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# The protective effect of Trillin LPS-induced acute lung injury by the regulations of inflammation and oxidative state

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ABSTRACT

Inflammation response and oxidative stress have been reported to be 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 purpose of our present study was to evaluate the anti-inflammatory efficacy of trillin (Tr) on ALI induced by lipopolysaccharide (LPS) in mice and explore the underlying mechanism. BALB/c mice received Tr (50, 100 mg/kg) intraperitoneally 1 h prior to the intratracheal instillation of lipopolysaccharide (LPS) challenge. Pretreatment with Tr at the dose of 50, 100 mg/kg markedly ameliorated lung wet-to-dry weight (W/D) ratio, myeloperoxidase (MPO) activity and pulmonary histopathological conditions. In addition, the protective efficacy of Tr might be attributed to the down-regulations of neutrophil infiltration, malondialdehyde (MDA), inflammatory cytokines and the up-regulations of super-oxide dismutase (SOD), catalase(CAT), glutathione(GSH), Glutathione Peroxidase(GSH-Px) in bronchoalveolar lavage fluid (BALF). Meanwhile, our study revealed some correlations between (NF-E2-related factor 2) Nrf2/heme oxygenase (HO)-1/nuclear factor-kappa B (NF-κB) pathways and the beneficial effect of Tr, as evidenced by the significant up-regulations of HO-1 and Nrf2 protein expressions as well as the down-regulations of p-NF-κB and p-inhibitor of NF-κB (IκB) in lung tissues. Taken together, our results indicated that Tr exhibited protective effect on LPS-induced ALI by the regulations of related inflammatory events via the activations of Nrf2, HO-1 and NF-κB pathway. The current study indicated that Tr could be a potentially effective candidate medicine for the treatment of ALI.



## 1. 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]. Thus, it is urgent to explore the effective medications and innovative therapies. The most common causes of ALI are considered to be trauma, pneumonia, acid aspiration and sepsis resulting from bacterial infection [4]. As a component of the Gram negative bacterial cell membrane, Lipopolysaccharide (LPS) can induce inflammatory response and immune dysfunction. The intratracheal administration of LPS has been widely used to study the pathogenesis and prevention of ALI in mice [5].

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 [6]. Thereby, the amelioration of the imbalance condition by enhancing cellular antioxidant capacity or scavenging ROS may make some

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difference for a variety of pathology and disease models [7].

Nuclear factor erythroid-2 related factor-2 (Nrf2), which generally exists in various cellulars and organisms as a Cap "n" Collar basic leucine zipper protective transcription factor, plays a crucial role in regulating cytoprotective and antioxidant genes triggered by oxidative stress [8]. Excessive ROS might lead to the disruption of Nrf2/Kelch-like ECH associated protein-1 (Keap1) complex and contribute to the Nrf2's translocation from the cytoplasm into the nucleus. After that, Nrf2 can combine with antioxidant response element (ARE) DNA sequence to eventually promote the expressions of ARE-related genes [9]. The downstream targets of Nrf2 are comprised of HO-1 and the key antioxidant enzymes including CAT and GPx. Furthermore, HO-1 participates in manifesting antioxidant and anti-inflammatory activities [10].

Complementary and alternative medicine has attracted increasing global attention owing to their widespread application for treatment of many diseases. Traditional Chinese medicine has been widely used by one-fifth of the world's population for centuries and is still considered as an important source of nature medicine [11,12]. In clinical practice, the rhizome of *Dioscorea nipponica* Makino (Dioscoreae Nipponicae Rhizoma or Japan Yam Rhizome) that has been consumed as an herbal medicine for more than four thousand years in China. It is used for ameliorating asthma, improving blood circulation, eliminating rheumatic aches and mitigating pain. The identified active components of *Dioscoreae Nipponicae* Rhizoma are steroidal saponins such as furostanol saponins and isospirostanol saponins. As a steroidal saponin extracted from *D. nipponica*, trillin has been confirmed to exhibit anthelmintic activity [13]. However, the literature on other biological effects of this component is still too limited. Hence, the purpose of the present study was to evaluate the therapeutic effect of trillin on ALI induced by LPS and elucidate the potential molecular mechanism.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Trillin and Dexamethasone (Dex) were obtained from the National Institutes for Food and Drug Control (Beijing, China). LPS was purchased from Beyotime Institute of Biotechnology (Nanjing, China). Mouse TNF-α, and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were supplied by Biolegend Inc. (San Diego, CA, USA). MPO kit was produced by Nanjing KeyGEN Biotech. CO., LTD. (Nanjing, China). The levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione (GSH) were evaluated using commercial kits provided by Jiancheng Institute of Biotechnology (Nanjing, China). All antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

### 2.2. Animals

Male BALB/c mice weighing 18–22 g were obtained from Jiangning Qinglongshan Animal Cultivation Farm (Nanjing, China). Mice were maintained in an animal room under standard conditions with water and standard chow *ad libitum*. All procedures in this study were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

### 2.3. Experimental protocol for acute lung injury model

All mice were randomly divided into five groups: control group, model group, dexamethasone (2 mg/kg) group, and Tr (50 and 100 mg/kg) group. 1 h before intratracheal instillation of 10 μg LPS instilled in 50 μl PBS, Tr (50 and 100 mg/kg) and dexamethasone (2 mg/kg) were given to mice orally. 6 h after LPS administration, all animals were sacrificed by diethyl ether asphyxiation.

### 2.4. Bronchoalveolar lavage

6 h after LPS challenge, mice were sacrificed by diethyl ether 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 3000 rpm at 4 °C for 10 min and the cell-free supernatants were kept at −80 °C for cytokine analysis. The sediment cells were resuspended in PBS for neutrophil counting with hemacytometer by the Wright–Giemsa staining method.

### 2.5. Measurement of wet-to-dry ratio of the lungs

The mice were euthanized at 6 h after LPS administration. The right lungs were excised to obtain the "wet" weight. The lungs were then placed at 60 °C for 48 h to record the "dry" weight. The index of pulmonary edema was calculated by the ratio of the wet weight to the dry weight of the lung tissues.

### 2.6. Measurement of MPO

Six hours after LPS stimulation, mice were killed and the right lungs were collected. 100 mg lung tissues were homogenized and fluidized with extraction buffer to obtain 5% homogenate. The activity of MPO was determined by commercial kits according to the instructions recommended by the manufacturers. The enzymatic activity was examined at 460 nm using a 96-well plate reader.

### 2.7. Determination of antioxidant system and lipid peroxidation products

The BALF was collected to assay SOD, CAT, MDA, GSH and GSH-Px levels. Centrifuged at the speed of 3000 rpm for 10 min at 4 °C, 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).

### 2.8. Cytokine assay

The contents of TNF-α and IL-6 in BALF were determined with ELISA kits according to the relevant kits. The optical density of each well was assayed at 450 nm with a microplate spectrophotometer. Finally, the contents were calculated according to the standard curves.

### 2.9. Pulmonary histopathology

6 h after LPS administration, the lungs were excised, fixed in 4% neutral buffered formalin for 24 h, embedded in paraffin and cut into 4-μm sections. Hematoxylin–eosin (HE) staining was carried out according to the standard protocol. After that, pathological conditions in the lung tissues were visualized under a light microscope.

### 2.10. Western blot assay

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 min at 4 °C and the supernatant was harvested. Then the protein concentration was