Effects of sevoflurane on ventilator induced lung injury in a healthy lung experimental model

A. Romero*, A. Moreno, J. García, C. Sánchez, M. Santos, J. García

Departamento de Anestesiología, Reanimación y Cuidados Críticos, Hospital Universitario Puerta de Hierro-Majadahonda, Majadahonda, Madrid, Spain

Received 10 January 2015; accepted 13 April 2015
Available online 1 October 2015

Abstract
Introduction and objective: Ventilator-induced lung injury (VILI) causes a systemic inflammatory response in tissues, with an increase in IL-1, IL-6 and TNF-α in blood and tissues. Cytoprotective effects of sevoflurane in different experimental models are well known, and this protective effect can also be observed in VILI. The objective of this study was to assess the effects of sevoflurane in VILI.

Material and method: A prospective, randomized, controlled study was designed. Twenty female rats were studied. The animals were mechanically ventilated, without sevoflurane in the control group and sevoflurane 3% in the treated group (SEV group). VILI was induced applying a maximal inspiratory pressure of 35 cmH2O for 20 min without any positive end-expiratory pressure for 20 min (INJURY time). The animals were then ventilated 30 min with a maximal inspiratory pressure of 12 cmH2O and 3 cmH2O positive end-expiratory pressure (time 30 min POST-INJURY), at which time the animals were euthanized and pathological and biomarkers studies were performed. Heart rate, invasive blood pressure, pH, PaO2, and PaCO2 were recorded. The lung wet-to-dry weight ratio was used as an index of lung edema.

Results: No differences were found in the blood gas analysis parameters or heart rate between the 2 groups. Blood pressure was statistically higher in the control group, but still within the normal clinical range. The percentage of pulmonary edema and concentrations of TNF-α and IL-6 in lung tissue in the SEV group were lower than in the control group.

Conclusions: Sevoflurane attenuates VILI in a previous healthy lung in an experimental subclinical model in rats.

© 2015 Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor. Published by Elsevier España, S.L.U. All rights reserved.

* Corresponding author.
E-mail address: antonromero@hotmail.com (A. Romero).
Introduction

Most patients undergoing general anesthesia and a large percentage of patients admitted to intensive care units receive mechanical ventilation in order to alleviate the work of breathing while lung function is restored. Incorrect use of mechanical ventilation can damage the lungs or aggravate an existing lung injury, and give rise to ventilator-induced lung injury (VILI)\(^1\),\(^2\) caused by an amplification and generalization of the systemic inflammatory response (biotrauma). During this inflammatory response, blood and tissue levels of proinflammatory cytokines such as the tumor necrosis factor (TNF-\(\alpha\)), Interleukin (IL) 1\(\beta\), IL-6 and lа IL-8 are increased.\(^3\)

VILI is mainly associated with phenomena such as the use of insufficient positive end-expiratory pressure (PEEP) to prevent cyclic alveolar collapse-reopening (atelectrauma), which increases perialveolar leukocyte infiltration\(^4\); the delivery of high alveolar pressure (barotrauma), which causes alveolar and perivascular edema\(^5\); and the use of high respiratory frequency, due to stress cycles.\(^6\) The damaging effect of high tidal volumes (volutrauma), which can induce overdistension of alveoli, has also been suggested as a cause of VILI.\(^7\),\(^8\)

A classic experimental VILI induction model involves the delivery of cyclic inflation pressures (>30 cmH\(\text{O}\)) at different time intervals.\(^7\)

Volatile anesthetics have been shown to have an anti-inflammatory action.\(^9\) Therefore, volatile anesthetics attenuate ischemia–reperfusion injury in the heart,\(^10\) the kidney,\(^11\) the liver\(^12\) and the lungs,\(^13\),\(^14\) and decrease the inflammatory response in in vivo\(^15\)-\(^17\) and in vitro\(^17\) sepsis-induced pulmonary injury models. Furthermore, recent studies have shown that sevoflurane could act as a pre- and postconditioning agent in endotoxin-induced lung injury models.\(^18\) Nevertheless, no studies have explored the possible protective effect of sevoflurane against VILI in previously healthy lungs.

The aim of this study is to evaluate the effect of sevoflurane on VILI, analyzing histopathological damage, the degree of pulmonary edema, and level of inflammatory cytokines in rats.

Materials and methods

We studied 20 Wistar rats with a mean weight of 215 ± 35 g. The animals were kept in groups of 6 in U-TEMP polyetherimide cages. They were given free access to food and water, with 12 h of light and 12 h of darkness, a temperature of 20 ± 2 °C and a relative humidity of 50–70%. The animals were kept in these conditions for at least 1 week to allow them to acclimatize before starting the experiments. The animals were handled in accordance with European and Spanish laws governing the protection of experimental animals (2010/63/EU and RD 53/2013), and the study was approved by the institutional Animal Experimentation Ethics Committee.
The animals were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and diazepam (5 mg/kg). When the target level of hypnosis had been reached, the animal was intubated using a 16 G polyethylene catheter (Abbott Ireland, Sligo, Republic of Ireland), using the otocope method\(^1\) to connect the catheter to the semi-closed anesthesia breathing system (Julian, Dräger, Lübeck, Germany).

Following this, pressure-controlled mechanical ventilation was started, with a peak inspiratory pressure (PIP) of 12 cmH\(_2\)O, PEEP of 3 cmH\(_2\)O, an FiO\(_2\) of 0.5, and a breathing rate of 30 rpm.

The trachea was dissected by means of a medial incision in the neck. The airway was tied off using suture thread, a line was placed in the left carotid artery (24 G polyethylene catheter, Abbott Ireland) to monitor heart rate (HR) and mean arterial pressure with a calibrated pressure transducer, and arterial blood samples were taken to determine pH, PaO\(_2\) and PaCO\(_2\).

A 24 G polyethylene catheter (Abbott Ireland) was placed in the tail vein to administer 10 mL/kg/h 0.9% physiological saline solution, and a temperature probe was placed in the rectum. An air heater set at 45 °C was used to maintain room temperature within physiological limits (37–38 °C).

Following this, the animals were randomized to one of two study groups: CONTROL (n = 10), which did not receive sevoflurane, and SEV (n = 10), which were ventilated with 3% sevoflurane vaporized in 11/min of oxygen.

After 30 min (BASELINE time), VILI was induced by increasing PIP to 35 cmH\(_2\)O with a driving pressure of 35 cmH\(_2\)O for 20 min (INJURY time). Following this, ventilation continued for 30 min at the initial setting of 12 cmH\(_2\)O peak pressure and 3 cmH\(_2\)O PEEP (POSTINJURY time) in order to allow time for proinflammatory cytokine levels in lung tissue to increase. During this process, the SEV group continued to receive 3% sevoflurane. At the end of each experiment, the animals were sacrificed with 50 meq/kg of intravenous potassium chloride and the lungs were removed to study cytokine levels, the wet/dry weight ratio, and to perform histopathological studies.

### Hemodynamic and blood gas parameters

HR, mean arterial pressure and arterial blood gases (pH, PaO\(_2\) and PaCO\(_2\)) were measured immediately prior to lung injury induction (baseline), 20 min after start of VILI, and at the end of the study (30 min POSTINJURY).

### Determination of the wet/dry lung weight ratio

The wet/dry weight ratio was calculated in the whole left lungs of all study animals. Lungs were dehydrated in a thermostat-controlled chamber at 80 °C for 72 h and the dry weight was measured. The wet/dry weight ratio was calculated to evaluate the percentage of pulmonary edema.

### Determination of TNF-α, IL-1β and IL-6 in lung tissue

The same lung segment (lower right lobe) was excised from all rats and stored at −80 °C until needed for study. TNF-α, IL-1β and IL-6 in lung tissue was measured. The segment was place in saline solution and centrifuged for 10 min at 10,000 rpm, at 4 °C. Cytokine levels were measured in the supernatant by means of an ELISA test.

### Histopathological analysis

The upper right lobes of all rats was excised and fixed in 10% formaldehyde. They were then stained with hematoxylin and eosin and embedded in paraffin for study by a histopathologist blinded to the study.

A 4-point qualitative ordinal variable injury scale (negative = 0, minor = 1, moderate = 2, severe = 3) was used to evaluate each of the following characteristics. The 4 main characteristics based on 15 individual items are: atelectasis, edema (septal edema, interstitial edema, lymphangiectasia, intra-alveolar exudate), inflammation (alveolar neutrophil infiltration, interstitial neutrophil infiltration, interstitial lymphocyte infiltration, granulocyte adhesion), and other (alveolar hemorrhage, hyperemia, thrombocyte aggregation, fibrin deposits, hyaline membrane formation, peeling of the bronchial and bronchiolar epithelium). A summary of the results obtained for each parameter in all animals is given below. The sum of all 15 parameters was then divided by the number of animals in each group to obtain the total injury score.

### Statistical analysis

Statistical analysis of study data was performed on SPSS\textsuperscript{a} v. 15.0 (SPSS Inc., Chicago, IL, USA). Based on the findings of earlier studies conducted by our group, we determined that each group should comprise 7 animals in order to detect a difference in HR of 50 bpm or over, with a p value of 0.05, a statistical power of 95% in a bilateral comparison, and a loss to follow-up or 0%.

All data were grouped and summarized as mean ± standard deviation. Hemodynamic and blood gas parameters were analyzed using Shapiro-Wilk normality testing, followed by repeated measures analysis of variance (ANOVA) testing and a Bonferroni multiple comparison test. For inter-group comparison of wet/dry weight ratio, TNF-α, IL-1β and IL-6, the unpaired t-test was used. Statistical significance was set at p < 0.05.

### Results

#### Hemodynamic and blood gas parameters

Arterial blood gas analysis showed respiratory alkalosis in both groups at VILI time vs baseline time (Table 1). Difference between CONTROLS and the SEV group were not statistically significant.

Mean arterial pressure in the CONTROL group was significantly higher at the 3 study time points vs the SEV group (Table 1). No statistically significant differences in HR were observed.
Table 1  Physiological parameters at baseline, VILI, and the end of the experiment in rats treated with (SEV) and without (CONTROL) sevoflurane.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>SEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.337 ± 0.047</td>
<td>7.287 ± 0.021</td>
</tr>
<tr>
<td>INJURY</td>
<td>7.482 ± 0.026</td>
<td>7.442 ± 0.123</td>
</tr>
<tr>
<td>30 min POSTINJURY</td>
<td>7.306 ± 0.041</td>
<td>7.268 ± 0.145</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>230 ± 16</td>
<td>232 ± 20</td>
</tr>
<tr>
<td>INJURY</td>
<td>258 ± 14a</td>
<td>256 ± 22a</td>
</tr>
<tr>
<td>30 min POSTINJURY</td>
<td>218 ± 28</td>
<td>206 ± 25</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>30.9 ± 3.7</td>
<td>26.4 ± 7.5</td>
</tr>
<tr>
<td>INJURY</td>
<td>17.3 ± 2.0b</td>
<td>17.1 ± 1.3b</td>
</tr>
<tr>
<td>30 min POSTINJURY</td>
<td>31.4 ± 4.1</td>
<td>26.8 ± 1.0</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>368 ± 45</td>
<td>342 ± 37</td>
</tr>
<tr>
<td>INJURY</td>
<td>357 ± 28</td>
<td>333 ± 35</td>
</tr>
<tr>
<td>30 min POSTINJURY</td>
<td>385 ± 27</td>
<td>362 ± 27</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>93 ± 15</td>
<td>80 ± 10b</td>
</tr>
<tr>
<td>INJURY</td>
<td>86 ± 11b</td>
<td>74 ± 13b</td>
</tr>
<tr>
<td>30 min POSTINJURY</td>
<td>101 ± 13</td>
<td>81 ± 19b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation.

a Statistically significant (p < 0.05) with respect to baseline (p < 0.05).
b Statistically significant (p < 0.05) with respect to CONTROL group (p < 0.05).

Determination of the wet/dry lung weight ratio

Wet/dry lung weight ratio as an indicator of the percentage of pulmonary edema was significantly lower in the SEV group vs CONTROLs (79.49% ± 1.78% vs 84.97% ± 1.69%; p < 0.0001) (Fig. 1).

Figure 1  Wet/dry lung weight ratio as a marker of the percentage of pulmonary edema following establishment of a mechanical ventilation-induced lung injury in rat models anesthetized with (SEV) or without (CONTROL) sevoflurane. *Statistically significant (p < 0.05) with respect to the CONTROL group.

Determination of TNF-α, IL-1β and IL-6 in lung tissue

TNF-α and IL-6 levels in lung tissue were significantly lower in the SEV group vs CONTROLs (144 ± 25 vs 243 ± 51 pg/mL for IL-6 [p < 0.0001] and 14 ± 4 vs 23 ± 4 pg/mL for TNF-α [p < 0.0001]) (Figs. 2 and 3); however, no statistically significant differences in changes in IL-1β levels in lung tissue were found between groups (128 ± 92 in CONTROL vs 115 ± 69 pg/mL in SEV) (p = 0.715) (Fig. 3).

Histopathological analysis

Histopathological findings were similar in the sevoflurane group (Fig. 4) and the CONTROL group (Fig. 5), and showed peribronchial inflammatory injury, edema, and centrilobular emphysema. The extent of injury was based on the score obtained from the 4-point qualitative ordinal variable scale:
In our study, we observed similar histopathological injuries using a driving pressure of 35 cmH₂O for 20 min, although in this case the injuries were not as severe as those reported in the foregoing studies. This shows that the time of exposure to high pressure (increased from 20 to 30 min) and the inflation pressure within this high pressure range (30, 35 or 40 cmH₂O) are both key factors in determining the severity of lung injury.

In our study, the quantification of pulmonary edema by means of the wet/dry lung weight ratio, and the quantification and measurement of proinflammatory cytokine levels were shown to be more sensitive early markers of inflation pressure-induced lung injury than histopathological injury, as this latter requires a more prolonged exposure to high pressures.

Reduction in the extent of pulmonary edema in the sevoflurane group coincides with the findings obtained from pulmonary ischemia-reperfusion injury and in sepsis-induced pulmonary injury models. In vitro, sevoflurane stimulates the Na⁺/K⁺-ATPase activity in injury-induced type II alveolar epithelial cells, one of the functions of which is to regulate transport of Cl⁻ and H₂O into the alveolar space. In vivo experiments, however, there is evidence that sevoflurane does not affect water reabsorption and edema resolution, but could contribute to edema formation.

The antiinflammatory effect of volatile anesthetics has been explained by their involvement in preconditioning. Pharmacological preconditioning is a protection mechanism that makes cells relatively resistant to the myocellular death resulting from ischemia-reperfusion injury. In the lung, volatile anesthetic-induced preconditioning protects against injury caused by both ischemia-reperfusion and sepsis. Both types of injury are characterized by activation of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6.

No significant differences in proinflammatory cytokines concentrations in bronchoalveolar lavage were found during mechanical ventilation with sevoflurane or pentobarbital; however, VILI-induced inflammatory response is characterized by activation of these proinflammatory cytokines. The pulmonary homogenate obtained in our study shows less proinflammatory cytokine secretion in all cytokine secreting alveolar cells (TNF-α and IL-6, but not IL-1β) in animals ventilated with sevoflurane. This confirms that the same anti-inflammatory and cell protective action of sevoflurane reported in other lung injury models is also found in healthy lungs, where it protects alveoli against damage induced by mechanical ventilation with high driving pressure.

Many alveolar cells involved in cytokine secretion, such as alveolar macrophages and polymorphonuclear leukocytes, can be affected by volatile anesthetics. Type II alveolar epithelial cells, together with inflammatory alveolar cells, form part of the intra-alveolar cytokine network, secreting IL-6, TNF-α, and other interleukins. Giraud et al. showed in vitro that volatile anesthetics in general, and halotane in particular, reduce secretion of TNF-α and IL-6 in type II epithelial cells. Subsequent in vitro experiments have shown the pre- and postconditioning with sevoflurane reduces expression of inflammatory mediators in alveolar epithelial cells. The present significant perivascular and alveolar edema after 30 min of delivery.

The CONTROL group had a mean score of 1.2 ± 0.33, with the SEV group scoring 0.8 ± 0.36, which describes a minor injury with no statistically significant difference between groups.

Discussion

Following induction of VILI with an inflation pressure of 35 cmH₂O for 20 min, neither study group showed any clinically significant severe histopathological injuries or oxygen deficit on examination. This indicates that neither the pressure nor the duration of delivery was sufficient to induce severe histopathological lung injury, or that postinjury time was not sufficient for these changes to show up on histopathological analysis. Nevertheless, the wet/dry lung weight ratio, which quantifies mechanical stress markers (pulmonary edema and IL-6 and TNF-α levels), shows significantly less pulmonary edema and inflammatory response in the SEV group vs CONTROLS.

Other experimental studies have shown that mechanical ventilation with a driving pressure of 14 cmH₂O does not induce histological changes in the lung, while subjects receiving driving pressures of between 30 and 45 cmH₂O
Effects of sevoflurane on ventilator induced lung injury

In contrast to our results, isoflurane and sevoflurane were found to reduce plasma concentrations of IL-1β in a sepsis-induced lung injury model. We believe that no significant intra-group differences in IL-1β levels were found in our study because samples were taken too soon to enable measurement of IL peaks in lung tissue (30 min POSTINJURY). This time frame was chosen to prevent loss of the IL-6 and TNF-α peaks, which occur earlier, because alveolar macrophages secrete IL-1β 8 h following lipopolysaccharide stimulation.

This study is subject to the limitation typical of any experimental study in rats. This means that the results cannot be directly extrapolated to clinical practice in humans, and more experimental studies are needed to confirm these preliminary findings. Ultimately, clinical trials should be conducted in human subjects to confirm our results. Furthermore, the VILI model used in our study was subject to a short injury exposure time (20 min). This means that although lung injury occurred, it had no significant clinical impact on gas exchange. Studies using higher pressure settings should be conducted, above all with longer exposure times, to show whether sevoflurane continues to exert an anti-inflammatory and cell protection effect even when lung damage is more severe and the clinical impact greater.

In conclusion, our study shows that sevoflurane attenuates VILI in health lungs in a subclinical rat model of VILI. Our findings suggest that further experimental and clinical studies are needed before the real clinical benefit of the cytoprotective effects of this anesthetic can be confirmed.

Funding

This study has been fully funded by departmental research funds managed by the Instituto de Investigación del Hospital Puerta de Hierro.

Conflict of interest

The authors declare they have no conflict of interest.

Acknowledgments

We sincerely thank Dr. Gerardo Tusman for his help in designing this study, and also Drs. Clara Salas and Mercedes Zurita for their wholly disinterested help in carrying out the histopathological and the immunohistochemical study, respectively.

References


