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Anti-inflammatory effects of eugenol on lipopolysaccharide-induced inflammatory reaction in acute lung injury via regulating inflammation and redox status

Xianfeng Huang *, Yuanyuan Liu, Yingxun Lu, Chunhua Ma *

School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou 213164, PR China

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ABSTRACT

Acute lung injury (ALI) represents a clinical syndrome that results from complex responses of the lung to a multitude of direct and indirect insults. This study aims to evaluate the possible mechanisms responsible for the anti-inflammatory effects of eugenol (EUL) on lipopolysaccharide (LPS)-induced inflammatory reaction in ALI. ALI was induced in mice by intratracheal instillation of LPS (0.5 mg/kg), and EUL (5, and 10 mg/kg) was injected intraperitoneally 1 h prior to LPS administration. After 6 h, bronchoalveolar lavage fluid (BALF) and lung tissue were collected. The findings suggest that the protective mechanism of EUL may be attributed partly to decreased production of proinflammatory cytokines through the regulating inflammation and redox status. The results support that use of EUL is beneficial in the treatment of ALI.

1. Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), represent a clinical syndrome that results from complex responses of the lung to a multitude of direct and indirect insults [1]. ALI and ARDS are characterized by an intense pulmonary inflammatory response, involving polymorphonuclear neutrophil (PMN) recruitment, interstitial edema, a disruption of epithelial integrity, and lung parenchymal injury [2]. The pathogenesis of ALI/ARDS disorders consists of oxidant/anti-oxidant imbalance and inflammation/anti-inflammation imbalance, up-regulation of adhesion molecules, and the increased production of chemokines [3,4]. Activated PMNs contribute to lung injury by releasing inflammatory cytokines, proteolytic enzymes, reactive oxygen species (ROS) and other proinflammatory mediators which promote key events in ALI and may represent a potential target for therapy. Inflammatory cytokines, such as interleukin 1β (IL-1β), IL-8, and IL-6, play a major role in mediating and amplifying ALI/ARDS by stimulating chemotaxis and activation of neutrophils. Although the causes of ALI and ARDS are numerous, endotoxin is thought to be the main pathogen that leads to the development of these disorders. Endotoxin or lipopolysaccharide (LPS), derived from the cell wall of gram-negative bacteria, induces a sepsis syndrome accompanied by key features of ALI, including the recruitment of inflammatory cells into the lung with subsequent increases in capillary permeability and alveolar edema [5]. It is often showed as hypoxemia, alveolar–capillary barrier damage, and pulmonary inflammation, and often associated with multiple organ failure in later stage. There is no specific medicine to control ALI or ARDS as yet, so searching for the new therapy target and exploring the new drug to treat ARDS have become the work of scientific research. Animal models of ALI are important tools for studying mechanisms relevant to ARDS in humans. Many methods developed in a variety of animal species can generate ALI, each with its own set of advantages and limitations. Since the most common cause of ALI is bacterial sepsis, many investigators have used intraperitoneal or intratracheal administration of LPS as a method of provoking ALI.

Nuclear factor-kappaB (NF-κB), a nuclear transcription factor, is a regulator of inflammatory processes. Chen et al. have reported that NF-κB plays an important role in the pathogenesis of lung diseases [6,7]. NF-κB is required for maximal transcription of numerous cytokines, including tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 [8]. These cytokines are thought to be important in the generation of ALI. Therefore, it has been suggested that inhibitors of NF-κB function may be useful as anti-inflammatory agents [9].

Eugenol (EUL) (4-allyl-1-hydroxy-2-methoxybenzene), a natural food-flavoring agent found in plant extracts of *Piper betle*, cinnamon, clove, basil, and nutmeg, has been found to ameliorate oxidative stress by preventing oxidative tissue damage in different experimental models [10–12]. However, no available study has evaluated the effects of EUL treatment on LPS-induced ALI.

* Corresponding authors.
E-mail addresses: hxfnju@163.com (X. Huang), machunhua25@126.com (C. Ma).

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2. Materials and methods

2.1. Reagents

Eugenol (99% purity) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Dexamethasone (Dex) was purchased from Xiansheng Drug Store (Nanjing, China). LPS was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Kits

Superoxide dismutase (SOD), myeloperoxidase (MPO), cholinesterase, PON1 and Wright–Giemsa solution staining were purchased from the Institute of Jiancheng Bioengineering (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits for determination of IL-6, TNF-α were produced by (R&D, Minneapolis, MN).

2.3. Animals

Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University. A total of 50 female BALB/c mice (18–22 g) were obtained from the Experimental Animal Center of China Pharmaceutical University (Nanjing, China). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to experiments, and provided with water and standard chow ad libitum. All experimental procedures were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of China.

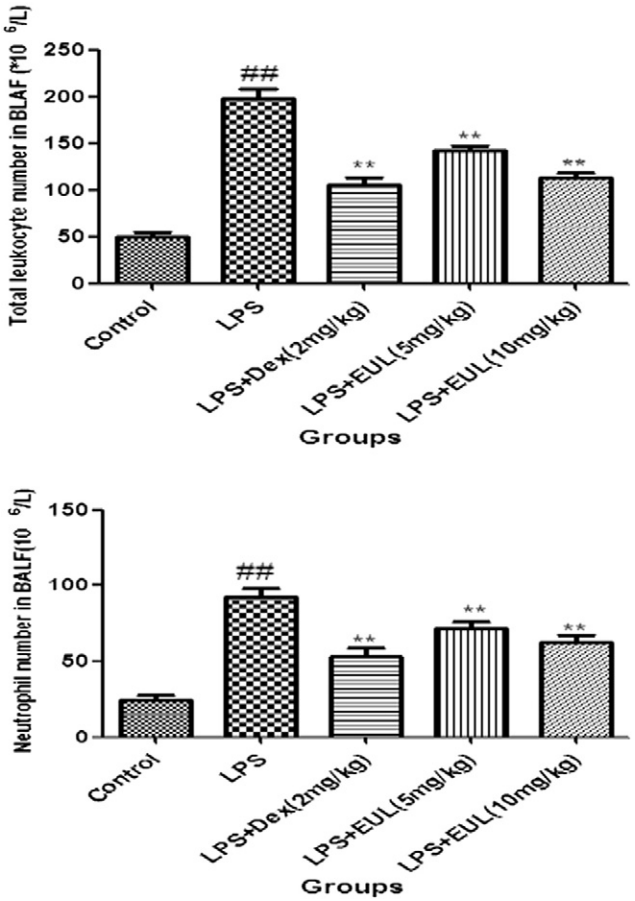

Fig. 1. Total leukocyte number and neutrophil number in BALF. Values are expressed as means ± SDs. Compared with control: #P < 0.05, ##P < 0.01; Compared with LPS: *P < 0.05, **P < 0.01.

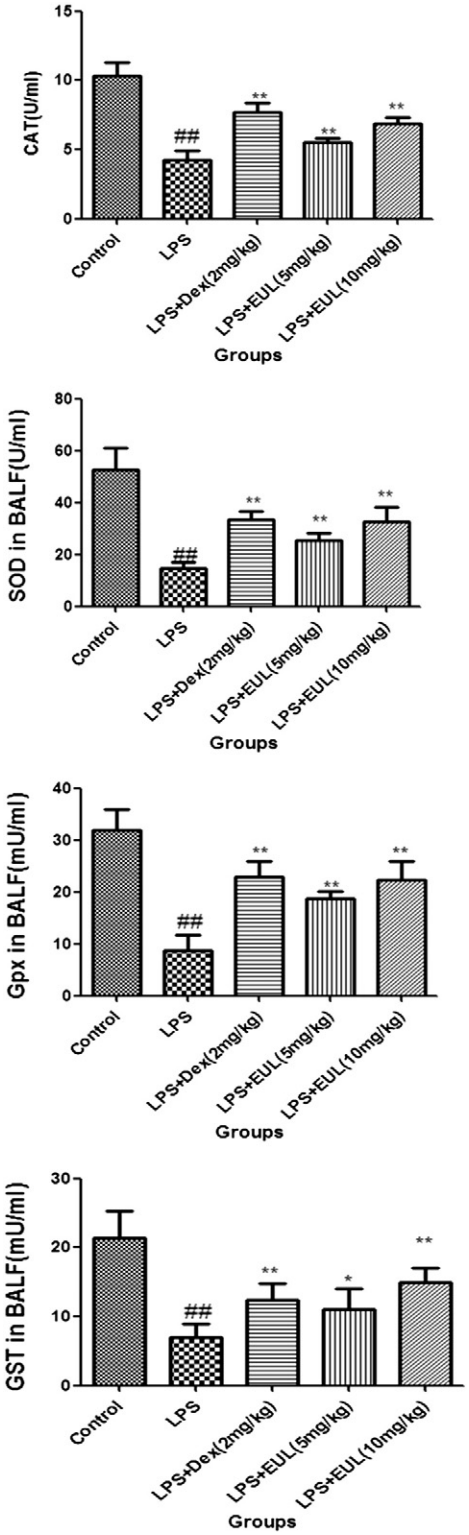

Fig. 2. Effects of EUL on antioxidant enzymes in BALF. Values are expressed as means ± SDs. Compared with control: #P < 0.05, ##P < 0.01; Compared with LPS: *P < 0.05, **P < 0.01.