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# Aucubin protects against lipopolysaccharide-induced acute pulmonary injury through regulating Nrf2 and AMPK pathways

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ABSTRACT

Aucubin (Ai), a natural compound isolated from plants, including Aucuba japonica and Eucommia ulmoides, shows significant anti-inflammatory and anti-oxidative bioactivities. Here, we attempted to explore the protect effects of Ai on LPS-induced acute lung injury (ALI). Our results indicated that Ai increased the survival rate and ameliorated pathogenic processes in lipopolysaccharide (LPS)-induced mice. However, nuclear factor erythroid 2-related factor 2 (Nrf2) deletion may impede protective effect of Ai. Additionally, Ai reduced oxidative stress by down-regulating malondialdehyde (MDA) and $O_2$· activity, and enhancing Nrf2-targeted signals, including heme oxygenase-1 (HO-1) and quinone oxidoreductase-1 (NQO-1). Also, Ai inhibited pro-inflammatory cytokines and phosphorylated-nuclear factor-κB (NF-κB) expression in LPS-administrated mice. However, these protective effects of Ai were suppressed in Nrf2-knockout mice. Importantly, Nrf2-deficiency showed no effects on phosphorylated AMP-activated protein kinase (p-AMPK) expression in mice treated with LPS and Ai. Similarly, in LPS-induced macrophages, Ai reduced reactive oxygen species (ROS) generation, elevated NQO-1 and HO-1 expression. LPS-stimulated pro-inflammatory cytokines and p-NF-κB were reversed by Ai. Of note, we found that Ai-induced Nrf2 activation was dependent on AMPK activation. Suppression of AMPK levels may inhibit Nrf2 activation, finally leading to up regulation of inflammatory response and oxidative stress. Thus, our findings indicated the crosstalk between Nrf2 and AMPK signaling pathways, and the interaction was essential for the anti-oxidant and anti-inflammatory effects of Ai in LPS-induced macrophages, which might be beneficial for finding new treatments against ALI.

## 1. Introduction

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is a clinical syndrome of severe lung failure [1]. Though mortality rates in ALI was decreased, the 60-day mortality rate indicated by ARDS Network clinical trials is about 25% even with best supportive care and lung-protective ventilation strategies [2]. Despite advances have been made in drug exploration and mechanisms understanding, to date effective pharmacotherapy for ALI is extremely limited [3].

Acute inflammatory response and cytokines accumulation have been implicated in pathogenesis of ALI. LPS, a well-known essential endotoxin, is a component in outer membranes of Gram-negative bacteria, which could initiate the local acute inflammation [4]. Further, LPS significantly enhanced the reactive oxygen species (ROS) in different cells, contributing to inflammatory responses. Thus, inhibition of the generation of intracellular ROS is suggested as a general mechanism by which the intracellular inflammation is blocked [5]. And in inflammation research, signaling pathway, related to ROS generation and clearance, has attracted accumulating attention. Inflammatory responses-related pathways are divided into pro-inflammatory and anti-inflammatory signaling pathways, including NF-κB and Nrf2, respectively. Phosphorylation of NF-κB promotes cytokines release and ROS production in various cell types, including macrophages [6]. However, Nrf2 could protect against inflammation, associated with the activation of anti-oxidants [7]. Of note, AMPK is reported as a crucial target for regulating inflammation and ROS generation. Its relationship with inflammation/ROS is linked to NF-κB and Nrf2 and has been noted before [8]. Nevertheless, the molecular mechanism is not fully understood in ALI.

Aucubin (Ai, Fig. 1A) is an iridoid glycoside found in several plants,

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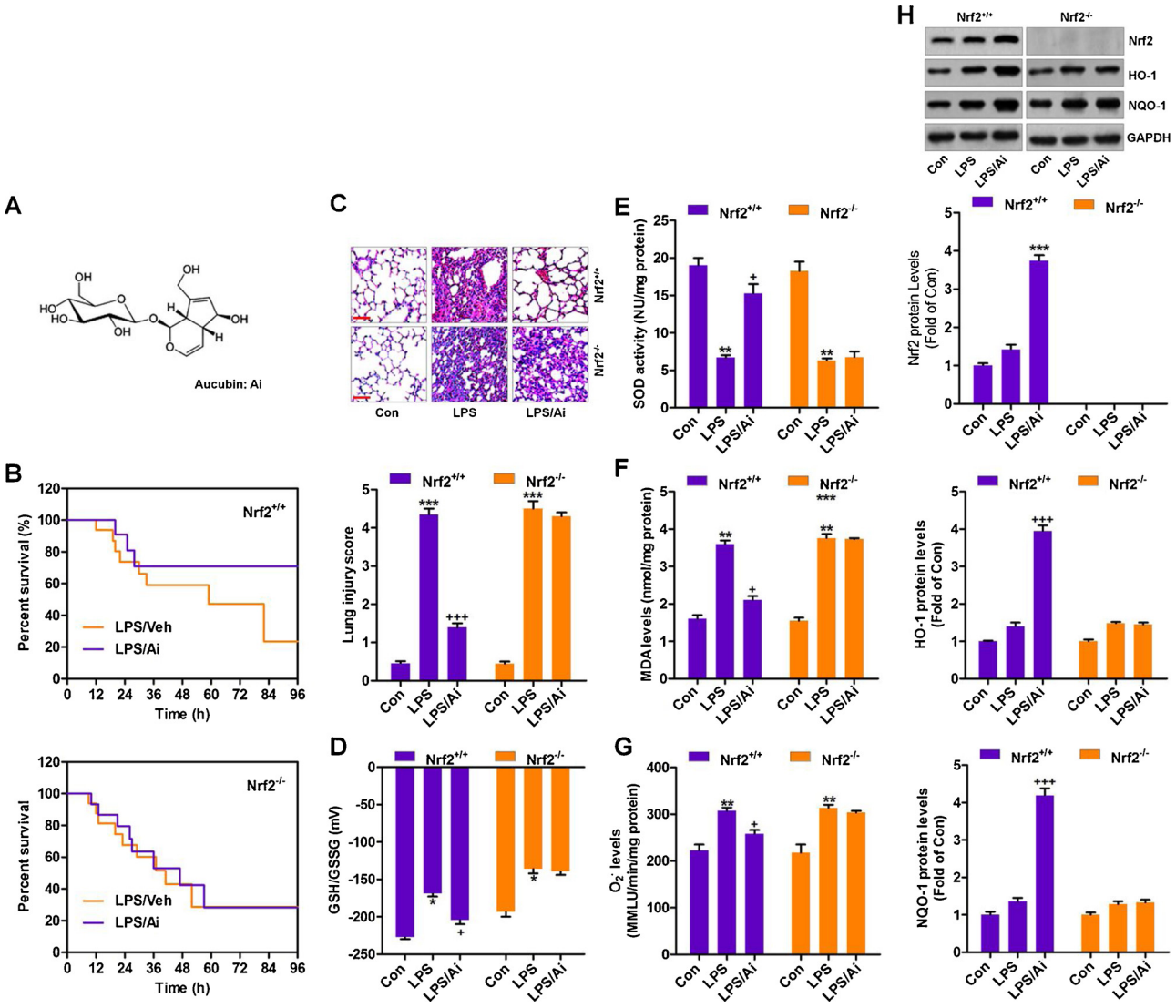


**Fig. 1.** Anti-oxidant effects of aucubin on LPS-induced ALI are Nrf2-dependent. (A) The chemical structure of aucubin. (B) Effects of aucubin on survival rate of mice. (C) H&E staining of lung tissue sections. (D) The ratio of GSH to GSSG in serum was determined. (E–G) SOD activity, MDA levels, and $O_2$· were calculated. (H) Western blot analysis of Nrf2, HO-1, and NQO-1. Data are represented as mean ± S.E.M. (n = 8). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the Con group; $^{+}P < 0.05$ and $^{+++}P < 0.001$ versus the LPS group.

such as Eucommia ulmoide and Rehmannia, possessing various pharmacological effects, such as angiogenesis, anti-inflammation and anti-oxidative stress [9]. Previous studies have investigated the therapeutic potential of Ai for many other disease, such as skin fibrosis, cardaic injury, diabetes and peripheral neuropathy [10–12]. However, its effects on ALI are little to be known. In our present study, the effects and the underlying mechanisms of Ai on LPS-challenged mice and macrophages were investigated. Our study indicated the significance of Nrf2 to suppress inflammation and oxidative stress regulated by Ai, and provided the fact that AMPK was strongly implicated in Nrf2 expression.

## 2. Materials and methods

### 2.1. Animals and treatments

The male, 6–8 week-old, wild type (WT) and Nrf-2 knockout (Nrf-$2^{-/-}$) C57BL/6 J mice weighing 20 ~ 25 g were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were required to adapt to the environment for 7 days before the experiments. They were housed in a specific pathogen-free, temperature and humidity-controlled environment (25 ± 2 °C, 50 ± 5% humidity) with a standard 12 h light/12 h dark cycle with food and water in their cages. All procedures were in line with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People's Republic of China. The Institutional Animal Care and Use Committee at the Department of Pediatric, Baoji Maternal and Child Health Hospital approved the animal study protocols. To calculate the safety of Ai, as well as its effects on LPS-induced lung injury, WT mice were divided into 3 groups (n = 8 in each group): Control (Con) group; Ai group (10 mg/kg); and Ai group (20 mg/kg) [13,14]. The i.p. injections of Ai once time a day were subjected to mice for consecutive 30 days. Then, all mice were sacrificed through eyeball extirpating, and the blood was collected for aminotransferases (AST) and alanine aminotransferase (ALT) measurements. The lung tissue samples were removed for further study. To calculate the role of Ai in the survival rate of mice, mice were randomly divided into 4 groups (n = 8 in each group): (1) LPS group of Nrf-$2^{+/+}$ mice; (2) LPS + Ai group of Nrf-$2^{+/+}$ mice; (3) LPS group of Nrf-$2^{-/-}$ mice; (4) LPS + Ai group of Nrf-$2^{-/-}$ mice. Then, LPS (35 mg/kg, Sigma-Aldrich, USA) was injected to Nrf-$2^{+/+}$ and Nrf-$2^{-/-}$ mice by i.p., along with three i.p. injections of Ai (20 mg/kg), at 2 h before and 12 h and 24 h after LPS injection [15,16].