.05 g kg^{-1}) and blank control group (with the same volume of 0.9% saline). Yujin extract or 0.9% saline were administered daily by gavage to rats for 4d, after once administration of full-day dose for 1 h on the fourth day, ether anaesthesia was given, and blood was taken from abdominal aortic in asepsis condition for preparation of medicated serum. HSC-T6 cells were divided into 5 groups: high dose medicated serum of Yujin group, medium dose medicated serum of Yujin group, low dose medicated serum of Yujin group, medicated serum of blank control group and negative control group (added with the same volume of PBS instead of rat serum), after 48 h of simultaneous acting on HSC-T6 cells in all groups by the medicated serum with a concentration of 10%, the mRNA expression level of TIMP-1, MMPs-13 and aI-collagen was analyzed with RT-PCR. Compared with the negative control group, the mRNA expression level of TIMP-1, MMPs-13 and aI-collagen in all experimental groups increased significantly. Compared with the control group, the mRNA expression of aI-collagen and TIMP-1 was obviously inhibited in all medicated serum of Yujin groups ($P < 0.01$), meanwhile, the mRNA expression level of MMPs-13 was effectively improved ($P < 0.05$). The medicated serum of Yujin had an effect on the production and degradation of Extracellular matrix (ECM) of HSC-T6 cell.

1. Introduction

Hepatic fibrosis (HF) is a pathological process with an excessive deposition of ECM caused by a variety of chronic pathogenic factors acting on the liver, a compensatory reaction in the tissue repair process, and an essential step for developing to liver cirrhosis. HSC is the cell from which are major source of ECM, whose proliferation and activation is the key step of HF (Friedman, 2004; Rockey, 2006; Huang et al., 2010; Ji et al., 2008; Friedman, 2008; Kisseleva and Brenner, 2006). Collagen is the main component of ECM, especially aI-collagen comprises a large proportion (Friedman, 2010; Lee and Friedman, 2011; Gao, 2005). Matrix metalloproteinases can decompose most collagens, however, after activation of HSC, the massive secretion of matrix metalloproteinases tissue inhibitor leads to rapid drop of activity of matrix metalloproteinases, unable to create decomposition, resulting in deposition of ECM (Cong et al., 2007; Jiang et al., 2008; Wang et al., 2012; Yoshiji et al., 2000). Currently, there are no effective chemical antifibrotics in the clinical context. Chinese herbal medicines have been demonstrated to be promising remedy for hepatic fibrosis and unique advantages and long-term clinical treatment in many aspects lead to its springing up in anti-hepatic fibrosis field, and many of its Chinese medicinal herbs, Chinese medicines prescription, Chinese herbal compounds or single components have already been proved to be highly effective in treatment of HF (Yuan et al., 2009; Popov and Schuppan, 2009; Li et al., 2016; Huang et al., 2013; Ding et al., 2011; Jiang et al., 2013; Du et al., 2013). Chinese medicinal herbs Yujin with obvious pharmacological activity, from the dry tuberous root of *Curcuma wenyujin Y.H. Chen et C. Ling, Curcuma longa L,*

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Curcuma kwangsiensis S.G. Lee et C.F. Liang, Curcuma phaeocaulis Val (all belong to the Zingiberaceae), has been used for about 1300 years in China, is a kind of Chinese medicinal herbs for treating hepatic diseases such as HF (Bisht et al., 2011; Vera-Ramirez et al., 2013; Lin et al., 2012; Zhang et al., 2014; Qin et al., 2010), contains a variety of chemical composition, such as curcumin etc. (Li et al., 2011). However, its mechanism of action in anti-hepatic fibrosis is not fully clear yet.

2. Materials

2.1. Animal, cell and herbs

Twenty adult male SD rats (SPF) (6 weeks of age, 180–220 g), were provided by Dongchuang Technology Service of Laboratory Animal, Changsha City, Hunan Province. All rats were kept in an environmentally controlled room (22 ± 2 °C, 55 ± 5% relative humidity, and 12 h light/dark cycle) and received food and water ad libitum. All rats experiments were conducted in accordance with Principles of Laboratory Animal Care.

HSC-T6 cell, an immortal cell line formed after rat hepatic stellate cell being transfected with SV40 virus, with the characteristics of unlimited passage was an activated hepatic stellate cell strain, and cryopreservation by Institute of Chinese medicinal herbs, Guangxi University of Chinese Medicine. Yujin were collected from Nanning City, Guangxi Province, and identified by Professor Wang Jian at Guangxi University of Chinese Medicine, belong to *Curcuma kwangsiensis S.G.Lee et C.F.Liang* in this study.

2.2. Major instruments and reagents

PCR (MJ, PTC-220); Gel imaging and analysis system (Bio-Rad, Gel Doc-2000); Trizol Reagent (Invitrogen corporation, USA); RevertAidRTTMFirst Strand cDNA Synthesis Kit (MBI, CAN); Taq (MBI, CAN); dNTP (TaKaRa, JPN); DMEM (GIBCO, USA); Primers were synthesized by Shanghai Generay Biotech Co. Ltd.

2.3. Primer

See Table 1.

3. Experimental method

3.1. Preparation process of Yujin extract

Yujin (1000 g) were extracted by reflux with distilled water (10 L) for 8 h, volatile oil and water extraction solution were collected, then the water extraction solution was filtered, the extraction process above mentioned was repeated for two times, the filtrates of two times were combined and concentrated, then concentrated to 2 g (equivalent to medicinal materials) $ml^{-1}$with the volatile oil, and kept in a refrigerator at 4 °C for use. Yujin concentration of mixed extracts was dissolved in distilled water to obtain the desired concentrations in experiments.

Table 1
Primer and its sequence.

Gene	Primer sequence	Length bP
TIMP-1 Sense Primer	5′-CCCTTTGCATCTCTGGCCT-3′	226 bP
Antisense Primer	5′-CATTTCCCACAGCGTCGA-3′	
MMPs-13 Sense Primer	5′-ACCATCCTGTGACTCTTGCG-3′	345 bP
Antisense Primer	5′-CAGCAGTGCCATCATGGA-3′	
aI-collagen Sense Primer	5′-ATGTTCAGCTTTGTGGACCTC-3′	261 bP
Antisense Primer	5′-TGGGCAGAAAGGACAGCA-3′	
β-actin Sense Primer	5′-CAATGAGCGGTTCCGATG-3′	155 bp
Antisense Primer	5′-TGCCACCAGACAGCACTG-3′	

3.2. Preparation of medicated serum

After one week's acclimatization, twenty rats were randomly divided into 4 groups (5 rats per group): one is blank control group (with the same volume of 0.9% saline), while other three groups received Yujin extract twice a day, at the dose of 16.2 g kg^{-1}, 8.1 g kg^{-1}, 4.05 g kg^{-1} body weight, named as high dose group, medium dose group, low dose group, continuous administration by gavage for 3d, and fasting deprivation for 12 h but water, after once administration of full-day dose for 1 h on the fourth day, ether anaesthesia was given, then blood was taken from abdominal aortic in asepsis condition, kept stationary at 37 °C for 24 h, centrifugalized at 3000 rpm for 10 min at 4 °C, and then the serum was separated, water bath at 56 °C for 30 min for inactivation, filtered using a 0.22 μm of filter, and stored at -80 °C for preservation.

3.3. mRNA expression of TIMP-1, MMPs-13 and aI-collagen

HSC-T6 cells were inoculated in a new culture bottle by 5×10^5 pcs ml^{-1}, 10 ml per culture bottle, after 24 h of adherence, they were cultured with serum-free DMEM complete culture solution for 24 h for synchronization, then the medicated serum was added. They were divided into 5 groups, with the medicated serum with a concentration of 10% for each group, including high dose medicated serum of Yujin group, medium dose medicated serum of Yujin group, low dose medicated serum of Yujin group, medicated serum of blank control group and negative control group (added with the same volume of PBS instead of rat serum), each group was set with 5 bottles, acting for 48 h, after trypsin digestion, centrifugalized at 1000 rpm for 5 min using a 15 ml of centrifuge tube, then, supernatants were sucked out and dumped, cells were collected, and total RNA was extracted, and synthesis of cDNA by reverse transcription was performed. 35 cycles were adopted for TIMP-1 using 94°C4 min, 94 °C 30 s, 57 °C 40 s, 72 °C 40 s and 72 °C 8 min; 36 cycles were adopted for MMPs-13 using 95 °C 4 min, 95 °C 30 s, 65 °C 40 s, 72 °C 1 min and 72 °C 8 min; 30 cycles were adopted for aI-collagen using 94 °C 5 min, 94 °C 30 s, 55 °C 30 s, 72 °C 45 s and 72 °C 8 min. Meanwhile, PCR was carried out for intrinsic parameter using β-actin.

3.4. Data processing

The results of all gel electrophoresis were analyzed using the Quantity One 4.6.2 software, and the average OD value of each group was calculated, and the genic OD value measured and ratio from reference of corresponding OD value of β-actin were regarded as its relative mRNA expression level in HSC-T6 cells. The experimental results were represented by the average ± standard deviation ($\overline{x} \pm s$), and statistical analysis was conducted using the analysis of variance of SPSS 11.5 software, and the comparison of average between groups was tested using the SNK method. It was statistically significant to represent the difference by $P < 0.05$.

4. Results

The results showed: Compared with the negative control group, the mRNA expression of TIMP-1, MMPs-13 and aI-collagen of HSC-T6 cell increased significantly; Compared with the blank control group, the mRNA expression of aI-collagen and TIMP-1 in each medicated serum of Yujin group decreased significantly ($P < 0.01$), while the mRNA expression of MMPs-13 in all

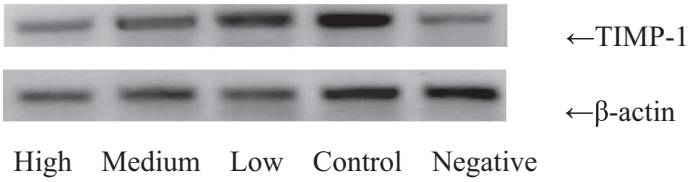

Fig. 1. mRNA expression of TIMP-1 of HSC-T6 cell in each group.

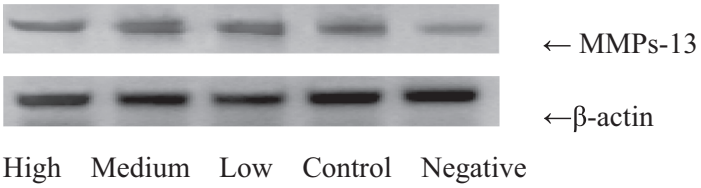

Fig. 2. mRNA expression of MMPs-13 of HSC-T6 cell in each group.

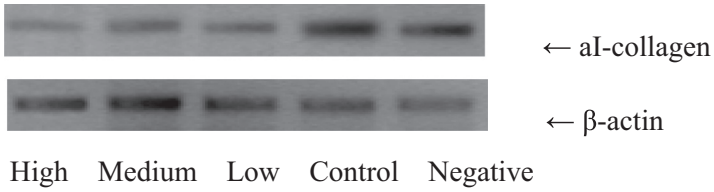

Fig. 3. mRNA expression of aI-collagen of HSC-T6 cell in each group.

Table 2
Comparison of mRNA expression of TIMP-1, MMPs-13 and aI-collagen of HSC-T6 cell in each group ($\bar{x} \pm s$) (n = 5).

Group	TIMP-1/β-actin	MMPs-13/β-actin	aI-collagen/β-actin
High dose group	0.8166 ± 0.02992★	0.7181 ± 0.04743☆	0.7611 ± 0.02222★
Medium dose group	0.8815 ± 0.03702★	0.6905 ± 0.03153☆	0.7433 ± 0.02332★
Low dose group	1.0160 ± 0.01252★	0.6868 ± 0.01903☆	0.8240 ± 0.02032★
Blank Control group	1.0768 ± 0.02181▲	0.6357 ± 0.04521▲	1.0772 ± 0.02884△
Negative control group	0.7920 ± 0.0811	0.5375 ± 0.0372	1.0448 ± 0.0103

Note: ▲ Compared with the negative control group: $P < 0.01$; △ Compared with the negative control group: $P < 0.05$; ★ Compared with the blank control group: $P < 0.01$; ☆ Compared with the blank control group: $P < 0.05$.

medicated serum of Yujin groups increased significantly ($P < 0.05$), however, the mRNA expression in all medicated serum of Yujin groups showed no positive correlation with the dose of medicated serum, indicating that reducing the mRNA expression of TIMP-1 and aI-collagen while improving the mRNA expression of MMPs-13 may be one of molecular mechanisms of anti-hepatic fibrosis action by Yujin (see Figs. 1–3 and Table 2).

5. Discussion

Similar to many studies (Chen and Guo, 2016; Wu et al., 2000; Guo et al., 2005; Zhao et al., 2010), in this experiment, the cell culture at earlier stage showed that the proliferation of HSC-T6 cells in blank control group (added with rat serum) was faster than that in negative control group (added with DMEM complete culture solution) due to homology. Thus, it can seen from the RT-PCR results, the mRNA expression of MMPs-13, TIMP-1 and aI-collagen in control group was greater than that in negative control group, proving that in the proliferation process of HSC-T6 cells, the mRNA expression level of MMPs-13, aI-collagen and TIMP-1 also improved. Among which, although the mRNA expression level of MMPs-13 improved, it cannot fully play the role in decomposition of ECM, which might be due to the fact that its activity was inhibited by TIMP-1. According to this experiment, it can be seen that the mRNA expression of MMPs-13 in all treatment groups improved ($P < 0.05$), while the mRNA expression of TIMP-1 in all treatment groups was reduced ($P < 0.01$), suggesting that the medicated serum of Yujin can not only improve the mRNA expression of MMPs-13, but also achieve its activity by inhibiting the mRNA expression of TIMP-1 so as to accelerate the decomposition of ECM, meanwhile, the mRNA expression level of aI-collagen in all treatment groups was obviously inhibited ($P < 0.01$), which can reduce the production of ECM. However, for the activation of HSC and increase of ECM, multiple cell signaling pathways can be used for adjusting, for example, TGF-β/Smad pathway, MAPK pathway, PPARγ pathway, Leptin pathway I, ntegrin pathway, NF-κB pathway and so on (Cheng et al., 2006; Wang et al., 2010; Qiang et al., 2006; Gianluca et al., 2001). As regard to whether or not Yujin may also have an effect on them, further study is needed.

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