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Protective effects of a traditional Chinese herbal formula Jiang-Xian HuGan on Concanavalin A-induced mouse hepatitis via NF-κB and Nrf2 signaling pathways

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ABSTRACT

Ethnopharmacological relevance: Jiang-Xian HuGan (JXHG) formulated by five natural products including Freshwater clam *(Corbicula fluminea)*, *Curcuma longa* L., *Ligustrum lucidum*, *Eclipta prostrata (L.)* L. and *Paeonia lactiflora Pall.*, has exhibited a great hepatoprotective effect.

Aim of this study: We investigated the effect of JXHG on concanavalin A (ConA)-induced acute live injury in mice, and to elucidate its underlying molecular mechanisms.

Materials and methods: Jiangkanling Capsule (900 mg/kg), low-dose JXHG (LJXHG, 700 mg/kg), high-dose JXHG (HJXHG, 1400 mg/kg) were administered to mice by oral gavage daily for 20 days prior to a single intravenous injection of ConA (20 mg/kg). Liver injury was evaluated by measuring the serum levels of enzymes and cytokines as well as liver histological analysis. We also measured the hepatic expression of cytokines at mRNA levels and the proteins related to NF-κB and Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathways.

Result: Our results showed that JXHG pretreatment significantly alleviated ConA-induced live injury as evidenced by decreased serum levels of glutamic-pyruvic transaminase (ALT) and glutamic oxalacetic transaminase (AST), and reduced hepatocyte apoptosis and mortality. Furthermore, JXHG was able to significantly reduce the serum levels of proinflammatory cytokines, down-regulate the mRNA expression of interleukin-6 (IL-6) and interferon-γ (IFN-γ), and up-regulate IL-10 as well as superoxide-dimutase-1 (SOD1), glutathione reductase (GSR) and Glutathione peroxidase 2 (GPX2) mRNA in the liver tissues after Con A injection. In addition, JXHG pretreatment dramatically suppressed the phosphorylation of NF-κB p65 (p65), increased Nrf2 expression, and decreased the expression ratio of cleaved caspase-3/caspase-3 in liver tissues.

Conclusion: These results suggest that JXHG protects against ConA-induced acute live injury through inhibiting NF-κB mediated inflammatory pathway and promoting Nrf2 mediated anti-oxidative stress signaling pathway.

1. Introduction

The liver is one of the most important organs in the human immune system, and plays an important role in immunoregulation, immunotolerance and immune response (Czaja et al., 2002). Liver injury can be caused by various stimuli such as drug abuse, infections, alcohol consumption and autoimmunity. Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease associated with liver failure. The morbidity rate of AIH seems to vary according to region and ethnicity. It appears to have the highest prevalence in North America (European Association for the Study of the liver, 2015). AIH is characterized by a severe disease course and a high tendency to develop into cirrhosis, which results in portal hypertension and hepatic insufficiency. Although corticosteroids and immunosuppressants are widely used for the treatments of AIH, there are widespread resistance and significant toxicity to these drugs (Ichiki et al., 2005). Therefore, we should pay special attention to its pathogenesis and prevention strategies.

In recent years, great importance has been attached to the use of

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natural products in treating liver diseases, because of their low poisonous effect and high safety (Corson and Crews, 2007; Ghosh et al., 2011). Jiang-Xian HuGan (JXHG) is formulated by five natural products including Freshwater clam (*Corbicula fluminea*), *Curcuma longa* L., *Ligustrum lucidum*, *Eclipta prostrata (L.)* L. and *Paeonia lactiflora Pall.* Freshwater clam is usually used as food and has been mentioned to have a hepatoprotective effect in ancient Chinese medical books. Previous studies reported that Freshwater clam extract can decrease the expression of proinflammatory cytokines, exhibiting a hepatoprotective effect in the model of chronic hepatic fibrosis induced by carbon tetrachloride (Hsu et al., 2010; Lin et al., 2012). *Ligustrum lucidum*, *Eclipta prostrata (L.)* L. and *Paeonia lactiflora Pall.* have been used for liver protection, antioxidation and delaying aging since ancient China (Gong et al., 2015; Ma-Ma et al., 1978; Seo et al., 2017; Yao et al., 2014). Pretreatment of Paeoniflorin, the main active component protects mice against Concanavalin A (Con A)-induced liver injury via inhibiting several inflammatory mediators and, at least in part, by suppressing $CD4^+$, $CD8^+$ and NKT cell infiltration in liver (Chen et al., 2015). Curcumin extracted from *Curcuma longa* L., has been reported to exhibit many pharmacological effects such as anti-tumor, anti-inflammation and antioxidation as well as hepatoprotective effects (Wang et al., 2012a). Previously, JXHG has been reported to have a protective effect on alcohol induced hepatic injury in mice (Zhou et al., 2017). In the present study, we investigated the protective effects of JXHG on ConA-induced acute live injury in mice, and further elucidate its underlying molecular mechanisms.

2. Materials and methods

2.1. Reagents

ConA was purchased from Sigma-Aldrich (St. Louis, USA). Alanine aminotransferase (ALT/GPT) and aspartate transaminase (AST/GOT) kits were purchased from Nanjing Jiancheng Biological Product (Nanjing, China). The cytokine enzyme-linked immunosorbent assay (ELISA) kits were from Dakewe (Shenzhen, China). Antibodies against Keap1, NRF2, caspase-3, cleaved caspase-3 NF-κB(p65) and p-NF-κB (p-p65) were from Cell Signaling Technology, Inc. (Beverly, MA, USA). Jiangkanling Capsule (JC) was purchased from Baiyunshan Guanghua Company (Guangzhou, China), and the SFDA approval number is Z44020001.

2.2. Preparation and quantity control of JXHG

JXHG was provided by Infinitus (China) Company Ltd (Guangzhou, China) prepared by mingling Freshwater clam (Corbicula fluminea) extracts, Curcuma longa L. extracts, and herbal extracts according to the weight ratio of 2:1:7. Freshwater clam (Corbicula fluminea) extracts were prepared by high temperature and high pressure extraction, sterilization, and spray drying similar as the previous report (Chijimatsu et al., 2015). The extraction rate was 4–5%. Curcuma longa L. extracts were prepared by the processing of 65–75% ethanol refluxing extraction, concentration and desiccation. The extraction rate was 4–6% and the content of curcumin was above 80%. Herbal extracts were prepared from Ligustrum lucidum, Eclipta prostrata (L.) L. and Paeonia lactiflora Pall. (2:2:1) by the processing of 60% ethanol under refluxing extraction for 2 times, filtration, vacuum concentration and desiccation. Their voucher specimens have been deposited in Infinitus R &D Center, Infinitus (China) Company Ltd.

To assure the quality and thereby warrant the safety and effectiveness of JXHG, the prepared JXHG was subjected to high performance liquid chromatography (HPLC) finger printing analysis in which curcumin and oleanolic acid were identified as the marker compounds for monitoring. The representative HPLC trace of APM is shown in Fig. 1. The chromatographic separation of curcumin was performed on a Shim-pack XR-ODS C18 column, which was protected by a high pressure column pre-filter (2 μm). The mobile phase was composed of acetonitrile–4% glacial acetic acid in water (48:52, v/v) at a flow rate of 1 mL/min. The temperature of column was maintained at 30 °C, respectively. The injection volume was 10 μL. The quality analysis of oleanolic acid was examined by using HPLC fingerprints. In HPLC system the mobile phase was methanol: water: acetic acid: triethylamine in the ratio of 265: 35: 0.1: 0.05 (v/v/v/v). Flushing solvent used for the experiment was mobile phase. UV detector was used at a wavelength of 210 nm. The temperature of column was set at 16–18 °C. Flow rate of 0.6 mL/min along with the injection volume as 10 μL were set as the chromatographic conditions. Cosmosil C18 column was used in this assay.

2.3. Animal treatment

Pathogen-free male BALB/c mice (6–8 weeks old, 20 ± 2 g) were obtained from the animal center of Fudan University (Shanghai, China). The mice were housed in a controlled temperature (25 ± 1 °C) and humidity (50 ± 5%) environment with a 12-h light/dark cycle and allowed free access to sterilized food and water. All animal experiments were conducted in strict accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Fudan University Animal Care Committee.

Mice were randomly divided into five groups: saline (Nor) group, ConA model (Mod) group, JC group (900 mg/kg/day), low-dose JXHG group (LJXHG, 700 mg/kg/day), high-dose JXHG group (HJXHG, 1400 mg/kg/day). For the first 3 weeks, the mice in the normal group and the model group were given the same amount of 0.5% CMC-Na solution and the mice in the treated groups were given the corresponding medicines once a day. At day 21, one hour after intragastric administration, ConA (20 mg/kg) was injected into the caudal veins of mice, except for the normal group, which received 0.9% physiological saline (0.05 mL/10 g). After 10 h or 24 h, mice were killed, and the serum and liver tissue were collected. All samples are stored at − 80 °C before measurement. Liver index (%) = liver mass / mouse body mass × 100%.

2.4. Serum aminotransferase analysis

After blood collection, all serum samples were separated by centrifugation at 3000 rpm for 15 min. The OD value of serum alanine aminotransferase (ALT/GOT) and aspartate aminotransferase (AST/GPT) were tested by spectrophotometric method using an automated chemistry analyzer (TECAN). (Absolute OD value = Measured OD value–Blank OD value). According to the standard curve, the corresponding ALT/GOT, AST/GPT vitality unit was obtained.

2.5. Liver histopathology analysis

The liver tissue samples were fixed in 10% paraformaldehyde and embedded in paraffin, cut at thicknesses of 5 μm, and stained with hematoxylin and eosin (H&E) and examined under light microscopy by two of the investigators in a blinded manner.

2.6. Serum cytokines assay

Blood was harvested at designated time-points after Con A challenge. The levels of serum cytokine TNF-α , interferon-γ (IFN-γ) , IL-4 and IL-6 were analyzed by the commercial ELISA kits.

2.7. Real-time quantitative PCR analysis

Liver samples were collected 24 h after Con A administration. Total RNA was extracted from liver tissues using Trizol reagent. RT-PCR kits (Perfect Real Time, SYBR® PrimeScriP™ TaKaRa) were used to test the expression levels of IFN-γ, IL-6, IL-10, SOD1, GSR and GPX2. The