JNX-IN-8 treatment alleviates lipopolysaccharide-induced acute lung injury via suppression of inflammation and oxidative stress regulated by JNK/NF-κB signaling

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Abstract. JNK serves critical roles in numerous types of inflammation- and oxidative stress-induced disease, including acute lung injury (ALI). JNK-IN-8 is the first irreversible JNK inhibitor that has been described. However, whether JNK-IN-8 can prevent lipopolysaccharide (LPS)-induced ALI by inhibiting JNK activation and its downstream signaling is poorly understood. The objective of the present study was to investigate the specific therapeutic effects of JNK-IN-8 on LPS-induced ALI and the molecular mechanisms involved. JNK-IN-8 attenuated myeloperoxidase activity, malondialdehyde and superoxide dismutase content and the lung wet/dry ratio, and improved the survival rate following lethal injection of LPS. Additionally, JNK-IN-8 decreased bronchoalveolar lavage fluid protein levels, lactate dehydrogenase activity, neutrophil infiltration and the number of macrophages (as demonstrated by flow cytometry), as well as the production of TNF-α, IL-6 and IL-1β (as evaluated via ELISA). In addition, reverse transcription-quantitative PCR and ELISA showed that JNK-IN-8 attenuated LPS-induced inflammatory cytokine production and oxidative stress in primary murine peritoneal macrophages and RAW264.7 cells in vitro. Furthermore, the present study demonstrated that the JNK/NF-κB signaling pathway was involved in the therapeutic effect of JNK-IN-8 against LPS-induced injury both in vivo and in vitro. In conclusion, these findings indicated that JNK-IN-8 had a therapeutic effect on LPS-induced ALI in mice. The mechanism may be associated with inhibition of the JNK/NF-κB signaling pathway. JNK-IN-8 may be a potential therapeutic agent for the treatment of ALI.

Introduction

Acute lung injury (ali) is a disease with high incidence and mortality rates and can cause acute respiratory distress syndrome (ARDS), which seriously threatens human health (1-3). A prospective epidemiological study in 1999-2000 estimated an annual incidence of ALI and ARDS of 190,000 adult patients in the United States of America (1). As a critical disease, ALI has attracted increasing attention in clinical practice. The primary manifestations of ALI are alveolar capillary system damage, increased pulmonary vascular permeability, overactivation of macrophages and neutrophils, excessive inflammatory response and increased reactive oxygen species (ROS) production, which eventually lead to respiratory function damage (4). Previous evidence has revealed that inflammation and oxidative stress are associated with ALI (5).

The mechanisms of inflammation and oxidative stress work in different ways. MAPKs serve as typical inflammation-associated signals and comprise ERK-1/2, p38 and JNK (6). JNK is primarily involved in inflammation and oxidative stress (6). JNK mediates activation of NF-κB signaling, the main upstream regulator of proinflammatory cytokines in ALI (7). Activated NF-κB/p65 translocates to the nucleus to regulate a number of inflammatory cytokines (8,9) and promote oxidative stress (10). Previous studies have demonstrated that, following paraquat poisoning, JNK is activated in the lung, and the JNK inhibitor SP600125 can alleviate ALI caused by paraquat (11,12). Another JNK inhibitor, JNK-IN-8 (13), has an inhibitory effect on the regulation of NF-κB signaling activation by JNK, and can inhibit inflammation and improve neurological function in ischemic brain injury (14). These studies (6-14) suggest that JNK inhibitors serve a major role in the molecular pathology of numerous types of disease. Nevertheless, to the best of our knowledge, the exact role of JNK-IN-8 in inflammation and oxidative stress associated with ALI is not clear.

JNK-IN-8, as an effective and specific JNK inhibitor, has a notable inhibitory effect on the JNK/NF-κB signaling pathway (14,15) which is associated with inflammation and oxidative stress in ALI. Therefore, its impacts may extend to ALI. However, to the best of our knowledge, the
in vitro and in vivo effects of JNK-IN-8 on lipopolysaccharide (LPS)-induced ALI have not been reported. Therefore, the aim of the present study was to investigate the protective effects of JNK-IN-8 in LPS-induced ALI, and the association between its molecular mechanism and inflammation and oxidative stress.

Materials and methods

Reagents and antibodies. JNK-IN-8 was purchased from MedChemExpress. Mouse TNF-α (cat. no. CSB-E04741m), IL-6 (cat. no. CSB-E04639m) and IL-1β (cat. no. CSB-E08054m) ELISA kits were purchased from Cusabio Technology LLC. Alexa Fluor® 488 anti-mouse Ly-6G (cat. no. 127625) and 594 anti-mouse F4/80 antibody (cat. no. 123140) were purchased from BioLegend, Inc. Specific primary antibodies against NF-κB p65 (cat. no. 8242), phosphorylated (p)-p65 (Ser536; cat. no. 3303), JNK (cat. no. 9252), p-JNK (Thr183/Tyr185; cat. no. 4668) and β-actin (cat. no. 3700) were purchased from Cell Signaling Technology, Inc.

Animals. A total of 75 animals (age, 8 weeks) received humane care in 2019, according to the Guide for the Care and Use of Laboratory Animals (16). Male specific pathogen-free C57BL/6 mice (weight, 18-22 g) were provided by Shanghai SLAC Laboratory Animal Co., Ltd., and were housed (5 mice/individually vented cage) using a 12-h light/dark cycle under optimal temperature (20-26°C) and humidity (40-70%). Mice were provided with free access to food and water. Prior to the experiment, the mice were habituated to the environment for ≥1 weeks. The specific criteria of humane endpoints were selected according to the Guide for the Care and Use of Laboratory Animals: Eighth Edition (17). The humane endpoints included dying (indicated by weak response to leg clamping, very faint heartbeat, irregular breathing, depression and hypothermia), loss of appetite (>24 h) and weakness (unable to feed, drink or stand). Euthanasia was performed according to the American Veterinary Medical Association Guidelines for the Euthanasia of Animals: 2013 Edition (18). The displacement rate used for CO2 inhalation euthanasia was 20% to avoid distress. Standard evidence of death included dilated pupils and absence of heartbeat, as well as failure to respond to a toe pinch or touch of the eye. Cervical dislocation was performed following euthanasia to ensure death. All animal experiments were approved by the Ethics Committee of Zhejiang University (approval no. ZJU20170913; Hangzhou, China).

Establishment of the ALI mouse model. The ALI mouse model was established by intratracheal administration of LPS (20 µg/50 µl PBS). A total of 24 mice were divided into four groups at random (n=6/group): Sham operation (equivalent PBS via intraperitoneal injection), JNK-IN-8, LPS and LPS + JNK-IN-8 group. Mice in the JNK-IN-8 and LPS + JNK-IN-8 groups received JNK-IN-8 (10 mg/kg) via intraperitoneal injection. After 1 h, mice in the LPS and LPS + JNK-IN-8 groups were given a lethal dose of LPS (20 mg/kg). Animals were monitored every 6 h and the survival rate was recorded every 12 h for 72 h, as previously described (19). In the survival experiment, 15 mice died due to LPS-induced ALI and two were euthanized upon reaching a humane endpoint of dying. All surviving mice were euthanized by CO2 inhalation followed by cervical dislocation after the experiment.

Inflammatory cell count in BALF. C57BL/6 mice used for the collection of BALF were euthanized by CO2 inhalation, after which an intermediate incision was made to open the chest to expose the trachea, as previously described (20). The alveoli were washed twice with 0.5 ml PBS during endotracheal intubation to collect 0.8 ml BALF. The numbers of inflammatory cells (specifically, total living cells, ly6g+ neutrophils and F4/80+ macrophages) were counted using flow cytometry (BD Biosciences). Cells in BALF (500 µl) were stained with ly6g for neutrophils and F4/80 for macrophages, as previously described (21,22). Subsequently, flow cytometry was used to count the cells. The ly6g+ and F4/80+ cells were considered to be ‘neutrophils’. The ly6g+ and F4/80+ cells were considered to be ‘macrophages’. The ly6g+ and F4/80+ cells were considered to be other cells. The sum of the three types of cells was the number of ‘total living cells’. Gates were artifically set according to the cell community location. Finally, the data were analyzed using FlowJo software (version 10; Tree Star, Inc.).

Wet/dry (W/D) weight ratio of lung tissue in the ALI model. Following euthanasia, the right lung tissue was collected and the wet weight was recorded. Subsequently, the lung tissue was covered with foil and placed in an incubator at 80°C until the weight remained stable to obtain the dry weight. Finally, the W/D ratio of the lung tissues was calculated to evaluate the degree of pulmonary edema.

Histology evaluation. At 6 h after LPS injection, the thoraces of the mice in the ALI model were opened under deep anesthesia, and the mice were perfused with 50 ml 0.01 M PBS (pH, 7.4) followed by 4% paraformaldehyde in 0.1 M PBS (pH, 7.4) through the ascending aorta. Subsequently, the left upper lung lobe was placed in 4% paraformaldehyde, dehydrated with ethanol and embedded in paraffin. Lung tissues were cut into slices (thickness, 4.5 µm) and then subjected to hematoxylin and eosin and periodic acid Schiff staining. Pathological changes were observed under an optical light microscope (magnification, x200) and two experienced pathologists blindly scored the changes, as previously described (23): 0, normal tissue; 1, minimal inflammatory change; 2, no obvious damage...
to the lung architecture; 3, thickening of the alveolar septae; 4, formation of nodules or areas of pneumonitis that distorted the normal architecture; and 5, total obliteration of the field.

**ELISA.** Expression levels of cytokines, including IL-1β, IL-6 and TNF-α, in BALF, lung tissue and cell supernatant were detected using ELISA kits according to the manufacturer's protocols.

**Cell culture.** Mouse macrophage (RAW264.7) cells were purchased from the American Type Culture Collection. RAW264.7 cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells were seeded into 96-well plates (10,000 cells per well) at 5% CO₂ and 37°C for 24 h. The experiments were performed in triplicate.

**Reverse transcription-quantitative (RT-q)PCR assay.** Total RNA from cells/tissue was extracted using the RNAeasy Mini kit (Qiagen). RT-qPCR was performed using an ABI Prism 7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Green QPCR Master Mix (Takara Bio, Inc.). The total volume (20 µl) of each PCR reaction consisted of 10 µl SYBR Green QPCR Master Mix, 6 µl ddH2O, 2 µl cDNA and 10 µM each of forward and reverse primers. RT-qPCR was performed using the following thermocycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 60°C for 20 sec and 72°C for a final extension at 72°C for 1 min. GAPDH served as the internal control. The murine primer sequences were as follows: GAPDH forward, 5'-ACC CAGAAGACTGGTATTGC-3' and reverse, 5'-CACCATT GGGGTTAGGAAACAC-3'; TNF-α forward, 5'-CCGGGCAA GTCTCATTGGG-3' and reverse, 5'-AACCCTGAGGCA TATCCCTCT-3'; IL-6 forward, 5'-AAGCATGATGCATT GCAGA-3' and reverse, 5'-TGTGACTCCAGTTATTCCTT TTGG-3'; and IL-1β forward, 5'-TGCCCCCTTGGACATGT GATG-3' and reverse, 5'-CAAGGTTGGAAGCAAGCCC-3'. The experiments were performed in triplicate.

**Western blot analysis.** Nucleoprotein and total protein were extracted from lung tissue or cells using RIPA reagent (Beijing Solarbio Science & Technology Co., Ltd.). A BCA protein assay kit (Thermo Fisher Scientific, Inc.) was used to determine the protein concentration of samples from lyzed lung tissues or cells. Each well of a 10% SDS-PAGE gel was loaded with an equal volume of protein, and the proteins were separated by SDS-PAGE, after which they were transferred to a PVDF membrane. After incubation with 5% milk at room temperature for 1 h, the membrane was incubated with primary antibodies (all 1:1,000; all Cell Signaling Technology, Inc.), including anti-NF-κB p65 (cat. no. 8242), anti-p-NF-κB p65 at Ser536 (cat. no. 3033), anti-β-actin (cat. no. 3700) and anti-Lamin B (cat. no. 12255), at 4°C overnight. Then, the membrane was incubated with corresponding secondary antibodies (1:2,000; cat. no. 6990; Cell Signaling Technology, Inc.) at room temperature for 1 h. The protein bands were visualized using an ECL western blot kit (Bio-Rad Laboratories, Inc.) and visualized on a ChemiDoc XRS System (Bio-Rad Laboratories, Inc.). Protein expression was calculated by measuring the optical density using Image Lab software (version 3.0, Bio-Rad Laboratories, Inc.). Target protein expression levels were normalized to those of β-actin.

**Statistical analysis.** Data are presented as the mean ± SD of three independent repeats. SPSS 16.0 software (SPSS, Inc.) was used for statistical analysis. Figures were created using Prism 5.0 software (GraphPad Software, Inc.). Survival data were analyzed with Kaplan-Meier plots and log-rank tests. Differences among the experimental groups were compared.
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Results

JNK-IN-8 decreases lung injury induced by LPS in mice.

Compared with the Sham and JNK-IN-8 groups, LPS caused marked pathological changes by increasing the accumulation of inflammatory cell damage in alveolar tissue (Fig. 1A and B). JNK-IN-8 treatment alleviated severe histopathological changes caused by LPS, including inflammatory cell infiltration, tissue destruction and glycogen deposition. In accordance with this finding, the lung injury score of the LPS + JNK-IN-8 treatment group was significantly lower than that of the LPS group (Fig. 1C). The W/D ratio of lung tissue and total protein levels in BALF (which represent the severity of pulmonary edema) were significantly higher in the LPS group than in the Sham and JNK-IN-8 groups, whereas pretreatment with JNK-IN-8 significantly decreased the W/D ratio and total protein levels (Fig. 1D and E). Furthermore, JNK-IN-8 treatment decreased LDH activity in the BALF of LPS-challenged mice (Fig. 1F). Pretreatment with JNK-IN-8 improved the survival rate within 72 h of lethal injection of LPS (Fig. 1G).

These findings suggest that JNK-IN-8 alleviated pathological damage of the lungs induced by LPS.

JNK-IN-8 decreases the inflammatory response and oxidative stress in the lungs of mice treated with LPS.

Subsequently, the role of JNK-IN-8 in the inflammatory response and oxidative stress in the lungs of mice challenged with LPS was determined. The mRNA expression levels of TNF-α, IL-6 and IL-1β in lung tissue were significantly decreased by JNK-IN-8 pretreatment (Fig. 2A), and this also decreased the serum levels of TNF-α, IL-6 and IL-1β (Fig. 2B). In addition, JNK-IN-8 treatment effectively inhibited the increase in MPO activity and MDA generation caused by LPS (Fig. 2C). It also significantly decreased LPS-induced SOD depletion (Fig. 2C), suggesting that JNK-IN-8 treatment decreased the inflammatory response and oxidative stress in mice with LPS-induced ALI.

Pretreatment with JNK-IN-8 significantly decreased the levels of TNF-α, IL-6 and IL-1β in BALF compared with those in the LPS group (Fig. 3A). Compared with the Sham group, the numbers of total cells, neutrophils and macrophages in BALF were increased following LPS stimulation, and this change was inhibited by pretreatment with JNK-IN-8 (Fig. 3B and C).

JNK-IN-8 decreases LPS-induced inflammatory cytokine production and oxidative stress in primary murine peritoneal macrophages and RAW264.7 cells in vitro.

Before assessing...
the effects of JNK-IN-8 on primary murine peritoneal macrophages, the cytotoxic effect of JNK-IN-8 on cells was assessed using a CCK-8 assay (Fig. 4A). No significant cytotoxic effects of JNK-IN-8 were observed at concentrations of ≤12.50 µM in primary macrophages (Fig. 4A). Based on these data, a maximal concentration of 10 µM was selected to analyze the effects of JNK-IN-8 in primary macrophages.

In order to investigate the anti-inflammatory effect of JNK-IN-8, primary macrophages were pretreated with JNK-IN-8 for 1 h and then stimulated with LPS (100 ng/ml) for 6 h. mRNA expression levels and secretion of TNF-α, IL-6 and IL-1β in the LPS group were significantly increased compared with those in the Sham group, but these were decreased in the LPS + JNK-IN-8 group compared with the LPS group (Fig. 4B and C). Subsequently, the role of JNK-IN-8 in oxidative stress was investigated. JNK-IN-8 pretreatment significantly decreased MDA content and inhibited the LPS-induced decrease in SOD activity (Fig. 4D) in primary macrophages.

The effects of JNK-IN-8 on the macrophage cell line RAW264.7 were assessed. In the CCK-8 assay, no cytotoxic effects of JNK-IN-8 were observed at concentrations ≤12.50 µM in RAW264.7 cells (Fig. 5A). RAW264.7 cells were cultured and treated with JNK-IN-8 in vitro. The trend was the same as that of primary macrophages. RAW264.7 cells were pretreated with JNK-IN-8 for 1 h and then stimulated with LPS (100 ng/ml) for 6 h. The gene expression levels and secretion of TNF-α, IL-6 and IL-1β were decreased by JNK-IN-8 pretreatment compared with those in the LPS group (Fig. 5B and C). JNK-IN-8 administration significantly decreased the MDA content and inhibited the LPS-induced decrease in SOD activity (Fig. 5D).

**JNK-IN-8 regulates JNK and NF-κB activation to affect inflammation and oxidative stress in LPS-treated cells.** In order to investigate the mechanism by which JNK-IN-8 inhibits inflammation, its effects on the JNK/NF-κB signaling pathway were analyzed. A significant increase was observed in the protein levels of p-JNK, as well as p-NF-κB p65, in LPS-stimulated lung tissues, and JNK-IN-8 treatment significantly inhibited this increase (Fig. 6A and B). JNK-IN-8 also decreased the phosphorylation of JNK and NF-κB p65, which was stimulated by LPS in a dose- and time-dependent manner in primary macrophages in vivo (Fig. 6C-F). Further
experiments revealed that JNK-IN-8 treatment exhibited a significant inhibitory effect on the nuclear translocation of p65 induced by LPS (Fig. 6G and H). In conclusion, these data provided evidence that JNK-IN-8 decreased LPS-induced injury via the JNK/NF-κB signaling pathway.

Discussion

Excessive inflammation and oxidative stress are key factors that regulate the development of ALI. The expression levels of proinflammatory regulators can be enhanced by oxidative stress (5). Furthermore, inflammatory cells induce the overproduction of ROS in a similar manner, thus forming a cycle to promote the occurrence and development of ALI (25-27). JNK-IN-8 was used in the present study to demonstrate that inhibition of JNK may be a potential therapy for the treatment of ALI. The present in vivo experiments revealed that inhibition of JNK by JNK-IN-8 attenuated ALI in an LPS-induced mouse model. Furthermore, in vitro experiments demonstrated that JNK-IN-8 inhibited secretion of inflammatory factors and oxidative stress in LPS-stimulated mouse peritoneal macrophages, as well as RAW264.7 cells, by inhibiting JNK/NF-κB signaling. Therefore, it was concluded that JNK-IN-8 inhibited the activation of JNK, as well as NF-κB, both in vivo and in vitro. This indicated that JNK contributed to the regulation of inflammation and oxidative stress. Furthermore, this could offer a novel strategy for treating ALI.

LPS is a well-known agent for inducing ALI (28). LPS stimulates macrophage activation and inflammatory cell infiltration via the JNK and NF-κB signaling pathways, causing uncontrolled release of inflammatory cytokines and oxidative stress (29-30). JNK signaling has been reported to be associated with inflammation and oxidative stress. As a result, JNK is a promising candidate for novel therapeutic targets of inflammation and oxidative stress (6). In inflammatory diseases, a number of JNK-associated synthetic inhibitors, such as the micromolecules SP600125 (31) and CC-930 (32), have been reported. Shen et al (12) reported that SP600125 treatment suppresses the JNK/AP-1 signaling pathway to decrease oxidative stress and the inflammatory response, thus decreasing the apoptosis of A549 cells. Notably, different JNK inhibitors exhibit numerous physiological characteristics due to the different types of JNKs (11-14). Therefore, further identification of selective JNK inhibitors is a key goal.

JNK-IN-8, the first irreversible JNK inhibitor to be described, suppresses IL-1β-stimulated c-Jun phosphorylation in IL-1R cells (13). A recent study revealed that JNK-IN-8 inhibits neuroinflammation in ischemic brain injury by inhibiting JNK/NF-κB (14). In the present study, JNK-IN-8 also effectively inhibited JNK/NF-κB signaling to suppress IL-1β,
TNF-α and IL-6 expression levels and release in primary peritoneal macrophages following LPS treatment. These data demonstrated that the JNK/NF-κB signaling pathway serves an important role in inflammatory disease. In addition, the inhibition of JNK-mediated NF-κB activation by JNK-IN-8 may be a potential target for treatment of inflammatory diseases.

Excessive oxidative stress is involved in the pathogenesis of ALI (29). When lung tissue is damaged in ALI, levels of MDA, a marker of lipid peroxidation, increase, whereas those of SOD, an antioxidant enzyme, decrease (26). The present results demonstrated that LPS increased the MDA content and decreased the activity of SOD in lung tissues and primary peritoneal macrophages.

Figure 4. JNK-IN-8 attenuates LPS-induced inflammatory cytokine production and oxidative stress in primary murine peritoneal macrophages in vitro. (A) Cytotoxic effects of JNK-IN-8 on primary macrophages were assessed using a Cell Counting Kit-8 cell viability assay at 24 h. TNF-α, IL-6 and IL-1β (B) mRNA levels in primary macrophages and (C) protein expression levels in the supernatant of primary macrophages were determined via reverse transcription-quantitative PCR and ELISA, respectively. (D) MDA activity and MPO content of primary macrophages were determined. Data are presented as the mean ± SD (n=3). *P<0.01 and **P<0.001 vs. Sham or 0.00; †P<0.05, ‡P<0.01 and §§P<0.001 vs. LPS. LPS, lipopolysaccharide; MDA, malondialdehyde; MPO, myeloperoxidase; SOD, superoxide dismutase.

Figure 5. JNK-IN-8 attenuates LPS-induced inflammatory cytokine production and oxidative stress in RAW264.7 cells in vitro. (A) Cytotoxic effects of JNK-IN-8 on RAW264.7 cells were assessed using a Cell Counting Kit-8 cell viability assay at 24 h. TNF-α, IL-6 and IL-1β (B) mRNA levels in RAW264.7 cells and (C) protein expression levels in the supernatant of RAW264.7 cells were determined by reverse transcription-quantitative PCR and ELISA, respectively. (D) MDA and SOD activity of RAW264.7 cells were determined. Data are presented as the mean ± SD (n=3). *P<0.01 and **P<0.001 vs. Sham or 0.00; †P<0.05, ‡P<0.01 and §§P<0.001 vs. LPS. LPS, lipopolysaccharide; MDA, malondialdehyde; SOD, superoxide dismutase.
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JNK-IN-8 inhibited the increase in MDA content and decreased the LPS-induced SOD activity in vivo and in vitro, indicating that JNK-IN-8 alleviated the oxidative stress induced by LPS and protected against lung damage.

In the present study, JNK-IN-8 contributed to the regulation of inflammation and oxidative stress following ALI. JNK-IN-8 treatment decreased LPS-induced inflammation and oxidative stress activation both in vivo and in vitro. TNF-α, IL-1β and IL-6 were upregulated following LPS stimulation, whereas treatment with JNK-IN-8 decreased LPS-induced upregulation of TNF-α, IL-1β and IL-6, suggesting that JNK-IN-8 treatment contributed to the decrease in inflammation. Compared with the control group, JNK-IN-8 inhibited JNK phosphorylation. This finding indicated that LPS-induced JNK activation was prevented by JNK-IN-8. In addition, JNK-IN-8 decreased NF-κB signaling activation. In summary, the present study demonstrated that targeting JNK-IN-8 is a potent prospective therapy for the treatment of ALI.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Authors' contributions

JD designed and performed the experiments, analyzed the data and wrote the manuscript. GW designed the experiments. GW, HL, NL and JX interpreted the data. JX conceptualized and supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Zhejiang University (approval no. ZJU20170913).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Matthy MA, Ware LB and Zimmerman GA: The acute respiratory distress syndrome. J Clin Invest 122: 2731-2740, 2012.
2. Li WW, Wang TY, Cao B, Liu B, Rong YM, Wang JJ, Wei F, Wei LQ, Chen H and Liu YY: Synergistic protection of matrine and lycopen against lipopolysaccharide induced acute lung injury in mol. Mol Med Rep 20: 455-462, 2019.
3. Yao H, Sun Y, Song S, Qi Y, Tao X, Xu L, Yin L, Han X, Xu Y, Li H, et al: Protective effects of dioscin against lipopolysaccharide-induced acute lung injury through inhibition of oxidative stress and inflammation. Front Pharmacol 8: 120, 2017.
4. Jiang K, Guo S, Yang C, Yang J, Chen Y, Shaukat A, Zou G, Wu H and Deng G: Barbaloin protects against lipopolysaccharide (LPS)-induced acute lung injury by inhibiting the ROS-mediated PI3K/AKT/NF-κB pathway. Int Immunopharmacol 64: 140-150, 2018.
5. Chen X, Zhang Y, Wang W, Liu Z, Meng J and Han Z: Mesenchymal stem cells modified with heme oxygenase-1 have enhanced paracrine function and attenuate lipopolysaccharide-induced inflammatory and oxidative damage in pulmonary microvascular endothelial cells. Cell Physiol Biochem 49: 101-122, 2018.
6. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K and Cobb MH: Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. Endocr Rev 22: 153-183, 2001.
7. Romashko JH, Horowitz S, Franek WR, Palatin T, Miller EU, Lin A, Borrer MJ, Scott W and Manielli LL: MAPK pathways mediate hyperoxia-induced oncostein cell death in epithelial cells. Free Radic Biol Med 35: 978-993, 2003.
8. Dong L, Zhou Y, Zhu ZQ, Liu T, Duan JX, Zhang J, Li P, Hammock BD and Guan CX: Soluble epoxide hydrolase inhibitor suppresses the expression of triggering receptor expressed on myeloid cells-1 by inhibiting NF-κB activation in murine macrophage. Inflammation 40: 13-20, 2017.
9. Chen XY, Dou XY, Luo DD, Zhang ZB, Li CL, Zeng HF, Su ZK, Xie JH, Lai XP and Li YC: β-Patchoulene from patchouli oil protects against LPS-induced acute lung injury via suppressing NF-κB and activating Nrf2 pathways. Int Immunopharmacol 50: 270-278, 2017.
10. Kuo MY, Liao MF, Chen FL, Li YC, Yang ML, Lin RH and Kuan YH: Luteolin attenuates the pulmonary inflammatory response involves abilities of antioxidantation and inhibition of MAPK and NFκB pathways in mice with endotoxin-induced acute lung injury. Food Chem Toxicol 49: 2660-2666, 2011.
11. Liu Z, Wang Y, Zhao H, Zheng Q, Xiao L and Zhao M: CB2 receptor activation ameliorates the proinflammatory activity in acute lung injury induced by paraquat. Biomed Res Int 2014: 971750, 2014.
12. Shen H, Wu N, Wang Y, Han X, Zheng Q, Cai X, Zhang H and Zhao M: JNK inhibitor SP600125 attenuates paraquat-induced acute lung injury: An in vivo and in vitro study. Inflammation 40: 1319-1330, 2017.
13. Zhang T, Inesta-Vaquera F, Niepel M, Zhang J, Ficarro SB, Machleidt T, Tie T, Marto JA, Kim ND, Sim T, et al: Discovery of potent and selective covalent inhibitors of JNK. Chem Biol 19: 140-154, 2012.
14. Zhou J, Dai Q, Han K, He R, Jia D, Mo Y, Lv Y, Tang H, Fu H and Geng W: JNK-IN-8, a c-Jun N-terminal kinase inhibitor, improves functional recovery through suppressing neuroinflammation in ischemic stroke. J Cell Physiol 235: 2792-2799, 2020.
15. Chen X, Li X, Zhang W, He J, Xu B, Lei B, Wang Z, Cates C, Rousselle T and Li J: Activation of AMPK inhibits inflammatory response during hypoxia and reoxygenation through modulating JNK-mediated NF-κB pathway. Metabolism 83: 256-270, 2018.
16. National Research Council: Guide for the Care and Use of Laboratory Animals - French version. The National Academies Press, Washington, DC, p134, 1996.
17. National Research Council: Guide for the Care and Use of Laboratory Animals. 8th edition. The National Academies Press, Washington, DC, p246, 2010.
18. Leary S: AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association, Schaumburg, IL, p201, 2013.
19. Wang F, Fu X, Wu X, Zhang J, Zhu J, Zou Y and Li J: Bone marrow derived M2 macrophages protected against lipopolysaccharide-induced acute lung injury through inhibiting oxidative stress and inflammation by modulating neutrophils and T lymphocytes responses. Int Immunopharmacol 61: 162-168, 2018.
20. Graller JJ, Haegeneder MD, Sarma JV, Zou Y, Xiao L and Ward PA: Induction of M2 regulatory macrophages through the β2-adrenergic receptor with protection during endotoxemia and acute lung injury. J Innate Immun 6: 607-618, 2014.
21. Zou Y, Bao S, Wang F, Guo L, Zhu J, Wang J, Deng X and Li J: β2-adrenergic blockade on pulmonary microvascular endothelial cells improves the outcome of sepsis-induced acute lung injury. Shock 49: 212-220, 2018.
22. Tan W, Zhang C, Liu J and Miao Q: Regulatory T-cells promote pulmonary repair by modulating T helper cell immune responses in lipopolysaccharide-induced acute respiratory distress syndrome. Immunology 157: 151-162, 2016.
23. Zhu X, Zou Y, Wang B, Zhu J, Chen Y, Wang L, Li J and Deng X: Blockade of CXC chemokine receptor 3 on endothelial cells protects against sepsis-induced acute lung injury. J Surg Res 204: 288-296, 2016.
24. Huang XT, Liu W, Zhou Y, Sun M, Yang HH, Zhang CY and Tang SY: Galectin-1 ameliorates lipopolysaccharide-induced acute lung injury via AMPK-Nrf2 pathway in mice. Free Radic Biol Med 146: 222-232, 2020.
25. Jiang W, Chunhua M and Shumin W: Effects of acteoside on lipopolysaccharide-induced inflammation in acute lung injury via regulation of NF-κB pathway in vivo and in vitro. Toxicol Appl Pharmacol 285: 128-135, 2015.
26. Jiang W, Luo F, Lu Q, Liu J, Li P, Wang X, Fu Y, Hao K, Yan T and Ding X: The protective effect of Trillium LPS-induced acute lung injury by the regulations of inflammation and oxidative state. Chem Biol Interact 243: 127-134, 2016.
27. Lin WC, Chen CW, Huang YW, Chao L, Chao J, Lin YS and LF: Kallistatin protects against sepsis-related acute lung injury via inhibiting inflammation and apoptosis. Sci Rep 5: 12463, 2015.
28. Chen H, Bai C and Wang X: The value of the lipopolysaccharide-induced acute lung injury model in respiratory medicine. Expert Rev Respir Med 4: 773-783, 2010.
29. Sarma JV and Ward PA: Oxidants and redox signaling in acute lung injury. Compr Physiol 1: 1365-1381, 2011.
30. Chen Y, Liu K, Zhuang J, Bai C, Wang Y, Wang H, Liu Q, Wang CCL, Yao J, Gao Y, et al: JNK phosphorylates the Neh6 domain Of Nrf2 and downregulates cytotoxic genes in acetaminophen-induced liver injury. Hepatology 2020.
31. Reich N, Tomcik M, Žerr P, Lang V, Dees C, Avouac J, Palumbo K, Reich G and Geng W: JNK-IN-8, a c-Jun N-terminal kinase inhibitor, improves functional recovery through suppressing neuroinflammation in ischemic stroke. J Cell Physiol 235: 2792-2799, 2020.

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