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Research Paper

Hepatoprotective effects of Yulangsan polysaccharide against isoniazid and rifampicin-induced liver injury in mice

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ABSTRACT

Ethnopharmacological relevance: Yulangsan polysaccharide (YLSPS) is often used in popular folk medicine in the Guangxi Zhuang Autonomous Region of China as a chief ingredient of *Millettia pulchra*, which is used as an hepatic protection, anti-aging and memory improving agent. In this study, the hepatoprotective effects of YLSPS against isoniazid (INH) or rifampicin and isoniazid (RFP+INH)-induced liver injury were investigated in mice.

Materials and methods: The liver injury was induced by intragastric administration of INH or RFP+INH daily for 10 days. During the experiment, the model group received INH or RFP+INH only, and the normal control group received an equal volume of saline. Treatment groups received not only INH or RFP+INH but also the corresponding drugs, DDB (200 mg/kg/day) or YLSPS (100, 200, and 400 mg/kg/day) 2 h after the administration of INH or RFP+INH.

Results: Analysis experiments showed that YLSPS significantly alleviated liver injury as indicated by the decreased levels of ALT and AST and the increased levels of SOD, GSH and GSH-Px. Moreover, YLSPS could effectively reduce the pathological tissue damage. The research on the mechanisms underlying the hepatoprotective effect showed that YLSPS was able to reduce lipid peroxidation and activate the antioxidative defense system.

Conclusion: Our results show that YLSPS is effective in attenuating hepatic injury in the INH or RFP+INH-induced mouse model, and could be developed as a new drug for treatment of liver injury.

1. Introduction

Tuberculosis is a fatal communicable disease that is easily spread among people. The WHO has declared that tuberculosis is a global emergency, with 9 million new cases and almost 2 million deaths per year worldwide (WHO, 2008). Multi-drug resistant strains of *Mycobacterium tuberculosis* have emerged. The administration of isoniazid (INH) or rifampicin and isoniazid (RFP+INH) is the most common medication prescribed against tuberculosis. These treatment protocols produce many metabolic and morphological aberrations in the liver because the liver is the main detoxifying site for these antitubercular drugs. Treatment with INH or RFP+INH can also induce hepatitis through a multiple step mechanism. This induction is characterized by a fall in serum albumin concentration and a rise in serum globulin concentration (Saad et al., 2010). These effects are related to the severity and duration of the disease. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of INH or RFP+INH. Antitubercular-drug-mediated oxidative damage is generally attributed to the formation of highly reactive oxygen species, which act as stimulators of lipid peroxidation and a source of destruction and damage to cell membranes (Georgieva et al., 2004; Santhosh et al., 2007). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components have been reported in INH- or RFP+INH-induced hepatotoxicity (Tasduq et al., 2005).

Marine polysaccharides have been shown to have a number of medicinal applications. Yulangsan (YLS) is the root of *Millettia Pulchra* (Benth.) Kurz var. *Laxior* (Dunn) Z. Wei (Lin et al., 2014). The extract of YLS has been demonstrated to be an effective antioxidant and is used in the treatment of neurological and cardiovascular diseases (Huang et al., 2003; Huang et al., 2008). Yulangsan polysaccharide (YLSPS) is the major effective ingredient in the extract of YLS (Lin et al., 2014), which is often used as an hepatic protection agent in folk medicine (Guangxi FDA, 2008; Dai, 2009). YLS is capable of both inhibiting peroxidation in vitro and suppressing the production of an excess of free radicals in vivo (Jiao et al., 2004). Furthermore, YLSPS alleviated the acute hepatic injury in mice and CCl_4 induced liver fibrosis in rats (Duan et al., 2007, 2008; Fu et al., 2009). Based on these reports,

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it would be of great interest to see if YLSPS has beneficial effects on antitubercular drug-induced hepatoxicity and to explore its underlying mechanism.

To test our hypothesis, a classic INH or RFP+INH-induced liver injury model was chosen to study the hepatoprotective effects of YLSPS in mice. The effect of YLSPS on liver injury was compared with that of dimethyl diphenyl bicarboxylate (DDB). DDB is a anti-hepatitis drug used in the therapy of chronic persistent hepatitis, chronic active hepatitis, drug-induced injury and other diseases (Hassan et al., 2010). DDB has been shown to directly protect hepatocyte DNA from oxidative damage, and it is capable of inhibiting tumor necrosis factor (TNF)-alpha mRNA expression in liver tissue. These effects of DDB have resulted in the prevention of liver damage (Gao et al., 2005). Park et al. (2005) have also demonstrated that DDB protects the liver from chemical-induced injury potentiated by glutathione (GSH) deficiency; DDB has the additional advantage of lowering plasma lipids. The markers of liver oxidative stress and anti-oxidative defense systems have also been examined for the investigation of the hepatoprotective mechanisms of YLSPS.

2. Materials and methods

2.1. Chemicals

YLSPS was prepared by a method described previously (Lin et al., 2014). The root of the *Millettia Pulchra* (Benth.) Kurz var. *Laxior* (Dunn) Z. Wei was dried, powdered, and extracted three times with boiling water. The polysaccharide in the filtrate was precipitated fractionally with alcohol. The protein in the product was removed by the Sevag method and further purified using DEAE ion exchange cellulose (DEAE-52). The components of the saccharide were determined by GC and TLC. The results showed that YLSPS was composed of D-glucose and D-arabinose in a molar ratio of 90.79% and 9.21%, with an average molecular weight of 14,301 Da.

Rifampicin (RFP) was purchased from Guangdong Southern China Pharmaceutical Group Co., Ltd (Guangdong, China); isoniazid (INH) was purchased from Shantou Jinshi General Pharmaceutical Factory (Shantou, China); dimethyl diphenyl bicarboxylate (DDB) was purchased from Guangzhou Xingqun Pharmaceutical Co., Ltd (Guangzhou, China); alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), Coomassie (Bradford) protein assay kits were acquired from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China); all other chemicals were of analytical grade.

2.2. Animals

Kunming mice of both sexes, weighing 20 ± 2 g, SPF, were provided by the Experimental Animal Center of Guangxi Medical University (Guangxi, China). The studies were conducted according to protocols approved by our institutional ethical committee. All mice were housed under controlled conditions with temperature of 25 ± 2 °C, relative humidity of 60 ± 10%, room air changes 12–18 times/h, and a 12-h light/dark cycle. Food and water were available ad libitum.

2.3. Treatment

INH-induced liver injury model: after an acclimatization period of 1 week, the animals were divided into six groups (five male mice and five female mice/group) and treated for 10 days as follows: (i) the normal control group, animals received saline intragastrically; (ii) the INH-treated model group, animals received INH (100 mg/kg/day) intragastrically for 10 days; (iii) the DDB-treated group (positive control group), animals received DDB (200 mg/kg/day) intragastrically 2 h after administration of INH (100 mg/kg/day) for 10 days; (iv) the low-, medium- and high-dosage YLSPS-treated groups, animals received YLSPS (100, 200 or 400 mg/kg) intragastrically 2 h after administration of INH (100 mg/kg/day) for 10 days. The drugs were dissolved in distilled water and diluted with physiologic saline for the animal tests.

RFP+INH-induced liver injury model: After an acclimatization period of 1 week, the animals were divided into six groups (five male mice and five female mice/group) and treated for 10 days as follows: (i) the normal control group, animals received saline intragastrically; (ii) the RFP+INH-treated model group, animals received RFP (100 mg/kg/day)+INH (100 mg/kg/day) intragastrically for 10 days; (iii) the DDB-treated group (positive control group), animals received DDB (200 mg/kg/day) intragastrically 2 h after administration of RFP (100 mg/kg/day)+INH (100 mg/kg/day) for 10 d; (iv) the low-, medium- and high-dosage YLSPS-treated groups, animals received YLSPS (100, 200 or 400 mg/kg) intragastrically 2 h after administration of RFP (100 mg/kg/day)+INH (100 mg/kg/day) for 10 d.

At the final stage of the experiment, the animals were fasted overnight and sacrificed on the next day by cervical dislocation immediately after withdrawal of blood from the retrobulbar vessels. Afterwards, the whole blood was centrifuged and serum samples were collected. Liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. One fraction of the liver samples was immediately stored at −80 °C for future analysis; another fraction was excised and fixed in a 10% formalin solution for histopathologic analysis.

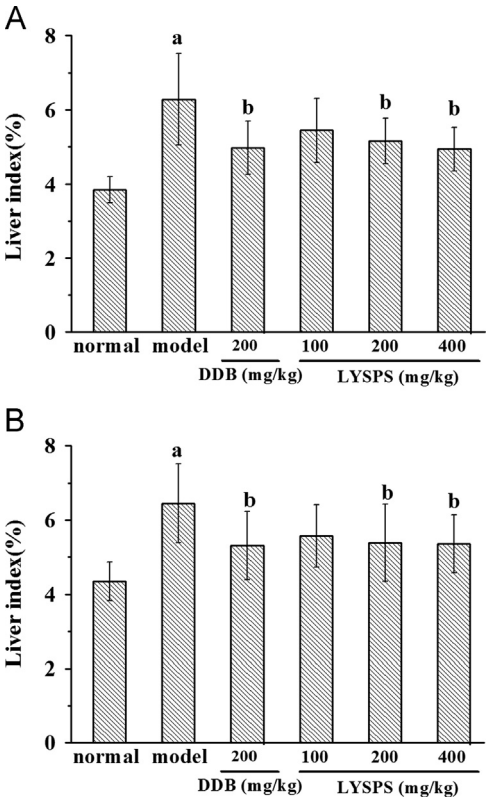

Fig. 1. (A) Effect of YLSPS on liver index in INH-induced hepatic injury mice. Results are presented as the mean ± S.E. (%) ($n=10$). $^{a}p<0.05$ compared with normal control group, $^{b}p<0.05$ compared with INH model group. (B) Effect of YLSPS on liver index in RFP+INH-induced hepatic injury mice. Results are presented as the mean ± S.E. (%) ($n=10$). $^{a}p<0.05$ compared with normal control group, $^{b}p<0.05$ compared with RFP+INH model group.