Biomedicine & Pharmacotherapy 99 (2018) 537–542

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Protective effect of acidic polysaccharide from *Schisandra chinensis* on acute ethanol-induced liver injury through reducing CYP2E1-dependent oxidative stress

Rongshuang Yuan[a], Xue Tao[a], Shuang Liang[a], Yan Pan[a], He Li[a], Jinghui Sun[a], Wenbo Ju[a], Xiangyan Li[b], Jianguang Chen[a,*], Chunmei Wang[a,*]

[a] Department of Pharmacology, College of Pharmacy, Beihua University, Jilin, Jilin, China
[b] Research and Development Center of Traditional Chinese Medicine and Biological Engineering, Changchun University of Chinese Medicine, Changchun, Jilin, China

ARTICLE INFO

Keywords:
Schisandra chinensis
Polysaccharide
Alcoholic liver disease
CYP2E1

ABSTRACT

Aim: *Schisandra chinensis* is a well-known traditional Chinese medicine used mainly as a recipe for hepatoprotection. Modern researches have revealed that the hepatoprotection is related to its lignans and crude polysaccharide. In this study, we examined the effect and mechanism of *Schisandra chinensis* acidic polysaccharide (SCAP) on the liver injury induced by ethanol.

Main methods: SCAP was extracted with water extraction and ethanol precipitation. Liver injury models of both mice and HepG2 cells were produced by ethanol. The liver index, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum of the mice and cell culture supernatant were examined; HE staining was performed for observing pathological changes of liver. The malondialdehyde (MDA) level and superoxide dismutase (SOD) activities in serum, liver tissue and HepG2 cells, and triglyceride (TG) content in liver tissue were tested. Western blot was conducted to determine cytochrome P450 2E1 (CYP2E1) expression in liver tissue of mice and HepG2 cells.

Key findings: SCAP significantly reduced serial AST and ALT levels in the injuried liver and HepG2 cells induced by ethanol and also decreased TG level in the liver tissue. SCAP also obviously improved the hepatopathological changes and decreased MDA level as well as increased SOD activities in the serum, liver tissue and HepG2 cells induced by ethanol. Furthermore, Western blot analysis indicated that SCAP significantly inhibited the upregulation of CYP2E1 protein.

Significance: SCAP has a protective effect on ethanol-induced liver injury in mice and cells, and the mechanism underlying may be via inhibiting the expression of CYP2E1 protein and then alleviating oxidative stress injury induced by ethanol.

1. Introduction

Alcoholic liver disease (ALD) is a disorder caused by a long-term heavy alcohol drinking, and can progress to alcoholic hepatitis and alcoholic liver fibrosis, finally even alcoholic liver cirrhosis and liver cancer [1,2]. In recent years, with the improvement of living standards, the incidence of ALD is rising year by year and ALD has become the second major cause of liver injury following viral hepatitis [3]. The pathogenesis of ALD is not completely clear, and it is considered now that its pathogenesis is mainly associated with oxidative stress and lipid peroxidation, cytokines and mitochondrial dysfunction, of which oxidative stress plays a key role in the occurrence and development of acute alcoholic liver injury [4,5]. Currently, the treatment of alcoholic liver injury is unsatisfied and there is no effective drug for its treatment in clinic yet [6].

Schisandra chinensis (turcz.) baill (*Schisandra chinensis*, *Schisandra*) is a traditional Chinese medicine and has been used in clinic for thousands of years, and usually used for the treatment of viral and chemical liver

Abbreviations: ALD, alcoholic liver disease; SCAP, *Schisandra chinensis* acidic polysaccharide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; MDA, malondialdehyde; SOD, superoxide dismutase; HE, hematoxylin and eosin; ADH, alcohol dehydrogenase; CYP2E1, cytochrome P450 2E1; MEOS, microsomal ethanol oxidizing system; ROS, reactive oxygen species; SD, standard deviation; ANOVA, analysis of variance

* Corresponding authors at: Department of Pharmacology, College of Pharmacy, Beihua University, No. 3999 Binjiang East Road, Fengman District, Jilin City 132013, Jilin Province, China.

E-mail addresses: yuanrs1993@163.com (R. Yuan), taoxue1120@126.com (X. Tao), lllsss1110@126.com (S. Liang), py941102@163.com (Y. Pan), yitonglh@126.com (L. He), sunjinghui2008@126.com (J. Sun), Juwenbo7061@aliyun.com (J. Wenbo), xiangyan-li1981@163.com (X. Li), chenjg@beihua.edu.cn (J. Chen), wangcm74@126.com (C. Wang).

https://doi.org/10.1016/j.biopha.2018.01.079
Received 28 November 2017; Received in revised form 26 December 2017; Accepted 12 January 2018
0753-3322/ © 2018 Elsevier Masson SAS. All rights reserved.

injuries, with an obvious therapeutic effect [7]. The main active constituents of *Schisandra* include lignans and polysaccharides, and most of the current studies focus on ethanol extracts and lignans of *Schisandra* [8,9]. Plant polysaccharides have attracted more and more attention due to their antioxidant, hypoglycemic, immunoregulation functions [10]. The content of polysaccharide accounts for about 10% of *Schisandra*, the results of our previous studies showed that *Schisandra* polysaccharide had a significant protective effect on the liver injury induced by both CCL_4 and high-fat diet, and a significant antioxidant effect, suggesting that *Schisandra* polysaccharide might be one of components that could play a protective effect of liver injury [11,12]. However, whether *Schisandra* polysaccharide has a protective effect on the liver injury induced by alcohol has not been reported yet. In this study, ethanol-induced mouse liver injury and liver cell injury models were established, then the preventive and therapeutic effect of *Schisandra chinensis* acidic polysaccharide (SCAP) on the alcoholic liver injury was investigated, and the underlying mechanism was further explored, to provide a theoretical basis for the further development of *Schisandra* new drugs used for the prevention and treatment of ALD and health foods with the auxiliary function.

2. Materials and methods

2.1. Reagents

Schisandra chinensis was purchased from Jian *Schisandra* Seedlings Base of Jilin Province and identified by Professor Lihua Zhang at the College of Pharmacy, Beihua University, according to the identification standard of the Pharmacopoeia of the People's Republic of China (2015 Edition). Kits for measuring serum and tissue levels of ALT, AST, TG, SOD and MDA were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA kit was purchased from Beyotime Institute of Biotechnology (Jiangshu, China). Human hepatic cancer cell lines HepG2 cells were obtained from American Type Culture Collection (ATCC, USA). DMEM medium and other culture reagents were obtained from Hyclone (Logan, Utah, USA). Polyclonal antibody against CYP2E1 was purchased from Abcam (Cambridge, MA, USA). Reagents for Western Blot were purchased from Bio-Rad (California Hercules, USA).

2.2. Extraction and characterization of SCAP

2.2.1. Extraction of SCAP

SCAP was prepared in The Center of Life Science, Northeast Normal University. Briefly, *Schisandra* was steeped in 10 times volume of distilled water overnight and extracted twice by boiling it in 100 °C hot water. The concentrated extract was added with 95% ethanol to be adjusted to one containing of 75% ethanol, precipitated overnight, and then centrifuged (4500 rpm, 15 min). The precipitation was collected and washed with 95% ethanol and absolute ethyl alcohol in turn, and then dried routinely to obtain a powder-like *Schisandra* polysaccharide, with a yield of 8.55% polysaccharide. A 0.5% *Schisandra* polysaccharide solution was prepared and loaded on a balanced DEAE-cellulose ion-exchange chromatographic column (20mL, Cl-type), eluted with distilled water to remove the neutral polysaccharide. Then 0–1.0 M NaCl solution was used for the linear gradient elution at a flow rate of 1 mL/min to obtain the eluent, which was concentrated and dried routinely to obtain the *Schisandra chinensis* acidic polysaccharide, with a yield about 2.8%.

2.2.2. Characterization of SCAP

Using D-glucose as the reference substance, total sugar content was determined with phenol-sulfuric acid method [13]. Using galacturonic acid as the reference, uronic acid content was measured with meta-hydroxydiphenyl method [14]. Each monosaccharide composition of SCAP was analyzed by PMP derivatization and high performance liquid chromatography (HPLC).

2.3. Animal experimentation

Male ICR mice, weighing 19–21 g, were provided by Changchun Yisi Laboratory Animal Technology Co. Ltd. (Changchun, China) [license number: SCXK (Ji) 2015-0001, SPF]. The mice were raised in separate cages at 18–22 °C (relative humidity 40-60%), under normal lighting conditions, and with ad libitum access to water. The normal diet for the experimental mice was purchased from Changchun Yisi Laboratory Animal Technology Co. Ltd (Changchun, China). All procedures were approved by the Ethics Committee for Use of Experimental Animals at Beihua University (Jilin, China).

Fifty male mice were randomly divided into normal control group (CON), model group (MOD), low-dose SCAP group (SCAP-L, 5 mg/kg), middle-dose SCAP group (SCAP-M,10 mg/kg) and high-dose SCAP group (SCAP-H, 20mg/kg), 10 in each group. Mice in the CON and MOD groups were administered equal volumes of water and those in SCAP-treated groups were intragastrically given 5, 10, 20 mg/kg of SCAP once daily successively for 15 days.

One hour after the last administration, mice in the MOD group and SCAP-treated groups were intragastrically given 50% ethanol solution (12 mL/kg), and those in the CON group were intragastrically given the same volume of solvent. All the mice were fasted, but with free access to water for 16 h, and anesthetized with ether, then 0.8–1.0 mL of blood was collected from each mouse by removing eyeballs. The serum was separated by centrifugation at 4000 rpm, 10 min and stored at −80 °C. The peritoneal cavity was opened along the abdominal middle line, and the liver carefully isolated and removed to calculate liver index (wet liver weight/body weight × 100%). The hepatic tissue was washed with cold saline and divided into three parts: the first part was fixed with 10% neutral formaldehyde for histopathological examination, the second part was prepared into homogenates for the detection of relative index, and the third part was preserved at −80 °C for analyzing the mechanism of drug action.

2.4. Cell culture and administration

HepG2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells were incubated in humidified atmosphere of 5% CO_2 at 37 °C and passaged according to the recommended procedures of ATCC, and used for experiments from the logarithmic phase of growth, seeded into 96-well plates (1×10^4 cells per well, 100 μL). Cells were exposed to different concentrations of ethanol (2%, 3%, 4%, 5%, 6%, v/v) or SCAP (3.12, 6.25, 12.5, 25, 50, 100 μg/mL) for 24 h for determining the concentration of ethanol and SCAP. The final concentration of ethanol was 3% for inducing liver cell injury model, and cells in SCAP-treated groups were treated with 3.12, 6.25 and 12.5 μg/mL of SCAP, respectively.

2.5. Determination of cell viability

Cell viability was determined by MTT assay method [15]. Briefly, MTT (5 mg/mL, 20 μL/per well) were added into the cell-seeded 96-well plates and incubated at 37 °C for 4 h. Then the solutions were removed and DMSO (100 μL/well) was added into the wells. The absorbance was measure at 490 nm using a 96-well plate reader.

2.6. Detection of ALT and AST

Cell culture supernatants were collected from different groups after the treatment for 24 h. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the mice's serum and cell culture supernatant were determined using the commercial kits.

Download English Version:

https://daneshyari.com/en/article/8526286

Download Persian Version:

https://daneshyari.com/article/8526286

Daneshyari.com