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Protective effects of Asiaticoside on acute liver injury induced by lipopolysaccharide/D-galactosamine in mice

Li Zhang [a,b,1], Hong-zhong Li [a,c,1], Xia Gong [a,d], Fu-ling Luo [a,e], Bin Wang [f], Ning Hu [g], Chang-dong Wang [a], Zhuo Zhang [a], Jing-yuan Wan [a,*]

[a] Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University, Chongqing 400016, People's Republic of China
[b] Department of Pathophysiology, Chongqing Medical University, Chongqing 400016, People's Republic of China
[c] Pharmacological and Toxicological Evaluation Center, Chongqing Pharmaceutical Research Institute, Chongqing 400061, People's Republic of China
[d] Department of Anatomy, Chongqing Medical University, Chongqing 400016, People's Republic of China
[e] Department of Pharmacy, The first Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China
[f] Department of Anesthesiology, The first Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China
[g] Department of Orthopedics, The first Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China

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ABSTRACT

Asiaticoside (AS), a triterpenoid product isolated from *Centella asiatica*, has been described to exhibit anti-inflammatory activities in several inflammatory models. However, the effects of AS on liver injury are poorly understood. The present study was undertaken to investigate whether AS is efficacious against Lipopolysaccharide (LPS) /D-galactosamine (D-GalN)-induced acute liver injury in mice and its potential mechanisms. AS (5, 10 and 20 mg/kg/d) was pretreated orally once daily for 3 days before LPS/D-GalN injected in mice. The mortality, hepatic tissue histology, plasma levels of Tumor necrosis factor-alpha (TNF-α) and alanine aminotransferase (ALT) and aspartate aminotransferase (AST), hepatic tissue TNF-α and caspase-3 activity were measured. Besides, western blotting analysis of phospho-p38 mitogen-activated protein kinase (phospho-p38 MAPK), phospho-c-jun N-terminal kinase (phospho-JNK) and phospho-extracellular signal regulated kinase (phospho-ERK) were determined. As a result, AS showed significant protection as evidenced by the decrease of elevated aminotransferases, hepatocytes apoptosis and caspase-3, alleviation of mortality and improvement of liver pathological injury in a dose-dependent manner. Further, we found that AS dose-dependently reduced the elevation of phospho-p38 MAPK, phospho-JNK, phospho-ERK protein and TNF-α mRNA expression in liver tissues and plasma TNF-α. These results suggest that AS has remarkable hepatoprotective effects on LPS/D-GalN-induced liver injury and the possible mechanism is related to inhibition of TNF-α and MAPKs.

Introduction

Acute hepatic failure characterized by hepatic encephalopathy, severe coagulopathy, jaundice, and hydroperitoneum is associated with high patient mortality, for which there is still no available therapy except liver transplantation limited by the chronic shortage of donor livers (Lee, 1994; Van Thiel et al., 2002). As the main pathogenic factor of Gram-negative bacteria, Lipopolysaccharide (LPS) inducing a systemic pro-inflammatory process which leads to multiple organ failure and death is implicated in the pathogenesis of liver injury (Jirillo et al., 2002).

LPS/D-GalN-induced liver injury in mice has been used frequently in the preparation of experimental animal models with endotoxemic shock and acute hepatic failure, which is similar to acute hepatic failure in clinic (Silverstein, 2004; Xiong et al., 1999). In this model, LPS exerts its effects by stimulating inflammatory cells and hepatic Kupffer cells to produce various pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12) and interferon-gamma (IFN-γ) (Batey and Wang, 2002; Malhi and Gores, 2008). Among these factors, TNF-α is the dominant mechanism of liver injury in this model. It induces activation of caspases and apoptosis in hepatocytes prior to secondary necrosis and release of transaminases by activation of TNF receptor-1 (de la Mata et al., 1990; Wullaert et al., 2007). With regard to the regulation of TNF-α, mitogen-activated protein kinases (MAPKs) family including p38 kinase, c-jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) are important mediators. MAPKs serve to regulate diverse cellular responses to extracellular stimuli, and modulate various cellular activities including gene expression, mitosis, differentiation and cell survival/apoptosis (Pearson et al., 2001).

Now in spite of an increasing need for agents to protect the liver from damage, modern medicine still lacks a reliable liver protective drug. Therefore numbers of natural substances have been studied to

* Corresponding author: Tel.: +86 023 6848 5038; fax: +86 023 8613 4172.
E-mail address: jywan@cqmu.edu.cn (J.-y. Wan).
[1] Contributed equally to this work.

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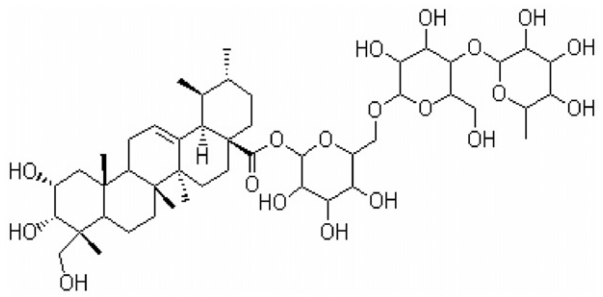

Fig. 1. Chemical structure of asiaticoside (AS).

evaluate the hepato-protective activity. The herb, *Centella asiatica*, a perennial creeper growing abundantly in moist areas and distributed widely in tropical and subtropical countries, has been used for centuries in Ayurvedic and traditional Chinese medicine to alleviate symptoms of wound, ulcer, arthritis, depression and anxiety (Brinkhaus et al., 2000; Jayathirtha and Mishra, 2004; Li et al., 2009). In South China, *C. asiatica* is even widely used as a dietary supplement and an ingredient of special tea to promote positive health and keep immunomodulation by establishing body equilibrium. Asiaticoside (AS) (Fig. 1), a major pentacyclic triterpenoid saponin component of *C. asiatica*, has been described to have wound healing, immunomodulatory and anti-inflammatory activities (Jia and Lu, 2008; Shukla et al., 1999). Our earlier study indicated that AS attenuated the inflammation on LPS-induced acute lung injury and collagen-induced arthritis in mice through reducing the level of cytokines such as TNF-α, IL-6 and PGE_2 (Li et al., 2007; Zhang et al., 2008). However, there are few reports on the effects and mechanisms of AS in treatment of acute liver injury.

In the present study, we utilized LPS/D-GalN model to evaluate the protective effects of AS on acute hepatic damage and then investigated whether TNF-α and MAPKs were involved in the potential mechanism.

Materials and methods

Animals

Balb/c mice (6–8 weeks old; weight range, 18-22 g) were obtained from the Laboratory Animal Center of Chongqing Medical University (Chongqing, PR China). All mice received human care according to the guidelines of the local institutes of health guide for the care and use of laboratory animals. They were maintained under controlled conditions (22°C, 55% humidity and 12 h day/night rhythm) and fed standard laboratory chow.

Reagents

LPS (Escherichia coli, 0111:B4), D-GalN and silymarin were purchased from Sigma (St. Louis, MO, USA). Asiaticoside ($C_{48}H_{78}O_{19}$, MW: 959.12, purity < 90%) determined by HPLC as previously described (Schaneberg et al., 2003) was purchased from Guangxi Changzhou Natural Products Development Co. Ltd. (Nanning, China). Rabbit anti-mouse phospho-p38 MAPK, phospho-JNK, phospho-ERK and Rabbit anti-mouse β-actin antibody was purchased from Cell Signaling Technology (Boston, MA, USA), and horseradish peroxidase-conjugated goat anti-rabbit antibody, bicinchoninic acid (BCA) protein assay kit and enhancer chemiluminescent (ECL) reagents were obtained from Pierce Biotechnology (Rockford, IL). alanine aminotransferase (ALT) and aspartate aminotransferase (AST) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Hoechst 33342 fluorescent dye and caspase 3 activity assay kits were obtained from Beyotime Institute (Shanghai, China). Mouse TNF-α enzyme-linked immunosorbent assay (ELISA) kit was purchased from Bender Med Systems (Vienna, Austria).

Experimental protocols

Mice were administered with AS (5, 10 and 20 mg/kg) dissolved in 0.5% sodium carboxymethyl cellulose or silymarin (50 mg/kg) or equal volume phosphate-buffered saline (PBS) once daily for 3 days prior to challenge experimentation. Mice were challenged intraperitoneally (i.p.) with a total volume of a combination of LPS (50 μg/kg) and D-GalN (800 mg/kg) dissolved in PBS. The doses of AS alone did not induce liver injury as determined by evaluating liver enzymes, cytokines, and liver histology (data not shown). Lethality was evaluated within 48 h after LPS/D-GalN administration. Liver samples for MAPKs, TNF-α analysis and histology were obtained at 0.5, 1.5 and 6 h after LPS/D-GalN administration, respectively.

Analysis of liver enzymes

Hepatocyte damage was assessed 6 h after LPS/D-GalN administration by measuring ALT and AST activities in serum and caspase-3 activity in hepatic tissue using corresponding detection kits according to the manufacturer's instructions.

Measurement of TNF-α Levels

Mouse serum samples were assayed for murine TNF-α by enzyme-linked immunosorbent assay as described by the manufacturer, while TNF-α in hepatic tissue was analyzed by were measured at 1.5 h after the LPS/D-GalN challenge (Endo et al., 1999).

Histologic analysis

Liver tissue was fixed in 10% neutral-buffered formalin and subsequently embedded in paraffin. Sections were stained with hematoxylin and eosin using a standard protocol and analyzed by light microscopy.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from hepatic samples using Trizol reagent according to the manufacturer's protocol. First-strand

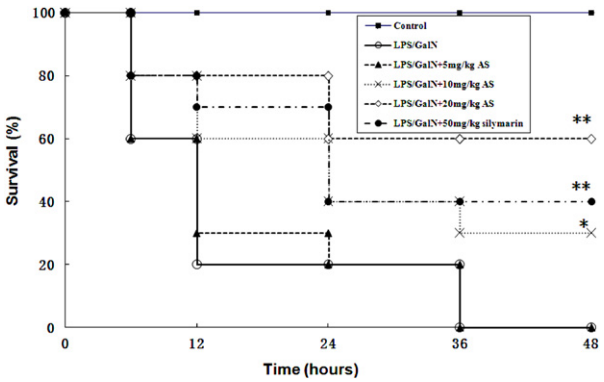

Fig. 2. Lethal toxicity induced by LPS/D-GalN in mice (n=10). Balb/c mice were pretreated orally with AS (5, 10 and 20 mg/kg) or silymarin (50 mg/kg) or equal volume phosphate-buffered saline (PBS) once daily for 3 days before LPS/ D-GalN administration. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with LPS/ D-GalN administration group.