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Journal of Ethnopharmacology 97 (2005) 7–13
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

The role of TGF-β1 and cytokines in the modulation of liver fibrosis by Sho-saiko-to in rat's bile duct ligated model

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Received 3 July 2003; received in revised form 21 April 2004; accepted 13 September 2004
Available online 10 December 2004

Abstract

Liver fibrosis is an over-accumulation of extra-cellular matrix (ECM) and the hepatic stellate cell (Ito cell) play a central role in the pathogenesis of liver fibrosis. There are a lot of growth factors and cytokines involved in the activation of hepatic stellate cell, including of transforming growth factor (TGF-α, TGF-β1), platelet-derived growth factor (PDGF), interleukin (IL-1α,β, IL-6) and tumor necrosis factor (TNF-α).

Sho-saiko-to (TJ-9; Xiao-Chai-Hu-Tang in Chinese) was the most popular herbal medicine for the treatment of chronic liver disease in Chinese and Japanese. Our aim of the current study was to examine whether TJ-9 regulated the growth factors and cytokines in the fibrogenesis of bile duct ligated model. Therefore, we assessed the TJ-9's potential in regulating TGF-β1, PDGF mRNA expression, the amount of IL-1α, IL-1β, IL-6, TNF-α and the fibrotic marker "PIII NP" in the serum. Then, using the immunohistochemical stain to observe the TGF-β1 expression in the tissue.

Our results showed that TJ-9 at a dose of 0.5 g/(kg day) significantly reduced the serum level of PIII NP, the mRNA expression of TGF-β1 and PDGF. For the cytokines involved in the activation of Ito cell, TJ-9 at a dose of 0.5 g/(kg day) significantly suppressed the increasing tendency of IL-1β and enhanced the production of TNF-α. Finally, we concluded that: (1) TJ-9 at a dose of 0.5 g/(kg day) significantly reduced the serum fibrotic marker PIII NP in the bile duct ligated model, and its mechanism was partly by means of downregulating the mRNA of TGF-β1 and PDGF. These results also confirmed by the immunohistochemical staining of TGF-β1. (2) TJ-9 at a dose of 0.5 g/(kg day) suppressed the increasing tendency of IL-1β and stimulated the production of TNF-α to inhibit Ito cell proliferation and collagen formation.

Keywords: Liver fibrosis; Bile duct ligation; Sho-saiko-to(TJ-9); TGF-β1; PDGF; Cytokine; PIII NP

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0378-8741/$ – see front matter
doi:10.1016/j.jep.2004.09.040

1. Introduction

Liver fibrosis is a result of chronic hepatocellular damage due to a variety of liver diseases including viral hepatitis, alcoholic hepatitis, primary and secondary biliary cirrhosis, primary sclerosing cholangitis, etc. Increased deposition of extracellular matrix and reduced matrix degradation are the major character of liver fibrosis. Ito cell, the central role in the process of liver fibrosis, is the major source of fibrillar and nonfibrillar matrix. During liver injury, Ito cell activate and go through the loss of vitamin A, perisinusoidal matrix degradation via induction of matrix metalloproteinase, cellular migration and proliferation, matrix production and accumulation, and contractility (Friedman, 1993). The key factors involve in the activation of Ito cell are divided into two groups. The first, mitogenic factors (stimulation of Ito cell proliferation) are TGF-α, PDGF, IL-1, TNF-α and insulin-like growth factor. The second, fibrogenic factors (induction of matrix protein) are TGF-β1 and IL-6 (Tsukamoto, 1999).

Among all of those cytokines, PDGF and TNF-α appear most critical in the early changes of Ito cell migration and proliferation. For the later phase of Ito cell activation, TGF-β1 is considered as a potent mediator to accumulate extracellular matrix, because it induces gene expression of matrix and inhibits of degradation via induction of tissue inhibitor metalloproteinase (TIMP) (Matsuoka et al., 1989). On the other hand, the release of IL-1, IL-6 and TNF-α is involved in liver inflammation and amplification of defensive system. IL-1 is one of the earliest inflammatory cytokine as a response of liver injury, and it determines the production of IL-6 and TNF-α (Trautwein et al., 1994). Another word, IL-6 and TNF-α are involved in the onset of lipid peroxidation, cell membrane disruption and replacement of the necrotic area with connective tissue.

Sho-saiko-to (TJ-9, Xiao-Chai-Hu-Tang, in Chinese) is the most popular herbal medicine in Chinese and Japanese, and it is widely administered to patients with chronic hepatitis and liver cirrhosis. Some researches indicate that the acute and subacute toxicity of Sho-saiko-to is comparatively low in rats, and daily dose of 2 g/kg produce no toxicologically significant effect (Sameshima et al., 1987; Minematsu et al., 1992). Several laboratories have proved its hepatoprotective effects on various liver injury experiments, such as CCl4 and D-galactosamine (Araki et al., 1988; Sakae et al., 1989). Furthermore, a lot of researchers demonstrated the preventive and therapeutic effect of TJ-9 on experimental hepatic fibrosis. Sakaida et al. (1998) had reported that TJ-9 prevented hepatic fibrosis, reduced the expression of type (III) procollagen mRNA and inhibited the activation of Ito cell. Shimizu (2000) also confirmed the antifibrotic effect of TJ-9 in reducing collagen type (I) synthesis and inhibiting the activation of Ito cell, via the suppression of oxidative stress in hepatocyte and Ito cell. Our previous research has proved that TJ-9 significantly reduced the collagen content by downregulating the mRNA expression of procollagen types (I), (III) and tissue inhibitor of metalloproteinase (Chen et al., 2004).

The aim of current study was to determine whether TJ-9 prevented liver fibrosis by regulating the cytokines in rat's bile duct ligated model. Therefore, we assessed the TJ-9's potential in the fibrotic marker "PIII NP" in the serum by radioimmunoassay (RIA), and in the regulation of TGF-β1, PDGF mRNA expression by reverse transcription polymerase chain reaction (RT-PCR). Another way, we estimated the amount of IL-1α, IL-1β, IL-6, TNF-α by ELISA. After that, using the immunohistochemical stain of TGF-β1 to observe the TGF-β1 expression in the tissue.

2. Materials and methods

2.1. Preparation of Sho-saiko-to(TJ-9) extract

Sho-saiko-to extract powder was kindly provided by Ko-Da Pharmaceutical co., Taiwan, ROC. The voucher specimens have been deposited in their herbarium. All of the herbs were authenticated by Dr. Shih-Chang Lee, Chinese Medical College, Taiwan. Briefly, 1000 g Chinese herbal drug, including 333 g root of *Bupleurum chinese* DC (Voucher No. 758), 133 g root of *Scutellaria baicalensis* Georgi (No. 812), 133 g root of *Panax ginseng* C.A. Meyer (No. 503), 67 g root of *Glycyrrhiza uralensis* Fischer (No. 609), 67 g rhizome of *Zingiber officinale* Roscoe (No. 610), 133 g tuber of *Pinellia ternata thunb.* Breit. (No. 607), 133 g fruit of *Zizyphus jujuba* Mill (No. 704) were decocted with 7 L of boiling water in stainless oven for 1 h. The decoction was filtered and decocted again for another 50 min. Then, the filtrate was concentrated under reduced pressure (60–80 mm Hg) at 55 °C by rotary vacuum evaporator, and freeze dried at −45 °C. Subsequently, the productive rate of TJ-9 extract was 25.47% and diluted it to the stock solution of 50 mg/ml. Previous phytochemical screening of Sho-saiko-to's components has shown presence of baicalin, glycyrrhizin, baicalein, saikosaponin-a, -c, -d, ginsenoside Rb1, Rg1, wogonin, viscidulin (Shimizu, 2000).

2.2. Animals

Eighty male Wistar albino rats weighing approximately 200–250 g were purchased from the National Animal Center and kept on a standard rat diet with free access to tap water, with a 12 h light-dark cycle. This experiment was reviewed by the committee of Ethics on animal experiment in Chiayi Christian Hospital. After accommodation for 2 weeks, the rats were randomly assigned to four groups: (A) sham-operation, (B) bile duct ligation (BDL), (C) bile duct ligation and treated with Sho-saiko-to (TJ-9) 0.5 g/(kg day) by intragastric gavage, (D) bile duct ligation and treated with Sho-saiko-to (TJ-9) 1.0 g/(kg day) by intragastric gavage. Rats were anaesthetized with 0.1 ml/100 g Zoletil (tietamine and zolezetam, Virbac, France) by intraperitoneal injection. A midline abdominal incision was made and the common bile duct was identified, doubly ligated with 3–0 black silk and