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Protective effect of zerumbone reduces lipopolysaccharide-induced acute lung injury via antioxidative enzymes and Nrf2/HO-1 pathway

Wai-Shing Leung [a], Ming-Ling Yang [b], Shiuan-Shinn Lee [c], Chi-Wen Kuo [d,e], Yung-Chyuan Ho [f], Rosa Huang-Liu [g], Hui-Wen Lin [h,i,1], Yu-Hsiang Kuan [j,k,*,1]

[a] Department of Emergency Medicine, Jen-Ai Hospital, Taichung, Taiwan.
[b] Department of Anatomy, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[c] School of Public Health, Chung Shan Medical University, Taichung, Taiwan
[d] Department of Pharmacology, Jen-Ai Hospital, Taichung, Taiwan
[e] Central Taiwan University of Science and Technology, Taichung, Taiwan
[f] School of Medical Applied Chemistry, Chung Shan Medical University, Taichung, Taiwan
[g] School of Nutrition, , Chung Shan Medical University, Taichung, Taiwan
[h] Department of Optometry, Asia University, Taichung, Taiwan
[i] Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan
[j] Department of Pharmacology, School of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan
[k] Department of Pharmacy, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

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ABSTRACT

Acute lung injury (ALI) is a serious disease with high morbidity and mortality rate. Although there are effective strategies for treatment of ALI; a widely accepted specific pharmacotherapy has not yet established. Zerumbone, the major active phytochemical compound from *Zingiber zerumbet* Smith, exhibits various beneficial biological and pharmacological activities, such as antioxidation, anti-inflammation, immunomodulation, and anti-cancer. We aimed to study the potential protective effects and mechanisms of zerumbone in mouse model of lipopolysaccharide (LPS)-induced ALI. Pretreatment with zerumbone inhibited the histopatholgical changes such as neutrophils infiltration, increased in alveolar barrier thickness, hemorrhage, and hyaline membrane formation occurred in lungs in LPS-induced ALI. In addition, not only LPS-induced activation of myeloperoxidase (MPO) and metallopeptidase-9 (MMP-9) was suppressed by zerumbone, but also lipid peroxidation in lungs was inhibited as well. Moreover, pretreatment with zerumbone reversed the antioxidative enzymes activities, including superoxide dismutase, catalase, and glutathione peroxidase, decreased by LPS and enhanced the expression of nuclear factor erythroid 2-related factor (Nrf2) and heme oxygenase (HO-1) induced by LPS. These results from present study suggested that the protective mechanisms of zerumbone on LPS-induced ALI were via up-regulation of antioxidative enzymes and Nrf2/HO-1 pathway.

1. Introduction

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome, are two serious illnesses associated with acute pulmonary inflammatory responses which can lead to respiratory failure [1]. A recent study performed in the King County of Washington estimates the crude incidence of ALI to be 78.9 per 100,000 person-years, with an age-adjusted incidence of 86.2 per 100,000 person-years [2]. Mortality rate for patients with ALI is about 40–60% and remains high although many new therapeutic strategies have been developed [1]. The development of ALI is triggered by several important environmental risk factors which can be divided into direct and indirect factors [3]. Sepsis and pneumonia are the common factors of causing indirect and direct lung injury respectively. Lipopolysaccharide (LPS) is a compound found in the outer cell membrane of gram-negative bacteria which has been recognized as playing the pivotal role in the pathogenesis of sepsis and pneumonia [4]. Therefore, LPS is a potent agent which induces inflammatory responses and acts as an important pathological factor in the development of ALI [5].

Abbreviations: ALI, Acute lung injury; AOE, antioxidative enzymes; BALF, bronchoalveolar lavage fluid; CAT, catalase; DMSO, dimethyl sulfoxide; GPx, glutathione peroxidase; HO-1, heme oxygenase; IP, intraperitoneal; IT, intratracheal; LPS, Lipopolysaccharide; MDA, malondialdehyde; MPO, myeloperoxidase; Nrf2, nuclear factor erythroid 2-related factor; SD, standard deviation; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

* Corresponding author at: Department of Pharmacology, School of Medicine, Chung Shan Medical University, Chung Shan Medical University Hospital, No. 110, Sec. 1, Jianguo N. Rd., Taichung 40201, Taiwan.
E-mail address: kuanyh@csmu.edu.tw (Y.-H. Kuan).
[1] These authors contributed equally to this work.

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After administration of LPS into the lungs, histopathological changes characterized by neutrophilic alveolitis, hyaline membrane formation, and increased in thickness of alveolar barrier occurred [6]. In the progression of ALI, neutrophils activation is the critical component of the innate immune system. Neutrophils engulf and kill the bacteria and fungi via phagocytosis, degranulation, and respiratory burst [7]. However, oxidative stresses are generated from degranulation and respiratory burst and result in neighborhood tissue damages that lead to inflammation in lung [6]. The mechanism of protection against oxidative stress injury in lung tissue involves antioxidative enzymes (AOEs) including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nuclear factor erythroid 2-related factor (Nrf2)/heme oxygenase (HO-1) antioxidant pathway [8,9].

Zerumbone is a monocyclic sesquiterpene and the major active phytochemical compound extracted from rhizome of *Zingiber zerumbet* Smith [10]. Zerumbone exhibits a wide range of beneficial biological activities and pharmacological effects which include antioxidative activity, anti-inflammation, immunomodulatory effect, and anti-cancer activity in several tumor cells such as blood, skin, liver, breast, colon, and gastric cancers [11]. Zerumbone reduces airway inflammation and exhibits antiallergic effect in the mouse model of ovalbumin-induced asthma [12]. In LPS-stimulated murine RAW264.7 macrophages, zerumbone inhibits production of proinflammatory mediators and enhances HO-1 expression [13]. At present study, we aimed to identify the protective mechanism of zerumbone via antioxidative enzymes and Nrf2/HO-1 pathway on in vivo animal model of LPS-induced ALI.

2. Materials and methods

2.1. Materials

Myeloperoxidase (MPO) content, CAT activity, SOD activity, and GPx activity assay kits were purchased from Cayman (Ann Arbor, MI, USA). Malondialdehyde (MDA) assay kit was manufactured by ZeptoMetrix (Buffalo, NY, USA). Antibodies against HO-1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were obtained from Jackson Immuno Research Laboratories (Baltimore, MD, USA). LPS from *Escherichia coli* Serotype 0111:B4, dimethyl sulfoxide (DMSO), and other reagents, unless specifically stated elsewhere, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Adult male pathogen-free ICR mice (25–30 g) were obtained from BioLASCO (Taipei, Taiwan). The mice were fed a standard laboratory diet and water ad libitum. All mice were housed under standard laboratory conditions at a constant temperature of 22 ± 1 °C and 50–60% humidity with 12 hour light-dark cycle. All procedures and care of mice were approved by the Institutional Animal Care and Use Committee of Chung Shan Medical University.

2.3. Murine model of LPS-induced ALI

The procedures for the induction of ALI by LPS in mice were performed as described in previous studies [14,15]. Forty-eight mice were divided into six groups randomly, which were one control group and five treatment groups. The mice of control group received intraperitoneal (IP) administration of solvent for 30 min followed by intratracheal (IT) administration of 50 μl saline; while the five treatment groups were pretreated with zerumbone at concentration of 0, 0.1, and 1 μmol/kg IP or dexamethasone at concentration of 1 mg/kg IP for 30 min respectively before IT injection of 100 μg/50 μl of LPS. After 6 h, all mice were sacrificed by pentobarbital and samples were collected.

2.4. Bronchoalveolar lavage fluid (BALF) collection

The lavage of lung was performed with 1 ml of sterile saline for three times through the tracheal cannula. The pooled BALF was collected on ice and centrifuged at 600*g* for 10 min at 4 °C. The supernatant was subsequently stored at −20 °C for MMP-9 activation analysis [14,15].

2.5. Histopathological analysis

After sacrifice, the lungs were excised by midsternal thoracotomy and fixed via tracheal cannula with 4% paraformaldehyde. Afterwards, the lungs were dehydrated and embedded in paraffin at 60 °C, 3 μm histological sections were procured using a rotatory microtome and stained with hematoxylin-eosin. Histopathological features of acute lung injury, including hemorrhage, infiltration of leukocytes, changes in the thickness of alveolar wall, and formation of hyaline membrane, were evaluated using light microscopy [16].

2.6. Measurement of MPO contents

The levels of MPO in lungs were measured as previously described [17]. MPO was extracted from tissues with phosphate buffer containing guaiacol and cetyltrimethylammonium bromide. After addition of hydrogen peroxide, the assay reactions were started. The contents of MPO, which is expressed as U/mg of the protein, in the lungs were measured at absorbance of 470 nm.

2.7. Zymography assay for MMP-9 activation

The activation of MMP-9 in BALF was measured using gelatin zymography assay and was performed according to previous study [18]. Briefly, protein samples from BALF were separated by SDS-PAGE containing gelatin. After electrophoresis, the gels were washed twice in 2.5% Triton X-100, incubated in reaction buffer at 37 °C for 16 h, then stained with Coomassie Brilliant R-250, and destained in the mix solution of 7.5% acetic acid and 5% methanol.

2.8. Measurement of lipid peroxidation

The levels of lipid peroxidation in lungs were measured by the thiobarbituric acid reactive substances (TBARS) assay as previously described [8]. The results of TBARS were expressed as MDA formation, which was presented as mmol/mg of the protein.

2.9. Measurement of antioxidative enzymes activities

The activities of SOD, CAT, and GPx were determined using commercially available assay kits following the manufacturer's instructions and previous study [19].

2.10. Western blot analysis for HO-1 and Nrf-2 expression

As previously described [20], lungs were harvested and homogenized in tissue protein extraction solution containing proteinase inhibitor cocktail and phosphatase inhibitor cocktail. Protein samples were separated by SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk (w/v) in phosphate buffer saline containing 0.1% Tween-20. After washing, the membranes were probed with antibodies including HO-1, Nrf-2, and β-actin; washed again, incubated with a 1:10,000 (v/v) dilution of horseradish peroxidase-labeled IgG, then the blots were developed using ECL western blotting reagents.