Original Article

Sub-anesthesia Dose of Isoflurane in 60% Oxygen Reduces Inflammatory Responses in Experimental Sepsis Models

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Abstract

Background: Sepsis is a major cause of mortality in Intensive Care Units. Anesthetic dose isoflurane and 100% oxygen were proved to be beneficial in sepsis; however, their application in septic patients is limited because long-term hyperoxia may induce oxygen toxicity and anesthetic dose isoflurane has potential adverse consequences. This study was scheduled to find the optimal combination of isoflurane and oxygen in protecting experimental sepsis and its mechanisms.

Methods: The effects of combined therapy with isoflurane and oxygen on lung injury and sepsis were determined in animal models of sepsis induced by cecal ligation and puncture (CLP) or intraperitoneal injection of lipopolysaccharide (LPS) or zymosan. Mouse RAW264.7 cells or human peripheral blood mononuclear cells (PBMCs) were treated by LPS to probe mechanisms. The nuclear factor kappa B (NF-κB) signaling molecules were examined by Western blot and cellular immunohistochemistry.

Results: The 0.5 minimum alveolar concentration (MAC) isoflurane in 60% oxygen was the best combination of oxygen and isoflurane for reducing mortality in experimental sepsis induced by CLP, intraperitoneal injection of LPS, or zymosan. The 0.5 MAC isoflurane in 60% oxygen inhibited proinflammatory cytokines in peritoneal lavage fluids (tumor necrosis factor-alpha [TNF-α]: 149.3 vs. 229.7 pg/ml, interleukin [IL]-1β: 12.5 vs. 20.6 pg/ml, IL-6: 86.1 vs. 116.1 pg/ml, and high-mobility group protein 1 [HMGB1]: 323.7 vs. 449.3 ng/ml; all $P < 0.05$) and serum (TNF-α: 302.7 vs. 450.7 pg/ml, IL-1β: 51.7 vs. 96.7 pg/ml, IL-6: 390.4 vs. 722.5 pg/ml, and HMGB1: 592.2 vs. 985.4 ng/ml; all $P < 0.05$) in septic animals. *In vitro* experiments showed that the 0.5 MAC isoflurane in 60% oxygen reduced inflammatory responses in mouse RAW264.7 cells, after LPS stimulation (all $P < 0.05$). Suppressed activation of NF-κB pathway was also observed in mouse RAW264.7 macrophages and human PBMCs after LPS stimulation or plasma from septic patients. The 0.5 MAC isoflurane in 60% oxygen also prevented the increases of phospho-IKKα/β, phospho-IκBα, and phospho-p65 expressions in RAW264.7 macrophages after LPS stimulation (all $P < 0.05$).

Conclusion: Combined administration of a sedative dose of isoflurane with 60% oxygen improves survival of septic animals through reducing inflammatory responses.

Key words: Inflammation; Isoflurane; Oxygen; Sepsis

Introduction

Sepsis is a complex pathology that arises from deregulated host inflammatory responses to systemic bacterial infection[1] and remains one of the leading causes of death in Intensive Care Units (ICUs).[2-4] Sepsis is characterized by an intravascular activation of the host's inflammatory pathways by which potent inflammatory mediators are released into the circulation[5] and is associated with septic shock,

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sequential multiple organ failure, and high-mortality rate. Typically, 50% of all sepsis cases start as an infection in the lungs.[6] Acute lung injury, especially adult respiratory distress syndrome, is a severe, life-threatening medical condition characterized by widespread inflammation in the lungs, with a high mortality of about 30%.[7]

It is common in a clinical setting that combined isoflurane with oxygen is applied for anesthesia of operation patients. The previous studies have reported that both hyperoxia[8-11] and anesthetic dose isoflurane[12-14] have significant protective effects on complex inflammation-mediated conditions including sepsis in various animal models of inflammation, and their application to the patients with critical diseases is limited because the use of anesthetic dose isoflurane in critically ill patients may have serious adverse consequences.[15-17] A large number of trials have demonstrated the safety of long-time lower doses of isoflurane for ICU sedation,[18,19] and long-term hyperoxia treatment can induce oxygen toxicity associated with the overproduction of reactive oxygen species (ROS).[20-23] Here, we demonstrated that combined administration of a low-dose isoflurane (0.5 minimum alveolar concentration [MAC]) with 60% oxygen reduced inflammatory responses to sepsis in animals and human peripheral blood mononuclear cells (PBMCs) and increased the 7-day survival rate of animals with experimental sepsis.

Methods

Animals

We used male C57BL/6 and ICR/Km (Institute of Cancer Research, National Institutes of Health, USA/Kunming Institute of Zoology, China) mice (specific pathogen-free, 20–25 g) and Sprague-Dawley (SD) rats (specific pathogen-free, 250–300 g) for these studies. One week before experimental manipulation, the animals were allowed to acclimatize to the experimental housing facilities. Animals were maintained in a constant 12-h light–dark cycle at 20°C–22°C with standard food and water available *ad libitum*. We performed all experiments according to the National Institutes of Health guidelines. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

Patients

Between July and September 2014, we collected blood samples from patients with or without sepsis who were admitted to Xijing Hospital, Fourth Military Medical University (Xi'an, China), as part of a study of the effects of oxygen and oxygen plus volatile anesthetics on experimental sepsis (ClinicalTrials.gov NCT02185118). Information of these patients is shown in Supplementary Tables 1 and 2. We obtained written informed consent from patients' proxy decision-makers before the study inclusion. After the individuals regained decision-making capacity, they were told about their participation, and re-consent was obtained according to the institutional policies. Inclusion and exclusion criteria were determined according to the diagnostic criteria for sepsis based on the literature.[24] The Institutional Review Board of Xijing Hospital, Fourth Military Medical University, approved all of the human protocols.

Cell culture

RAW264.7 cell lines were obtained from the Department of Microbiology, School of Basic Medicine, The Fourth Military Medical University, and maintained in Dulbecco's modified Eagle's medium (Gibco, New York, USA) supplemented with 10% (volume/volume) heat-inactivated fetal calf serum and penicillin and streptomycin.

Human PBMCs were isolated from heparinized venous blood under endotoxin-free conditions through Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ, USA). The purity of $CD14^+$ monocytes [Figure 1] was always more than 90% as assessed by immunofluorescence staining and counting.[25,26]

Sepsis models

For cecal ligation and puncture (CLP)-induced sepsis, animals were anesthetized with 10% chloral hydrate (3 ml/kg). Briefly, the lower quadrant of the abdomen was disinfected, and a longitudinal skin midline incision

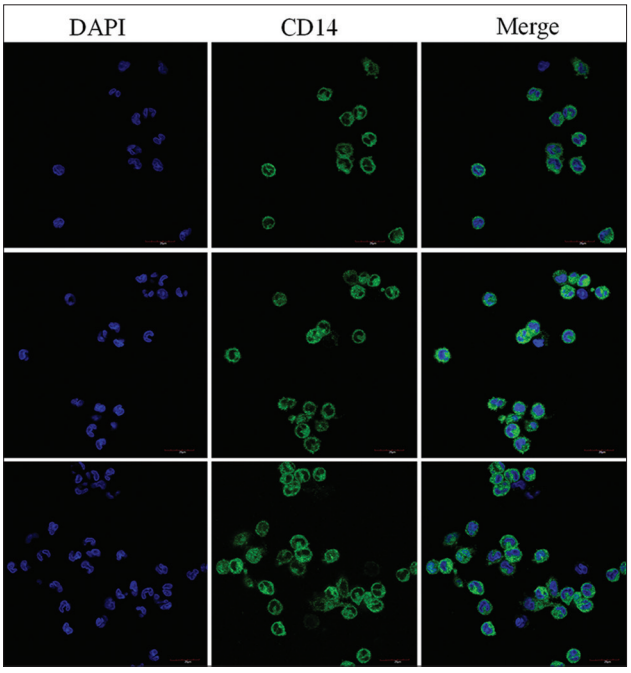

Figure 1: Identification of $CD14^+$ monocytes in human peripheral blood mononuclear cells. Blood was collected from nonseptic patients and anticoagulated with heparin. PBMCs were separated by a Ficoll-Hypaque density gradient centrifugation, added to plastic flasks, and incubated for 6 h at 37°C. Adherent cells were obtained by washing the flasks for three times with Hank's solution and subjected to fluorescence staining to detect CD14 using Alexa-F488-conjugated mouse anti-human CD14 antibody. The purity of $CD14^+$ monocytes in human PBMCs was >90%. Fluorescence images of cells were captured using confocal laser scanning microscopy (original magnification ×90). PBMCs: Peripheral blood mononuclear cell cells; DAPI: 4',6-diamidino-2-phenylindole.

was made to expose the cecum. For the induction of mid-grade sepsis (survival rate about 40%), the cecum was ligated half the distance between the distal pole and base of the cecum. High-grade sepsis (100% lethality) comprised ligation of 75% of the cecum. We punctured the cecum ("through-and-through") from the mesenteric toward the antimesenteric direction with a 21-gauge needle, and animals in the sham groups underwent surgery without the CLP procedure. Finally, the abdominal incision was closed in two layers by applying simple interrupted sutures. After surgery, the mice were resuscitated in a warm cage.

Lipopolysaccharide (LPS)-induced sepsis was induced by an intraperitoneal injection of LPS (50 mg/kg for ICR/Km mice and 30 mg/kg for C57BL/6 mice) (Sigma Chemical, St. Louis, MO, USA), with normal saline (NS) as control. Induction of an *in vitro* sepsis was performed on RAW264.7 cell lines with exposure to LPS. A clinical *in vitro* sepsis was induced in human PBMCs by LPS or plasma from septic patients.

Zymosan-induced sterile sepsis was induced by intraperitoneal injection of zymosan (Sigma Chemical Co., St. Louis, MO, USA) at 1 g/kg as was described previously;[9] sham group was injected with NS.

Treatment with oxygen or isoflurane in oxygen

Animals were placed in a sealed plexiglass chamber with an inflow and an outflow. Oxygen and isoflurane (Lunan Pharmaceutical Co., Ltd., Shandong, China) were delivered to the chamber through a tube, and carbon dioxide was removed from the chamber gases with Baralyme. The concentration of oxygen and isoflurane in the outflow hose of the chamber was continuously monitored with a gas analyzer (Bruel and Kjaer, Naerum, Denmark). The concentration of oxygen or isoflurane was maintained at the predetermined level during the treatment. The temperature of the room and the chamber was maintained at 20°C–22°C. The treatment time was in accordance with the previous study.[13]

The cells were transferred to a sealed hypoxia modular incubator chamber (MIC-101, Billups-Rothenberg, San Diego, California, USA) containing a mixture of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen at 37°C, or normal culture conditions (control) for 2 h, while the chamber was continuously monitored with a gas analyzer (Bruel and Kjaer, Naerum, Denmark).

Histological analysis

Twenty-four hours after CLP, animals were anesthetized, and the lungs were collected for histological observation with a microscope (Olympus, Tokyo, Japan). The histological slides were read in a blinded manner and assessed by two experienced pathologists.

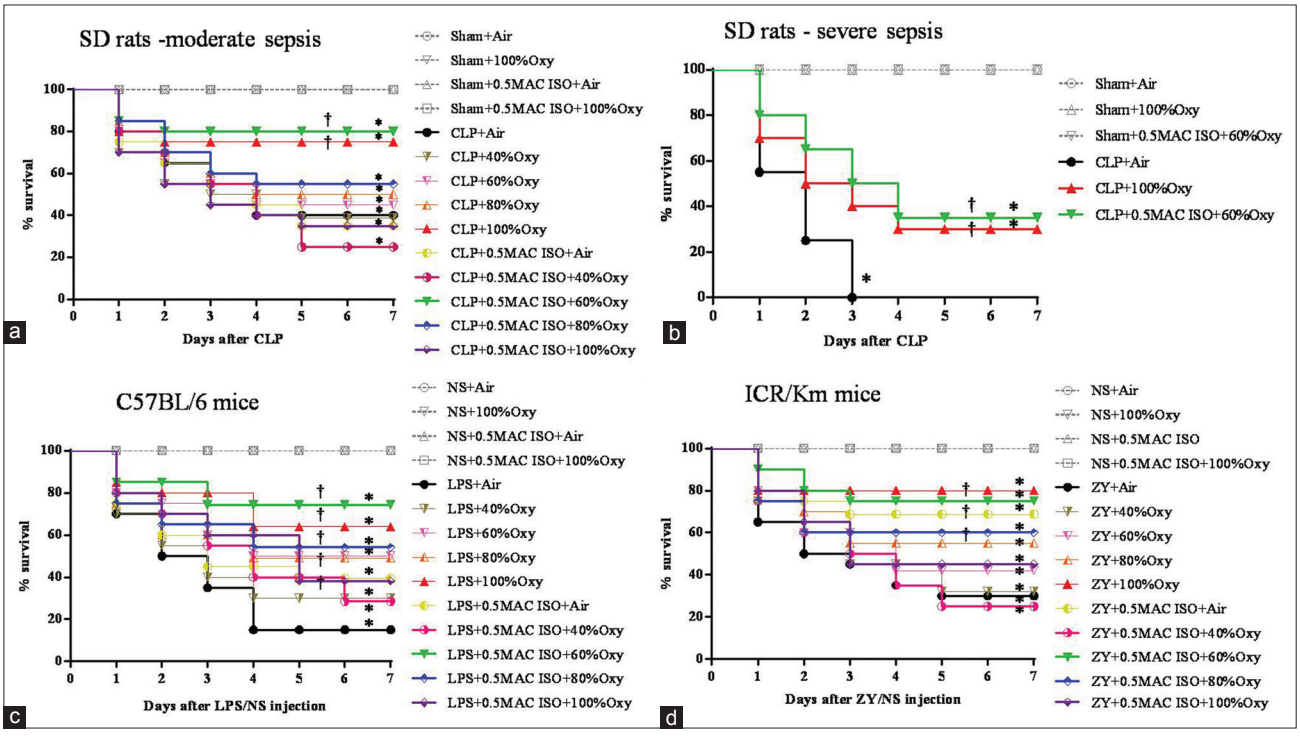

Figure 2: Inhalation of 0.5 minimum alveolar concentration isoflurane in 60% oxygen protected against sepsis-induced lethality. Sepsis was induced by CLP or intraperitoneal injection of LPS, zymosan. Various concentrations of oxygen or 0.5 MAC isoflurane in various concentrations of oxygen was inhaled for 1 h at 1 and 6 h after the challenge, respectively. The survival rate was observed for 7 days. The values are expressed as the survival percentage ($n = 20$ for each group). (a) SD rats with CLP-induced moderate sepsis. (b) SD rats with CLP-induced severe sepsis. (c) C57BL/6 mice with LPS-induced sepsis. (d) ICR/Km mice with zymosan-induced sterile sepsis. *$P < 0.05$ versus NS/Sham+Air group; †$P < 0.05$ versus ZY/LPS/CLP+Air group, respectively. CLP: Cecal ligation and puncture; LPS: Lipopolysaccharide; MAC: Minimum alveolar concentration; NS: Normal saline; SD: Sprague-Dawley; ISO: Isoflurane; Oxy: Oxygen; ZY: Zymosan.

Arterial blood gas analysis

Arterial blood samples were collected from the carotid artery (mice) or femoral artery (rats). The arterial blood gas analysis was performed in all groups using a GEM Premier 3000 gas analyzer (Instrumentation Laboratory, Milan, Italy).

Lung wet to dry weight ratio

To quantify the magnitude of the pulmonary edema, we evaluated the lung wet to dry (W/D) weight ratio. The harvested wet lung was weighed. It was then placed in an oven for 24 h at 80°C and re-weighed when it was dried. The lung W/D weight ratio was recorded.

Cell number count and protein assay of the bronchoalveolar lavage fluid

Bronchoalveolar lavage fluid (BALF) was obtained by cannulating the trachea with a 20-gauge catheter in mice and a 16-gauge catheter in rats. Animals were anesthetized, and the trachea and lung were exposed by thoracotomy. Phosphate-buffered saline (PBS) (pH 7.4) was instilled with a syringe and allowed to stay in the lung for 30 s, which was repeated three times using the same solution. Lavage samples were centrifuged (12,000 ×*g* for 10 min, 4°C). The supernatant was stored at −80°C. The cell pellet was diluted in PBS, and the total cell number was counted with a hemocytometer after staining with trypan blue (Beyotime Biotechnology, Shanghai, China). Neutrophils were counted in cytocentrifuge preparations (Cytospin 3; Shandon Scientific, Cheshire, UK) stained with Diff-Quik stain (Baxter Diagnostics, McGaw Park, IL, USA). Total protein was measured in the cell-free supernatant using the bicinchoninic acid (BCA) method. Bovine serum albumin was used as a standard.[27]

Assay of serum biochemical parameters

At the predetermined time points, animals were

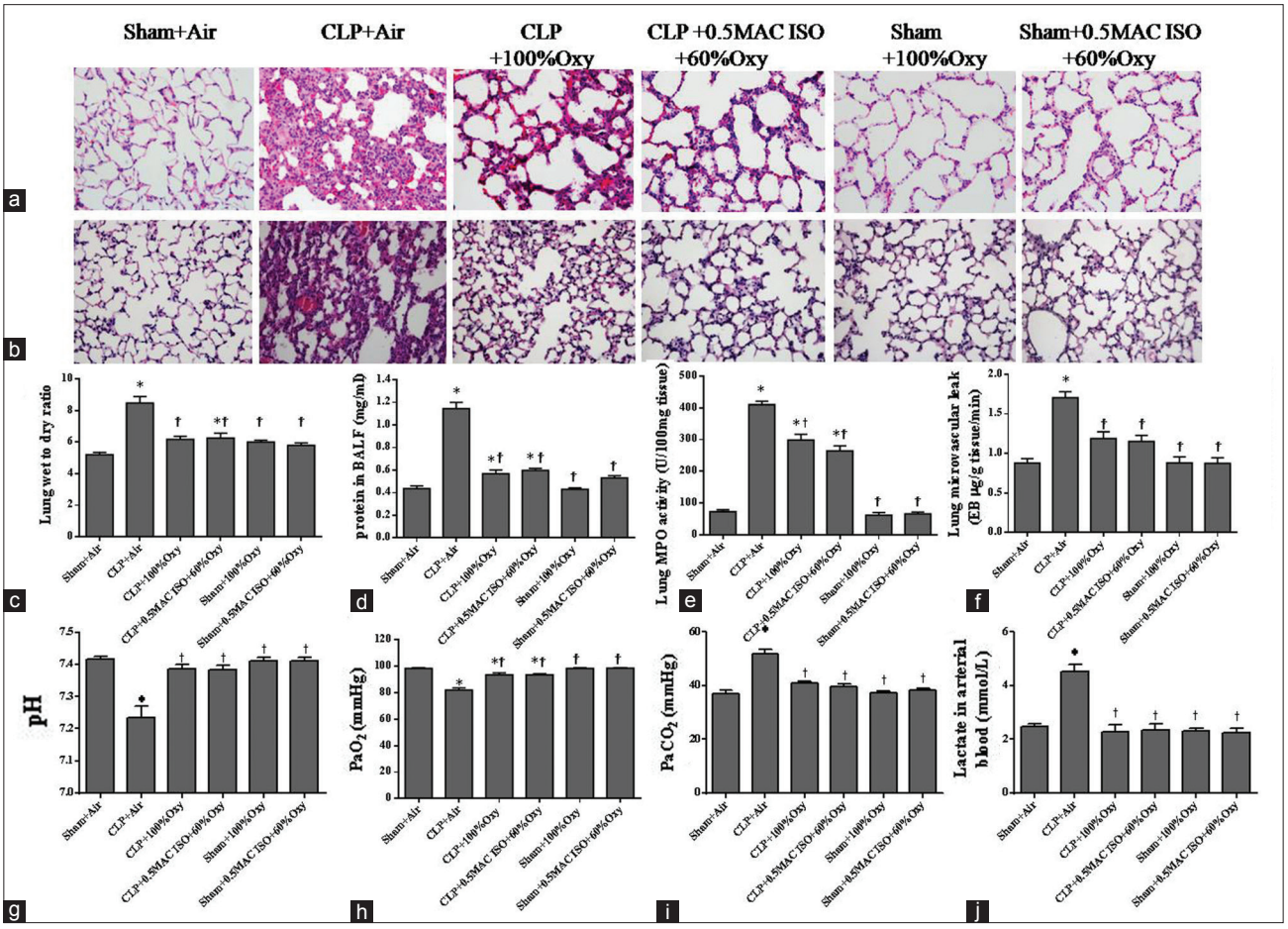

Figure 3: Inhalation of 0.5 minimum alveolar concentration isoflurane in 60% oxygen reduced cecal ligation and puncture-induced lung injury. CLP-induced sepsis was performed on ICR/Km mice or SD rats. Treatment was conducted by inhalation of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after CLP, respectively. Lungs were harvested 24 h after CLP for histological observation under optical microscopy (original magnification ×40). Photomicrographs are representative of data obtained from lung sections derived from six animals. (a) Histological assessment of lungs in rats. (b) Histological assessment of lungs in ICR/Km mice. Parameters reflecting lung injury were also determined 24 h after CLP. Data are expressed as the mean ± standard error (n = 6 in each group). (c) Lung wet to dry weight ratio. (d) Protein production in the BALF. (e) Lung MPO activity. (f) Lung microvascular albumin leak. (g) Arterial pH value. (h) Arterial partial pressure of oxygen (PaO_2). (i) Arterial partial pressure of carbon dioxide ($PaCO_2$). (j) Lactate in arterial blood. *$P < 0.05$ versus Sham+Air group; †$P < 0.05$ versus CLP+Air group. CLP: Cecal ligation and puncture; SD: Sprague-Dawley; MAC: Minimum alveolar concentration; BALF: Bronchoalveolar lavage fluid; MPO: Myeloperoxidase; ISO: Isoflurane; Oxy: Oxygen.

anesthetized, and blood samples were collected by cardiac puncture for determining serum levels of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine using a biochemistry autoanalyzer (Hitachi Autoanalyzer 7150; Hitachi, Tokyo, Japan). In addition, serum lactic dehydrogenase was determined spectrophotometrically using a commercially available kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of lung myeloperoxidase activity

The activity of myeloperoxidase (MPO), an indicator of neutrophil infiltration in lung tissue, was detected in homogenized lung supernatants and measured as previously reported[28] using commercial kits purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

Microvascular albumin leakage in the lung

Evans blue (EB) (MP Biomedicals, Shanghai, China) (2%, 4 ml/kg) was injected into the tail vein 2 h before CLP. At 24 h after CLP, lung tissue was homogenized in ice-cold PBS, incubated with formamide at 60°C for 16 h, and centrifuged at 7000 ×*g* for 25 min. The absorbance (A_{620}) of the supernatant was determined, and the tissue EB content was calculated.[29]

Assay of inflammatory cytokines

The levels of high-mobility group protein-1 (HMGB1), interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) in cell culture supernatant or serum or plasma or intraperitoneal lavage fluid were detected using specific mouse or human enzyme-linked immunosorbent assay kits (R&D Systems Inc., Minneapolis, Minnesota, USA) with a microplate reader (Denley Dragon, Wellscan MK3, Thermo, Finland). All of the standards and samples were run in triplicate.

Western blot analysis

The protein from RAW264.7 cell samples was directly extracted based on the manufacturer's standard protocols (Beyotime Biotechnology). The cytoplasmic and nuclear protein fractions were extracted using Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology) according to the manufacturer's protocol. Cytoplasmic/nuclear protein extracts or whole protein extracts were used for Western blot analysis. The primary rabbit antibodies for nuclear factor kappa B (NF-κB) p65 subunit (p65), phosphorylated NF-κB p65 (p-p65), phosphorylated inhibitor of kappa B alpha (p-IκBα), and phosphorylated IκB kinase-α/β (p-IKKα/β) (Cell Signaling Technology,

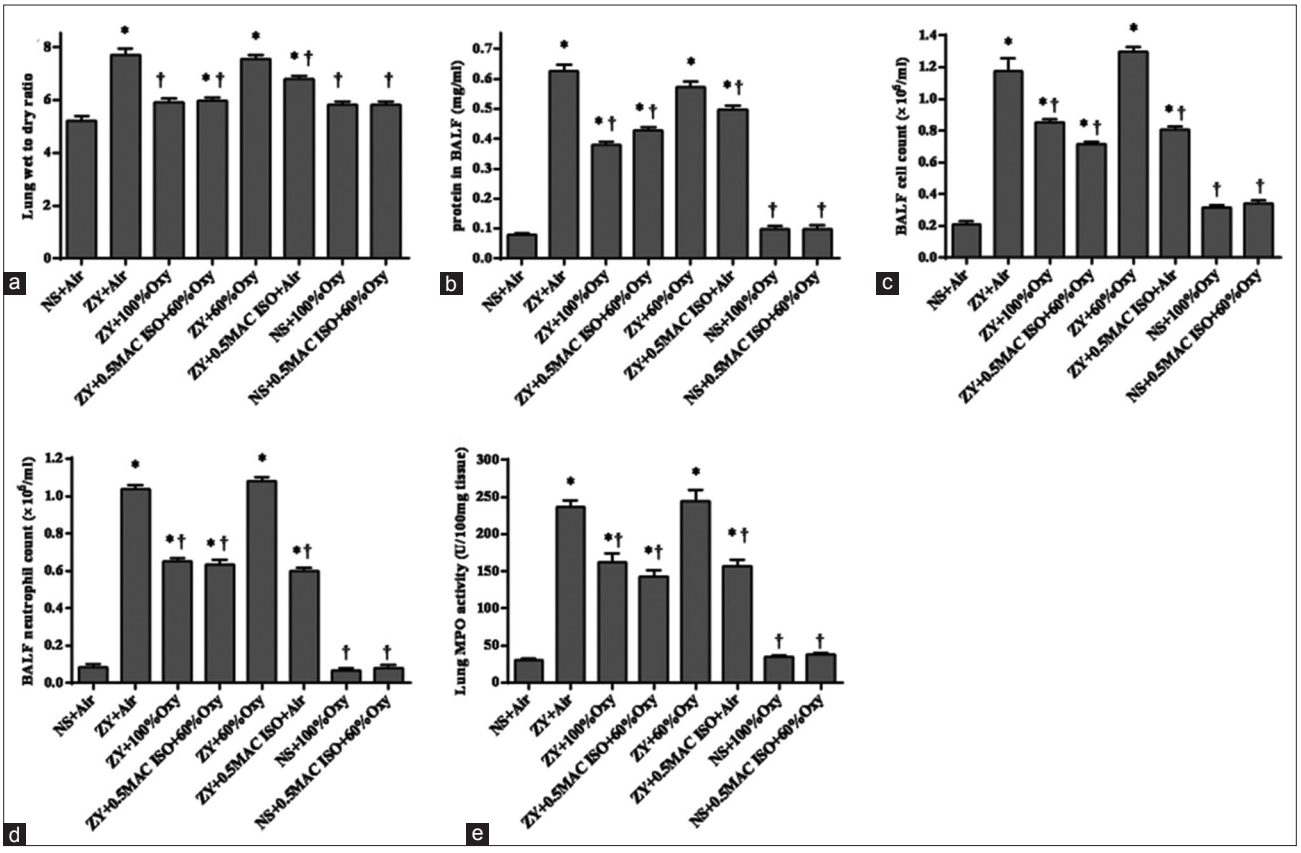

Figure 4: Inhalation of 0.5 minimum alveolar concentration isoflurane in 60% oxygen protected mice against zymosan-induced lung injury 24 h after zymosan injection. Treatment was performed in ICR/Km mice by inhalation of 100% oxygen, 60% oxygen, 0.5 MAC isoflurane, or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after zymosan/NS injection, respectively. (a) Lung wet to dry weight ratio. (b) Protein production in BALF. (c) Whole cell numbers in BALF. (d) Neutrophil numbers in BALF. (e) Lung MPO activity. Data are expressed as the mean ± standard error (n = 6 in each group). *P < 0.05 versus NS+Air group; †P < 0.05 versus ZY+Air group. MAC: Minimum alveolar concentration; BALF: Bronchoalveolar lavage fluid; MPO: Myeloperoxidase; ISO: Isoflurane; NS: Normal saline; Oxy: Oxygen; ZY: Zymosan.

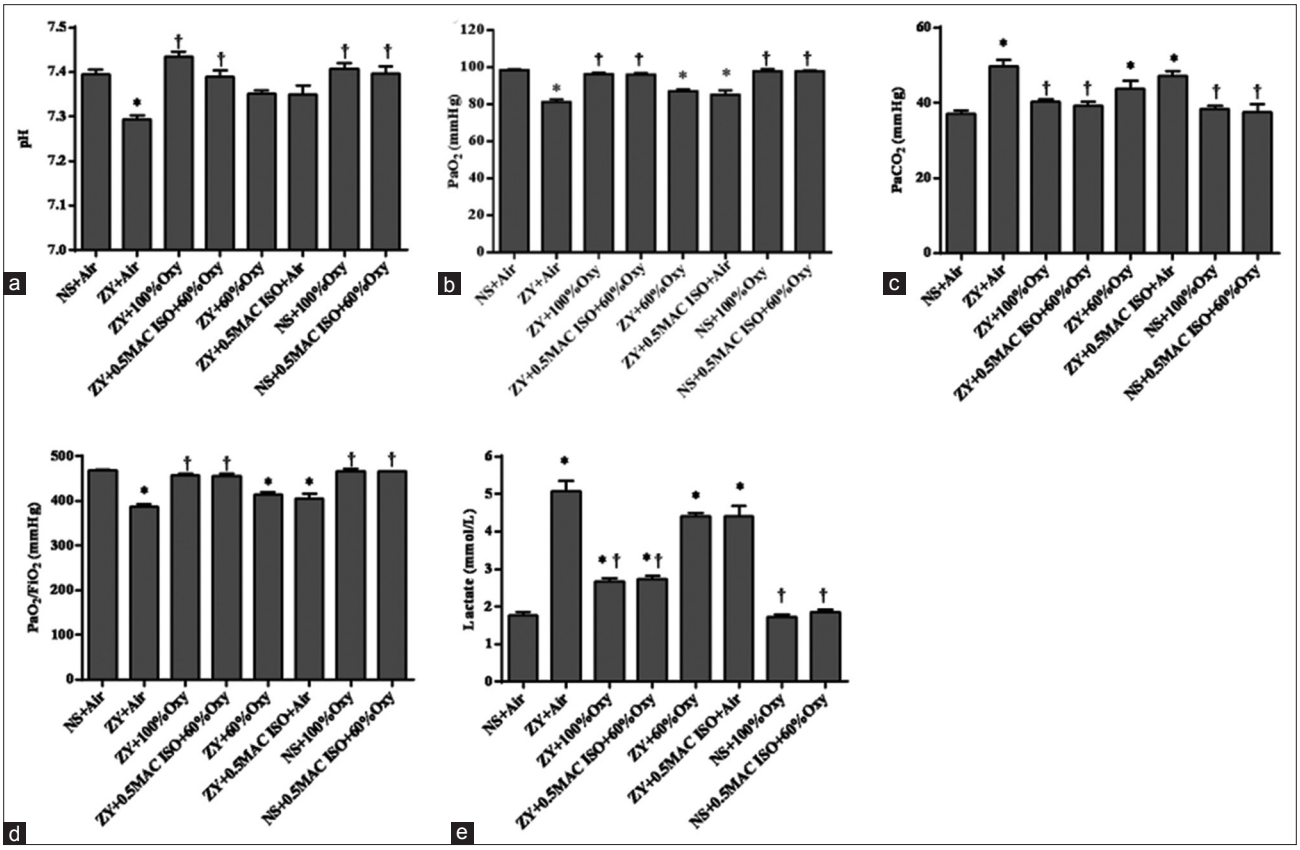

Figure 5: Inhalation of 0.5 minimum alveolar concentration isoflurane in 60% oxygen improved tissue oxygenation in zymosan-challenged mice. Treatment was performed in ICR/Km mice by inhalation of 100% oxygen, 60% oxygen, 0.5 MAC isoflurane, or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after zymosan/NS injection, respectively. Arterial blood gas analysis was conducted 24 h after zymosan/NS injection. (a) Arterial pH value. (b) Arterial partial pressure of oxygen (PaO_2). (c) Arterial partial pressure of carbon dioxide ($PaCO_2$). (d) Oxygenation indices (PaO_2/FiO_2). (e) Lactate in arterial blood. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus NS+Air group; †$P < 0.05$ versus ZY+Air group. MAC: Minimum alveolar concentration; NS: Normal saline; FiO_2: Fraction of inspired oxygen; ISO: Isoflurane; Oxy: Oxygen; ZY: Zymosan.

Boston, USA) proteins were used for the detection of p65, p-p65, p-IκBα, and p-IKKα/β protein expression. Moreover, the primary rabbit antibodies for proliferating cell nuclear antigen (PCNA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), IKKα, IKKβ, IκBα proteins (Cell Signaling Technology) were used for the detection of PCNA, GAPDH, IKKα, IKKβ, and IκBα proteins, which were used as the control. Immunostained proteins were detected by electrochemiluminescence (CWBio Co., Ltd., Beijing, China).

Immunofluorescence staining

For the immunofluorescence assays, RAW264.7 cells or freshly isolated PBMCs were seeded on coverslips in 24-well plates and cultured. Immunofluorescence staining of the cells was performed using the NF-κB p65 nuclear translocation kit (Beyotime Biotechnology) manufacturer's instruments. The 4',6-diamidino-2-phenylindole staining was used for counterstaining of the nucleus. Fluorescent images of coverslips were obtained by confocal microscopy (Olympus, Tokyo, Japan).

Statistical analysis

The measurement data are expressed as mean ± standard error (SE). Intergroup differences in the levels of biochemical parameters and inflammatory cytokines were tested by one-way analysis of variance (ANOVA), followed by Dunnett's *t*-test for multiple comparisons. Survival studies were analyzed using the log-rank test. The survival rates are expressed as a percentage. The intergroup differences of histopathologic scores were tested by Kruskal–Wallis *H* method, followed by Nemenyi test for multiple comparisons. The statistical analysis was performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

Combined administration of 0.5 minimum alveolar concentration isoflurane with 60% oxygen protected against lung injury and sepsis induced by cecal ligation and puncture

In CLP-challenged rats with moderate mortality, the 7-day survival rate was markedly decreased to about 40%, and a significant improvement in the 7-day survival rate was observed only in those treated with inhalation of 100% oxygen (75% vs. 40%, $P < 0.05$) or 0.5 MAC isoflurane

in 60% oxygen (80% vs. 40%, $P < 0.05$) [Figure 2a]. Inhalation of 40% oxygen, 60% oxygen, 80% oxygen or 0.5 MAC isoflurane in air, 0.5 MAC isoflurane in 40% oxygen, 0.5 MAC isoflurane in 80% oxygen, and 0.5 MAC isoflurane in 100% oxygen did not have similar protective effects. We also observed that inhalation of 100% oxygen (30% vs. 0%, $P < 0.05$) or 0.5 MAC isoflurane in 60% oxygen (35% vs. 0%, $P < 0.05$) improved the 7-day survival rate of rats with a higher mortality induced by CLP [Figure 2b].

We further observed similar histological changes in lungs from the two animal models [Figure 3a and 3b]. In sham-operated animals, uniform and small alveoli were seen, while alveolar collapse, interstitial edema, congestion, alveolar wall thickening, consolidation involving half of the lung, and heterogeneous alveolar size were observed in animals from the CLP+Air group. Treatment with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen resulted in improvement of the CLP-induced lung impairments. In addition, lung W/D weight ratio (8.48 vs. 5.21, $P < 0.05$), lung MPO activity (410.4 vs. 72.8 U/100 mg, $P < 0.05$), lung microvascular EB leak (1.71 vs. 0.88 μg/g, $P < 0.05$), and BALF protein (1.15 vs. 0.44 mg/ml, $P < 0.05$) as well as arterial partial pressure of carbon dioxide (52.0 vs. 37.0 mmHg, $P < 0.05$; 1 mmHg = 0.133 kPa) and arterial blood lactate (4.53 vs. 2.48 mmol/L, $P < 0.05$) were significantly increased at 24 h after CLP. The pH value (7.23 vs. 7.42, $P < 0.05$) and arterial partial pressure of oxygen (81.9 vs. 98.3 mmHg, $P < 0.05$) were significantly decreased at 24 h after CLP. Treatment of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen significantly improved these impairments in SD rats with the CLP challenge [Figure 3c-3j].

The above results imply that combined administration of 0.5 MAC isoflurane with 60% oxygen is the best combination of oxygen and isoflurane for protecting against CLP-induced sepsis.

Combined administration of 0.5 minimum alveolar concentration isoflurane with 60% oxygen protected against lethality in mouse models of sepsis induced by intraperitoneal injection of lipopolysaccharide or zymosan

In C57BL/6 mice, intraperitoneal injection of LPS resulted in a marked decrease of the 7-day survival rate to 15%, which was improved significantly by inhalation of 60% oxygen (50.0% vs. 15.0%, $P < 0.05$), 80% oxygen (49.1% vs. 15.0%, $P < 0.05$), 100% oxygen

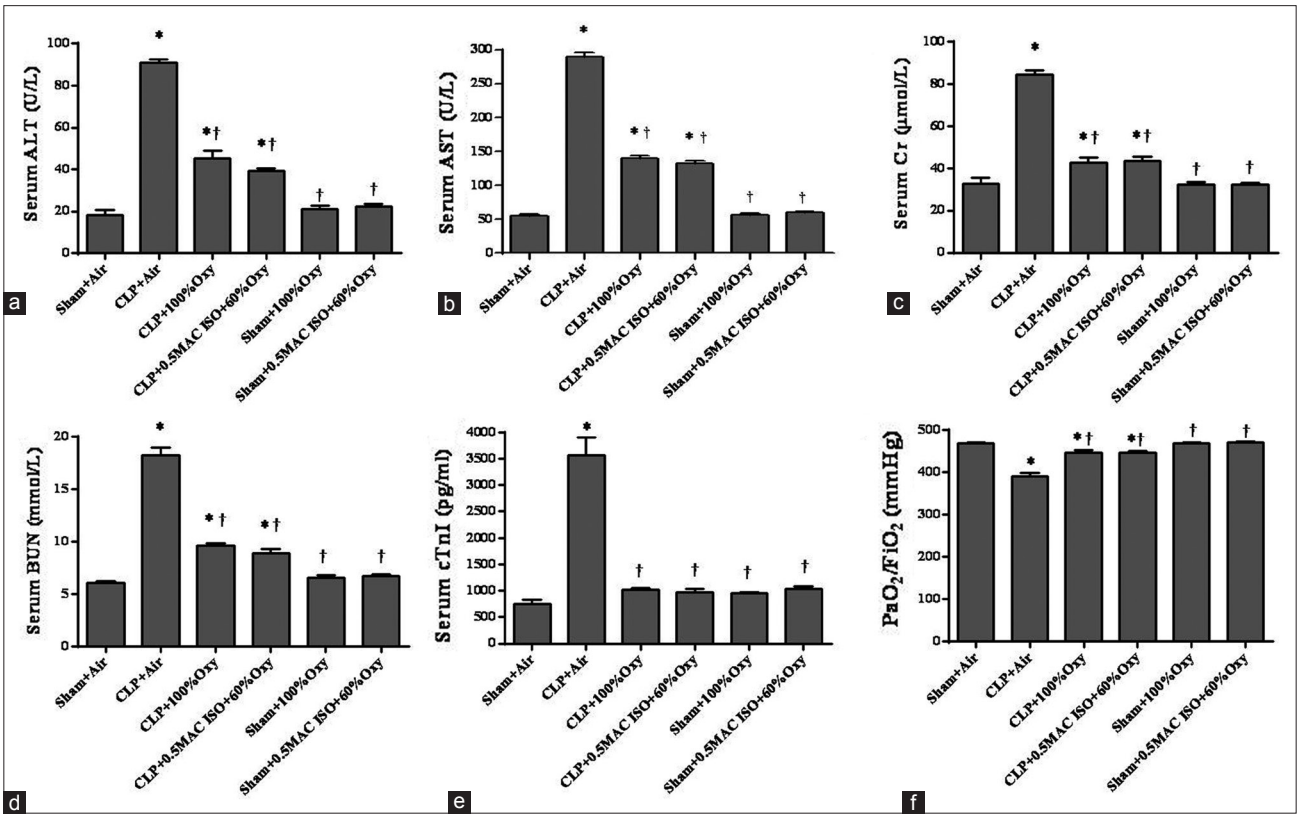

Figure 6: Assessment of organ function in cecal ligation and puncture-challenged rats with or without treatment of 0.5 minimum alveolar concentration isoflurane in 60% oxygen. Treatment was performed in SD rats by inhalation of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after CLP, respectively. Parameters for the assessment of heart, lung, liver, and kidney functions were examined 24 h after CLP. (a) Serum ALT levels. (b) Serum AST levels. (c) Serum Cr levels. (d) Serum BUN levels. (e) Serum cTnI levels. (f) Oxygenation parameters. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus Sham+Air group; †$P < 0.05$ versus CLP+Air group. 1 mmHg = 0.133 kPa. SD: Sprague-Dawley; MAC: Minimum alveolar concentration; CLP: Cecal ligation and puncture; FiO_2: Fraction of inspired oxygen; ISO: Isoflurane; Oxy: Oxygen; PaO_2: Arterial partial pressure of oxygen; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Cr: Creatinine; BUN: Blood urea nitrogen; cTnI: Cardiac troponin I.

(64.0% vs. 15.0%, $P < 0.05$) or 0.5 MAC isoflurane in 60% oxygen (74.4% vs. 15.0%, $P < 0.05$), 0.5 MAC isoflurane in 80% oxygen (54.2% vs. 15.0%, $P < 0.05$) [Figure 2c]. In zymosan-challenged ICR/Km mice, the 0.5 MAC isoflurane in 60% oxygen demonstrated the most effective combination of oxygen and isoflurane for improving the 7-day survival of animals (75.0% vs. 30.0%, $P < 0.05$) [Figure 2d]. We also observed that the 0.5 MAC isoflurane in 60% oxygen significantly attenuated lung inflammation and lung injury in zymosan-challenged animals [Figures 4 and 5]. These findings revealed that combined administration of 0.5 MAC isoflurane with 60% oxygen is the best combination of oxygen and isoflurane for protecting against sepsis induced by LPS or zymosan. Furthermore, 0.5 MAC isoflurane in 60% oxygen protected heart, liver, and kidney function in animals with experimental sepsis [Figures 6 and 7].

The 0.5 minimum alveolar concentration isoflurane in 60% oxygen inhibited peritonitis and systemic inflammation after cecal ligation and puncture

We determined changes of proinflammatory cytokines TNF-α, IL-1β, IL-6, and HMGB1 in serum or peritoneal lavage fluids 24 h after CLP in rats. CLP induced increases in TNF-α (450.7 vs. 116.7 pg/ml, 229.7 vs. 90.8 pg/ml, all $P < 0.05$), IL-1β (96.7 vs. 38.5 pg/ml, 20.6 vs. 12.0 pg/ml, all $P < 0.05$), IL-6 (722.5 vs. 290.4 pg/ml, 116.1 vs. 34.5 pg/ml, all $P < 0.05$), and HMGB1 levels (985.4 vs. 364.7 ng/ml, 449.3 vs. 215.0 ng/ml, all $P < 0.05$) in serum and peritoneal lavage fluids, respectively [Figure 8]. Both treatments with 100% oxygen and 0.5 MAC isoflurane in 60% oxygen significantly reduced the levels of these cytokines in serum and peritoneal lavage fluid in CLP-challenged animals; for 0.5 MAC isoflurane in 60% oxygen treatment, TNF-α (302.7 vs. 450.7 pg/ml, 149.3 vs. 229.7 pg/ml, all $P < 0.05$), IL-1β (51.7 vs. 96.7 pg/ml, 12.5 vs. 20.6 pg/ml, all $P < 0.05$), IL-6 (390.4 vs. 722.5 pg/ml, 86.1 vs. 116.1 pg/ml, all $P < 0.05$), and HMGB1 levels (592.2 vs. 985.4 ng/ml, 323.7 vs. 449.3 ng/ml, all $P < 0.05$) in serum and peritoneal lavage fluids were significantly inhibited, respectively. These results suggested that 0.5 MAC isoflurane in 60% oxygen inhibited peritonitis and systemic inflammation induced by CLP.

Nuclear factor kappa B signaling pathway participated in the protective action against sepsis by 0.5 minimum alveolar concentration isoflurane in 60% oxygen in RAW264.7 cells

An *in vitro* sepsis was induced in RAW264.7 macrophages by 100 ng/ml LPS for at least 2 h based on our preliminary

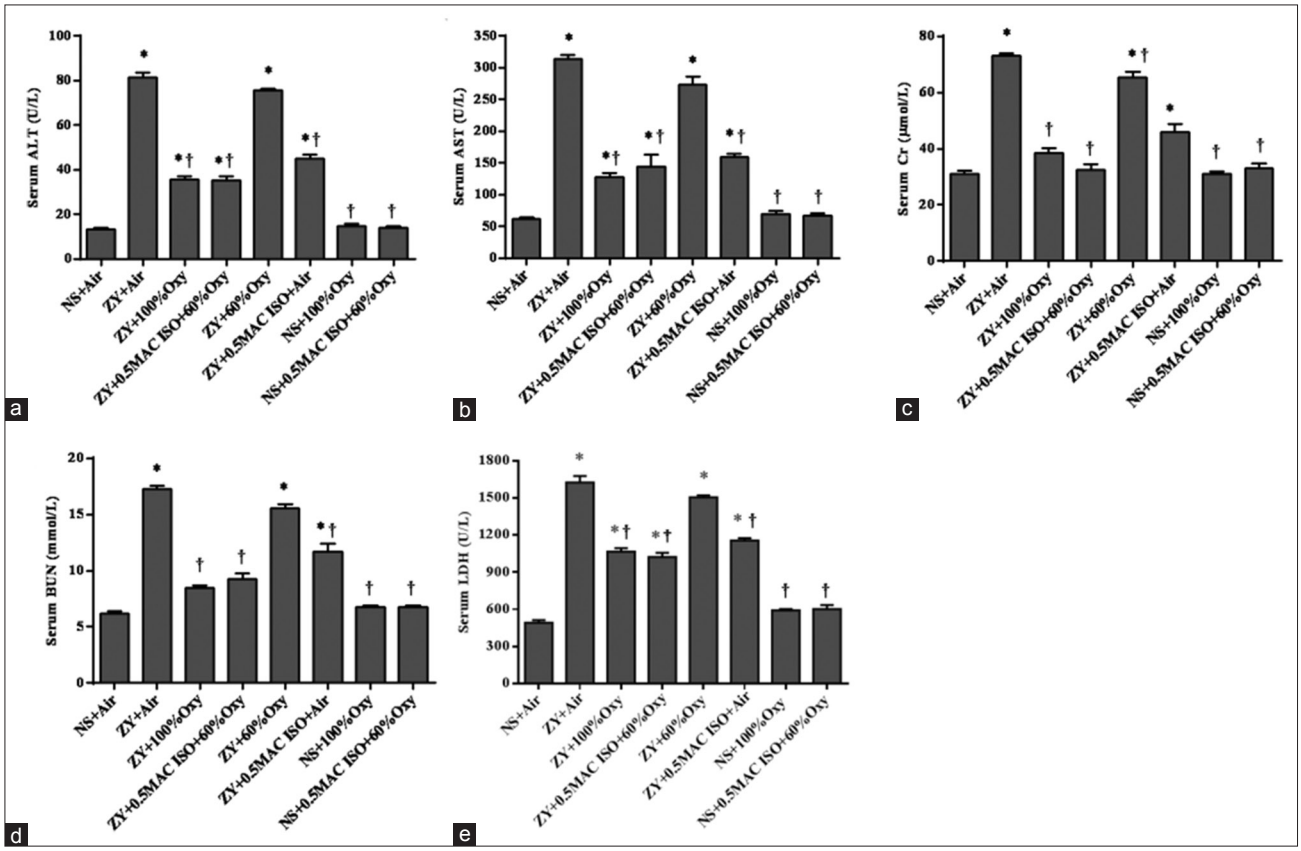

Figure 7: Serum levels of biochemical parameters in zymosan-challenged mice with or without treatment with 0.5 minimum alveolar concentration isoflurane in 60% oxygen. ICR/Km mice were treated with 100% oxygen, 60% oxygen, 0.5 MAC isoflurane, or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after LPS/NS injection, respectively. Serum levels of biochemical parameters were examined 24 h after zymosan/NS injection. (a) Serum ALT levels. (b) Serum AST levels. (c) Serum Cr levels. (d) Serum BUN levels. (e) Serum LDH levels. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus NS+Air group; †$P < 0.05$ versus ZY+Air group. MAC: Minimum alveolar concentration; LPS: Lipopolysaccharide; NS: Normal saline; ISO: Isoflurane; Oxy: Oxygen; ZY: Zymosan; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Cr: Creatinine; BUN: Blood urea nitrogen; LDH: Lactic dehydrogenase.

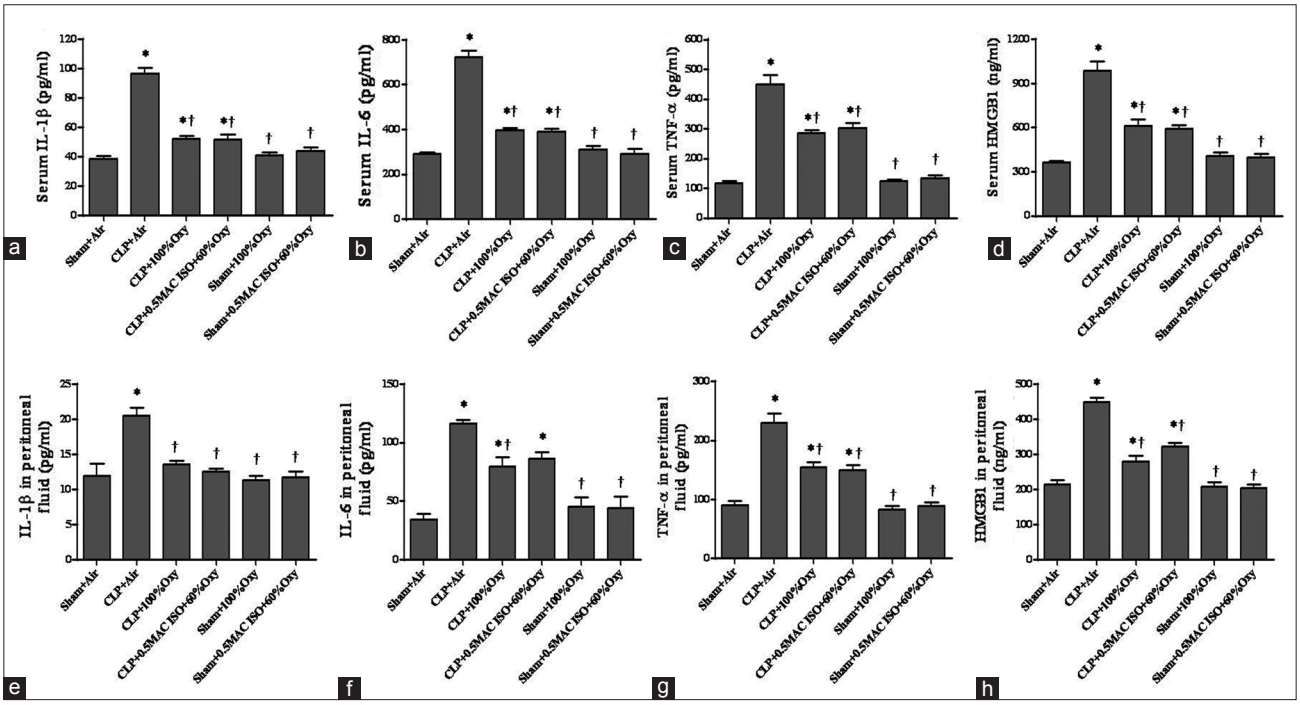

Figure 8: Treatment with 0.5 minimum alveolar concentration isoflurane in 60% oxygen attenuated increases of pro-inflammatory cytokines in serum or peritoneal lavage fluid in cecal ligation and puncture challenged rats. Treatment was performed in SD rats by inhalation of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 1 h at 1 and 6 h after CLP, respectively. The levels of inflammatory factors in serum and peritoneal lavage fluid were examined 24 h after CLP. (a) IL-1β in serum. (b) IL-6 in serum. (c) TNF-α in serum. (d) HMGB1 in serum. (e) IL-1β in peritoneal lavage fluid. (f) IL-6 in peritoneal lavage fluid. (g) TNF-α in peritoneal lavage fluid. (h) HMGB1 in peritoneal lavage fluid. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus Sham+Air group; †$P < 0.05$ versus CLP+Air group. SD: Sprague-Dawley; MAC: Minimum alveolar concentration; CLP: Cecal ligation and puncture; ISO: Isoflurane; Oxy: Oxygen; IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; HMGB1: High-mobility group box 1.

results as shown in Figure 9. Cells were treated by exposure to 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 2 h initiated either simultaneously or at 1 h after the addition of LPS. A significant increase in TNF-α in the cell culture supernatant was observed at least 2 h after LPS stimulation (TNF-α, 195.7 vs. 65.4 pg/ml, 294.5 vs. 121.2 pg/ml, all $P < 0.05$), which was inhibited by 100% oxygen (TNF-α, 96.5 vs. 195.7 pg/ml, 155.1 vs. 294.5 pg/ml, all $P < 0.05$) or 0.5 MAC isoflurane in 60% oxygen (TNF-α, 89.5 vs. 195.7 pg/ml, 134.1 vs. 294.5 pg/ml, all $P < 0.05$) started simultaneously or at 1 h after the addition of LPS. We also found that LPS stimulation induced a significant increase in nuclear NF-κB p65 subunit expression, which was partly reversed by treatment with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. LPS stimulation led to nuclear translocation of NF-κB p65 subunit, which was blocked by 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. We further detected changes of p-IKKα/β, p-IκBα, and p-p65 protein expression in LPS-stimulated RAW264.7 cells with or without treatment of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. LPS stimulation induced higher expression levels of p-IKKα/β, p-IκBα, p-p65 proteins at cellular levels, which were inhibited by treatment of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. The above results indicated that 0.5 MAC isoflurane in 60% oxygen reduces the inflammatory responses in RAW264.7 cells by inhibiting activation of NF-κB signaling pathway [Figure 10].

The 0.5 minimum alveolar concentration isoflurane in 60% oxygen protected human peripheral blood mononuclear cells against *in vitro* sepsis induced by lipopolysaccharide or by plasma from septic patients

In LPS-stimulated human PBMCs, TNF-α (92.4 vs. 43.5 pg/ml, $P < 0.05$) and IL-1β (55.7 vs. 25.0 pg/ml, $P < 0.05$) concentrations in the cell culture supernatant were increased after the addition of LPS, which were inhibited significantly by 100% oxygen (TNF-α, 45.0 vs. 92.4 pg/ml. IL-1β, 14.7 vs. 55.7 pg/ml, all $P < 0.05$) or 0.5 MAC isoflurane in 60% oxygen (TNF-α, 52.0 vs. 92.4 pg/ml. IL-1β, 16.6 vs. 55.7 pg/ml, all $P < 0.05$) [Figure 11a], and LPS stimulation also induced the nuclear translocation of NF-κB p65 subunit, which was blocked by 100% oxygen or 0.5 MAC isoflurane in 60% oxygen [Figure 11b]. In human PBMCs stimulated by plasma from septic patients, plasma from septic patients also led to nuclear translocation of NF-κB p65 subunit, which was also blocked by 100% oxygen or 0.5 MAC isoflurane in 60% oxygen [Figure 11c]. The above findings revealed that 0.5 MAC isoflurane in 60% oxygen reduces the inflammatory responses in human PBMCs by inhibiting nuclear translocation of NF-κBp65 subunit.

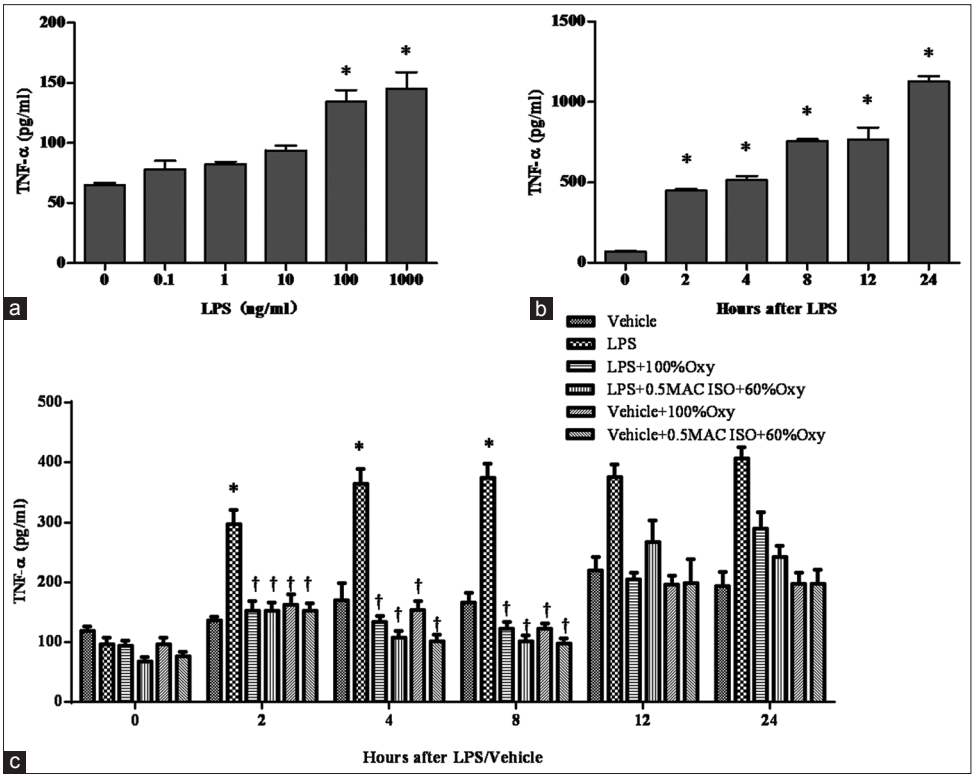

Figure 9: Changes in tumor necrosis factor-alpha concentrations of cell culture supernatant from lipopolysaccharide stimulated RAW264.7 cells with or without treatment with 100% oxygen or 0.5 minimum alveolar concentration isoflurane in 60% oxygen. TNF-α concentration in the culture supernatant was detected using an enzyme-linked immunosorbent assay kit. (a) Dose-dependent effects of LPS stimulation for 24 h on TNF-α concentrations. TNF-α level was increased significantly after the stimulation with 100 ng/ml and 1000 ng/ml LPS. (b) Time-dependent effects of 100 ng/ml LPS on TNF-α concentrations. TNF-α level was increased significantly at least 2 h by 100 ng/ml LPS. (c) Treatment with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen significantly inhibited LPS-induced increases of TNF-α concentrations in cell culture supernatants. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus vehicle group; †$P < 0.05$ versus LPS group. TNF-α: Tumor necrosis factor-alpha; LPS: Lipopolysaccharide; MAC: Minimum alveolar concentration; ISO: Isoflurane; Oxy: Oxygen.

Discussion

Sepsis is a complex pathology that arises from deregulated host inflammatory responses to systemic bacterial infection.[1] To date, animal models of sepsis have been used as part of the development of novel therapeutic agents. In the present study, we found the 0.5 MAC isoflurane in 60% oxygen was the best combination of oxygen and isoflurane for reducing organ damage, especially lung injury and mortality resulting from sepsis caused by CLP, intraperitoneal injection of LPS, or zymosan. The 0.5 MAC isoflurane in 60% oxygen also significantly inhibited lung inflammation, peritonitis, and systemic inflammation to sepsis. The systemic inflammatory responses and the consequent multiple organ failure syndrome are the most severe manifestations of bacterial infections.[30] CLP, which is a murine model of bacterial peritonitis, has been regarded as the "gold standard" animal model of sepsis.[31-34] Bacterial LPS is the major component of the outer membrane of Gram-negative bacteria which are considered one of the predominant causative organisms in sepsis; in experimental animals, LPS challenge leads to pathophysiological changes similar to the human septic shock syndrome.[35] Zymosan, a substance derived from the cell wall of the yeast *Saccharomyces cerevisiae*, has been used as a tool to induce animal models with sterile sepsis/multiple organ dysfunction syndrome (MODS) in many studies.[9,13,36] Several clinical trials have demonstrated a mortality ranging from 40% to 75% in patients with MODS arising from sepsis.[37] In earlier studies, hyperoxia treatment was reported to preserve gut morphology and to improve gut barrier function, thus decreasing the amount of bacterial translocation.[8-10] In addition, the beneficial effects of safe subtoxic regimens of normobaric hyperoxia were observed in various animal models of sepsis including gut-derived mouse sepsis,[38] zymosan-induced mouse sterile sepsis,[9] and early hyperdynamic porcine fecal peritonitis.[10] However, prolonged exposure to hyperoxia leads to the generation of excessive ROS, which can cause acute inflammatory lung injury.[20-23] Some investigators observed that anesthetic dose isoflurane protects animals against septic shock.[12,39,40] However, in sheep with LPS-induced sepsis, isoflurane anesthesia blunts cardiovascular compensatory mechanisms and aggravates lung and renal dysfunction in sepsis,[15-17] which indicates that the use of anesthetic dose isoflurane in critically ill patients has serious adverse consequences on outcome. Recently, a number of trials have demonstrated the safety of long-time lower doses of isoflurane for ICU sedation.[18,19] The above statements supported the protective

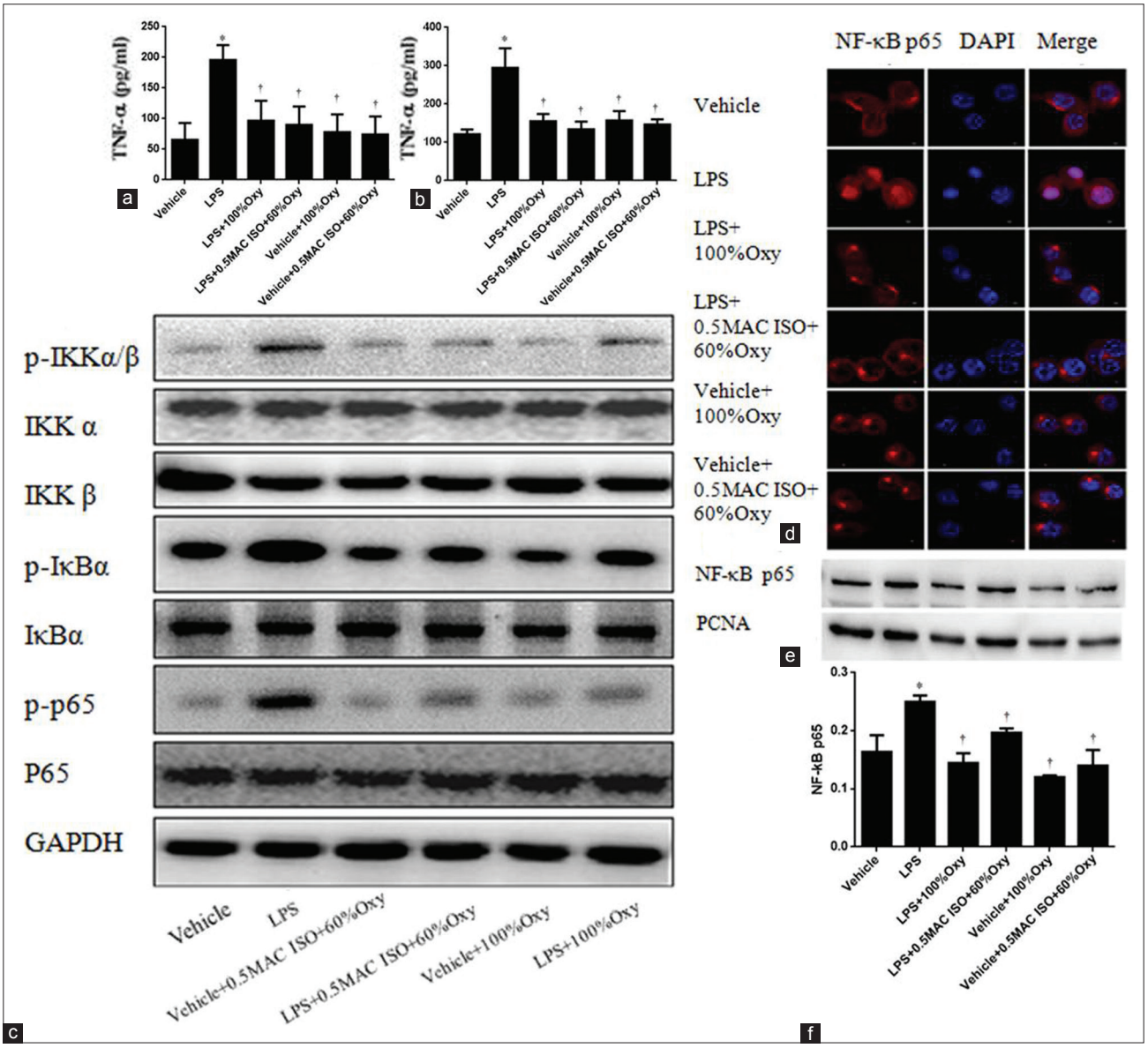

Figure 10: Treatment with 0.5 minimum alveolar concentration isoflurane in 60% oxygen protected against lipopolysaccharide induced *in vitro* sepsis through inhibition to activation of nuclear factor kappa B pathway. An *in vitro* sepsis was induced in the RAW264.7 macrophage cell line through incubation with 100 ng/ml LPS for at least 2 h. Cells were treated with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 2 h beginning 1 h after LPS stimulation or simultaneously with LPS stimulation. TNF-α concentration in the culture supernatant was detected using an enzyme-linked immunosorbent assay kit. Expression of NF-κB p65 subunit protein in cell nuclei was examined by Western blot. The expression of PCNA in cell nuclei was used as the control. Expressions of cellular p-IKKα/β, p-IκBα, p-p65, as well as IKKα/β, IκBα, p65 and GAPDH proteins were determined by Western blot. Of all, the expressions of IKKα/β, IκBα, p65, and GAPDH in cell were used as the controls. (a) TNF-α concentration in the culture supernatant of cells treated with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 2 h applied simultaneously with LPS stimulation. (b) TNF-α concentration in the culture supernatant in cells treated with 100% oxygen or 0.5 MAC isoflurane in 60% oxygen for 2 h applied 1 h after LPS stimulation. (c) Expressions of cellular p-IKKα/β, p-IκBα, p-p65, as well as IKKα/β, IκBα, p65, and GAPDH proteins. Each represents three independent experiments. (d) Fluorescence images of the nuclear translocation of the NF-κB p65 subunit captured using a confocal laser scanning microscope. (e and f) Expression of NF-κB p65 subunit protein in cell nuclei. Data are expressed as the mean ± standard error ($n = 6$ in each group). *$P < 0.05$ versus vehicle group; †$P < 0.05$ versus LPS group. LPS: Lipopolysaccharide; MAC: Minimum alveolar concentration; NF-κB: Nuclear factor kappa B; PCNA: Proliferating cell nuclear antigen; p-IKKα/β: Phosphorylated IκB kinaseα/β; p-IκBα: Phosphorylated inhibitor of nuclear factor kappa Bα; p-p65: Phosphorylated p65 subunit of nuclear factor kappa B; IKKα/β: IκB kinaseα/β; IκBα: Inhibitor of nuclear factor kappa Bα; p65: p65 subunit of nuclear factor kappa B; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; TNF-α: Tumor necrosis factor-alpha; DAPI: 4',6-diamidino-2-phenylindole; ISO: Isoflurane; Oxy: Oxygen.

effects on sepsis by the 0.5 MAC isoflurane in 60% oxygen which would be a good therapy for sepsis with improved clinical safety and broader clinical applications. However, the mechanism regarding the interaction between oxygen and isoflurane remains to be further studied. The above results suggested that interaction existed in the protective effects on sepsis by oxygen and by isoflurane. The interaction between oxygen and isoflurane was also reported in a recent study.[41]

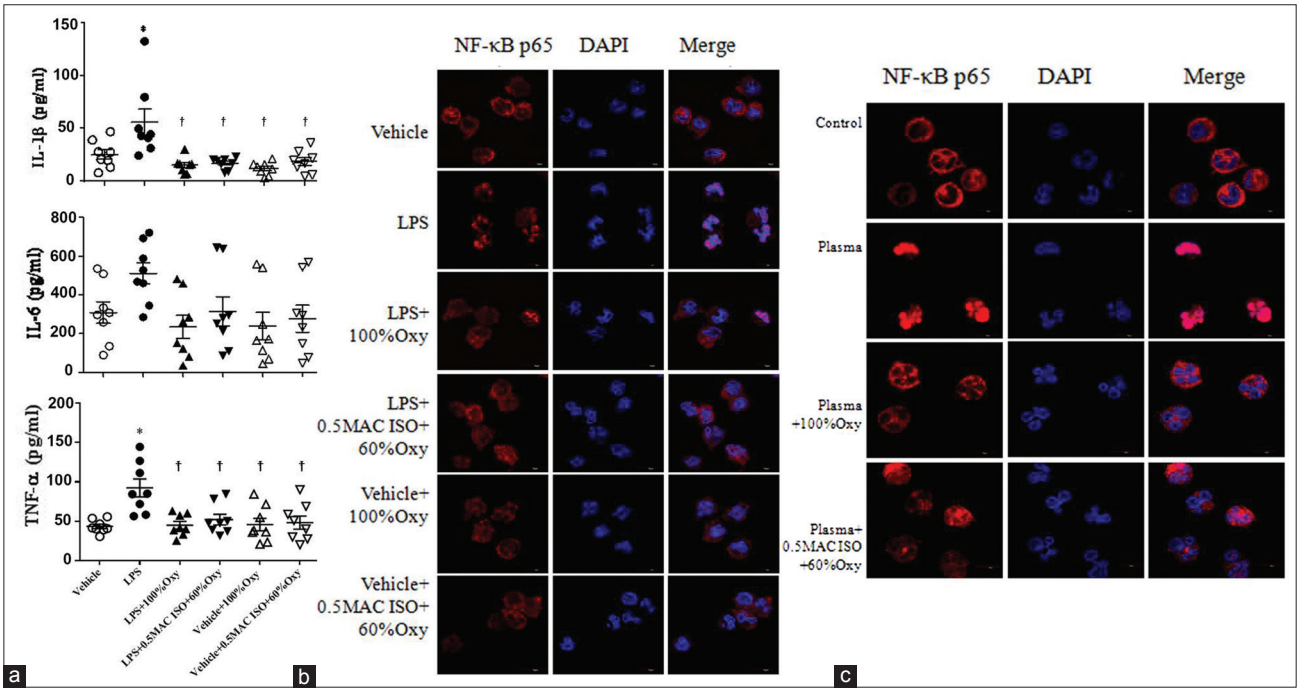

Figure 11: The 0.5 minimum alveolar concentration isoflurane in 60% oxygen protected human peripheral blood mononuclear cells against *in vitro* sepsis induced by lipopolysaccharide or plasma from septic patients. An *in vitro* sepsis was induced in human PBMCs through incubation with 100 ng/ml LPS or by plasma from patients with sepsis. Cells were treated with 100% oxygen or 0.5 MAC isofluranein 60% oxygen for 2 h applied 1 h after the challenge with LPS or plasma. (a) IL-1β, IL-6, and TNF-α concentrations in the cell culture supernatant. Data are expressed as the mean ± standard error ($n = 8$ in each group). *$P < 0.05$ versus vehicle group; †$P < 0.05$ versus LPS group. (b and c) Fluorescence images of the nuclear translocation of the NF-κB p65 subunit were captured using a confocal laser scanning microscope in *in vitro* sepsis induced by lipopolysaccharide or plasma from septic patients, respectively (original magnification ×240). PBMC: Peripheral blood mononuclear cell; LPS: Lipopolysaccharide; MAC: Minimum alveolar concentration; IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; DAPI: 4',6-diamidino-2-phenylindole; ISO: Isoflurane; NF-κB: Nuclear factor kappa B; Oxy: Oxygen.

Blood monocytes and macrophages serve as the first line of host defense and are equipped to recognize and respond to infection by triggering an immune-inflammatory response in sepsis.[42] The present study demonstrated that the 0.5 MAC isoflurane in 60% oxygen also protected RAW264.7 cells and human PBMCs against *in vitro* sepsis induced by LPS or plasma from septic patients. The NF-κB pathway is central to the regulation of inflammation. In sepsis, activation of NF-κB through its translocation to the nucleus leads to significant circulating levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.[43-45] Moreover, the severity of sepsis is correlated with the elevation of HMGB1.[46] Our results showed that LPS stimulation led to nuclear translocation of NF-κB p65 subunit in RAW264.7 macrophages and in human PBMCs, and plasma from septic patients also led to nuclear translocation of NF-κB p65 subunit in human PBMCs, which were blocked by 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. We also found that LPS stimulation induced higher expressions of phospho-IKKα/β, phospho-IκBα, and phospho-p65 proteins in RAW264.7 macrophages, which were inhibited by treatment of 100% oxygen or 0.5 MAC isoflurane in 60% oxygen. According to the literature,[47-49] the increased NF-κB activity and its polymorphism are associated with increased mortality in patients with sepsis, and the heightened expression of phospho-IκBα is believed as an indicator of NF-κB activation in sepsis monocytes.[42] Earlier studies reported that inhalation of 70% oxygen (48 h) fails to attenuate markers of lung inflammation, while intermittent 100% oxygen exerts favorable effects on markers of inflammation.[50] Isoflurane anesthesia was also reported to have an inhibitory effect on renal inflammation and systemic inflammation both in murine septic peritonitis[12,39] and in rat endotoxemia.[50] The above-mentioned findings demonstrated the important role of NF-κB inflammatory pathway in the sepsis protective effects of 0.5 MAC isoflurane in 60% oxygen, thus providing evidence for the safety of clinical application of combined administration of 0.5 MAC isoflurane with 60% oxygen.

In conclusion, combined administration of 0.5 MAC isoflurane with 60% oxygen is the optimal combination of oxygen and isoflurane for reducing lung injury and mortality of septic animals. This combination reduces inflammatory responses to sepsis in animals and human PMBCs, suggesting that combined therapy with a sedative dose isoflurane and 60% oxygen would be a novel, safer, and effective therapeutic measure for septic patients.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

References

1. Hotchkiss RS, Coopersmith CM, McDunn JE, Ferguson TA. The sepsis seesaw: Tilting toward immunosuppression. Nat Med 2009;15:496-7. doi: 10.1038/nm0509-496.
2. Huet O, Chin-Dusting JP. Septic shock: Desperately seeking treatment. Clin Sci (Lond) 2014;126:31-9. doi: 10.1042/cs20120668.
3. ProCESS Investigators, Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, *et al.* A randomized trial of protocol-based care for early septic shock. N Engl J Med 2014;370:1683-93. doi: 10.1056/NEJMoa1401602.
4. Seymour CW, Cooke CR, Heckbert SR, Spertus JA, Callaway CW, Martin-Gill C, *et al.* Prehospital intravenous access and fluid resuscitation in severe sepsis: An observational cohort study. Crit Care 2014;18:533. doi: 10.1186/s13054-014-0533-x.
5. Wallet F, Loïez C, Herwegh S, Courcol RJ. Usefulness of real-time PCR for the diagnosis of sepsis in ICU-acquired infections. Infect Disord Drug Targets 2011;11:348-53.
6. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369:2063. doi: 10.1056/NEJMc1312359.
7. National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network, Wheeler AP, Bernard GR, Thompson BT, *et al.* Pulmonary-artery versus central venous catheter to guide treatment of acute lung injury. N Engl J Med 2006;354:2213-24. doi: 10.1056/NEJMoa061895.
8. Calzia E, Asfar P, Hauser B, Matejovic M, Ballestra C, Radermacher P, *et al.* Hyperoxia may be beneficial. Crit Care Med 2010;38 10 Suppl: S559-68. doi: 10.1097/CCM.0b013e3181f1fe70.
9. Hou L, Xie K, Li N, Qin M, Lu Y, Ma S, *et al.* 100% oxygen inhalation protects against zymosan-induced sterile sepsis in mice: The roles of inflammatory cytokines and antioxidant enzymes. Shock 2009;32:451-61. doi: 10.1097/SHK.0b013e31819c391a.
10. Barth E, Bassi G, Maybauer DM, Simon F, Gröger M, Oter S, *et al.* Effects of ventilation with 100% oxygen during early hyperdynamic porcine fecal peritonitis. Crit Care Med 2008;36:495-503. doi: 10.1097/01.ccm.0b013e318161fc45.
11. Waisman D, Brod V, Rahat MA, Amit-Cohen BC, Lahat N, Rimar D, *et al.* Dose-related effects of hyperoxia on the lung inflammatory response in septic rats. Shock 2012;37:95-102. doi: 10.1097/SHK.0b013e3182356fc3.
12. Lee HT, Emala CW, Joo JD, Kim M. Isoflurane improves survival and protects against renal and hepatic injury in murine septic peritonitis. Shock 2007;27:373-9. doi: 10.1097/01.shk.0000248595.17130.24.
13. Mu J, Xie K, Hou L, Peng D, Shang L, Ji G, *et al.* Subanesthetic dose of isoflurane protects against zymosan-induced generalized inflammation and its associated acute lung injury in mice. Shock 2010;34:183-9. doi: 10.1097/SHK.0b013e3181cffc3f.
14. Bedirli N, Demirtas CY, Akkaya T, Salman B, Alper M, Bedirli A, *et al.* Volatile anesthetic preconditioning attenuated sepsis induced lung inflammation. J Surg Res 2012;178:e17-23. doi: 10.1016/j.jss.2011.12.037.
15. Wagner F, Radermacher P, Stahl W. Anesthesia and the immune response: Evidence for an "isoflurane paradox"? Shock 2010;34:437-8. doi: 10.1097/SHK.0b013e3181d883ab.
16. Frithiof R, Soehnlein O, Eriksson S, Fenhammar J, Hjelmqvist H, Lindbom L, *et al.* The effects of isoflurane anesthesia and mechanical ventilation on renal function during endotoxemia. Acta Anaesthesiol Scand 2011;55:401-10. doi: 10.1111/j.1399-6576.2011.02406.x.
17. Soehnlein O, Eriksson S, Hjelmqvist H, Andersson A, Mörgelin M, Lindbom L, *et al.* Anesthesia aggravates lung damage and precipitates hypotension in endotoxemic sheep. Shock 2010;34:412-9. doi: 10.1097/SHK.0b013e3181d8e4f5.
18. Bellgardt M, Bomberg H, Herzog-Niescery J, Dasch B, Vogelsang H, Weber TP, *et al.* Survival after long-term isoflurane sedation as opposed to intravenous sedation in critically ill surgical patients: Retrospective analysis. Eur J Anaesthesiol 2016;33:6-13. doi: 10.1097/eja.0000000000000252.
19. Jerath A, Beattie SW, Chandy T, Karski J, Djaiani G, Rao V, *et al.* Volatile-based short-term sedation in cardiac surgical patients: A prospective randomized controlled trial. Crit Care Med 2015;43:1062-9. doi: 10.1097/ccm.0000000000000938.
20. Folz RJ, Abushamaa AM, Suliman HB. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. J Clin Invest 1999;103:1055-66. doi: 10.1172/jci3816.
21. Altemeier WA, Sinclair SE. Hyperoxia in the Intensive Care Unit: Why more is not always better. Curr Opin Crit Care 2007;13:73-8. doi: 10.1097/MCC.0b013e32801162cb.
22. Rodríguez-González R, Martín-Barrasa JL, Ramos-Nuez Á, Cañas-Pedrosa AM, Martínez-Saavedra MT, García-Bello MÁ, *et al.* Multiple system organ response induced by hyperoxia in a clinically relevant animal model of sepsis. Shock 2014;42:148-53. doi: 10.1097/shk.0000000000000189.
23. Helmerhorst HJ, Roos-Blom MJ, van Westerloo DJ, de Jonge E. Association between arterial hyperoxia and outcome in subsets of critical illness: A systematic review, meta-analysis, and meta-regression of cohort studies. Crit Care Med 2015;43:1508-19. doi: 10.1097/ccm.0000000000000998.
24. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, *et al.* Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013;39:165-228. doi: 10.1007/s00134-012-2769-8.
25. D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste M, *et al.* Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J Exp Med 1992;176:1387-98.
26. Nicholson GC, Malakellis M, Collier FM, Cameron PU, Holloway WR, Gough TJ, *et al.* Induction of osteoclasts from CD14-positive human peripheral blood mononuclear cells by receptor activator of nuclear factor kappaB ligand (RANKL). Clin Sci (Lond) 2000;99:133-40.
27. Aggarwal NR, D'Alessio FR, Tsushima K, Files DC, Damarla M, Sidhaye VK, *et al.* Moderate oxygen augments lipopolysaccharide-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 2010;298:L371-81. doi: 10.1152/ajplung.00308.2009.
28. Jeyaseelan S, Chu HW, Young SK, Worthen GS. Transcriptional profiling of lipopolysaccharide-induced acute lung injury. Infect Immun 2004;72:7247-56. doi: 10.1128/iai.72.12.7247-7256.2004.
29. Gill SE, Taneja R, Rohan M, Wang L, Mehta S. Pulmonary microvascular albumin leak is associated with endothelial cell death in murine sepsis-induced lung injury *in vivo*. PLoS One 2014;9:e88501. doi: 10.1371/journal.pone.0088501.
30. Victor VM, Rocha M, Esplugues JV, De la Fuente M. Role of free radicals in sepsis: Antioxidant therapy. Curr Pharm Des 2005;11:3141-58.
31. Fink MP. Animal models of sepsis and its complications. Kidney Int 2008;74:991-3. doi: 10.1038/ki.2008.442.
32. Remick DG, Newcomb DE, Bolgos GL, Call DR. Comparison of the mortality and inflammatory response of two models of sepsis: Lipopolysaccharide vs. cecal ligation and puncture. Shock 2000;13:110-6.
33. Rittirsch D, Hoesel LM, Ward PA. The disconnect between animal models of sepsis and human sepsis. J Leukoc Biol 2007;81:137-43.

doi: 10.1189/jlb.0806542.
34. Deng D, Li X, Liu C, Zhai Z, Li B, Kuang M, *et al.* Systematic investigation on the turning point of over-inflammation to immunosuppression in CLP mice model and their characteristics. Int Immunopharmacol 2017;42:49-58. doi: 10.1016/j.intimp.2016.11.011.
35. van der Poll T, Opal SM. Host-pathogen interactions in sepsis. Lancet Infect Dis 2008;8:32-43. doi: 10.1016/s1473-3099(07)70265-7.
36. Volman TJ, Hendriks T, Goris RJ. Zymosan-induced generalized inflammation: Experimental studies into mechanisms leading to multiple organ dysfunction syndrome. Shock 2005;23:291-7.
37. Deutschman CS, Tracey KJ. Sepsis: Current dogma and new perspectives. Immunity 2014;40:463-75. doi: 10.1016/j.immuni.2014.04.001.
38. Gennari R, Alexander JW. Effects of hyperoxia on bacterial translocation and mortality during gut-derived sepsis. Arch Surg 1996;131:57-62.
39. Fuentes JM, Talamini MA, Fulton WB, Hanly EJ, Aurora AR, De Maio A. General anesthesia delays the inflammatory response and increases survival for mice with endotoxic shock. Clin Vaccine Immunol 2006;13:281-8. doi: 10.1128/cvi.13.2.281-288.2006.
40. Yin N, Peng Z, Li B, Xia J, Wang Z, Yuan J, *et al*. Isoflurane attenuates lipopolysaccharide-induced acute lung injury by inhibiting ROS-mediated NLRP3 inflammasome activation. Am J Transl Res 2016;8:2033-46.
41. Kim GH, Lee JJ, Lee SH, Chung YH, Cho HS, Kim JA, *et al.* Exposure of isoflurane-treated cells to hyperoxia decreases cell viability and activates the mitochondrial apoptotic pathway. Brain Res 2016;1636:13-20. doi: 10.1016/j.brainres.2016.01.052.
42. Shalova IN, Lim JY, Chittezhath M, Zinkernagel AS, Beasley F, Hernández-Jiménez E, *et al.* Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1α. Immunity 2015;42:484-98. doi: 10.1016/j.immuni.2015.02.001.
43. Endale M, Park SC, Kim S, Kim SH, Yang Y, Cho JY, *et al.* Quercetin disrupts tyrosine-phosphorylated phosphatidylinositol 3-kinase and myeloid differentiation factor-88 association, and inhibits MAPK/AP-1 and IKK/NF-κB-induced inflammatory mediators production in RAW 264.7 cells. Immunobiology 2013;218:1452-67. doi: 10.1016/j.imbio.2013.04.019.
44. Blackwell TS, Yull FE, Chen CL, Venkatakrishnan A, Blackwell TR, Hicks DJ, *et al.* Multiorgan nuclear factor kappa B activation in a transgenic mouse model of systemic inflammation. Am J Respir Crit Care Med 2000;162(3 Pt 1):1095-101. doi: 10.1164/ajrccm.162.3.9906129.
45. Htwe SS, Harrington H, Knox A, Rose F, Aylott J, Haycock JW, *et al.* Investigating NF-κB signaling in lung fibroblasts in 2D and 3D culture systems. Respir Res 2015;16:144. doi: 10.1186/s12931-015-0302-7.
46. Hou LC, Qin MZ, Zheng LN, Lu Y, Wang Q, Peng DR, *et al*. Severity of sepsis is correlated with the elevation of serum high-mobility group box1 in rats. Chin Med J 2009;122:449-54.
47. Paterson RL, Galley HF, Dhillon JK, Webster NR. Increased nuclear factor kappa B activation in critically ill patients who die. Crit Care Med 2000;28:1047-51.
48. Böhrer H, Qiu F, Zimmermann T, Zhang Y, Jllmer T, Männel D, *et al.* Role of NFkappaB in the mortality of sepsis. J Clin Invest 1997;100:972-85. doi: 10.1172/jci119648.
49. Adamzik M, Schäfer S, Frey UH, Becker A, Kreuzer M, Winning S, *et al.* The NFKB1 promoter polymorphism (-94ins/delATTG) alters nuclear translocation of NF-κB1 in monocytes after lipopolysaccharide stimulation and is associated with increased mortality in sepsis. Anesthesiology 2013;118:123-33. doi: 10.1097/ALN.0b013e318277a652.
50. Waisman D, Brod V, Wolff R, Sabo E, Chernin M, Weintraub Z, *et al.* Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2003;285:H643-52. doi: 10.1152/ajpheart.00900.2002.

Supplementary Table 1: Basic information and signs of patients with sepsis

Characteristics	Case 1	Case 2	Case 3
Hospital number	B85313	B86324	B86505
Gender	Female	Male	Female
Age (years)	44	77	49
Temperature (°C)	37	36	36.5
Heart rate (beats/min)	112	72	104
Respiratory rate (beats/min)	26	15	26
Mental status	Sober	Sober	Sober
Plasma glucose (mmol/L)	5.60	5.61	5.78
Leukocyte ($\times 10^9/mm^3$)	11.92	5.62	7.67
CRP (mg/L)	20.9	–	32.5
PCT (ng/ml)	–	–	–
Platelet ($\times 10^9/mm^3$)	327	155	227
Blood pressure (mmHg)	94/49	134/92	149/79
Creatinine level (μmol/L)	141	116	–
Coagulation (INR)	1.05	1.47	1.15
Bowel sounds	Normal	Normal	Normal
Plasma total bilirubin (μmol/L)	–	26.9	11.2
PaO_2 (mmHg)	47.2	63.7	32.6
$PaCO_2$ (mmHg)	40.3	36.5	79.2
BE (mmol/L)	5	–1	12.7
Lactate (mmol/L)	1.17	1.19	1.22
Diagnosis	Severe pneumonia Respiratory failure Chronic obstructive pulmonary disease with acute exacerbation Electrolyte imbalance Acute renal failure	Type 1 respiratory failure Pulmonary infection Stage 3 hypertension Renal dysfunction Abnormal liver function	Bronchiectasis with infection Type 2 respiratory failure NYHA functional Class III Hypoalbuminemia

1 mmHg = 0.133 kPa. CRP: C-reactive protein; PCT: Procalcitonin; INR: International normalized ratio; $PaCO_2$: Arterial blood partial pressure of carbon dioxide; PaO_2: Arterial partial pressure of oxygen; BE: Base excess; NYHA: New York Heart Association.

Supplementary Table 2: Basic information of patients without sepsis

Case	Sampling date	Gender	Age (years)	Hospitalization number	Diagnosis
1	July 31, 2014	Male	41	B88233	Thoracic 12 spinal nerve sheath tumors
2	July 31, 2014	Female	69	B89937	Left knee degenerative joint disease; left knee flexion contracture
3	August 4, 2014	Female	51	B91431	Left knee degenerative joint disease
4	August 5, 2014	Female	65	B86689	Lumbar disc herniation, type 2 diabetes; left leg varicose veins
5	August 7, 2014	Female	70	B92047	Double knee degenerative joint disease; Stage 2 hypertension
6	August 8, 2014	Female	40	B92305	Bilateral femoral head necrosis; hypertension, nephritis
7	August 11, 2014	Male	43	B93003	Lumbar disc herniation
8	August 11, 2014	Female	63	B91859	Lumbar spinal stenosis, lumbar disc herniation, Stage 1 hypertension