Bisabolol attenuates sepsis-induced acute lung injury through inhibiting NF-κB-mediated inflammatory response

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Abstract: Acute lung injury (ALI) is characterized by excessive inflammatory responses in lung tissue and the anti-inflammatory agent is proposed to be a therapeutic drugs for ALI. Bisabolol has been demonstrated to have anti-inflammatory activity through inhibiting the activation of NF-κB (nuclear factor kappa B). However, it is still unknown whether the bisabolol possesses the protective effect on ALI. In this study, we assessed the effect of bisabolol on the lung inflammation caused by sepsis in mice. We found that bisabolol pretreatment could markedly improve the histology of the lung and suppress the lung inflammation, such as the production of inflammatory cytokines in bronchoalveolar lavage fluid (BALF). Meanwhile, the lung wet/dry ratio, myeloperoxidase (MPO) activity, total inflammatory cells and production of nitric oxide (NO) in lung tissue, induced by sepsis, were reduced by bisabolol treatment. Additionally, bisabolol treatment also inhibited the degradation of IκB-α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), subsequently blocked the activation of NF-κB pathway caused by sepsis in vivo. Bisabolol could attenuate sepsis-induced lung injury in mice through inhibiting NF-κB signaling in macrophages, suggesting bisabolol might be a potential therapeutic candidate for the treatment of sepsis-induced lung injury.

Keywords: Bisabolol, sepsis, acute lung injury, inflammation, NF-κB pathway

Introduction

Despite significant advances in the understanding of the pathogenesis of sepsis and its management, the mortality rate of severe sepsis remains unacceptably high [1]. The ultimate cause of death in patients with severe sepsis is multiple organ failure. The lung is frequently the principal failing organ during the sequential development of multiple organ dysfunction in sepsis [2, 3]. Acute lung injury (ALI), characterized by oedema, inflammatory cell infiltration and, in consequence, impaired gas exchange, clinically manifests as acute respiratory distress syndrome is the primary cause of death under these conditions [3]. About 40% of septic patients develop ALI [4].

Sepsis is a potentially fatal whole-body inflammation (a systemic inflammatory response syndrome or SIRS) caused by severe infection [5, 6]. Sepsis develops when the initial host response to an infection is amplified and becomes damaging to the host [7]. Some structural components of bacteria (pathogen-associated molecular patterns-PAMPs), are recognized by pattern recognition receptors (PRRs) expressed in phagocytes and other cell types [8] and are responsible for the initiation of the septic process and the process could result in activation of NF-κB and transcription of several pro-inflammatory genes [8], including TNF-α, IL-6, and IL-1β.

Chronic and acute inflammation are multiple processes that are mediated by activated inflammatory or immune cells [9]. Macrophages play a crucial role in managing many different immunopathological phenomena, such as the over-production of pro-inflammatory cytokines and inflammatory mediators, including ROS (Reactive oxygen species) and NO [10, 11]. In the case of oxidative stress, NO and ROS affect virtually every step of the development of
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Macrophages mediate the inflammatory process through the release of cytokines, such as TNF-α, IL-1β and IL-6. Additionally, ALI is also characterized by up-regulation of a host of inflammatory mediators, including an increase in the expression and activity of inducible nitric oxide synthase (iNOS), resulting in increased NO production. Therefore, there is an unmet need for searching effective anti-inflammatory and anti-oxidative agents as a drug for acute lung injury.

The use of natural products as antioxidant or anti-inflammatory agents has a long history that began with folk medicine. Bisabolol is a natural monocyclic sesquiterpene present in the essential oil has generated considerable interest in the chemical and pharmaceutical industries and currently in use in various formulations, mainly in cosmetics [12]. Bisabolol is the most powerful natural anti-inflammatory [13, 14] and antioxidative agent [15, 16]. Different studies also demonstrate that it has anti-inflammatory activities [17] and anti-bacterial activity [18]. Meanwhile, bisabolol could inhibit the expression of inflammatory mediators and cytokines, such as iNOS, TNF-α, IL-1β, IL-6 and GM-CSF through inhibition of NF-κB activation in LPS-stimulated RAW264.7 cells [19].

Therefore, in this study, first we determined the therapeutic effect of bisabolol on the sepsis-induced lung injury. We next demonstrated that bisabolol treatment could improve the histology of the lung tissues and decrease the wet/dry ratio, inflammatory cell counts and cytokines production in BALF. In summary, our results indicated that bisabolol could attenuate sepsis-induced acute lung injury through its potential antioxidative activity and ability to reduce the production of inflammatory cytokines via blocking the NF-κB signal pathway in vivo.

Materials and methods

Animals

Eighty (female = 40, male = 40), specific-pathogen-free C57BL/6 mice (8-10 weeks old, weight: 18-22 g) were purchased from the SLRC Laboratory (Shanghai, China). The mice were housed under barrier conditions and provided with water and standard chow ad libitum and kept at 22-25°C with a 12-hour light/dark cycle. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of China. All experiments were approved by the local Animal Care and Use Committee.

Animal experiment

Before the induction of sepsis, mice were fasted overnight but allow water ad libitum. Mice were anesthetized using sodium pentobarbital (intraperitoneally, 40 mg/kg). And then the ventral neck, abdomen, and groin were shaved and washed with 10% povidoneiodine. Sepsis was induced by cecal ligation and puncture as previously described [20]. Briefly, the lower abdomen area was shaved and disinfected, a median 0.5-1.0 cm incision was made in the lower abdomen. After careful dissection, the cecum was ligated below the ileocecal valve, followed by a single ‘through and through’ perforation (21-gauge needle); The caecum was replaced in the abdomen, and the incision was closed. After the surgery, the animals were returned to their cages and were allowed access to food and water ad libitum.

Mice groups and administration of bisabolol

The animals were randomly divided to four groups: Sham-operated animals (control group) underwent the same procedure with the exception that the cecum was neither ligated nor punctured; The cecum of the sepsis group (CLP group), vehicle treated sepsis group (CLP + vehicle group) and bisabolol treated sepsis group (CLP + Bisabolol group) was ligated to establish the sepsis model to induce acute lung injury. In addition, Bisabolol-treated group and vehicle-treated group mice received bisabolol and vehicle (30 mg/kg, dissolved in phosphate buffered saline (PBS)) via oral treatment 2 days before ALI induction.

Histopathology

One lobe of right lung was fixed in 4% paraformaldehyde, dehydrated in grade ethanol and embedded in paraffin. Six to ten 5-μm-thick tissue sections were prepared in a noncontiguous way and dyed with hematoxylin-eosin; stained areas were viewed using an optical microscope. In order to determine the severity of the lung injury, the histological images were evaluated by an investigator who was initially blinded to
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these different treatment groups. The lung injury was scored depending on the following principle: (1) alveolar congestion, (2) hemorrhage, (3) infiltration or aggregation of neutrophils in the airspace or vessel wall, and (4) thickness of the alveolar wall/hyaline membrane formation. The average value of the lung injury obtained were considered a semi-quantitative histological index of lung injury.

Myeloperoxidase (MPO) activity

MPO activity in tissues was determined by a procedure similar to that described by Hillegass et al [21]. Samples of lung tissues were homogenized in 50 μM potassium phosphate buffer (PB), with PH 6.0, and centrifuged at 15000 rpm for 20 min at 4°C. The pellets were then suspended in 50 μM PB containing 0.5% hexadecyltrimethylammonium bromide. After three freeze-and-thaw cycles, with sonication between cycles, the samples were centrifuged at 15000 rpm for 20 min at 4°C. The liquid supernatants (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 μM PB, o-dianisidine, and 20 nM H₂O₂ solution. The change in absorbance was measured at 460 nm using a spectrophotometer (U-2000; Hitachi Instruments, Webster, NY). MPO activity was derived from the observed change in absorbance per minute. The MPO activity was normalized further to the total protein content of the supernatant, which was measured by the micro-Lowry technique. Activity is expressed as units of MPO activity per milligram of protein. All the samples were assayed in triplicate.

Bronchoalveolar lanage (BAL) examination

The trachea was exposed and cannulated with a catheter. The left lung was lavaged for 3 times with sterile PBS in a volume of 0.5 ml/wash. The fluid recovered after lavage was greater than 90% on average. The BAL fluid (BALF) was centrifuged at 2000 rpm for 10 min at 4°C, and the supernatant was stored at -80°C for cytokine and protein analysis, while the cell pellet was resuspended in PBS for cell counting.

ELISA

The production of inflammatory cytokines and mediators, such as TNF-α, IL-6, IL-1β, and MIP-2 in BALF were determined using a commercially available ELISA kit (R&D System, Minneapolis, Minn, USA). These concentrations were interpolated from the standard curves for recombinant TNF-α, IL-6, IL-1β and MIP-2, respectively. All the samples were assayed in triplicate.

NOS activity

The frozen lung tissue samples were homogenized in six volumes (w/v) of homogenization buffer (PH, 7.4; 10 mM HEPES buffer; 0.1 mM EDTA; 1 mM dithioreitol; 1 mg/ml phenylmethylsulfonfluoride; 0.32 mM sucrose; 10 mg/ml leupeptin; 10 mg/ml aprotinin; 10 mg/ml pepstatin A). The crude homogenates were centrifuged at 4°C for 15 min at 10000 rpm. The supernatant (50 μl) was added to prewarmed (37°C) 10-ml tubes containing 100 μl of reaction buffer of the following composition: 50 mM KH₂PO₄, 60 mM valine, 1.5 mM NADPH, 10 mM FAD, 1.2 mM MgCl₂, 2 mM CaCl₂, 1 mg/ml bovine serum albumin, 1 mg/ml calmodulin, 10 mM tetrahydrobiopterin, and 25 μl of 120 μM stock L-[2,3-³H]arginine (150 to 200 cpm/pM). The samples were incubated for 30 min at 37°C and the reaction was terminated by the addition of cold (4°C) stop buffer (PH, 5.5; 100 mM HEPES; 12 mM EDTA). To obtain free L-[³H]citrulline for the determination of enzyme activity, 2 ml of Dowex 50 w resin (8% cross-linked, Na⁺ form) were added to eliminate excess L-[2,3-³H]arginine. The supernatant was assayed for L-[³H]citrulline by using liquid scintillation counting. Enzyme activity was expressed in piconoles of L-citrulline produced/min/mg total protein. Protein concentration was measured by the Bradford technique with bovine serum albumin as standard. Total NOS activity was calculated as the difference between the differentially treated ALI group mice.

Determination of nitrite

The amount of NO was examined by measuring the intermediate and end products, NOX. The supernatants were added with two volumes of ethanol at 4°C for 2 h to precipitate protein. Then the NOX in samples were reduced to NO by adding a reducing agent (0.8% VCl₃ in 1 N HCl). Then the NO was determined by a Sievers Nitric Oxide Analyzer (Sievers 280 NOA, Sievers, Boulder, CO, USA). Nitrate concentration was calculated by comparison with a standard solution of sodium nitrate. All the samples were assayed in triplicate.
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Quantitative realtime PCR assay

Total cellular or tissue RNA was extracted from isolated mice macrophages or snap-frozen lung tissues. RNA was ethanol precipitated and dissolved in diethyl pyrocarbonate-treated water and total RNA concentration was determined by spectrophotometry. Four micrograms of RNA reverse-transcribed into cDNA with a Takara RNA PCR kit (Takara, China). RT-PCR detection was performed with SYBR Green Premix Ex Taq (Takara, Japan) in a sequence detection system (ABI7900 fast real-time detection system). The following primer pairs were used in the analysis: mouse HPRT, 5'-CTGGTGAAAAGGACCTCTCG-3' (Forward) and 5'-TGAAGTACTCATTATAGTCAAGGGCA-3' (Reverse); mouse IL-6, 5'-CCAGAAACCGCTATGAAGTTCC-3' (Forward) and 5'-TCACCAGCATCAGTCCCAAG-3' (Reverse); mouse TNF-α, 5'-CTCCAGGCAGGTTGCCTATGT-3' (Forward) and 5'-GAAGAGCTGGTGGCCC-3' (Reverse); mouse MIP-2, 5'-TCCAGAGCTTGAGTGTGACG-3' (Forward) and 5'-TCAGGTACGATCCAGGCTTC-3' (Reverse).

Western blotting

The lung tissues or macrophages of differentially treated ALI mice were homogenized, washed with PBS, incubated in lysis buffer, and added with a protease inhibitor cocktail (Sigma, St. Louis, MO, USA) to obtain extracts of tissue or cell proteins. The protein concentration in the supernatant was determined by Bradford assay. Then the lysate proteins were subjected to SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% milk, the membrane was incubated with indicated primary antibodies, including phosphorylated NF-κB P65, β-actin, and IkB-α, and then protein bands were visualized by the ECL system after hybridization with a horseradish peroxidase-conjugated secondary antibody. All the samples were assayed in triplicate.
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Statistical analysis

All the data were analyzed using SPSS13.0 software and expressed as Means ± SEM. Significant differences were assessed by one-way analysis of variance (ANOVA) followed by Fisher protected least significant difference test. All results were conformed by Bonferroni correction. A probability value of less than 0.05 was considered to indicate a statistical significance.

Results

Bisabolol treatment reduced pathological damages in sepsis-induced ALI mice

In order to investigate the effect of bisabolol on the lung injury caused by sepsis, we induced acute lung injury by CLP surgery. Then, histological analysis following H&E staining revealed that the lung of sepsis mice (Figure 1B) exhibited dramatically changes compared with those control group mice (Figure 1A), including patchy hemorrhage, thickened alveolar interstitium and heavy infiltration of inflammatory neutrophils and lymphocytes into the intra-alveolar and interstitial space. We also observed that the mice in the normal control group (Figure 1A) showed no significant morphologic damages, indicating that the sham-operated procedure, the cecum was neither ligated nor punctured, did not induce additional inflammation reaction in this protocol (Figure 1A). Additionally, we found that bisabolol treatment could improve the histology of lung tissue (Figure 1D) compared with the sepsis group (Figure 1B) or vehicle treated sepsis group (Sepsis + vehicel group) (Figure 1C).

To further assess the degree of pulmonary damage and inflammatory reaction, the lung injury index, MPO activity and total inflammatory cell infiltration were determined. Compared with the sepsis-induced ALI group (Sepsis group) or vehicle treated sepsis group (Sepsis + vehicel group), the administration of bisabolol decreased the lung injury index (Figure 1E), MPO activity (Figure 1F) in lung homogenate and total inflammatory cells count (Figure 1G) in BALF. These results suggested that bisabolol has a potentially therapeutic effect on the sepsis-induced acute lung injury.

Bisabolol treatment decreased the production of inflammatory cytokines in BALF

To determine the production of inflammatory cytokines in the differentially treated sepsis mice, TNF-α, IL-6, IL-1β, and MIP-2 concentrations were detected in the BALF using ELISA. As shown in Figure 2, those inflammatory cytokines, such as TNF-α (Figure 2A), IL-6 (Figure 2B), IL-1β (Figure 2C), and MIP-2 (Figure 2D), in the bisabolol treated septic ALI mice lung BALF (Sepsis + bisabolol group) were found to be dramatically decreased compared with the

Figure 2. Proinflammatory cytokines in BALF of differentially treated ALI mice. (A) TNF-α; (B) IL-6; (C) IL-1β and (D) MIP-2 in BALF of ALI mice following different type of treatment. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicel group.
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To further evaluate the effect of bisabolol on the production of NO in vitro, macrophages were pretreated with the indicated reagents of bisabolol or 1400 W for 1 h before stimulation for 24 h or no stimulation with LPS. The production of nitrite in the medium was measured. *P<0.05; **P<0.01. NS, not significant. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicle group.

**Figure 3.** The determination of inflammatory mediators in differentially treated ALI mice. The production of NO (A), activity of NOS (B) and expression of iNOS (C) in differentially treated ALI mice. Macrophages were pretreated with the indicated reagents of bisabolol or 1400 W for 1 h before stimulation for 24 h or no stimulation with LPS. (D) The production of nitrite in the medium was measured. *P<0.05; **P<0.01. NS, not significant. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicle group.

sepsis-induced ALI group (Sepsis group) or vehicle treated group (Sepsis + vehicle group). These results indicated that bisabolol intervention prevented the release of those inflammatory cytokines in BALF.

**Bisabolol treatment reduced the production of NO, iNOS and inhibit the activity of NOS induced by sepsis**

As an anti-inflammatory agent, a number of previous studies indicated that bisabolol could inhibit the LPS-induced NO and iNOS expression by blocking the NF-κB signaling pathway (19). Our results demonstrated that bisabolol administration blocked the upregulation of NO (Figure 3A) and iNOS (Figure 3C) production caused by sepsis in lung tissues, compared with the vehicle treated control group. Afterwards, we detected the NOS activity in the lung tissues of differentially treated ALI mice. We found that bisabolol also inhibited the activity of NOS in lung tissue (Figure 3B). This results were in agreement with the levels of NO and iNOS in the differentially treated ALI mice lung tissues.

To further understand the detail inhibited mechanisms of bisabolol on inflammatory reac-
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In sepsis-induced acute lung injury mice, we next examined the activation of NF-κB signaling pathway in the lung of differentially treated ALI mice. The results from Western Blot assay showed that sepsis induction reduced the content of IκB-α (Figure 4A and 4B). However, the IκB-α content was dramatically increased in the lung of bisabolol treated ALI mice compared with those the vehicle control group (Figure 4A and 4B). Meanwhile, compared with the sepsis group or vehicle treated sepsis group, bisabolol treatment also decreased the phosphorylation of NF-κB P65 in vivo (Figure 4C and 4D). These data was consistent with the level of inflammatory cytokines and mediators in BALF.

**Bisabolol treatment inhibited the expression of pro-inflammatory cytokines in lung tissue and macrophages**

Above data suggested that bisabolol could suppress the activation of NF-κB signaling in vivo. As we known, NF-κB play a key role in transcription of inflammatory cytokines and activation of inflammatory reactions. So, we detected the expression of inflammatory cytokines, including IL-6, TNF-α, and MIP-2, in lung tissue or macrophages of differentially treated ALI mice. As Figure 5 showed, compared with the sepsis or vehicle treated sepsis group, the expression of IL-6 (Figure 5A), TNF-α (Figure 5B), and MIP-2 (Figure 5C) were inhibited in the lung of bisabolol treated ALI mice. We next measured the levels of mRNA in the macrophages which isolated from differentially treated ALI mice, we found that bisabolol treatment could inhibit the expression of IL-6 (Figure 5D) and TNF-α (Figure 5E) in macrophages compared with sepsis or vehicle treated control group, but the level of MIP-2 mRNA was no change between differentially treated mice (Figure 5F). This data was supported by the previous studies on the RAW264.7 cell line.

**Bisabolol improved the survival rate of sepsis-induced ALI in mice**

Survival rate, which is a key indication of therapeutic benefit of bisabolol, was examined in differentially treated ALI mice according to reports by others [24]. As Figure 6 shows, the survival rate was significantly improved by bisabolol treatment, compared with these sepsis group or vehicle treated sepsis group (Figure 6).

**Discussion**

Previous studies demonstrated that bisabolol have potential antioxidant and anti-inflammation effect in vitro. Furthermore, inflammatory response play an essential role in the pathogenesis of acute lung injury. So, in the present study, we demonstrated that pre-treatment with bisabolol significantly attenuated the
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The expression of inflammatory cytokines in lung tissues and macrophages from differentially treated ALI mice. A: The mRNA level of IL-6 in lung tissues from differentially treated ALI mice; B: The mRNA level of TNF-α in lung tissues from differentially treated ALI mice; C: The mRNA level of MIP-2 in lung tissues from differentially treated ALI mice; D: The mRNA level of IL-6 in macrophages from differentially treated ALI mice; E: The mRNA level of TNF-α in macrophages from differentially treated ALI mice; F: The mRNA level of MIP-2 in macrophages from differentially treated ALI mice. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicle group.

Progressive multiorgan failure is the most common cause of death following sepsis, a systemic inflammatory response to infection, with the lung usually representing the first organ to fail [25]. Conversely, sepsis is the most common clinical setting in which pathologic alterations of the lung defined as ARDS develop [26]. As we known, acute lung injury (ALI) is a diffuse heterogeneous lung injury characterized by hypoxemia, non cardiogenic pulmonary edema, low lung compliance and widespread capillary leakage. ALI is caused by any stimulus of local or systemic inflammation, principally sepsis [27].

Figure 5. The expression of inflammatory cytokines in lung tissues and macrophages from differentially treated ALI mice. A: The mRNA level of IL-6 in lung tissues from differentially treated ALI mice; B: The mRNA level of TNF-α in lung tissues from differentially treated ALI mice; C: The mRNA level of MIP-2 in lung tissues from differentially treated ALI mice; D: The mRNA level of IL-6 in macrophages from differentially treated ALI mice; E: The mRNA level of TNF-α in macrophages from differentially treated ALI mice; F: The mRNA level of MIP-2 in macrophages from differentially treated ALI mice. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicle group.

The cecal ligation and puncture (CLP) method is a widely used procedure for modeling sepsis in vivo. As the cecum is full of bacteria, its puncture results in polymicrobial peritonitis, translocation of bacteria into the blood, septic shock, multi-organ dysfunction and, ultimately, death [28]. It is generally accepted that CLP reflects clinical reality more accurately than previous techniques, such as injection of endotoxin or even purified bacteria into rodents, thus, CLP is considered the gold standard [29] for the
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Figure 6. Bisabolol treatment delayed the death of mice with sepsis-induced lung injury. The Kaplan-Meier survival curves of mice (n = 10) with indicated treatment were monitored. Data are expressed as mean ± SEM of the values of 10 mice of each group. *P<0.05 compared with the sepsis group; #P<0.05 compared with the sepsis + vehicle group.

experimental induction of sepsis or sepsis-induced ALI.

Clinically, many cytokines, including tumor necrosis factor, interleukins and inflammatory mediators, are produced during sepsis as a result of stimuli by bacteria and bacterial endotoxins. Interactions between these various cytokines result in the activation of a cascade reaction [30]. In sepsis, multiple inflammatory pathways such as the cytokine network and coregulation cascade are triggered as a result of the complex interactions between the body and infectious factors, such as bacteria [31]. So in the acute lung injury caused by sepsis, TNF-α, IL-6 and IL-1β are released in massive amounts and inappropriate immune activation is stimulated, resulting in the damage of lung tissue.

In our present study, we found that the treatment of bisabolol not only improved the histology of the lung (Figure 1), but also impaired the lung damage compared with the sepsis group or vehicle treated sepsis group. Meanwhile, bisabolol treatment also suppressed the inflammatory reactions in sepsis-induced ALI mice. Compared with the sepsis or vehicle control group, the MPO activity (Figure 1F), inflammatory cells count (Figure 1G), production of inflammatory cytokines, such as TNF-α (Figure 2A), IL-6 (Figure 2B), IL-1β (Figure 2C), and MIP-2 (Figure 2D), were decreased in bisabolol treated ALI mice.

Previous data suggested that bisabolol could potentially inhibit the Nitric Oxide production and expression of pro-inflammatory cytokines in LPS-stimulated RAW264.7 cells [19]. So, in this study we detected the production of NO, iNOS and the activity of NOS in serum and lung tissues of differentially treated ALI mice respectively. We found bisabolol treatment decreased the production of NO (Figure 3A) and the expression of iNOS (Figure 3C) in vivo and reduced the NOS activity (Figure 3B) in lung tissue compared with the vehicle control group. Moreover, the expression of inflammatory cytokines was also inhibited in bisabolol treated ALI mice (Figure 5). These results indicated that bisabolol have a potentially protective effect on the sepsis-induced ALI through its antioxidative and anti-inflammatory activity.

We know, NF-κB signaling pathway plays a critical role in the regulation of inflammatory responses, so we next measured the content of IκB-α and the phosphorylation of NF-κB P65 in lung tissues from differentially treated ALI mice. We observed that the level of IκB-α in the lung tissues from bisabolol treated ALI mice was decreased (Figure 4A and 4B) and the phosphorylation of NF-κB P65 (Figure 4C and 4D) was also inhibited by bisabolol treatment in the lung tissue of ALI mice. These suggested that bisabolol block the inflammatory response in ALI mice via regulating the activation of NF-κB pathway.

In summary, in sepsis-induced ALI mice, bisabolol treatment could improve lung histology, prolong survival time, and decrease the levels of proinflammatory cytokines in lung tissues. This potentially protective effect appears to be mediated through inhibition of NF-κB signaling pathway. Thus, bisabolol can be used as a candidate of therapeutic drug for sepsis-induced ALI.

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Disclosure of conflict of interest

None.

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