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Protective effects of asiaticoside on septic lung injury in mice

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ABSTRACT

Asiaticoside (AS), a major triterpenoid saponin component isolated from *Centella asiatica*, has been described to exhibit antioxidant and anti-inflammatory activities. The present study aimed to determine the protective effects and the underlying mechanisms of AS on septic lung injury induced by cecal ligation and puncture (CLP). Mice were pretreated with the AS (45 mg/kg) or AS as well as GW9662 at 1 h before CLP, the survival, lung injury, inflammatory mediators and signaling molecules, and Peroxisome proliferator-activated receptor-γ (PPAR-γ) were determined 24 h after CLP. The results showed that AS significantly decreased CLP-induced the mortality, lung pathological damage, the infiltration of mononuclear, polymorphonuclear (PMN) leucocytes and total proteins. Moreover, AS inhibited CLP-induced the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB), the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) protein in lung tissues, and the production of serum tumor necrosis factor (TNF-α) and interleukin-6 (IL-6). Interestingly, the expression of PPAR-γ protein in lung tissue was up-regulated by AS. Furthermore, GW9662 (the inhibitor of PPAR-γ) significantly reversed these beneficial effects of AS in septic mice. These findings suggest that AS could effectively protect from septic lung injury induced by CLP and the underlying mechanisms might be related to up-regulation of PPAR-γ expression to some extent, which inhibits MAPKs and NF-κB pathway.

1. Introduction

Although advances in understanding the mechanism of the pathophysiology of sepsis and its treatment, sepsis remains the major cause of mortality, particularly in intensive care units (Altemeier et al., 2005; Guo et al., 2004; Lee et al., 2006; Vincent and Abraham, 2006). At the onset of sepsis, bacteria trigger the inflammatory cascades, resulting in systemic inflammatory response syndrome (SIRS), and multiple organ failure (MOF). During the development of MOF in sepsis, the lung is the most commonly damaged organ and easily develops into acute respiratory distress syndrome (ARDS) (van Griensven et al., 2002; Zhao et al., 2005). The occurrence of ARDS rises to 42% in cases of sepsis and mortality rate ranges from 30% to 50% (Hudson and Steinberg, 1999; Richardson et al., 2008). Therefore, it is urgent for searching potential therapeutic drugs to improve the survival of septic lung injury.

Previous many attempts to treat sepsis relied on the inhibition of production of pro-inflammatory mediators, but endogenous anti-inflammatory molecules were not mentioned. Recently, there is an increasing notion in studying the roles of anti-inflammatory molecules in sepsis. Peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear hormone receptor superfamily of ligand-dependent transcription factors, has been found that play important roles on inflammation, lipid metabolism, and cell growth (Tontonoz et al., 1994; Desvergne and Wahli, 1999; Elbrecht et al., 1996). Studies have shown that PPAR-γ activated by 15-deoxy-delta (12,14)-PGJ_2 or thiazolidinediones had anti-inflammatory effects (Yi et al., 2008; Zhao et al., 2006). In addition, PPAR-γ mainly expresses in monocytes and macrophages, and it exerts anti-inflammatory effects by decreasing the production of pro-inflammatory mediators such as tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6) and the inducible nitric oxide synthase (iNOS) (Alleva et al., 2002; Wang et al., 1999).

Asiaticoside (AS) (Fig. 1), a major triterpenoid component isolated from *Centella asiatica*, has been described to have wound healing, immunomodulatory and anti-inflammatory activities (Tajima et al., 2006; Liang et al., 2008; Maquart et al., 1990; Maquart

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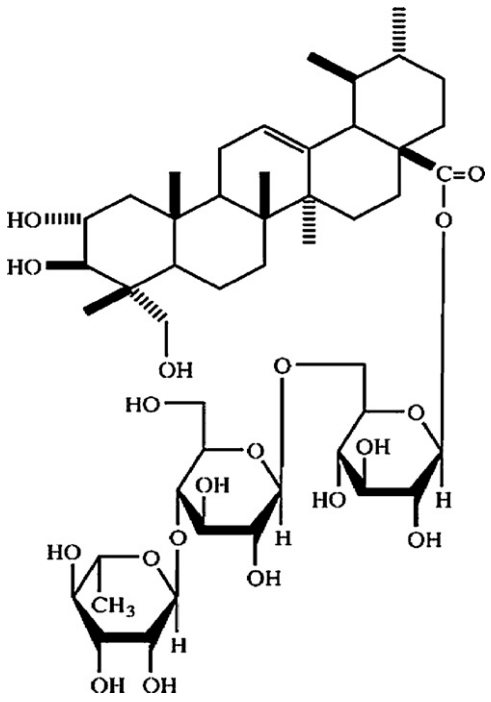

Fig. 1. The chemical structure of AS isolated from the herb *Centella asiatica*.

et al., 1999). Previous studies have shown that AS inhibited iNOS activity and NO production during gastric ulcer healing, alleviated Gram-negative bacteria-caused infection (Guo et al., 2004; Altemeier et al., 2005). Recently, our study indicated that AS attenuated the inflammation on LPS-induced acute lung injury and acute liver injury in mice through reducing the production of cytokines such as TNF-a, IL-6 (Zhang et al., 2008; Zhang et al., 2010). However, the effects of AS on septic lung injury are not known.

In present study, we attempted to determine whether AS could serve as an effective therapeutic drug in the treatment of septic lung injury. Moreover, we also investigated whether AS could regulate the expression of PPAR-γ and whether such a mechanism involving in PPAR-γ might help to explain the therapeutic effects of AS on septic lung injury.

2. Materials and methods

2.1. Animals

Male KunMing mice (6–8 weeks of age, 20–25 g body weight) were obtained from the Experimental 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. Mice were housed under optimum conditions (25 ± 2 °C, 55% humidity and 12 h day/night rhythm) and fed with a standard laboratory diet and water. They were acclimatized for at least 1 week before use. All experimental procedures involving animals were approved by the Animal Care and Use Committee of Chongqing Medical University.

2.2. Reagents

AS ($C_{48}H_{78}O_{19}$, MW: 975.15, purity ≥ 98%) was purchased from Guangxi Changzhou Natural Products Development Co. Ltd. (Nanning, China). Bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA). TNF-α and IL-6 ELISA kits were obtained from the Bender MedSystems (Vienna, Austria). Rabbit anti-cyclooxygenase-2 (COX-2), iNOS and PPAR-γ antibodies were obtained from Abcam (Cambridge, MA, UK). Phospho-inhibit κB (I-κB), phospho-extracellular signal-regulated kinase (p-ERK), phospho-c-Jun N-terminal kinase (p-JNK), phospho-p38 mitogen-activated protein kinase (p-p38 MAPK) and β-actin antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). GW9662 was obtained from the Alexis Biochemicals (Farmingdale, NY, USA).

2.3. Experimental protocols

The cecal ligation and puncture (CLP) protocol was started with the abdominal wall clipped of hair and cleansed with ethyl alcohol. Under sodium pentobarbital (30 mg/kg i.p.) anesthesia, a 1-cm long midline incision was made and the cecum was exposed. The cecum was ligated by silk 4–0 and punctured twice with an 18-gauge needle, and then the cecum was gently squeezed to extrude a small amount of feces. The cecum was repositioned, after which the abdomen was closed in two layers with 4–0 silk thread. Finally, 1 ml of saline was administered s.c. for resuscitation. Control group mice underwent the same surgical procedures (i.e., laparotomy and resuscitation), but the cecum was neither ligated nor punctured. After surgery, animals were put back to their home cages with free access to water.

KunMing mice ($n = 100$) were randomly assigned to four groups: Control group ($n = 25$), CLP Model group ($n = 25$), CLP-AS group ($n = 25$) and CLP-AS-GW9662 group ($n = 25$). The control and the CLP groups were pretreated with an intraperitoneal (i.p.) injection of sterile saline 200 μl 1 h before operation. The CLP-AS group was injected i.p. with the same volume of AS (45 mg/kg) 1 h before CLP. The CLP-AS-GW9662 group was received an i.p. injection of same volume of the mixed drugs of AS (45 mg/kg) 1 h and GW9662 (2 mg/kg) before CLP. Mortality was monitored every 12 h during 24 h after laparotomy or CLP. The survivors of mice were anesthetized at 24 h after CLP. In one set of experiments, six mice were chosen, blood sample was collected by eyeball enucleated, and then mice were sacrificed, the lung was used for collecting bronchoalveolar lavage fluid (BALF) in which leukocytes and proteins were quantified. In the other separate set of experiments, five mice were chosen and sacrificed. The right lung was ligated and excised; one part was fixed in formaldehyde for histology, and the remaining lung tissue was weighed, snap-frozen in liquid nitrogen, and stored at −80 °C for later Western blot. The dose of AS or GW9662 alone did not induced lung injury (data not shown).

2.4. Histopathology analysis

The Lung specimens were immersion-fixed for 1 week in 10% formaldehyde at room temperature, and then embedded in paraffin. Serial paraffin sections (4 μm) were stained with hematoxylin-eosin routinely for conventional morphological evaluation under a light microscope (Olympus, Tokyo, Japan).

2.5. Bronchoalveolar lavage fluid (BALF)

BALF was obtained by washing the airways three times with 1.5 ml of saline through a tracheal cannula. This procedure consistently produced 1.0 ml of lavage fluid. BALF was centrifuged at 4 °C, 1500 × *g* for 10 min. The supernatant was harvested for total protein analysis using the BCA protein assay kit and the pellet was smeared onto slides for cell classification and counting in BALF with a modified Giemsa stain.

2.6. Serum cytokines analyses

Blood was collected for serum TNF-α and IL-6 assay. The blood sample was centrifuged at 3000 rpm for 10 min. Then the serum