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Liuweiwuling tablets attenuate BDL-induced hepatic fibrosis via modulation of TGF-β/Smad and NF-κB signaling pathways

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ABSTRACT

Ethnopharmacological relevance: Liuweiwuling (LWWL) tablets contain a six-herb Chinese formula and are commonly prescribed to facilitate nourishment of the liver and kidneys, clear away toxic materials and activate blood circulation. Administration of LWWL is well known for its protective effects on the liver and its capacity to confer long-term stability in patients exhibiting reduced transaminase levels. Clinical studies have reported that LWWL can also be used for the treatment of liver fibrosis with associated treatment regimens resulting in a concomitant reduction in transforming growth factor β1 (TGF-β1) levels in the serum of patients with hepatic fibrosis. TGF-β1 plays a prominent role in stimulating liver fibrogenesis and this effect is mediated by myofibroblasts (MFB) derived from hepatic stellate cells (HSCs). It is likely that this phenomenon underpins the antifibrotic effects associated with LWWL.

Aim: The purpose of this study was to investigate the antifibrotic effects and mechanisms pertaining to LWWL.

Methods: Hepatic fibrosis was induced in rats following bile duct ligation (BDL). Rats that underwent BDL received daily gavage administration of colchicine (0.2 mg/kg per day), LWWL (0.4, 1.6, 6.4 g/kg per day) or PBS (for the control group). Pathological changes in hepatic tissue were examined using hematoxylin and eosin (HE) and sirius red staining. Immunohistochemical analysis was performed to monitor α-SMA and type I collagen (Collagen I) protein expression. Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) and Western blot analyses were used to monitor the expression of genes and proteins in the TGF-β/Smad signaling pathway, including TGF-β1, bone morphogenic protein and activin membrane-bound inhibitor (Bambi), Smad3, phosphorylated Smad3 (p-Smad3) and Smad7. We also monitored the expression of genes and proteins in the nuclear factor-κB (NF-κB) signaling pathway, including NF-κB p65, IκBα and phosphorylation of IκBα (p-IκBα), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1β (IL-1β).

Results: LWWL dose-dependently inhibited BDL-induced liver injury and hepatic fibrosis in rats. Furthermore, LWWL reduced liver tissue collagen deposition, hydroxyproline content, liver dysfunction and α-SMA expression in BDL-induced hepatic fibrosis rats. Moreover, LWWL markedly prevented activation of the TGF-β/Smad signaling pathway by inhibiting expression of Smad2/3 and phosphorylation of Smad3, and upregulating the expression of Bambi and Smad7. In addition, LWWL regulated the expression of the inflammatory cytokines IL-1β, TNF-α and IL-6 by inhibiting the activation of NF-κB p65 and the phosphorylation of IκBα, and increasing the expression of IκBα.

Conclusions: LWWL can attenuate BDL-induced hepatic fibrosis in rats, and this effect may be due to modulation

Abbreviations: LWWL, Liuweiwuling tablets; BDL, bile duct ligation; HE, hematoxylin and eosin; Collagen I, collagen type I; RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; p-smad3, phosphorylation of Smad3; ECM, extracellular matrix; MFB, myofibroblasts; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; TGF-β1, transforming growth factor β1; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitor of metalloproteinases; TCM, traditional Chinese medicine; ALT, alanine transaminase; AST, aspartate aminotransferase; ELISA, enzyme-linked immunosorbent assay, BCA, bicinchoninic acid; cDNA, complementary DNA; ANOVA, analysis of variance

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of the NF-κB-dependent inflammatory response and activation of HSC and TGF-β/Smad-mediated synthesis and degradation of Collagen I.

1. Introduction

Hepatic fibrosis, a common pathological consequence of many chronic liver diseases, is characterized by excessive deposition of extracellular matrix (ECM) proteins and inflammatory reactions (Friedman, 2008a). HSCs play a central role in the process of hepatic fibrosis (Friedman, 2000). Quiescent HSCs can transdifferentiate into α-SMA-positive, proliferative, fibrogenic and contractile MFB with increased capacities for ECM production. Macrophages play an important role in regulating inflammatory signaling pathways and promoting the survival of activated HSCs (Pradere et al., 2013). Survival and activation of HSCs is regulated by several intracellular signaling cascades. Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are mainly responsible for the maintenance of HSC activation and proliferation (Fallowfield, 2011a). However, TGF-β1 is the most effective profibrosis cytokine. This cytokine induces increased synthesis of ECM by activating Smad signaling (TGF-β/Smads) in HSCs (Gressner et al., 2002). It was observed that TGF-β1 promotes the activation of HSCs through autocrine and paracrine signaling (Friedman, 2008b). As a consequence of TGF-β1-mediated activity, type I and II TGF-β1 receptors form a complex and induce receptor autophosphorylation and activation. The activated receptors then phosphorylate the signaling molecule Smad2/3, which forms a homo-oligomer with Smad4. This homo-oligomer enters the nucleus and activates the transcription of genes encoding collagen I, MMPs and TIMPs, causing excessive production of ECM proteins (Iredale et al., 1993; Iredale, 1997; Derynck and Zhang, 2003), which will lead to liver fibrosis. Smad7, which negatively regulates TGF-β1 signal transduction, competes with Smad2/3 to competitively bind receptors. Smad7 also recruits E3 ubiquitin ligase to degrade the receptors, thereby inhibiting the activation of Smad2/3. These activities effectively prevent TGF-β/Smad signal transduction (Shi and Massagué, 2003). Bambi, a pseudoreceptor of TGF-β signaling, not only impairs TGF-β type I and type II receptor heterocomplex formation, but also enhances the binding of Smad7 to the TGF-β type I receptor. Consequently, Bambi is capable of attenuating TGF-β signaling (Onichtchouk et al., 1999; Yan et al., 2009).

In recent years, extensive research has revealed that liver fibrosis is reversible. Research also suggests that cirrhosis is potentially reversible (Malekzadeh et al., 2004). Reports have confirmed that HSC apoptosis can effectively eliminate the formation and deposition of ECM, thereby effectively reversing liver fibrosis (Wright et al., 2001; Iredale, 2003). Fiona et al. reported that the NF-κB signal transduction pathway can promote the activation of HSCs and inhibit the expression of pivotal proteins and genes involved in the pathway. These reactions can induce HSC apoptosis, thereby accelerating the regression of liver fibrosis (Oakley et al., 2005). In addition, NF-κB can promote the development of liver fibrosis by increasing the inflammatory response (Sunami et al., 2012). The p65:p50 heterodimer is the prominent form of NF-κB in HSCs. In the majority of mammalian cells, NF-κB forms an inactive complex in the cytoplasm by binding to IκB. A variety of signal activation mechanisms associated with NF-κB, including activation of IκBα by IκB kinase (IKK) and phosphorylation of IκBα by ubiquitination and degradation, result in NF-κB nuclear transcription and concomitant regulation of target genes (Perkins, 2007). Furthermore, once IκBα degradation is inhibited, activation of HSCs is significantly reduced (Elsharkawy et al., 1999). In addition, the TLR4-NF-κB pathway plays a crucial role in hepatic fibrogenesis by enhancing TGF-β-mediated HSC activation and the secretion of inflammatory cytokines in macrophages (Seki et al., 2007).

LWWL is a Chinese Medicine formula which has been used to decrease aminotransferase levels induced by chronic viral liver disease. This formula is approved by the Chinese State Food and Drug Administration (CFDA). LWWL is prepared from the following six traditional Chinese herbs: Schisandrae chinensis fructus, Ligustri lucidi fructus, Forsythiae fructus, Curcumae rhizoma, Perennial sow thistle and Ganoderma spore in a ratio of 3.5:2.5:1.5:1:1.5:1, respectively. Schisandrae chinensis fructus results in astringency, replenishing and promoting the synthesis of body fluids and tonifying the kidney to relieve mental strain; Ligustri lucidi fructus nourishes the liver and kidneys; Forsythiae fructus and Perennial sow thistle are involved in clearing heat and detoxifying; Curcumae rhizoma relieves blood and promotes Qi, relieving food stasis and analgesia; Ganoderma spore invigorates Qi and tranquilization, thereby relieving coughing and asthma. Manufacturers of this medicine have adopted internationally advanced technologies for Traditional Chinese Medicine (TCM) preparation to improve drug bioavailability. LWWL has been extensively used for many years in the 302 Military Hospital of China. Clinical studies have confirmed that LWWL, at a standard dose of 0.06 g/kg, can be used to treat drug-induced liver injury (Gao and Yan-Ling, 2012; Lei et al., 2015b), alcohol-induced liver disease (Rong et al., 2009), liver fibrosis and cirrhosis (Xin et al., 2009; Ji-Liang, 2011; An et al., 2014).

The mechanisms underpinning LWWL-mediated activity in the treatment of hepatic fibrosis have not yet been elucidated. The purpose of this study was to investigate the antifibrotic efficacy and mechanisms associated with LWWL activity using BDL-induced hepatic fibrosis models.

2. Materials and methods

2.1. Reagents and experimental drugs

LWWL was purchased from Shandong Shibojindu Pharmaceutical Company (Batch no.: 141203, 141205, 150303, 150509, 151105) (Contents of LWWL see Table 1) and pure reagent (AR) grade Colchicine (Lin et al., 2012) was purchased from Sigma, USA. Alanine transaminase (ALT) and aspartate aminotransferase (AST) testing kits were purchased from Nanjing Jiancheng Co., Ltd., China. Rat TGF-β1 and TNF-α ELISA kits were purchased from Multi Sciences (Lianke) Biotechnology Co., Ltd., China. PCR primers for TGF-β1, Smad3, Acta2

Table 1
Contents of Liuweiwuling tablets (LWWL).

Chinese name	Botanical name	Common name	Part used
Wu Wei Zi	*Schisandra chinensis* (Turcz.) Baill.	Schisandrae chinensis fructus	fruit
Nv Zhen Zi	*Ligustrum lucidum* Ait.	Ligustri lucidi fructus	fruit
Lian Qiao	*Forsythia suspensa* (Thunb.) Vahl	Forsythiae fructus	fruit
E Zhu	*Curcuma phaeocaulis* val., *curcuma kwangsiensis* S. G.Lee et C. F. Liang or *Curcuma wenyujin* Y. H. Chen et C. ling.	Curcumae rhizoma	Rhizome
Ju Mai Cai	*Sonchus arvensis* Linn.	Perennial sow thistle	Herb
Ling Zhi Bao Zi Fen	*Ganoderma lucidum* (Leyss. Ex Fr.) Karst or *Ganoderma sinense* Zhao, Xu et Zhang.	Ganoderma spore	Seed