International Immunopharmacology 31 (2016) 140–148

Contents lists available at ScienceDirect

International Immunopharmacology

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

Angelica sinensis polysaccharide attenuates concanavalin A-induced liver injury in mice

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ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 4 December 2015
Accepted 16 December 2015
Available online xxxx

Keywords:
Angelica sinensis polysaccharide
Concanavalin A
JNK
NF-κB
Oxidative stress
Proinflammatory cytokine

ABSTRACT

Angelica sinensis polysaccharide (ASP), extracted from the roots of *A. sinensis* (Oliv.) Diels, is a β-D-pyranoid polysaccharide with an average molecular weight of 72,900 Da. In this study, we investigated the protective effects of ASP against concanavalin A-induced liver failure and the underlying mechanisms. Concentrations of ASP ranging from 5 to 125 μg/mL could inhibit concanavalin A (ConA)-induced lymphoproliferative response. The potential hepatoprotective activity of ASP was demonstrated by the significant decrease in serum transaminase (ALT and AST) levels and the attenuation of liver inflammation damage exhibited by H&E stain of the liver. Furthermore, ASP pretreatment significantly decreased proinflammatory cytokines (TNF-α, IFN-γ, IL-2 and IL-6) and alleviated oxidative stress by reducing MDA and ROS levels and by enhancing SOD activity after ConA administration in mice. Results of Western blot analysis indicated that ASP attenuated Caspase-3-dependent apoptosis by Caspase-8 and JNK-mediated pathway and inhibited the activation of IL-6/STAT3 and NF-κB signaling pathways in ConA-induced liver damage in mice. In conclusion, ASP pretreatment could attenuate concanavalin A-induced liver injury through its anti-inflammatory and anti-oxidant actions in mice.

1. Introduction

Liver injury, which can be easily induced by various factors including viral infections, alcohol, drug abuse and autoimmune attack of hepatocytes, results in a public health problem worldwide results in a worldwide health problem in human [1,2]. Autoimmune and viral hepatitis can progress into cirrhosis and no effective therapies exist at present, resulting in a significant health issue. Concanavalin A (ConA), a lectin derived from the seeds of jack beans (*Canavalia ensiformis*), has been widely used to establish experimental murine model of autoimmune or viral hepatitis to mimic many pathological features of viral and autoimmune hepatitis in human [3]. Previous studies have reported that ConA is a T-cell mitogen which activates T cells to proliferate and produce proinflammatory cytokines, including tumor necrosis factor TNF-α, interferon IFN-γ and interleukin IL-6 [4].

Polysaccharides are biomacromolecules with diverse functions and have been reported to play a vital role in sustaining life functions such as respiration, cellular mechanical strength and stress tolerance. Therefore, polysaccharide-rich plants, fungi and algae are used for centuries around the world as food components for disease prevention and medical application [5]. *Angelica sinensis* (Oliv.) Diels, a kind of Chinese herbal medicine, has been widely used for thousands of years in prescriptions for anemia, rheumatism, cardiovascular disease, hepatic fibrosis [6]. It is well documented that ASP is the main effective chemical substance of *A. sinensis* and has various important bioactivities, such as hematopoietic, antiviral, antitumor, radioprotective and hypoglycemic activity [7]. Furthermore, studies on phytochemistry and pharmacology have demonstrated that ASP exhibits hepatoprotective effects against carbon tetrachloride (CCl4)-induced liver injury and anti-oxidation effects by using myocardial ischemia/reperfusion (I/R) rat [8].

Several active components have been developed as liver-protection drugs. However, long-term use of those drugs may give rise to obvious complications and side effects, such as elevated blood sugar, water and sodium retention plus concurrent infection. Our previous studies found that ASP could protect the integrity of cellular shape and improve hepatic steatosis in the diabetic mice by selectively triggering liver tissue, indicating the potential hepatoprotective activity against hepatic injury [9]. Therefore, in the present study, we isolated ASP from

Abbreviations: ASP, *Angelica sinensis* polysaccharide; ConA, concanavalin A; HPGPC, high performance gel permeation chromatography; TNF-α, tumor necrosis factor alpha; SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species; CASPASE, cysteine-aspartic acid specific protease; JNK, c-Jun N-terminal kinase.

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http://dx.doi.org/10.1016/j.intimp.2015.12.021
1567-5769/

A. sinensis and investigated whether ASP could prevent liver failure in mice provoked by ConA.

2. Materials and methods

2.1. Materials and reagents

ConA was purchased from Sigma Aldrich (St. Louis, MO, USA). Assay kits for the determination of AST, ALT, MDA, ROS and SOD were obtained from Jiancheng Bioengineering Institute (Jiangsu, China). Enzyme-linked immunosorbent assay (ELISA) kits of IFN-γ and TNF-α were purchased from eBioscience (San Diego, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kits of IL-2 and IL-6 were purchased from NeoBioscience Technology Co., Ltd. (Shenzhen, China). Primary antibodies against NF-κB, IKKα, p-IκBα, Caspase-3, Caspase-8, cleaved Caspase-8, p-JNK, Bax, Bcl-2, p-Akt, STAT3 and p-STAT3 were purchased from Cell Signaling Technology (Danvers, MA, USA). All of the other chemicals and reagents were of analytical grade.

2.2. Extraction and purification of the plant polysaccharide

The raw *A. sinensis* polysaccharide was achieved from the fresh roots of *A. sinensis* (Oliv.) Diels by boiling water extraction and alcohol precipitation method as previously described [10,11]. Briefly, the dried roots (200 g) were defatted thoroughly with 95% alcohol and then extracted with distilled water (*w*/*v*, 1:10) at 100 °C for two times, 1 h each time. Subsequently, the combined extracts were pooled, filtered, concentrated to half of the original volume and treated with $Ca(OH)_2$ at pH 12. The supernatant was separated by centrifugation at 4000 rpm for 10 min. After adding dilute sulfuric acid to adjust to pH 5–6, the supernatant was concentrated to 100–150 mL. An equal volume of 95% alcohol was added while stirring to precipitate the crude polysaccharide. After standing at 4 °C overnight, the precipitate was obtained by centrifugation at 6000 rpm for 15 min and dried at 60 °C. Finally, the Sephadex G-50 column was used to get the purified *A. sinensis* polysaccharide. The refined polysaccharide, named ASP, was received by freeze-drying.

2.3. General analytical methods

As described previously, total sugar content, monosaccharide composition and molecular weight were determined by phenol-sulfuric acid colorimetric method, GC–MS and HPGPC. Proteins and nucleic acids in the polysaccharide were detected, combined with the method of UV absorption on a UV-spectrophotometer (UV-1750). The FT-IR spectra of ASP in the frequency range of 4000–500 cm^{-1} were obtained using a Fourier-transform infrared spectrometer (Bruker-VERTEX 70, Germany).

2.4. Animals

Male Balb/c mice (6–8 weeks old and weighing 18.0–22.0 g) were purchased from the Center for Experimental Animal Research in Hubei province, China for the present study. Mice were kept in cages under controlled conditions of 22 ± 0.5 °C, 50 ± 2.0% RH and maintained with free access to food and water under a normal day/night cycle at least 6 days before experiments. The procedures for the present study were approved by the Animal Care and Use Committee of Hubei Province, China.

2.5. Establishment of the ConA-induced hepatitis model and experimental groups

Animals were randomly divided into five groups (n = 10 each): the normal control group, the ASP (6 mg/kg) group, the ConA group, the ConA + ASP (1.5 mg/kg) group, and the ConA + ASP (6 mg/kg) group. ConA and ASP were completely dissolved in pyrogen-free PBS before passing through a 0.22 μm cell strainer. The ConA + ASP (1.5 mg/kg) group and the ConA + ASP (6 mg/kg) group were intravenously administered single doses of 1.5 and 6 mg/kg body weight of ASP per day for two weeks, respectively. A single intravenous (i.v.) administration of a dose (6 mg/kg body weight) of ASP was given to the ASP (6 mg/kg) group per day for two weeks. The normal control group and the ConA group were intravenously administered with the same volume of PBS per day for two weeks. The ConA group, the ConA + ASP (1.5 mg/kg) group and the ConA + ASP (6 mg/kg) group were challenged intravenously with ConA (15 mg/kg body weight) on the 15th day, while the normal control group and the ASP (6 mg/kg) group were challenged intravenously with the same volume of PBS on the 15th day. Blood samples were collected 8 h after ConA or PBS injection. After 20 h of ConA challenge, all mice were sacrificed, and the liver tissues were collected.

2.6. Hematoxylin and eosin (H&E) staining

Liver tissues fixed in 4% paraformaldehyde were embedded in paraffin using a tissue procedure, and 4-μm-thick sections were cut and stained with hematoxylin and eosin (H&E) reagent. Photomicrographs were taken with a light microscope equipped with a microscope camera.

2.7. Assessment of liver injury

Serum AST, ALT and MDA contents and liver ROS levels and liver SOD activities were analyzed using a colorimetric test. Serum TNF-α, IFN-γ, IL-2 and IL-6 were determined by ELISA kits.

2.8. Western blot

The total cellular proteins were extracted from frozen liver tissues and equal amounts (60 μg per lane) of proteins were subjected to electrophoresis on SDS-PAGE. The separated proteins were electroblotted onto nitrocellulose membranes (Hybond, Escondido, CA, USA). After blocked with 5% fat-free milk in TBS-T, the membranes were incubated overnight at 4 °C with primary antibodies (1:1000 diluted; Cell Signaling Technology, Danvers, MA, USA), respectively. After washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h, protein bands were visualized with an enhanced chemiluminescence (ECL) detection kit and immediately exposed to X-ray films. Protein expression levels were normalized as the ratio of the target protein to β-actin or GAPDH.

2.9. Splenocytes preparation and proliferation assays

Spleen single cell suspensions were prepared, depleted of red blood cells, washed and collected by standard techniques as described earlier from Balb/c mice [12,13]. Viable cells counts were performed by trypan blue exclusion test. Spleen cell suspensions were cultivated using pyrogen-free RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin–streptomycin and 10% fetal serum (Gibco, Paisley, UK). Triplicate cultures were performed in 96-well flat-bottom plates (Corning Inc., Corning, NY) in a final volume of 100 μL per well approximately containing 1×10^6 cells. A volume of 20 μL of ConA (20 μg/mL, dissolved with PBS) was added either in the absence or in the presence of 100 μL ASP (5–125 μg/mL). After passing through a 0.22 μm cell strainer, different concentrations of ASP (5–125 μg/mL, dissolved with PBS) were added to the ASP control group so that the final volume was 220 μL. A volume of 120 μL of complete medium was added to the normal control group. Cultures were then incubated at 37 °C, 5% CO_2 for 3 days. The MTT assay was done as described earlier, and the absorbance was read at 570 nm [14].