Sevoflurane improves gaseous exchange and exerts protective effects in lipopolysaccharide-induced lung injury in mice models

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INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) cases are seen frequently in intensive care units (ICUs) [1]. Although there has been tremendous progress in science and technology, and new therapeutic methods have been introduced, ARDS-associated mortality still remains very high (30 – 40 %) [1-3]. So far, only low-tidal-volume ventilation has been reported to positively affect mortality in ARDS. Patients requiring...
mechanical ventilation in ICUs are often administrated intravenous sedatives which include but are not limited to propofol and midazolam [4,5]. However, with recent advancements in science, it has become possible to sedate the patients through volatile anesthesia with the help of Anesthetic Conserving Device [6].

 Unlike intravenous sedatives, volatile anesthetics exhibit a number of beneficial effects which improve the health of the patient. Volatile anesthetics have also been reported to exhibit anti-inflammatory activities [6–9]. Sevoflurane, which is one of the commonly used sedatives acts as pre-conditioning as well as post-conditioning agent [10] by the protecting organs in ALI models through inhibition of pro-inflammatory expressions. Moreover, ALI leads to increased accretion of ROS and myeloperoxidase (MPO), and reduces the levels of ROS scavengers such as GSH and superoxide dismutase (SOD), which confer protection against oxidative damage in vivo [11]. Information related to the immune-modulatory activity of volatile anesthetics comes mostly from ischemia–reperfusion injury studies. In the present study, the effect of sevoflurane on gaseous exchange, antioxidant defense system and LPS-induced lung tissue injuries was investigated in a mice model of ALI.

EXPERIMENTAL

Experimental animals

Seven week-old female BalB/C mice weighing 21 ± 2 g were used. The mice had free access to pellet diet and drinking water, and were housed in well-ventilated rooms with controlled light/dark cycle, at a temperature of 24 ± 2 °C and humidity of 40 - 62 %. The animal protocols for the study were approved by the animal ethical committee of Shandong Provincial Hospital to Shandong University (approval no. SPH-009876/2016/A45), and international guidelines for animal/human studies were followed [12].

Animal grouping and acute lung injury model

The mice were randomly divided into 5 groups (10 mice / group): group I consisted of normal control mice that were administered normal saline, while group II mice received sevoflurane (300 μL) only, dissolved in phosphate-buffered saline (PBS). Group III mice were administered LPS only (0.5 mg/kg, dissolved in saline); mice in group IV received LPS plus sevoflurane, while group V mice were administered LPS (0.5 mg/kg body weight) + dexamethasone (5 mg/kg body weight dissolved in saline). Sevoflurane was given with the aid of AnaCoDa system. The sevoflurane expiratory content was estimated with a multi-gas analyzer and the content of sevoflurane was 1–2 vol % of the gas.

Lung tissue and BALF extraction

Extractions of broncho-alveolar lavage fluid (BALF) and lung tissue were carried out as reported earlier [13]. The lung tissues were frozen in liquid nitrogen, or fixed in situ at 25 cm H2O with 4 % paraformaldehyde prior to analysis.

Determination of lung water content and cell count

To determine lung water content, the lung tissues were first weighed and then dried in line with the protocol described previously [14]. The lung water content was calculated as the ratio of H2O to body weight. The cell count of the pooled lung lavage fluid was determined by hemocytometer (n = 4). Cells in the BALF were pelleted by centrifugation at 1,000 x g for 15 min, and the protein content of the cell-free BALF was determined by the bicinchoninic acid method. The supernatant samples were used immediately or stored at –80 °C for later use.

Cytokine and chemokine analysis

The levels of IL-6, IL-8 and anti-inflammatory cytokine IL-10 were quantified using commercially available kits according to manufacturer’s protocol.

Myeloperoxidase assay

Myeloperoxidase (MPO) activity was determined to evaluate infiltration of neutrophils in the lung tissues of LPS-induced ALI mouse [15-17]. To assay MPO activity, mice lungs were chopped and then homogenized in PBS containing 0.5 % hexadecyl trimethyl ammonium bromide. The cell homogenate was centrifuged, and the supernatant was added with mixing, to phosphate buffer, pH 6.0, 0.167 mg/ml O-dianisidine hydrochloride and 0.0005 % H2O2. The absorbance of the resultant solution was read spectrophotometrically at 460 nm. The MPO activity was then determined and expressed as U/g as described previously [18].

Antioxidant assays

Catalase (CAT), SOD, T-AOC and GSH were determined by well-established protocols as reported previously [19,20].
Histopathological examination

Lungs were PBS-washed two times and fixed in 4% paraformaldehyde overnight at 4 °C. Thereafter, the lung tissues were embedded in paraffin and then sectioned. The sectioned tissues were subjected to hematoxylin and eosin (H&E) staining and examined under a Ti–S bright field microscope [20].

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). One-way ANOVA followed by Tukey’s test were used for statistical analysis with the aid of GraphPad Prism 7 software. The results were considered significant at p < 0.05 and p < 0.01.

RESULTS

Sevoflurane effected gaseous exchange

LPS administration to rats (group III) caused a significant decrease in PaO2/FIO2, when compared to normal rats in groups I and II (Figure 1). The LPS + sevoflurane-treated rats (group IV) had a significantly higher PaO2/FIO2 after 6 h of LPS injury (p < 0.01). However, there were no significant differences between the normal (group I) and sevoflurane-treated (group II) rats.

Effect of sevoflurane on lung water content

To evaluate the effect of sevoflurane on lung edema triggered by LPS, the lung water content was measured 24 h after the intra-nasal administration of LPS (Figure 2). A noticeable rise in water contents of the lungs was observed in group III animals (LPS-treated), relative to group I (saline-treated), and group II mice treated with sevoflurane only. The water contents in the lungs of group IV (LPS + sevoflurane) and group V (LPS + dexamethasone) were more or less similar, but significantly higher than that of group II animals (p < 0.01).

Effect of sevoflurane on lung inflammation and cell count

Lung inflammation in relation to neutrophil counts triggered by LPS was assessed by determining cell profile of BALF 24 h succeeding intra-nasal instillation. The LPS treatment caused high escalation in the total cells in group III animals (Figure 3A) and neutrophil counts (Figure 3B), when compared to group I animals. However, total cells (Figure 3A) and neutrophil counts (Figure 3B) in the sevoflurane-treated mice were similar to that of group I animals. In group IV and group V, the cell counts were more or less similar, but significantly lower (p < 0.01) than in group II animals (Figure 3A and Figure 3B, respectively).
< 0.05 (group I vs group III); #p < 0.01 (group III vs group IV and group V)

**Sevoflurane altered pro-inflammatory and anti-inflammatory cytokines**

Sevoflurane treatment decreased the LPS-induced high levels of IL-6 and IL-8 in the lungs of the ALI model mice (Figure 4). Furthermore, treatment with LPS significantly ($p < 0.05$) reversed the LPS-triggered suppression of IL-10 in the lung tissues in group III animals, relative to group 1 animals. However, the BALF levels of IL-6, IL-8 and IL-10 in group IV and group V were almost similar.

**Sevoflurane modified myeloperoxidase activity**

Neutrophil infiltration was determined by estimating the MPO activity in homogenized lung tissues of ALI mice (Figure 4). It was observed that LPS increased MPO activity at least 2 folds in group III mice, when compared with group I (saline-treated). However, sevoflurane significantly reduced the MPO activity in group IV mice, when compared with group III (LPS-treated) animals. These results suggest that sevoflurane prevents neutrophil migration into the alveolar space.

**Sevoflurane reduced oxidative stress**

The effect of sevoflurane on several anti-oxidant biomarkers, such as GSH/GSSG ratio, T-AOC and the activities of CAT and SOD were evaluated. Significant reductions in GSH/GSSG ratio and activities of T-AOC, CAT and SOD were observed in the LPS-treated mice, when compared with group I (saline-treated) control (Figure 5). Moreover, sevoflurane treatment significantly attenuated the LPS-triggered oxidative stress. However, LPS administration significantly reduced T-AOC (Figure 5B), CAT (Figure 4C) and SOD (Figure 5D) in lung tissue of ALI mice model, but GSH/GSSG ratio remained unaffected (Figure 5A).

**Histological features**

The LPS treatment resulted in diffused interstitial edema, alveolar thickening, extensive leukocyte infiltration into the interstitium and alveoli, and a significant reduction in air spaces of alveoli in group III animals (Figure 6A-D). However, treatment with sevoflurane in group IV significantly attenuated these pathological changes in ALI mice when compared to group III (Figure 6E).
from mice of different groups: Group I (A), Group II (B), Group III (C) Group IV (D) and (E) (H & E, x 200)

DISCUSSION

Sevoflurane is one of the commonly used sedatives and acts as pre-conditioning as well as post-conditioning agent by protecting organs in ALI models through inhibition of pro-inflammatory expressions [10]. In the present study, the effects of sevoflurane on gaseous exchange, antioxidant defense system and LPS-induced lung tissue injuries were investigated in a mice model of ALI. The results indicated that sevoflurane significantly improved gaseous exchange, as was evident from PaO2/FIO2 Lipopolysaccharide (LPS) is often associated with pulmonary edema. Therefore, lung water content was estimated 24 h after the intra-nasal instillation of LPS or saline to mice.

Sevoflurane administration to LPS-treated mice significantly improved water content of the LPS-treated mice when compared to the saline-treated control mice, indicating the role of sevoflurane in relieving lung edema. No significant difference in lung water content was seen between groups IV and V animals that were treated with dexamethasone, which suggests that sevoflurane and dexamethasone exert similar protective effects in ALI mice model. Lung edema, and endothelial/epithelial injuries are associated with influx of neutrophils into the interstitium and broncho-alveolar space. Neutrophils have been reported to play important roles in the progression of ALI and ARDS [22]. This is so because stimulation and transmigration of neutrophils are characteristic steps in the development of ALI and ARDS. Thus, the total cell count and number of neutrophils in the BALF of normal and control mice were monitored. The results also indicated that sevoflurane prevented neutrophil migration into the lung alveolar space.

Several laboratory and clinical studies have reported major roles for an intricate set of inflammatory cytokines and chemokines in the initiation of inflammation-triggered ALI from aspiration, sepsis, pneumonia, and shock [23]. The results obtained in the present study revealed that the pro-inflammatory cytokines (IL-6 and IL-8) were augmented in the LPS groups when compared to control groups, but IL-10 was reduced. The results also indicated that treatment LPS enhanced the levels of MPO, indicating that inflammation triggered by LPS administration plays a significant role in the pathogenesis of lung injury in mice. Myeloperoxidase (MPO) is a widely used marker for neutrophil activity. Oxidative stress also triggers inflammatory responses which in turn induce the generation of ROS. Oxidative stress and inflammation have been reported to be interconnected events both of which are involved in the pathogenesis of ALI [24].

In earlier studies, it was reported that intra-nasal LPS instillation triggered the release of high levels cytokines, chemokines and ROS, thereby inducing in mice ALI which has similar pathological characteristics with human ALI [25]. Loss of homeostasis between pro-oxidants and antioxidants is often linked to the etiology of oxidative stress [26]. Hence, the current research was also designed to further explore the impact of sevoflurane on several antioxidant biomarkers, such as GSH/GSSG ratio, CAT and SOD. Considerable reduction in the GSH/GSSG ratio, CAT and SOD were seen in LPS-challenged mice, relative to control mice. However, the LPS-triggered reduction in these anti-oxidative biomarkers was significantly diminished by sevoflurane administration.

CONCLUSION

Administration of sevoflurane to ALI mice confers protection against acute LPS-induced lung injury. This protective effect may be attributed to its potential anti-oxidant and anti-inflammatory properties. Therefore, the results of the present study point to the potential clinical application of sevoflurane in the prevention of ALI.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

The main experiment was done by Wei-Min Shen under supervision of Yong-Liang Chi. Chen Li did...
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REFERENCES


