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Inhibition of lung inflammatory responses by bornyl acetate is correlated with regulation of myeloperoxidase activity

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ABSTRACT

Background: Bornyl acetate is a bicyclic monoterpene present in numerous conifer oils. In this study, we aimed at clarifying the potential anti-inflammatory function and mechanism of bornyl acetate by using lipopolysaccharide (LPS)-induced acute lung injury murine model and RAW 264.7 cells.

Materials and methods: RAW 264.7 cells were pretreated with bornyl acetate 1 h before LPS stimulation and cell-free super supernatants were collected to measure cytokine concentrations. To induce acute lung injury, BALB/c mice were injected intranasally with LPS and treated with bornyl acetate 1 h before LPS stimulation. Seven hours after administration, the bronchoalveolar lavage fluid (BALF) was collected for measuring the cell count and cytokine production. We collected lungs for assaying wet-to-dry weight ratio, myeloperoxidase activity, and histologic changes. The extent of phosphorylation of mitogen-activated protein kinases and nuclear factor κB was detected by Western blot.

Results: Our results showed that bornyl acetate downregulated the levels of proinflammatory cytokines *in vitro* and *in vivo*; reduced the number of total cells, neutrophils, and macrophages in BALF; attenuated the histologic alterations in the lung; decreased the wet-to-dry weight ratio in BALF; and suppressed NF-kappa-B inhibitor alpha, extracellular regulated protein kinases, c-JunN-terminal kinase, p38 mitogen-activated protein kinase activation.

Conclusions: These findings suggested that bornyl acetate may be developed as a preventive agent for lung inflammatory diseases.

1. Introduction

Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) are major causes of mortality in intensive care units, and characterized by hypoxemia, pulmonary infiltration, absence of an elevated pulmonary capillary wedge pressure, pulmonary neutrophil sequestration, intravascular coagulation, disruption of pulmonary capillary integrity leading to edema, and increased shunt fraction [1]. The most severe form of ALI is ARDS, which is a major cause for admission to critical care units [2]. Most of these pathologic features of human ALI/ARDS have also been observed in experimental animals, in

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response to systemic infusions of live bacteria or endotoxin of gram-negative bacteria [3,4]. Endotoxin, which is an intrinsic component of the outer membrane of gram-negative bacteria, is composed of lipopolysaccharide (LPS) and is known to cause ALI [5]. LPS provokes damage to the alveolar–capillary membrane and the adhesion, activation, and sequestration of polymorphonuclear neutrophils, which result in the deterioration of gas exchange [6]. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 are potent proinflammatory cytokines that play a role in the initiation and amplification of inflammatory responses [7]. Proinflammatory cytokines also are regularly expressed by activated polymorphonuclear neutrophils traveling to the lung [8]. They appear to be a relevant source of IL-1β, favoring the subsequent release of other mediators, such as TNF-α, macrophage inflammatory protein-2, and IL-8 [9–11]. Although recent data have shown a reduction in mortality since the implementation of lung-protective ventilation strategies [12], there are no effective therapeutic measures or medicines to treat ALI.

The essential oil, which imparts the specific flavor and odor, is the aromatic portion of various herbs. It is known that the essential oil has various biological activities, especially antimicrobial and antioxidant capacity, and has been widely used as composition in skin products, wound care, cosmetics, and natural antiseptic agents. Bornyl acetate (Fig. 1) is the main volatile constituent in numerous conifer oils and some Chinese traditional herbs, such as *Fructus Amomi*, *Houttuynia Cordata*, and so forth. Several researches have shown that bornyl acetate had a sedative effect when inhaled, antidiarrheal, dephlogistication, depressing spasm, analgesic effects, and an antiabortive effect on pregnant mice through modulation of the immunologic balance at the maternal–fetal interface [13–16]. So far, there have been very few reports on anti-inflammatory effects of bornyl acetate, and the detailed mechanism of its anti-inflammatory activities. Therefore, the present study was aimed to investigate the effects of bornyl acetate on ALI induced by LPS and the underlying mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Bornyl acetate (purity >99%, Fig. 1) was purchased from the National Institute for Food and Drug Control (Beijing, China).

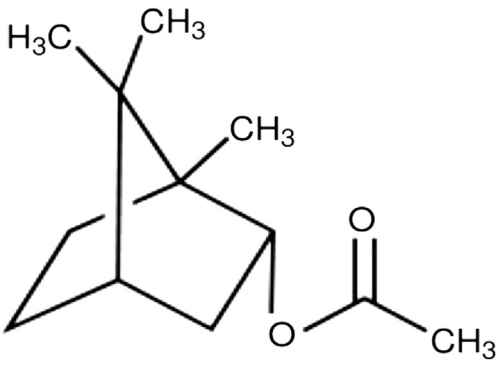

Fig. 1 – The chemical structure of bornyl acetate.

LPS (*Escherichia coli* 055:B5), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co (St Louis, MO). Dulbecco's modified Eagle medium, fetal bovine serum, penicillin, and streptomycin for cell culture use were obtained from Invitrogen-Gibco (Grand Island, NY). Mouse TNF-α, IL-1β, and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biolegend (San Diego, CA). The myeloperoxidase (MPO) determination kit was provided by the Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China). Rabbit mAb extracellular regulated protein kinases (ERK), c-JunN-terminal kinase (JNK), and p38, and mouse mAb NF-kappa-B inhibitor alpha (IκB) were purchased from Cell Signaling Technology Inc (Beverly, MA). Horseradish peroxidase–conjugated goat anti-rabbit and goat anti-mouse antibodies were provided by GE Healthcare (Buckinghamshire, UK). All other chemicals were of reagent grade.

2.2. Animals

Male BALB/c mice, 6–8 wk and weighing 18–20 g, were obtained from the Center of Experimental Animals of Baiqiuen Medical College of Jilin University (Jilin, China). The mice were kept in microisolator cages. All mice were fed with a standard laboratory diet and water *ad libitum*. They were maintained in a controlled environment at a temperature of 24 ± 1°C, 40%–80% relative humidity, and acclimatized for at least 2–3 d before experimentation. All studies were approved by the Animal Use Committee of Jilin University and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Bethesda, MD).

2.3. In vitro study

2.3.1. Cell culture and sample treatment

RAW 264.7 cells, a murine macrophage cell line, were obtained from the China Cell Line Bank (Beijing, China). Cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 3 mmol/L of glutamine, antibiotics (100 U/mL of penicillin and 100 U/mL of streptomycin) at 37°C in a humidified incubator containing 5% CO_2. Bornyl

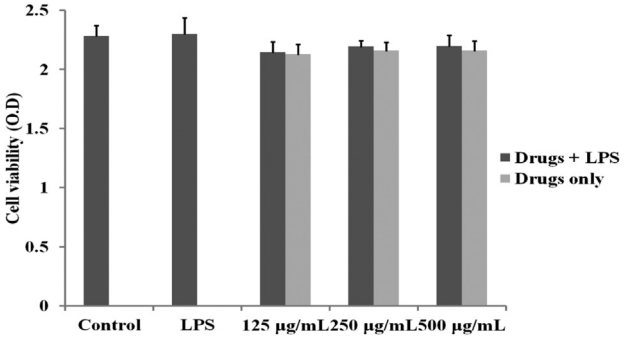

Fig. 2 – Effects of bornyl acetate on macrophage viability. RAW 264.7 cells were cultured with bornyl acetate (0–500 μg/mL) in the absence or presence of LPS (1 mg/L) for 18 h. Cell viability was assessed by MTT reduction assays. Data are presented as mean ± standard error of the mean of three independent experiments.