Protective Effect of Acanthopanax giraldii Polysaccharide on Immune Injury in Rat Hepatocytes and Its Mechanism Postprint

Authors: Li Qingqing, Wu Jiang, Chen Yong, Zheng Birong, Li Ruyue, Wang Shuxian, Zhu Yaoqiang

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Abstract

Acanthopanax giraldii belongs to the Araliaceae family, most members of which exhibit bidirectional immunomodulatory effects. Acanthopanax giraldii polysaccharide is a single component extracted from the traditional Chinese medicine Acanthopanax giraldii, and there are currently few reports on its anti-inflammatory effects. This study aimed to investigate the protective effect of Acanthopanax giraldii polysaccharide AHP-II against LPS-induced immune injury in rat hepatocytes and its underlying mechanism. The experiment was divided into five groups: a control group, a model group (LPS, 40 $g \cdot mL^{-1}$), and low, medium, and high dose groups of AHP-II (25, 50, 100 $g \cdot mL^{-1}$). The rat hepatocyte immune injury model was induced using LPS (40 $g \cdot mL^{-1}$). ELISA was employed to detect TNF-α secretion levels, and flow cytometry was used to measure ROS content, thereby investigating the inhibitory effects of different AHP-II doses on inflammatory factors. Furthermore, Western blotting was utilized to detect P-JNK protein levels to further explore the inhibitory mechanism of AHP-II. The results demonstrated that AHP-II at low, medium, and high doses all decreased TNF-α content in hepatocytes after injury. Simultaneously, AHP-II at medium and high doses reduced ROS secretion in injured hepatocytes, with the high-dose group exhibiting the strongest inhibitory effect on ROS. Following AHP-II treatment, the phosphorylation level of JNK2 protein decreased in a dose-dependent manner, with the high-dose AHP-II group showing the most potent inhibition. These findings indicate that Acanthopanax giraldii polysaccharide AHP-II can exert immunoprotective effects by reducing P-JNK protein content to inhibit the levels of the inflammatory factor TNF-α and ROS.

Full Text

Preamble

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Title: Protective Effect of Acanthopanax giraldii Polysaccharide on Hepatocyte Immune Injury in Rats and Its Mechanism
Authors: LI Qingqing, WU Jiang, CHEN Yong*, ZHENG Birong, LI Ruyue, WANG Shuxian, ZHU Yaoqiang
Affiliation: Weifang Medical University, Weifang 261053, Shandong, China

Abstract

Acanthopanax giraldii belongs to the Araliaceae family, most members of which exhibit bidirectional immunomodulatory effects. Acanthopanax giraldii polysaccharide (AHP-II) is a single component extracted from this traditional Chinese medicine, though few reports have documented its anti-inflammatory effects. This study investigated the protective effects of AHP-II against LPS-induced hepatocyte immune injury in rats and explored the underlying mechanisms. The experiment consisted of five groups: blank control, model (LPS, 40 $g \cdot mL^{-1}$), and low-, medium-, and high-dose AHP-II groups (25, 50, and 100 $g \cdot mL^{-1}$, respectively). An LPS-induced hepatocyte immune injury model was established, and ELISA was used to measure TNF-α secretion while flow cytometry assessed ROS content. Western blotting was employed to detect P-JNK protein levels to further elucidate the inhibitory mechanism of AHP-II. The results demonstrated that all three doses of AHP-II reduced TNF-α levels in damaged hepatocytes. Both medium and high doses decreased ROS secretion, with the high-dose group showing the strongest effect. AHP-II treatment led to a dose-dependent reduction in JNK2 phosphorylation, again most pronounced in the high-dose group. These findings indicate that AHP-II exerts immunoprotective effects by suppressing inflammatory factors TNF-α and ROS through downregulation of P-JNK protein expression.

Keywords: *Acanthopanax giraldii* polysaccharide; lipopolysaccharide; liver injury; JNK

Introduction

Acanthopanax giraldii is a perennial shrub of the Araliaceae family, with its medicinal component being the hairy stem bark known as *Acanthopanax giraldii* cortex. Most Araliaceae plants possess therapeutic properties including dispelling wind-dampness, unblocking joints, tonifying liver and kidney, and strengthening bones and tendons. Previous research has shown that *Acanthopanax senticosus* water extract significantly enhances mononuclear-macrophage phagocytic function in mice, increases delayed-type hypersensitivity reactions, and elevates spleen and thymus indices, suggesting immune-enhancing effects associated with macrophage activation (Wang et

al., 2013). Other studies have found that intraperitoneal injection of *A. senticosus* extract markedly inhibits H_2O_2 production by mouse peritoneal macrophages stimulated with LPS & IFN-γ and PMA, as well as NO production induced by LPS & IFN-γ and O_2^- generation triggered by ZYM, indicating anti-inflammatory efficacy through suppression of inflammatory mediators from activated macrophages (Lin, 2007). These findings suggest that most active components from Araliaceae exhibit dual functions in both suppressing inflammatory responses and promoting immune cell activity (Chien et al., 2015; Sun et al., 2012).

Our research group previously isolated a single polysaccharide component, AHP-II, from *A. giraldii* and demonstrated its immunostimulatory activity. However, few studies have reported on the anti-inflammatory effects of this polysaccharide. To further investigate AHP-II' s potential hepatoprotective effects, we established an LPS-induced injury model in rat hepatocyte BRL cells to simulate human immune-mediated liver injury and examined whether this single component possesses liver-protective properties.

Materials and Methods

1.1 Experimental Materials

Reagents and materials included: RPMI 1640 medium and fetal bovine serum (Gibco), penicillin-streptomycin solution (Beijing Solarbio Technology), LPS (Sigma), TNF-α ELISA kit (eBioscience), reactive oxygen species detection kit (Beyotime), phospho-JNK rabbit anti-mouse antibody (Cell Signaling Technology), HRP-conjugated goat anti-rabbit secondary antibody (Cell Signaling Technology), chemiluminescence kit (Thermo), AHP-II polysaccharide prepared by our research group, and BRL rat hepatocyte cell line purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

1.2.1 Cell Culture

BRL rat hepatocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 incubator and passaged every other day. Cells were seeded in culture plates according to experimental requirements.

1.2.2 Cytotoxicity Assessment of AHP-II

BRL cells were seeded in 96-well plates at a density of 10^3 cells per well. Experimental groups included: (1) blank control, (2) low-dose AHP-II (25 $g \cdot mL^{-1}$), (3) medium-dose AHP-II (50 $g \cdot mL^{-1}$), and (4) high-dose AHP-II (100 $g \cdot mL^{-1}$). The blank group received serum-free RPMI 1640 medium, while treatment groups received respective drug concentrations. After 24-hour incubation at 37°C in 5% CO_2, CCK-8 assay was performed to assess AHP-II' s cytotoxic effects on cell proliferation.

1.2.3 ELISA Detection of TNF-α

BRL cells were seeded in 6-well plates at 10^6 cells per well and cultured as described in Section 1.2.2. An additional LPS model control group (40 $g \cdot mL^{-1}$) was included. Following drug treatment, cells were incubated for 24 hours at 37°C in 5% CO_2. Supernatants were collected and TNF-α levels were measured according to the ELISA kit instructions.

1.2.4 Flow Cytometry Detection of ROS

BRL cells were seeded in 6-well plates at 10^6 cells per well and cultured for 24 hours as described in Section 1.2.3. Cells were then harvested and ROS content was quantified by flow cytometry.

1.2.5 Western Blot Analysis of JNK Phosphorylation

Cells were treated according to the method described in Section 1.2.3. After 1 hour of drug stimulation, total protein was extracted and JNK protein and phosphorylation levels were determined by Western blotting.

1.3 Statistical Analysis

Data were analyzed using SPSS 17.0 software and expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Multiple group comparisons were performed using LSD-t test.

Results

2.1 Effects of Different AHP-II Concentrations on Hepatocyte Toxicity

To exclude the influence of cell number on inflammatory factors, CCK-8 assay was used to assess the cytotoxic effects of AHP-II at various working concentrations. The results demonstrated that AHP-II at low, medium, and high concentrations exhibited no cytotoxic effects on cell proliferation (Table 1).

2.2 Effects of Different AHP-II Concentrations on Cytokine Secretion After Hepatic Injury

TNF-α is a critical factor in acute and chronic liver injury, exerting cytotoxic effects that indirectly or directly cause hepatocyte immune damage by activating T and B lymphocytes and enhancing NK cell cytotoxicity. TNF-α also induces massive secretion of cytokines including IL-8 and IL-6, mediating hepatocyte dysfunction and exacerbating liver injury. To establish an immune-mediated liver injury model, we stimulated hepatocytes with LPS and verified model establishment by measuring TNF-α secretion. Compared with the blank group, LPS stimulation significantly increased TNF-α levels ($P < 0.05$), confirming that LPS (40 $g \cdot mL^{-1}$) successfully induced a hepatocyte injury model. To

investigate AHP-II' s protective effects, we compared low-, medium-, and high-dose AHP-II groups with the LPS model group, finding significant differences ($P < 0.05$). All AHP-II dose groups reduced TNF-α levels in injured hepatocytes (Table 2).

2.3 Effects of Different AHP-II Concentrations on ROS Secretion After Hepatic Injury

Uncontrolled switching between hepatocyte proliferation and quiescence significantly impacts normal liver development, regeneration, and hepatocarcinogenesis. Current research indicates that reactive oxygen species (ROS) play a dual regulatory role in cell proliferation: high ROS levels induce apoptosis and necrosis, while low levels promote cell proliferation. Flow cytometry results showed that ROS content was significantly elevated in the LPS model group compared with the blank group ($P < 0.05$), consistent with ELISA findings and confirming that LPS (40 $g \cdot mL^{-1}$) induced an inflammatory model in BRL hepatocytes. AHP-II at 50 and 100 $g \cdot mL^{-1}$ significantly reduced ROS secretion in damaged hepatocytes compared with the model group ($P < 0.05$), with the 100 $g \cdot mL^{-1}$ dose showing the strongest inhibitory effect (Figures 1 and 2).

[Figure 1: see original paper]
[Figure 2: see original paper]

2.4 Effects of AHP-II on the JNK Signaling Pathway

Research has demonstrated close associations between the JNK signaling pathway and liver diseases, with JNK activation observed in various forms of hepatic stress and injury. Our results showed significant differences between the LPS group and blank group ($P < 0.05$), indicating that LPS stimulation induced JNK2 protein phosphorylation to initiate inflammatory responses. However, AHP-II treatment led to a dose-dependent decrease in JNK2 phosphorylation, with significant differences observed at 50 and 100 $g \cdot mL^{-1}$ compared with the LPS model group. The 100 $g \cdot mL^{-1}$ AHP-II dose exhibited the strongest inhibitory effect (Figures 3 and 4).

[Figure 3: see original paper]
[Figure 4: see original paper]

Discussion

Fulminant hepatic failure (FHF) is a fatal complication of acute liver injury characterized by poor prognosis and high mortality. While viral infection is the most common cause of FHF in developing countries, drug-induced acute liver injury has become the primary etiology in Western nations. The pathogenic mechanisms of pathogen-induced FHF involve two aspects: first, direct destruction of hepatocyte structure and organelles triggers signaling cascades that disrupt cellular function and induce apoptosis and necrosis; second, damaged hepato-

cytes release endogenous damage-associated molecular patterns (DAMPs) that activate the immune system, stimulating immune cells to release inflammatory cytokines that further exacerbate hepatocyte injury and ultimately lead to FHF. Despite significant progress in understanding FHF pathogenesis and diagnosis, no effective treatments currently exist. Liver transplantation remains the only available therapy but is limited by donor shortage. Consequently, increasing research efforts focus on identifying more effective therapeutic agents for FHF (Sultan et al., 2017).

Numerous compounds have demonstrated hepatoprotective and enzyme-lowering effects, including flavonoids (silymarin, baicalin, quercetin) (Zhang et al., 2014; Zhang and Ci, 2012; Weng et al., 2015), terpenoids (andrographolide, betulinic acid) (Wang, 2016; Xu and Yan, 2017), alkaloids (matrine) (Gao et al., 2013), polyphenols (kuding tea polyphenols) (Zhao et al., 2017), and polysaccharides (angelica polysaccharide, goji polysaccharide) (Song, 2016; Zhang et al., 2011). To maximize the value of traditional Chinese medicine resources, we extracted AHP-II from *A. giraldii* and combined it with rat hepatocyte culture techniques to establish an *in vitro* immune-mediated liver injury model using LPS challenge, which closely mimics human pathophysiological mechanisms, to investigate the protective effects of this polysaccharide against immune-mediated liver injury.

Most previous studies on hepatoprotective effects of Araliaceae polysaccharides have focused on alcoholic and chemical liver injury, with few reports on immune-mediated liver injury. Lu et al. (2016) found that *A. senticosus* polysaccharide protects against liver injury through metabolic pathways including cysteine and methionine metabolism, bile secretion, and citric acid cycle. Zhang et al. (2011) reported that *Acanthopanax gracilistylus* fruit ethanol extract protects against chemical liver injury by reducing serum ALT and AST activities and hepatic MDA content while increasing SOD activity. Hao et al. (2012) demonstrated that acanthopanax acid protects against alcoholic liver injury by elevating ALT, AST, and TNF-α activities and inhibiting hepatocyte DNA damage. Ju et al. (2005) found that *A. giraldii* polysaccharide exerts hepatoprotective effects by reducing excessive NO levels.

Numerous studies have shown that LPS triggers various pathological events, including production of inflammatory cytokines such as TNF-α, which mediates hepatocyte necrosis and induces other cytokines including IL-1β and IL-6, playing crucial roles in liver injury pathogenesis. Inhibition of TNF-α expression can alleviate liver damage. Our study found that LPS treatment significantly increased intracellular TNF-α levels, confirming successful induction of a liver injury model. Additionally, AHP-II significantly reduced inflammatory cytokine levels to near-normal, suggesting hepatoprotective effects through anti-inflammatory mechanisms. ROS participates in liver cirrhosis pathogenesis and represents a risk factor for hepatocellular carcinoma. Moderate to high ROS concentrations induce apoptosis and necrosis through oxidative stress. Our investigation examined ROS level changes after liver injury to explore AHP-II' s

critical role in the injury process. The results demonstrated that LPS stimulation significantly elevated cellular ROS levels, while AHP-II treatment markedly reduced ROS, indicating hepatoprotective effects potentially mediated through antioxidant mechanisms.

The MAPK signaling pathway participates in immune responses, regulates inflammatory reactions, and is involved in various pathophysiological processes including cell growth, development, and apoptosis. However, few studies have investigated the MAPK pathway in LPS-induced immune-mediated liver injury (Wang et al., 2017; Tian et al., 2016; Zhu et al., 2006). JNK activation exhibits dual regulatory functions: excessive activation induces apoptosis pathways, while low-level expression supports normal cellular differentiation and proliferation. Thus, JNK plays crucial regulatory roles in various hepatic inflammatory conditions and injuries. To further investigate AHP-II' s protective mechanism against LPS-induced liver injury, we examined JNK phosphorylation status. The results revealed that LPS induced hepatocyte injury by promoting JNK protein phosphorylation, while medium and high concentrations of AHP-II suppressed inflammatory responses by inhibiting JNK phosphorylation. In summary, *Acanthopanax giraldii* polysaccharide AHP-II exerts immunoprotective effects by reducing JNK phosphorylation and subsequently inhibiting inflammatory factors TNF-α and ROS.

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Note: Figure translations are in progress. See original paper for figures.