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Alpinetin ameliorates inflammatory response in LPS-induced endometritis in mice

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ABSTRACT

Alpinetin has been reported to have anti-inflammatory effects. However, whether alpinetin has anti-inflammatory effects in LPS-induced endometritis has not been thoroughly elucidated to date. The aim of this study was to investigate the protective effects of alpinetin on LPS-induced endometritis in mice. A mouse model of endometritis was induced by LPS and alpinetin was given 1 h before LPS treatment. According to the results, alpinetin protected mice against LPS-induced endometritis by attenuating uterine histological changes and myeloperoxidase (MPO) activity. The LPS-induced inflammatory response was inhibited by alpinetin as confirmed by the inhibition of TNF-α, IL-1ß, and IL-6 production. Furthermore, LPS-induced TLR4 expression and NF-κB activation were significantly suppressed by alpinetin. In addition, the expression of PPAR-γ was dose-dependently increased by the treatment of alpinetin. Taken together, the results of this study showed that alpinetin had protective effects against LPS-induced endometritis in mice, and the beneficial effects were occurred through the activation of PPAR-γ and inhibition of the TLR4 signaling pathway.

1. Introduction

Endometritis, the inflammation of the uterine lining, often causes infertility in women [1]. Previous studies have shown a relationship between endometritis and infertility [2, 3]. Endometritis can be caused by many factors and bacteria is one of the most important factors leading to endometritis [4, 5]. Among these bacteria, *Gram-negative* bacteria, such as *Escherichia coli* and *Fusobacterium necrophorum*, have been known as the major bacteria that lead to endometritis [6]. LPS is the major activator of inflammatory cells [7]. During the development of endometritis, LPS can induce the activation of neutrophils and lead to the release of inflammatory cytokines [8]. Recent studies have suggested that suppression of these inflammatory cytokines has potential therapeutic effects for endometritis [9].

Antibiotics are widely used for the treatment of endometritis. The use of antibiotics is likely to cause bacterial resistance. Therefore, new agents to treat endometritis are urgently needed. Alpinetin has been known to have anti-inflammatory effects. Alpinetin was found to suppress LPS-induced inflammatory cytokine production in RAW264.7 cells [10]. Additionally, alpinetin can attenuate the production of inflammatory mediators in LPS-stimulated THP-1 cells [11]. In vivo, alpinetin had protective effects on LPS-induced mastitis in mice [12]. Alpinetin can also attenuate DSS-induced acute colitis in mice by suppressing the TLR4 signaling pathway [13]. In addition, alpinetin can inhibit LPS-induced acute kidney injury in mice [14]. However, whether alpinetin can protect against LPS-induced endometritis is unknown. This study is aimed at determining the protective effects of alpinetin on LPS-induced endometritis in vivo.

2. Materials and methods

2.1. Reagents

Alpinetin (purity > 98%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). The PPAR-γ antibody was purchased from Santa Cruz (TX, USA), and the TLR4 and NF-κB signaling pathway antibodies were purchased from Biolegend (CA, USA). LPS was purchased from Sigma (St. Louis, MO, USA). ELISA kits were obtained from R&D systems (Minneapolis, MN, USA).

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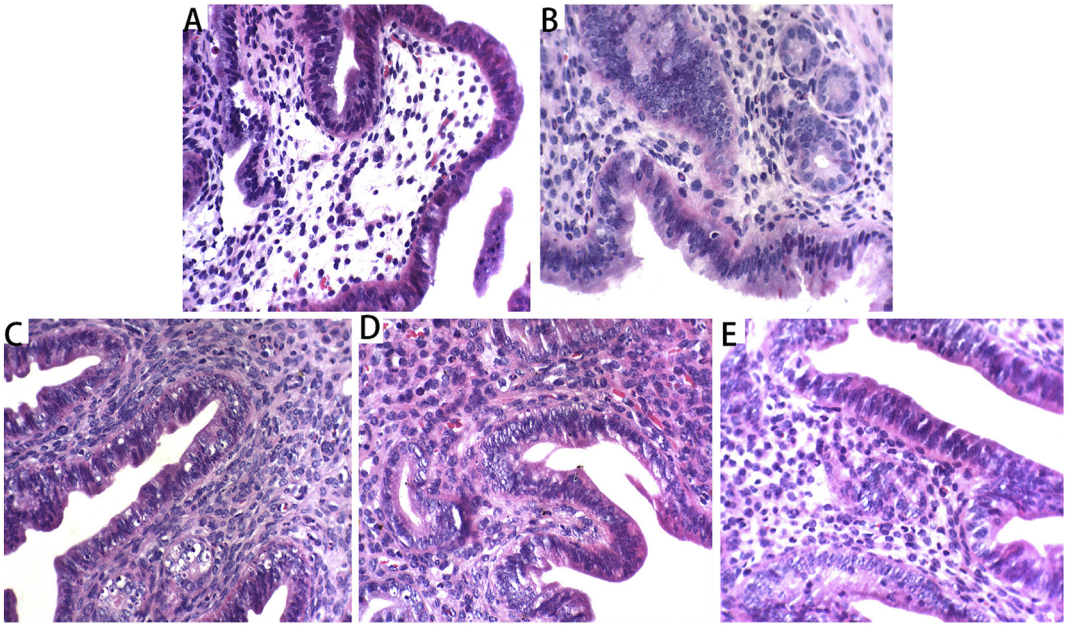

Fig. 1. Alpinetin attenuates LPS-induced uterine histopathological changes (magnification 200×). A: Control group, B: LPS group, C–E: LPS + alpinetin (10, 20, 40 mg/kg) groups.

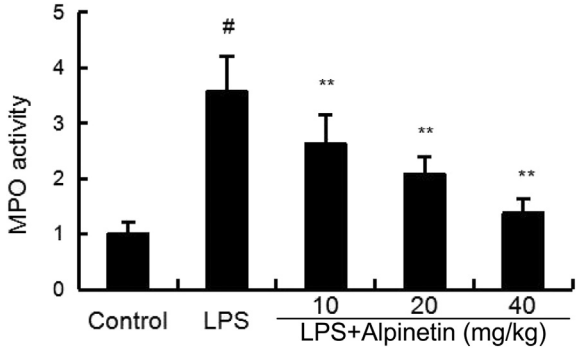

Fig. 2. Alpinetin inhibits LPS-induced MPO activity. The data of this study are presented as mean ± SEM of three parallel measurements. p# < 0.01 vs. control group, p** < 0.01 vs. LPS group.

2.2. Animals

Female C57BL/6 mice, weighing 18–22 g, were purchased from Southeast University. The experiment was approved by the Ethical Committee of Southeast University.

2.3. Experimental groups

Sixty mice were randomly divided into five groups: Control group, LPS group, and LPS + alpinetin (10, 20, 40 mg/kg) groups. LPS-induced endometritis was induced by giving 50 μL of LPS (1 mg/mL) to each side of the mouse uterus through the vagina using a 100 μl syringe (patent no.: 201410391820.8). Alpinetin was given intraperitoneally 1 h before LPS treatment. 24 h later, the mice were sacrificed and the uterine tissues were collected for subsequent experiments.

2.4. Histological analysis

The uterine tissues were collected and washed. The tissues were then fixed in 10% formalin, dehydrated, and embedded in paraffin. The uterine tissues were cut into 5 μm sections using a microtome and stained with hematoxylin and eosin (H&E).

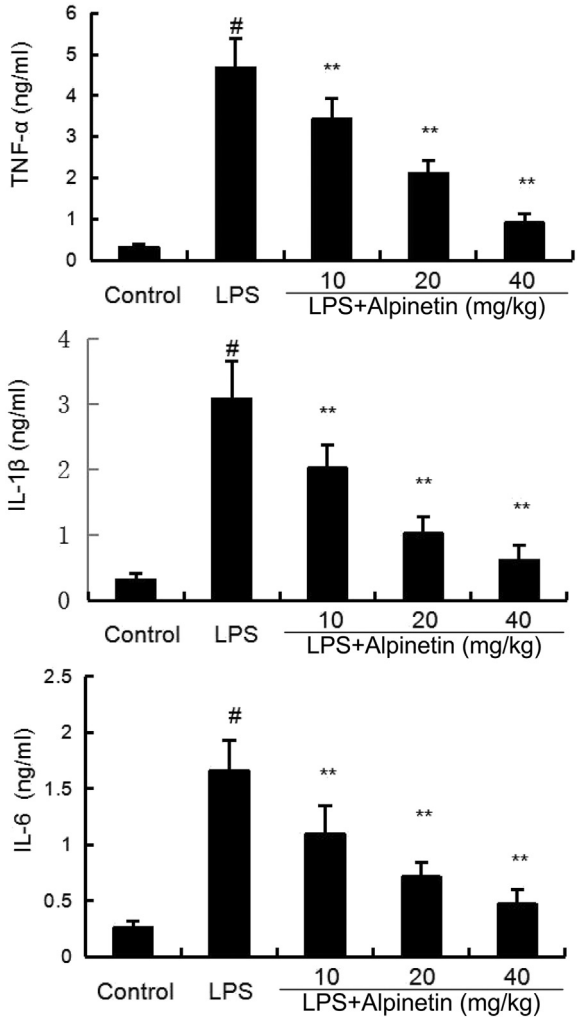

Fig. 3. Effects of alpinetin on LPS-induced TNF-α, IL-6, and IL-1β production. The data of this study are presented as mean ± SEM of three parallel measurements. p# < 0.01 vs. control group, p** < 0.01 vs. LPS group.