Protective Effect of Artesunate on LPS-induced Acute Lung Injury by Anti-oxidant Through AKT/PI3K Pathway

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Abstract. Oxidative stress are involved in the pathogenesis of acute lung injury (ALI). Accordingly, anti-inflammatory treatment is proposed to be a possible efficient therapeutic strategy for ALI. The aim of the study was to evaluate the antioxidant efficacy of Artesunate (Ar) on ALI induced by lipopolysaccharide (LPS) in mice and explore the underlying mechanism. BALB/c mice received Ar (2, 4 mg/kg) intraperitoneally 1h prior to the in intratracheal instillation of lipopolysaccharide (LPS) challenge. Malondialdehyde (MDA) was detected to evaluate the anti-oxidant efficacy of Artesunate. Content of Superoxide Dismutase (SOD) and Catalase (CAT) were assayed by enzyme linked immunosorbent assay (ELISA). Western blot was employed to determine the protein expression of P-PI3K, PI3K, P-AKT and AKT. The results showed the severity of lung injury was attenuated by Ar (40 mg/kg) and Dex (2 mg/kg) and the levels of MDA in Ar (20, 40 mg/kg) and Dex (2mg/kg) groups were obviously lower than those in LPS group. The treatment with Ar (20, 40 mg/kg) and Dex (2 mg/kg) remarkably increased the levels of SOD and CAT. The protein expressions of signaling showed the protein expressions of PI3K and AKT significantly enhanced compared with those in LPS group after Ar treating with Ar (20 and 40 mg/kg) and Dex (2 mg/kg). In conclusion, this study revealed that Artesunate could exert protective effect of on LPS-induced acute lung injury by anti-oxidant through AKT/PI3K pathway.

Introduction

Acute Lung Injury (ALI), a clinical severe respiratory disorder, remains the leading cause of morbidity and mortality in critically ill patients [1]. It is characterized by the pulmonary edema, neutrophils infiltration, uncontrolled oxidative stress and inflammatory process [2]. Despite extensive studies about the relevant pathogenesis has been reported to date, there has been no effective medicine for ALI [3].

Oxidative stress is defined as a status of an imbalance between cellular anti-oxidative capacity and reactive oxygen species (ROS) formation caused by the dysregulation of antioxidant system [4]. Thereby, the amelioration of the imbalance condition by enhancing cellular antioxidant capacity or scavenging ROS may make some difference for a variety of pathology and disease models [5].

Artesunate, the water-soluble semisynthetic derivative of artemisinin, has been a standard treatment for cerebral malaria and all kinds of other severe malaria [6]. In addition, numerous studies also pointed out that artemesate had widespread pharmacological activities, such as antiparasite, antitumor, antimicrobial, anti-inflammatory, antioxidant, impeding angiogenesis, protecting the BBB, and immunoregulatory effect[7].

Although there are numerous studies showed artemesate has many pharmacological effects, its protective effect and mechanism on acute lung injury has not yet reported. The purpose of the present study was to evaluate the therapeutic effect of artemesate on ALI induced by LPS and elucidate the potential molecular mechanism.
Materials and Methods

Chemicals and Reagents

Artesunate was purchased from the National Institutes for Food and Drug Control (Beijing, China). LPS was purchased from Beyotime Institute of Biotechnology (Nanjing, China). SOD and CAT enzyme-linked immunosorbent assay (ELISA) kits were supplied by Biolegend Inc. (San Diego, CA, USA). All antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

Animals and Protocol for Acute Lung Injury Model

Male BALB/c mice (20±2g) were purchased from Xi’an Jiaotong University (Xi’an, China). All mice were randomly divided into five groups: control group, model group dexamethasone (2mg/kg) group, and Ar (2 and 4mg/kg) group. 1 h before intraperitoneally instillation of 10 µg LPS instilled in 50µl PBS, Ar (2 and 4mg/kg) and dexamethasone (2mg/kg) were given to mice orally. 6 hours after LPS administration, all animals were sacrificed by diethylether asphyxiation.

Collection of Bronchoalveolar Lavage

6 h after LPS challenge, mice were sacrificed by diethylether asphyxiation. The collection of BALF was performed three times with 500 µl of sterile PBS (7.2) three times (total volume 1.5 ml). The recovery ratio of the fluid was about 85±2 %. After that, the bronchoalveolar lavage fluid (BALF) was immediately centrifuged at 3,000 rpm at 4°C for 10 min and the cell-free supernatants were kept at -80°C for cytokine analysis.

Determination of Antioxidant System and Lipid Peroxidation Products

The BALF were centrifuged at the speed of 3,000 rpm for 10 min at 4°C, then the supernatant was transferred and stored at -80°C. Subsequently, the following operations were conducted according to the instructions of commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China).

Western Blotting

Lung tissues were homogenized in RIPA buffer containing proteinase and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). The lysate was centrifuged at 12,000 rpm for 15 minutes at 4 ºC and the supernatant was harvested. Total protein was extracted from the resulting supernatant and the concentration quantified by the BCA assay.

Equal amounts (30 μg) of protein were separated by 10% SDS-polyacrylamide gel, followed by transfer onto PVDF membranes. After blocking, the membranes were treated overnight at 4°C with rabbit monoclonal anti-human P-PI3K, PI3K, P-AKT, AKT and GAPDH primary antibodie. This was followed by incubation with appropriate horseradish Peroxidase (HRP)-conjugated secondary antibodies (1h, room temperature and detection with an enhanced chemiluminescence (ECL) kit (GE Healthcare).

Statistical Analysis

The data were presented as mean value ± SD. Differences between groups were analyzed by one-way ANOVA with Tukey multiple comparison test using GraphPad Prism 5.0 Software, p<0.05 was considered as significant difference.

Results

Effect of Ar on LPS-induced Pathological Changes of the Lung

Hematoxylin and Eosin (H&E) staining was performed to evaluate the protective effect of Ar on physiological impairment. As shown in the Fig.1, there are no pathological changes was observed in lung specimens in control group. Lung tissues of the group of administered LPS presented clear and distinct pathological changes, such as alveolar hemorrhage and infiltration of inflammatory cell. However, the severity of lung injury was attenuated by Ar (40 mg/kg) and Dex (2 mg/kg).
Effect of Ar on LPS-induced pathological changes of the lung tissues. 1h before intracheal instillation of LPS, Ar (20 and 40 mg/kg) and dexamethasone (2 mg/kg) were given to mice orally. All animals were sacrificed at 6 hours after LPS administration. (A. ConArol; B. LPS-induced acute lung injury; C. Dexamethasone (2 mg/kg); D. Ar (20 mg/kg); E. Ar (40 mg/kg).

Effect of Ar on LPS-induced MDA Content of the Lung

It was shown that the mice exposed to LPS presented a significant rise in MDA. On the contrary, the levels of MDA in Ar (20, 40 mg/kg) and Dex (2 mg/kg) groups were obviously lower than those in LPS group. (Fig.2)

Effects of Ar on Enzyme Content of SOD and CAT in BALF

Enzyme content of SOD and CAT were assessed to verify the anti-oxidative activity of Ar on oxidative stress in LPS-stimulated mice. It was shown that LPS stimulation decreased the contents of SOD and CAT. The treatment with Ar (20, 40 mg/kg) and Dex (2 mg/kg) remarkably increased the levels of SOD and CAT in BALF (Fig.3).
Figure 3. Enzyme content of SOD and CAT in BALF. 1 h before intracheal instillation of LPS, Ar (20 and 40 mg/kg) and dexamethasone (2 mg/kg) were given to mice orally and bronchoalveolar lavage were collected and enzyme content of SOD and CAT of the lung were detected.

The Protein Expressions of Signaling In Lungs

PI3K/AKT signaling are the well-known cytoprotective target mediating oxidative stress and inflammation. Exposure to LPS notably increased the protein expressions of P-PI3K and decreased PI3K. While after treating with Ar (20 and 40 mg/kg) and Dex (2 mg/kg), the protein expressions of PI3K significantly enhanced compared with those in LPS group (Fig. 4A). In addition, LPS challenge contributed to the up regulations of PAKT, while the treatment with Ar (20 and 40 mg/kg) and Dex (2 mg/kg) effectively down regulated the levels of AKT.
Figure 4. The protein expressions of PI3K, AKT the phosphorylations of PI3K and AKT in lung tissues. 1h before intracheal instillation of LPS, Ar (20 and 40 mg/kg) and dexamethasone (2 mg/kg) were given to mice orally. All animals were sacrificed at 6 hours after LPS administration. A. The protein expressions of PI3K and the phosphorylations of PI3K in lung tissues. B. The protein expressions of AKT and the phosphorylations of AKT in lung tissues.

Discussion

A disturbance of the alveolar-capillary barrier concerning several clinical diseases possibility lead to ALI. It is essential that ALI be prevented because of its high morbidity and mortality rates due to the deficiency of effective drugs in clinical Arials [8]. As a basic component of the Gram-negative bacteria outer membrane, LPS can enter the blood sAream and initiate inflammatory reaction which may develop to shock and even ultimately to death [9]. In this study, Lung tissues of the group of administered LPS presented clear and distinct pathological changes, such as alveolar hemorrhage and infiltration of inflammatory cell. However, the severity of lung injury was attenuated by Ar (40 mg/kg) and Dex (2mg/kg). These results suggested that LPS-induced pathological changes were attenuated by Ar to an intensive degree.

As a trusty index of oxidative stress, MDA was applied to reflect the level of cell damage resulting from reactive oxygen metabolites [10]. In the present study the mice exposed to LPS presented a significant rise in MDA. On the contrary, the levels of MDA in Ar (20, 40 mg/kg) and Dex (2 mg/kg) groups were obviously lower than those in LPS group.

Oxidative stress is defined as a status of an imbalance between cellular anti-oxidative capacity and reactive oxygen species (ROS) formation caused by the dysregulation of antioxidant system [4]. SOD and CAT are the main enzymes in the scavenging of ROS. LPS stimulation decreased the contents of SOD and CAT. The treatment with Ar (20, 40 mg/kg) and Dex (2 mg/kg) remarkably increased the levels of SOD and CAT in BALF.

It is reported that ROS-mediated enhancements in self-renewal and neurogenesis were dependent on PI3K/Akt signaling [11]. PI3K /AKT signaling are the well-known cytoprotective target mediating oxidative sAress and inflammation. Exposure to LPS notably increased the protein expressions of P-PI3K and decreased PI3K . While after treating with Ar (20 and 40 mg/kg) and Dex (2 mg/kg), the protein expressions of PI3K significantly enhanced compared with those in LPS group (Fig.4 A). In addition, LPS challenge contributed to the up regulations of PAKT, while the treatment with Ar (20 and 40 mg/kg) and Dex (2 mg/kg) effectively down regulated the levels of AKT.
In conclusion, this study revealed that Artesunate could exert protective effect of on LPS-induced acute lung injury by anti-oxidant through AKT/PI3K pathway.

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