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Alpinetin inhibits lipopolysaccharide-induced acute kidney injury in mice

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ABSTRACT

Alpinetin, a novel plant flavonoid isolated from *Alpinia katsumadai Hayata*, has been demonstrated to have anti-inflammatory and antioxidant effects. However, the effects of alpinetin on lipopolysaccharide (LPS)-induced acute kidney injury have not been reported. In the present study, we investigated the protective effects and the underlying mechanism of alpinetin against LPS-induced acute kidney injury in mice. The results showed that alpinetin inhibited LPS-induced kidney histopathologic changes, blood urea nitrogen (BUN) and creatinine levels. Alpinetin also inhibited LPS-induced ROS, MDA, and inflammatory cytokines TNF-α, IL-6 and IL-1β production in kidney tissues. Meanwhile, Western blot analysis showed that alpinetin suppressed LPS-induced TLR4 expression and NF-κB activation in kidney tissues. In addition, alpinetin was found to up-regulate the expression of Nrf2 and HO-1 in a dose-dependent manner. In conclusion, alpinetin protected LPS-induced kidney injury through activating Nrf2 and inhibiting TLR4 expression.

1. Introduction

Sepsis, a clinical syndrome caused by injury or infection, is characterized by the whole-body inflammatory response [1]. Acute kidney injury (AKI) is a devastating condition of sepsis [2]. LPS has been identified as one of the most important factors that leads to AKI [3,4]. LPS could up-regulate the production of inflammatory cytokines TNF-α, IL-6 and IL-1β [5,6]. These cytokines lead to the development of AKI [7]. AKI is a worldwide heath problem and there are no drugs for the treatment of AKI at present [8]. Nowadays, the mortality rate of AKI remains high and reaches to 30% [9]. Thus, it is urgent to find novel compounds to treat AKI.

Alpinetin is a natural flavonoid abundantly present in *Alpinia katsumadai Hayata* [10]. Previous studies showed that alpinetin had various biological properties, inducing anti-inflammatory and antioxidant effects [11]. Alpinetin was found to inhibit LPS-induced inflammatory cytokines production in RAW264.7 cells [12] and THP-1-derived macrophages [13]. Alpinetin also had the ability to protect against LPS-induced acute lung injury in mice [12]. Furthermore, recent study showed that alpinetin inhibited LPS-induced mastitis in mice [14]. However, the protective effects of alpinetin against LPS-induced AKI remain unclear. Therefore, in the present study, we investigated the protective effects and the possible mechanism of alpinetin against LPS-induced AKI in mice.

2. Materials and methods

2.1. Reagents

LPS (*Escherichia coli* 0111:B4) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Alpinetin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Inflammatory cytokines TNF-α, IL-6 and IL-1β ELISA kits were obtained from BioLegend (San Diego, CA, USA). Antibodies against TLR4, Nrf2, HO-1, NF-κB, IκBα, β-actin, and Lamin B were purchased from Santa Cruz Biotechnology (Autogen, Bioclear, UK).

2.2. Animals

Male BALB/c mice, 6–8 weeks old, were purchased from the Center of Experimental Animals of Huazhong University of Science and Technology (Wuhan, China). The mice were housed in an animal care facility with the temperature 23 ± 2 °C. The mice were allowed free access to food and water. All animal experiments were performed in accordance with the Health's Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

2.3. Experimental design and grouping

Sixty mice were randomly divided into five groups: control group, LPS group, and LPS + alpinetin (12.5, 25 and 50 mg/kg) groups. The mice were injected intraperitoneally (i.p.) with 15 mg/kg body weight of LPS in 50 μl PBS to induce kidney injury. Alpinetin (12.5, 25 and 50 mg/kg) was given intraperitoneally (i.p.) 1 h after LPS treatment.

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24 h after LPS challenge, the mice were euthanized and the blood and kidney tissues were collected.

2.4. Histopathological analysis

24 h after LPS challenge, the kidney tissues were collected and fixed in 10% formalin then embedded in paraffin wax. After dehydrated in a series of graded alcohols for staining, the sections were stained with hematoxylin and eosin (H&E) stain. Then, the pathological changes were observed using a light microscope.

2.5. Serum creatinine and BUN assay

24 h after LPS challenge, the blood were collected to obtain serum. Serum creatinine and BUN levels were detected by using an autoanalyzer according to the manufacturer's instructions.

2.6. ROS and MDA assay

24 h after LPS challenge, the kidney tissues were collected. The level of kidney MDA was measured by MDA test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The level of ROS was measured based on the oxidation of DCFH-DA to DCF as described previously [15].

2.7. ELISA assay

The productions of inflammatory cytokines TNF-α, IL-6, and IL-1β, in serum and kidney tissues were measured using ELISA kits (BioLegend, CA, USA) according to the manufacturer's instructions.

2.8. Western blot analysis

Nuclear and cytoplasmic proteins of kidney tissues were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Pierce, Rockford, IL, USA) according to the manufacturer's protocol. Protein concentration was determined by Bradford method. Equal amounts of proteins were separated by 10% SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% nonfat dry milk at room temperature for 2 h and probed with specific primary antibody at 4 °C overnight. After washing three times, the membrane was probed with the secondary antibody at room temperature for 1 h. Finally, the blots were developed using ECL Western blotting reagents.

2.9. Cell culture and cell viability assay

Human renal proximal tubular epithelial cells (HK-2 cells) were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium F-12 supplemented with 10% fetal bovine serum at 37 °C in a humidified incubator under 5% CO_2.

The effects of alpinetin on cell viability was determined by MTT assay. Briefly, HK-2 cells were seeded in 96 well plates and treated with various concentrations of alpinetin (0–200 μg/ml) for 24 h. 20 μl MTT (5 mg/ml) was added to each well, and the cells were further incubated for an additional 4 h. The resulting formazan crystals were dissolved in DMSO (150 μl/well). Absorbance was determined at 540 nm.

2.10. Statistical analysis

Data are expressed as means ± S.E.M. of three independent experiments. Differences between mean values of normally distributed data were analyzed using one-way ANOVA (Dunnett's t-test) followed by Dunnett's test. Statistical significance was accepted $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Effects of alpinetin on LPS-mediated kidney histopathologic changes

To investigate the protective effects of alpinetin on LPS-induced AKI, the kidney histological changes were evaluated by H&E stain. As shown in Fig. 1, the control group showed normal structure of the cortex and medulla. Kidney tissues of LPS group were significantly damaged, including the damage in the renal cortex, outer medulla, denudation of epithelium, and infiltration of inflammatory cells. Treatment with alpinetin markedly ameliorated the kidney injury (Fig. 1C–E).

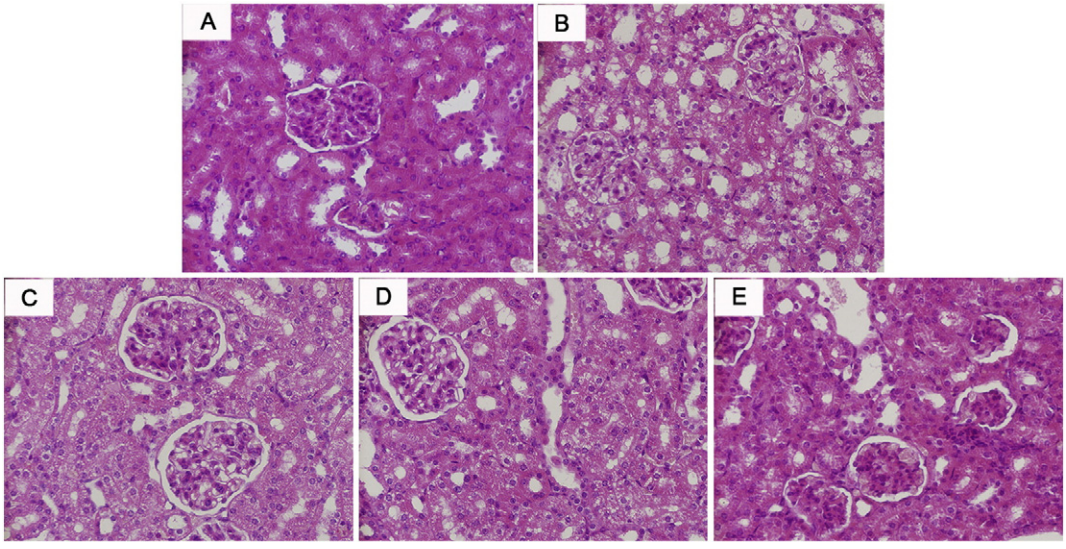

Fig. 1. Effects of alpinetin on histopathological changes in kidney tissues in LPS-induced AKI mice. Alpinetin (12.5, 25 and 50 mg/kg) was given intraperitoneally (i.p.) 1 h after LPS treatment. 24 h after LPS challenge, kidney tissues from each experimental group were processed for histological evaluation. Representative histological changes of kidney obtained from mice of different groups. A: control group, B: LPS group, C: LPS + alpinetin (12.5 mg/kg) group, D: LPS + alpinetin (25 mg/kg) group, and E: LPS + alpinetin (50 mg/kg) group (hematoxylin and eosin staining, magnification 200×).