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Original article

The protective effect and mechanism of sevoflurane on LPS-induced acute lung injury in mice

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Abstract: Acute lung injury (ALI) is a disturbance caused by infectious or non-infectious inflammation and lipopolysaccharide (LPS) could induce an artificial pathological ALI process. Sevoflurane has been demonstrated to be an inhaled anesthetic having anti-inflammatory and protective effects on inflammatory injury. To study the protective effects and mechanisms of sevoflurane on LPS-induced acute lung injury in mice. By assessing W/D ratio, sevofluranecan counteract the edema induced by LPS. The ELISA results showed that sevoflurane reduced IFN-γ production and increased IL-10 level. Elevation of PGE2 induced by sevofluraneand LPS in peritoneal macrophages was inhibited by NS-398, an inhibitor of the PGE2 regulator COX-2, indicating that NS-398 blocked COX-2 mediated PGE_2 synthesis. NS-398 itself did not cause lung inflammation and mitigated the protective effect of sevoflurane on LPS-induced ALI in mice. LPS changes immune homeostasis, resulting in acute lung inflammatory injury. Inhaled sevoflurane regulates immune homeostasis, thereby playing a protective role in alleviating LPS-induced ALI.

Keywords: Sevoflurane, acute lung injury, IFN-γ, IL-10, PGE2

Introduction

Sevoflurane is an inhaled halogenated hydrocarbon anesthetic used for general anesthesia. Widely used in clinical practice, it is characterized by its rapid induction, its sustained anesthesia andits fast and full awakening. The existing literature has demonstrated that inhaled anesthetics such as sevoflurane,exertanti-inflammatory and protective effects on inflammatory injuries caused by sepsis or ischemia/reperfusion in the heart, brain, kidneys and other vital organs [1-4].

Acute lung injury (ALI) is the result of a cascade of cellular responses caused by infectious or non-infectious inflammation, including non-cardiac causes, such as severe infection, trauma and shock [5]. Gram-negative bacterial infections arethe most common cause of ALI [6], and lipopolysaccharide (LPS), the main cell wall component of gram-negative bacilli, is the primary pathogenic antigen of these bacteria. LPS can directly or indirectly damage alveolar epithelial and pulmonary endothelial cells. This damage leads to a cascade of reactions, including increased capillary permeability, neutrophil infiltration and the accumulation of inflammatory mediators, which induce acute hypoxic respiratory deficiency, other pulmonary pathological and physiological changes and ALI [5, 7]. Therefore, the pathological process of LPS-induced ALI is to that of clinical ALI, andLPS-induced ALI has consequently been widely used as a research model [8].

In this study, we established a mouse model of ALI by intraperitoneal LPS injection. Specifically, we investigated the effect of inhaled sevoflurane on LPS-induced inflammatory reactions in mice by observing changes in the wet weight to dry weight (W/D) ratio, the histopathological features of lung specimens and the serum interferon (IFN)-γ and interleukin (IL)-10 levels. We also discussed potential mechanisms by which sevoflurane may protect mice from LPS-induced ALI.

Materials and methods

Animals

In this study, 10-week-old C57L/B6 male mice of clean grade were used. The mice weighed 30

g to 35 g, were purchased from the Shanghai Experimental Animal Center housed at the Experimental Animal Center of Suzhou University. The mice were acclimated for 1 week before the experiment.

Methods

Groups: Thirty mice were randomly assigned into 1 of the 3 following groups of 10 mice: Sevo + LPS (sevoflurane inhalation followed by LPS injection), O_2 + LPS (oxygen inhalation followed by LPS injection) and O_2 + phosphate-buffered saline (PBS) (oxygen inhalation followed by PBS injection) (Part 1).

Establishment of an animal model

Each group of mice was placed in a sealed glass vesselthat contained a thin layer of soda lime at the bottom. The following experimental conditions were then introduced. Sevo + LPS: the sevoflurane evaporation canister was adjusted so that the concentration of sevoflurane was 3.4%. After 40% O_2 was passed through the evaporation canister at 1 L/min, the gas mixture was introduced into the sealed glass vessel for 2 hr, followed by an intraperitoneal injection of 2.5 mg/kg LPS. O_2 + LPS: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed by an intraperitoneal injection of 2.5 mg/kg LPS. O_2 + PBS: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed an by intraperitoneal injection of 1 mL of PBS. An anesthetic gas monitor was used to monitor the concentrations of sevoflurane and O_2 in the vessel. The general condition of mice was observed, and visible pulmonary changes were noted. Mice were sacrificed 6 hr after the start of gas inhalation, and blood samples werethen collected from the hearts of mice from each group and stored at 4°C overnight for serum preparation. Additionally, lung tissue wash arvested for further experiments.

Collection of peripheral blood samples

The mice were sacrificed by cervical dislocation and secured supine on an operating table. The chest, abdominal skin and sternum were then quickly cut open to expose the heart. A 1-mL syringe was used to puncture the ventricle and draw blood, which was placed into an Eppendorf tube and stored at 4°C overnight. After coagulation, the blood samples were centrifuged at 10,000 rpm for 5 min. The serum was removed, aliquoted, and stored at -80°C for later use.

Collection and processing of lung tissue specimens

The chest was opened along the midline, the trachea was dissected and ligated, and the entire lung was removed. Five lung specimens were taken from each group, surface blood was dabbed dry, and the wet weight W/D ratio was determined. In addition, 5 lung specimens from each group were cut into small pieces of 1.5 mm x 1.5 mm × 1.5 mm and then fixed in 4% paraformaldehyde (PFA) at 4°C overnight.

Pathological examination

Mouse lung tissue was stained with hematoxylin andeosin (HE), and the slides were examined by a pathologist to note any pathological changes.

Determination of lung water content

Surface water and blood on lung specimens weredabbed dry with absorbent paper, and the wet weight was then measured. Next, the lung specimens were placed at 80°C for 72 hours, and the dry weight was measured to calculate the W/D ratio. Enzyme linked immunosorbent assay (ELISA) to detect peripheral IFN-γ and IL-10 levels.

Groups

A total of 80 mice were randomly assigned into 1 of 8 groups of 10 mice in each, which are described as follows: Group 1 (S-N group): sevoflurane/O_2 inhalation, followed by an injection of *N*-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS-398); Group 2 (S-L-N group): sevoflurane/O_2 inhalation, followed by an injection of LPS and NS-398; Group 3 (S-L group): sevoflurane/O_2 inhalation, followed by an injection of LPS; Group 4 (S-P Group): sevoflurane/O_2 inhalation, followed by an injection of PBS; Group 5 (O-N group): O_2 inhalation, followed by an injection of NS-398; Group 6 (O-L-N group): O_2 inhalation, followed by an injection of LPS and NS-398; Group 7 (O-L group): O_2 inhalation, followed by an injection of LPS; and Group 8 (O-P group): O_2 inhalation, followed by injection of PBS *(Part 2).*

Establishment of different groups

The mice in each group wereplaced in a sealed glass vessel that containeda thin layer of soda lime at the bottom. The following experimental conditions were then introduced.

Group 1: the sevoflurane evaporation canister was adjusted so that the concentration of sevoflurane was 3.4%. After 40% O_2 was passed through the evaporation canister at 1 L/min, the gas mixture was introduced into the sealed glass vessel for 2 hr, followed by an intraperitoneal injection of 10 mg/kg NS-398.

Group 2: the sevoflurane evaporation canister was adjusted so that the concentration of sevoflurane was 3.4%. After 40% O_2 was passed through the evaporation canister at 1 L/min, the gas mixture was introduced into the sealed glass vessel for 2 hr, followed by an intraperitoneal injection of a mixture of 2.5 mg/kg LPS and 10 mg/kg NS-398.

Group 3: the sevoflurane evaporation canister was adjusted so that the concentration of sevoflurane was 3.4%. After 40% O_2 was passed through the evaporation canister at 1 L/min, the gas mixture was introduced into the sealed glass vessel for 2 hr, followed by an intraperitoneal injection of 2.5 mg/kg LPS.

Group 4: the sevoflurane evaporation canister was adjusted so that the concentration of sevoflurane was 3.4%. After 40% O_2 was passed through the evaporation canister at 1 L/min, the gas mixture was introduced into the sealed glass vessel for 2 hr, followed by an intraperitoneal injection of 1 mL of PBS.

Group 5: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed by an intraperitoneal injection of 10 mg/kg NS-398.

Group 6: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed by an intraperitoneal injection of a mixture of 2.5 mg/kg LPS and 10 mg/kg NS-398.

Group 7: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed by an intraperitoneal injection of 2.5 mg/kg LPS.

Group 8: 40% O_2 was introduced into the vessel at 1 L/min for 2 hr, followed by an intraperitoneal injection of 1 mL of PBS.

A Datex-Ohmeda gas monitor was used to monitor the concentrations of sevoflurane and O_2 in each vessel. The general condition of mice was observed, and mice were sacrificed 6 hr after the start of gas inhalation to harvest lung tissue and peritoneal macrophages.

Sample collection

Collection and processing of lung tissue specimens: Lung specimens were harvested from 5 randomly selected mice from each group, and the W/D ratio was determined. In addition, lung specimens were collected from the remaining 5 mice in each group for pathological examination, and peritoneal macrophages were collected and cultured to determine prostaglandin E2 (PGE_2) levels in the medium (see Part 1 collection and processing of lung tissue specimens).

Determination of lung water content (see Part 1 determination of lung water content)

Pathological examination of lung tissue (see Part 1 pathological examination): Collection and processing of peritoneal macrophages: A 10-mL syringe was used to wash the peritoneum with PBS. Next, the peritoneal irrigation fluid was drawn, cooled in an ice bath, centrifuged at 4°C and 1800 rpm for 5 min. The supernatant was then discarded. Magnetic-activated cell sorting (MACS) was employed with anti-CD11b crosslinked to beads to sort and obtain peritoneal macrophages, which were then washed with ice-cold calcium- and magnesium-free PBSandresuspendedto a concentration of 3×10^4 cells/mL in RPMI-1640 medium containing 10% fetal calf serum (FCS). One milliliter of this suspension was then seeded in 24-well plateswhich were placed in an incubator at 37°C under 5% CO_2 for 24 hr. The supernatant was taken and stored at -20°C for later use, and ELISA was used to detect the PGE_2 levels in peritoneal macrophages.

Statistical analysis

SPSS17.0 software was used for the statistical analysis. The data are expressed as the mean ± standard deviation (mean ± SD). A 1-way analysis of variance (ANOVA) was performed to compare groups, and the q test was performed for pairwise comparison. $P < 0.05$ was considered statistically significant.

Results

The effect of sevoflurane on LPS-induced pulmonary edema in mice

Gross examination

Mice in the O_2 + LPS group began to appear restless, as evidenced byarched backs and

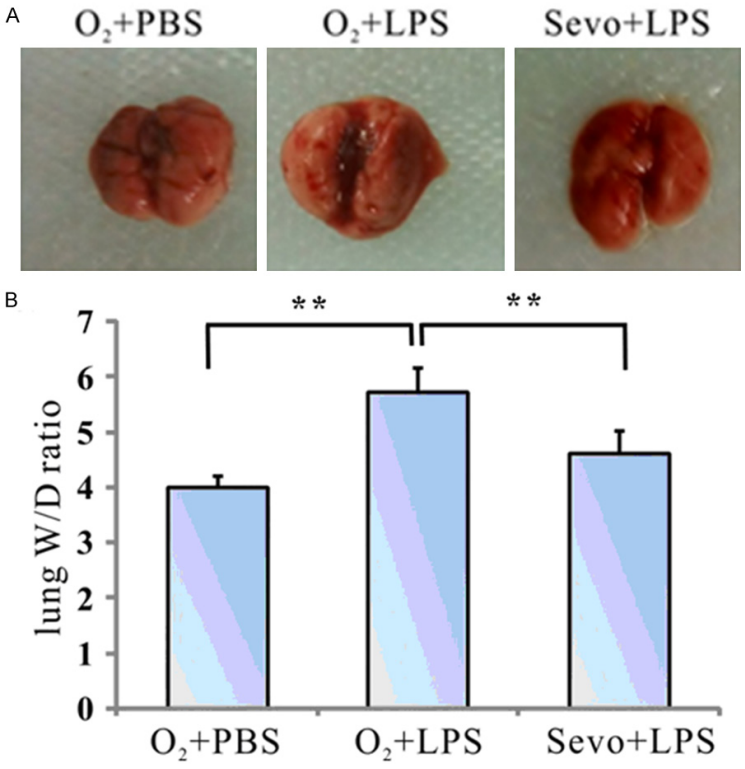

Figure 1. Gross examination and W/D ratio of lung tissue ($\bar{x}$±s, *n*=5, ***P* < 0.01). (A: O_2 + PBS: oxygen inhalation followed by PBS injection; O_2 + LPS: oxygen inhalation followed by LPS injection; LPS + Sevo: sevoflurane inhalation followed by LPS injection; B: O_2 + PBS: oxygen inhalation followed by PBS injection; O_2 + LPS: oxygen inhalation followed by LPS injection; LPS + Sevo: sevoflurane inhalation followed by LPS injection). (from left to right).

Table 1. W/D ratio andserum IFN-γ, IL-10 levels ($\bar{x}$±s, *n*=5)

Group	W/D ratio	IFN-γ (pg/ml)	IL-10 (pg/ml)
O_2 + PBS	4.23 ± 0.184*	34.0 ± 15.0	36.9 ± 10.0
O_2 + LPS	5.32 ± 0.300*	2355 ± 180**	682 ± 86.0**
Sevo + LPS	4.32 ± 0.353*	1833 ± 266**,#	865 ± 83.1**,#

*Compared with each group. P < 0.01; **P < 0.01, vs O_2 + PBS group; #P < 0.05, vs O_2 + LPS group.

shortness of breath, 30 min after LPS injection. In the Sevo + LPS group, the symptoms were significantly milder at the same time point, and the mice appeared normal in the O_2 + PBS group. After the mice were sacrificed, a gross examination of isolated lung tissue showed that this tissuewas significantly swollen in the O_2 + LPS group and somewhat swollen in the Sevo + LPS group relative to the O_2 + PBS group (**Figure 1A**).

W/D ratio

Isolated lung tissue was weighed, yieldingwet weights of 0.125 ± 0.015 g in the O_2 + PBS group, 0.191 ± 0.030 g in the O_2 + LPS group and 0.145 ± 0.016 g in the Sevo + LPS group. The lung tissue was then dried and weighed. The W/D ratio was 3.96 ± 0.23 in the O_2 + PBS group and 5.73 ± 0.43 in the O_2 + LPS group, and this difference was significant (P < 0.01). In the Sevo + LPS group, the W/D ratio was 4.62 ± 0.41, which was significantly lower than that in the O_2 + LPS group (P < 0.01) (**Figure 1B**, **Table 1**).

Light microscopic examination of lung tissue

A Light microscopic examination of lung tissue showed clear alveolar structure free of alveolar exudate in the O_2 + PBS group. However, in the O_2 + LPS group, alveolar wall congestion and thickening, pulmonary interstitial and alveolar edema, a large amount of inflammatory cells and alveolar exudate were observed. Significantly milder lung interstitial and alveolar edema was observed in the Sevo + LPS group relative to the O_2 + LPS group (**Figure 2**).

The effect of sevoflurane on serum IFN-γ and IL-10 levels in mice

Serum IFN-γ level

An ELISA revealed a serum IFN-γ level of 34 ± 15 pg/mL in the O_2 + PBS group, which was significantly lower than that in the O_2 + LPS group (2,355 ± 180 pg/mL; P < 0.01). The serum IFN-γ level was 1,830 ± 266 pg/mL in the Sevo + LPS group, which was also significantly lower than that in the O_2 + LPS group (P < 0.05) (**Figure 3A**, **Table 1**).

Serum IL-10 level

An ELISA revealed a serum IL-10 level of 37 ± 12 pg/mL in the O_2 + PBS group, which was sig-

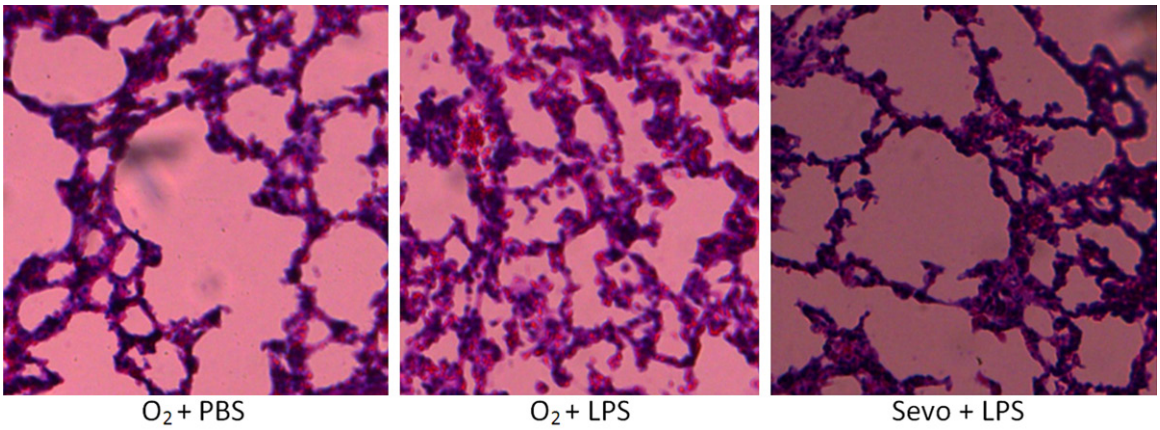

Figure 2. Histological changes of lung tissue in mice (HE×20). (O_2 + PBS: oxygen inhalation followed by PBS injection; O_2 + LPS: oxygen inhalation followed by LPS injection; LPS + Sevo: sevoflurane inhalation followed by LPS injection). (from left to right).

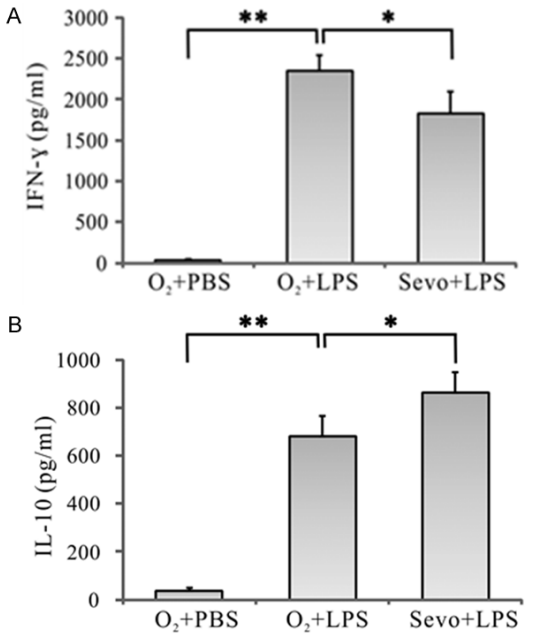

Figure 3. The concentration of serum IFN-γ and IL-10 ($\bar{x}$±s, n=5; $^{**}P < 0.01$; $^{*}P < 0.05$). (A: O_2 + PBS: oxygen inhalation followed by PBS injection; O_2 + LPS: oxygen inhalation followed by LPS injection; LPS + Sevo: sevoflurane inhalation followed by LPS injection; B: O_2 + PBS: oxygen inhalation followed by PBS injection; O_2 + LPS: oxygen inhalation followed by LPS injection; LPS + Sevo: sevoflurane inhalation followed by LPS injection). (from left to right).

nificantly lower than that in the O_2 + LPS group (682 ± 86 pg/mL; $P < 0.01$). The serum IL-10 level was 865 ± 83 pg/mL in the Sevo + LPS group, which was also significantly higher than that in the O_2 + LPS group ($P < 0.05$) (**Figure 3B**, **Table 1**).

The effect of sevoflurane on PGE_2 levels inmouse peritoneal macrophages

An ELISA revealed significantly different PGE_2 levels in peritoneal macrophages of 12,648 ± 443.2 pg/mL in the O_2 + LPS group (Group 7, O-L) and 11,580 ± 718.4 pg/mL in the O_2 + PBS group (Group 8, O-P) ($P < 0.05$), suggesting that LPS induced a mild increase in the PGE_2 level in peritoneal macrophages. The PGE_2 level was 19,656 ± 831.2 pg/mL in the Sevo + LPS group (group 3, S-L), which was significantly higher than that in the O_2 + LPS group (group 7, O-L) (12,648 ± 443.2 pg/mL; $P < 0.01$), suggesting that sevoflurane induced a marked increase in the PGE_2 level in LPS-stimulated peritoneal macrophages. However, the PGE_2 levels in peritoneal macrophagesdid not significantly differ among the groups injected with NS-398 (Groups 1, 2, 5 and 6) ($P > 0.05$), but the PGE_2 levels in these 4 groups were lower than those in Group 3 (S-L) and Group 7 (O-L) ($P < 0.01$). This result suggested that sevoflurane- and LPS-induced increases in the PGE_2 levels in peritoneal macrophages were inhibited by NS-398, an inhibitor of the PGE_2 regulator cyclooxygenase COX-2 (**Figure 4**, **Table 2**).

NS-398 altered the protective effect of sevoflurane on LPS-induced ALI in mice

Gross examination

Themice were sacrificed, and their lung tissue was removed. A gross examination showed normal lung tissue without edema in mice that had not been injected with LPS. However, mice that

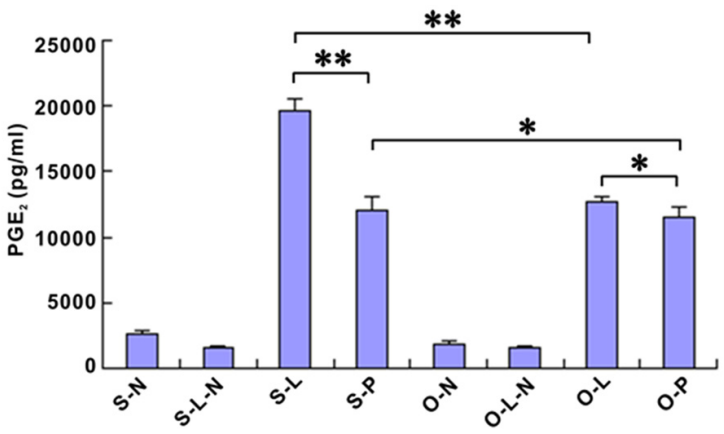

Figure 4. PGE_2 levels of peritoneal macrophages inmice ($\bar{x}$±s, *n*=5; **P < 0.01, *P < 0.05), (from left to right).

Table 2. The W/D ratio and PGE_2 levels in-mouse peritoneal macrophages $\bar{x}$±s, *n*=5)

Groups	W/D ratio	PGE_2 (pg/ml)
Group 1 (S-N)	3.92 ± 0.218	2660 ± 211.8
Group 2 (S-L-N)	6.00 ± 0.219	1561 ± 176.9
Group 3 (S-L)	4.38 ± 0.314	19656 ± 831.2
Group 4 (S-P)	3.90 ± 0.209	11988 ± 1048.2
Group 5 (O-N)	3.95 ± 0.491	1907 ± 166.2
Group 6 (O-L-N)	6.05 ± 0.440	1652 ± 121.1
Group 7 (O-L)	5.95 ± 0.344	12648 ± 443.2
Group 8 (O-P)	3.88 ± 0.304	11580 ± 718.4

had been injected with LPS exhibited pulmonary edema, especially mice in Group 2 (S-L-N), Group 6 (O-L-N), and Group 7 (O-L). In contrast, pulmonary edema was milder in Group 3 (S-L), suggesting that sevoflurane mitigated LPS-induced pulmonary edema (**Figure 5**).

W/D ratio

Fresh mouse lung specimens were weighed to obtain their wet weights; once dried, the specimens were weighted again to obtain their dry weight before calculating the W/D ratio (**Figure 5**, **Table 2**).

Among mice that had been injected with LPS, the W/D ratio was 5.95 ± 0.344 in Group 7 (O-L), which was higher than that in Group 3 (S-L) (4.20 ± 0.271; *P* < 0.01), suggesting that sevoflurane reduced LPS-induced pulmonary edema. The W/D ratio was 6.00 ± 0.219 in Group 2 (S-L-N), which was higher than that in Group 3 (S-L) (4.20 ± 0.271; *P* < 0.01), suggesting that NS-398 countered the mitigating effect of sevoflurane on pulmonary edema in mice with ALI. Among mice that did not receive an LPS injection, the W/D ratio was 3.92 ± 0.218 in Group 1 (S-N), 3.79 ± 0.409 in Group 4 (S-P), 4.00 ± 0.463 in Group 5 (O-N) and 3.88 ± 0.304 in Group 8 (O-P). These differences were all insignificant (*P* > 0.05), suggesting that NS-398 itself not affect the lung water content.

Light microscopic examination of lung tissue

A light microscopic examination of mouse lung specimens showed alveolar wall congestion and thickening, as well as pulmonary interstitial and alveolar edema, with a large amount of inflammatory cells and alveolar exudate in mice that had been injected with LPS (Groups 2, 3, 6 and 7). However, pulmonary interstitial and alveolar edema was significantly milder in Group 3 (S-L) than in the other groups (Groups 2, 6 and 7) (**Figure 6**). Moreover, a light microscopic examination showed clear alveolar structures free of alveolar exudate in mice that did not receive an LPS injection (Groups 1, 4, 5 and 8) (**Figure 6**).

Discussion

This study showed that sevoflurane reduced LPS-induced ALI in mice by changing the equilibrium of cytokines such as IFN-γ and IL-10. Specifically, sevoflurane increased the PGE_2 levels in macrophages of mice with ALI, thereby regulating the equilibrium of cytokines and reducing the pulmonary damage caused by inflammation.

ALI is the result of a cascade of cellular responses caused by infectious or non-infectious inflammation [7]. For example,sepsis associated with gram-negative bacterial infection is a common cause of ALI, where in LPS, the main component of the cell wall of gram-negative bacteria, is the main pathogenic factor [9]. Therefore, the pathology of LPS-induced ALI is similar to that of clinical ALI, and LPS-induced ALI has consequently been widely used as a research model [8].

Some researchers believe that the lung is an important target organ of inflammatory media-

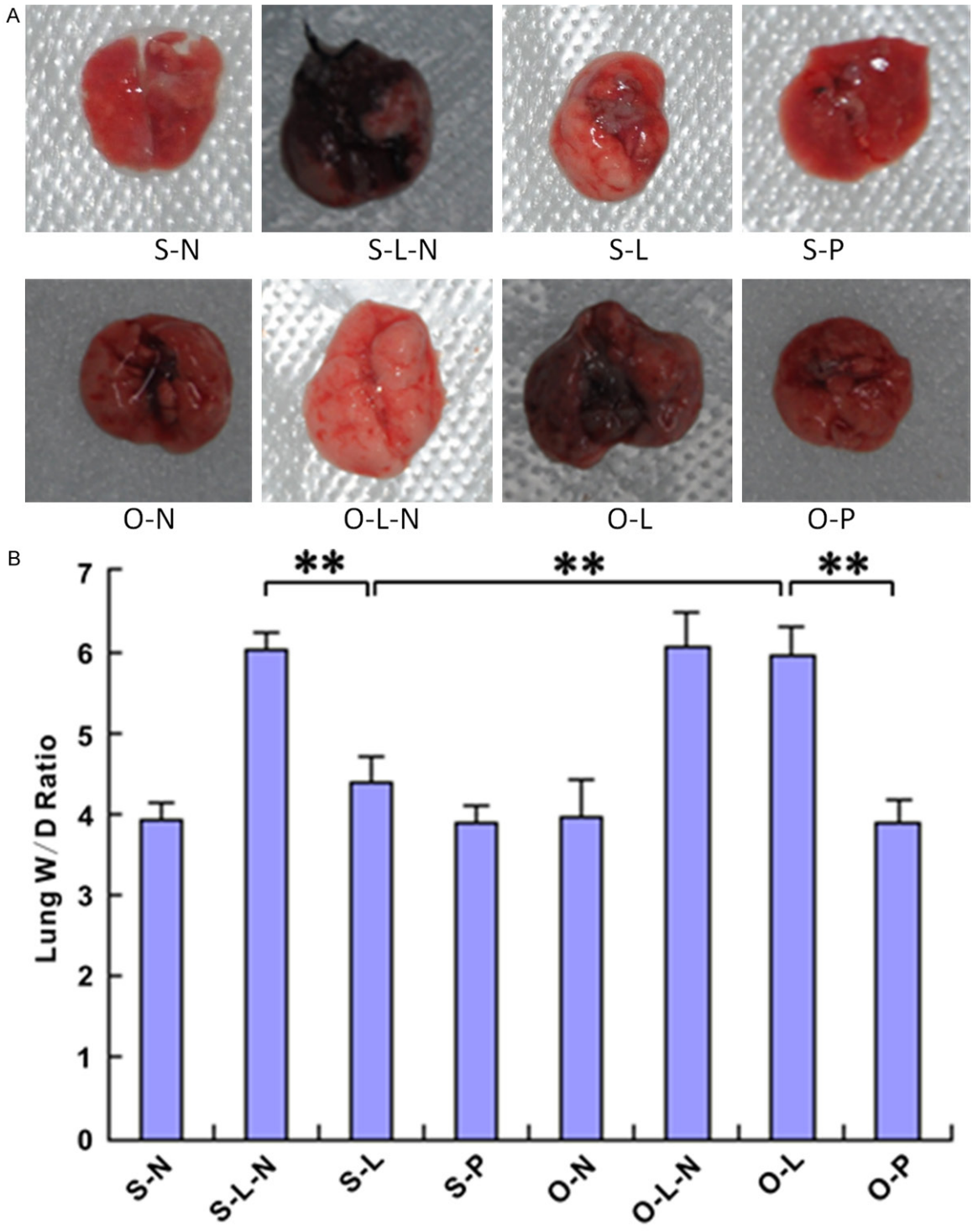

Figure 5. Gross examination and W/D ratio of lung tissue of part 2 ($\bar{x}\pm s$, n=5,** P < 0.01). (A: Up row: group 1-4; down row: group 5-8; B: W/D ratio of lung tissue of part 2) (from left to right).

tors. During sepsis, imbalances between pro- and anti-inflammatory mediators are a key step for ALI development and progression [10]. Therefore, in this study, we selected 2 representative cytokines to investigate changes in the cytokine equilibrium during LPS-induced inflammation and the effect of sevoflurane on this process.

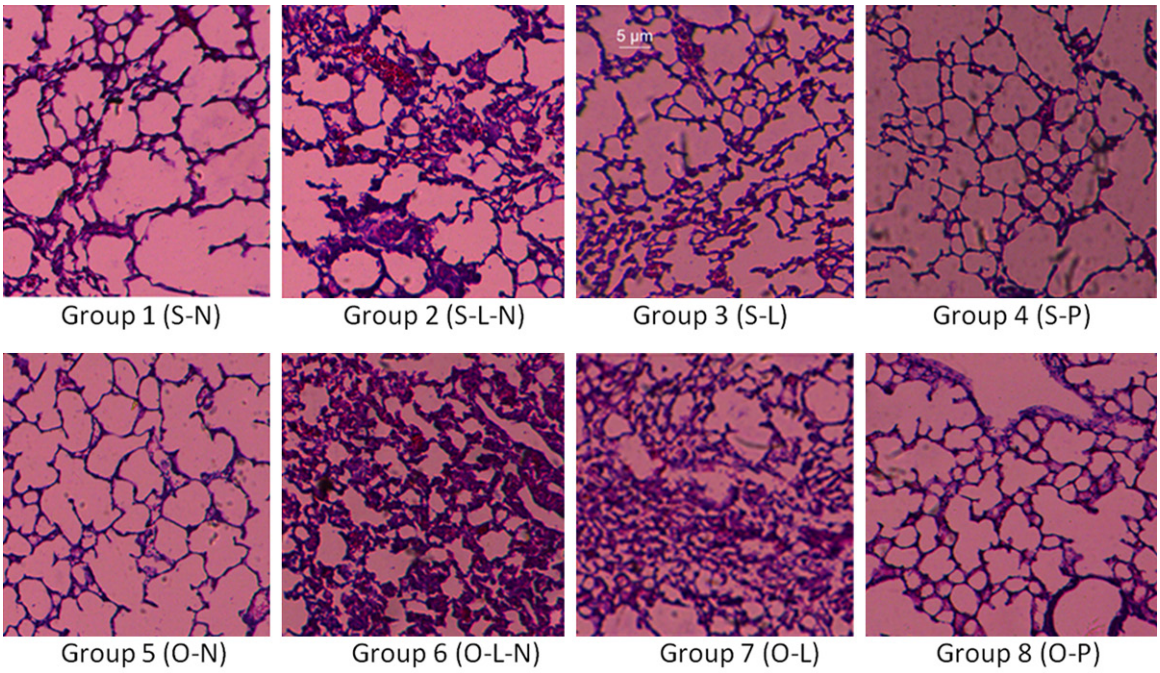

Figure 6. Histological changes of lung tissue in mice in the 8 groups from part 2 (HE×20).

IFN-γ is an important inflammatory mediator. It is a positive regulator of immune responses and the most important cytokine to activate mononuclear macrophages [11]. IFN-γ, a type 1 T helper (Th1)-type cytokine, induces conversion to a Th1-type immune response and suppresses type 2 T helper (Th2)-type immune responses. Specifically, IFN-γ is an effector of Th1 cell secretion and playsan important role in regulating Th1/Th2 differentiation and equilibrium. Transformation and differentiation from native T cell (T0) to Th1 cells increases IFN-γ production, which inhibits the T0 to Th2 transformation and increases the number of Th1 cells and their cytotoxicity.Therefore, this process plays anti-viral and anti-bacterial roles [12].

IL-10 is a cytokine that exerts a wide range of effects, to inducemany immunosuppressive processes. Generally, IL-10 inhibits inflammatory reactions and specific cellular immunity and enhances immune tolerance. Specifically, IL-10 exertsdirect and indirect effects (viaantigen-presenting cells) on T cells. IL-10 affects Th0 cell transformation and promotes Th0 to Th2 transformation, thereby changing the Th1/Th2 balance. IL-10 also inhibits the synthesis of inflammatory cytokines such as IFN-γ, by Th1 cells; suppresses antigen presentation by Th1 cells; and inhibits the proliferation of antigen-specific T cells. IL-10 exerts similar effects on granulocytes and mononuclear macrophages: itinhibits the generation and release of pro-inflammatory cytokines and induces the generation of anti-inflammatory cytokines, thereby blocking neutrophil-mediated tissue injury [13-16].

Studies on the effect of IL-10 on acute lung inflammation have shown that IL-10 inhibits inflammation by suppressing the activity of mast cells and recruiting neutrophils [17, 18]. Additionally, low IL-10 levels in the bronchoalveolar lavage fluid (BALF) of patients withacute respiratory distress syndrome (ARDS) is associated with high a mortality rate, which suggests that IL-10 plays a vital role in the equilibrium of inflammatory cytokines [19].

The present study showed that the levels of serum IFN-γ and IL-10 (an anti-inflammatory molecule), were significantly increased in mice injected with of LPS, indicating an immune imbalance and potential immune-related injury during acute inflammation. Sevoflurane inhalation significantly improved LPS-induced pulmonary edema and related pulmonary pathological changes. Additionally, sevoflurane reduced IFN-γ production and increased the IL-10 level,

suggesting that sevoflurane may be involved in the regulation ofthe immune equilibrium of cytokines. Thus, sevoflurane may playa protective role in LPS-induced ALI.

Previous studies showed that the equilibrium of IL-10 and IFN-γ is primarily maintained bythe interaction and equilibrium of Th subsets. During the functional differentiation of T cells, antigen-presenting cells play a vital regulatory role. Specifically, mononuclear macrophages and dendritic cells are important antigen presenting cells in the body. In this study, we established a mouse model of ALI using anintraperitoneal injection of LPS, wherein large numbers of peritoneal macrophages recognizes the antigen and presents antigen information to T cells during the anti-inflammatory immune response. This process affects the type, intensity and duration of the immune response. Therefore, we hypothesized that sevoflurane may affect the immune regulatory function of macrophages to influence the balance of Th subsets, leading to changes in IL-10 and IFN-γ.

Recent studies have shown that macrophages are an important source of PGE_2, which is an important cytokine for maintaining internal hemostasis. In particular, PGE_2 regulates several steps of the inflammatory reaction and coordinates the balance of pro- and anti-inflammatory responses; thus, it is thus an important mediator of the immune response during inflammation: PGE_2 inhibits the generation of the pro-inflammatory cytokine IFN-γ and promotes the generation of the anti-inflammatory cytokine IL-10 [20-22].

This study showed that among mice intraperitoneally injected with LPS, the PGE_2 levels in peritoneal macrophages were higher in the sevoflurane group than in the O_2 group. This result suggested that sevoflurane promoted the production of PGE_2 in peritoneal macrophages in mice with ALI, tipping the equilibrium of cytokines towards anti-inflammatory cytokines, such as IL-10, and decreasing the level of pro-inflammatory cytokines, such as IFN-γ. This shift helped alleviate tissue and organ damage due to the cytokine storm that occurs during severe inflammation, there by protecting the lung and other vital organs.

Moreover, sevoflurane promotes PGE_2 production in macrophages. Thus, we also investigated the role of PGE_2 in the protective effect of sevoflurane on inflammatory injury. The synthesis of prostaglandins (PGs) starts with a 20-carbon unsaturated fatty acid known as arachidonic acid, which is released from the cell membrane following the catalytic effect of phospholipase, and completes after a series of reactions catalyzed by Cosand related synthases. Thus, PG production is determined by the activity of COX, whichexhibits both COX and peroxidase activities. COX exists in 2 distinct isoforms (COX-1 and COX-2), which both play an important role in the self-regulation and hemostasis of PGs and promote the release of PGs during inflammation [23, 24].

NS-398 is specific COX-2 inhibitor [25]. In this study, the PGE_2 levels in macrophages did not significantly differ amongthe 4 groups of mice that had been injected with NS-398 injection ($P > 0.05$), but the PGE_2 levels among these groups were significantly lower than those of the 4 groups that had not been injected with NS-398 ($P < 0.05$). Together, these results suggestthat NS-398 blocked COX-2 mediated PGE_2 synthesis.

In this study, pulmonary edemadid not significantly differ between Groups S-N, O-N, S-P, and O-P. Additionally, the W/D ratio also did not significantly differamong these 4 groups ($P > 0.05$), suggesting that NS-398 itself did not cause lung inflammation. However, Group S-L-N showed more severe pulmonary edema than Group S-L and a severity of pulmonary edema similar to that observed in Group O-L. Moreover, Group S-L-N presented a significantly higher W/D ratio than Group S-L ($P < 0.05$) and a W/D ratio similar to that of Group O-L ($P > 0.05$). These ratios suggested that NS-398 mitigated the protective effect of sevoflurane on LPS-induced ALI in mice and that PGE_2 played a vital role in the protective effect of sevoflurane on inflammatory injury.

NS-398 is a specific COX-2 inhibitor, and COX-2 plays an important role in PGE_2 synthesis during an inflammatory reaction. Subsequently, PGE_2 regulates inflammatory cytokines by inhibiting IFN-γ production and increasing the IL-10 levels [14]. Thus, during inflammatory reactions, sevoflurane altered PGE_2 levels in macrophages and changed the equilibrium between the pro-inflammatory cytokine IFN-γ and the anti-inflammatory cytokine IL-10, and

this shift alleviated pulmonary injury due to the cytokine storm that occursduring inflammatory reaction. Therefore, sevoflurane protected the lung and other vital organs from inflammatory injury [26].

Conclusion

In summary, LPS changes immune homeostasis, resulting in acute lung inflammatory injury. Conversely, inhaled sevoflurane regulates immune homeostasis, which helps to alleviate LPS-induced ALI.

Disclosure of conflict of interest

None.

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