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Abstract

BACKGROUND: Acute lung injury is a common complication in critically ill patients. Several studies suggest that volatile anesthetics have immunomodulating effects. The aim of the current study was to assess possible postconditioning with sevoflurane in an in vivo model of endotoxin-induced lung injury.

METHODS: Rats were anesthetized, tracheotomized, and mechanically ventilated. Lipopolysaccharide (saline as control) was administered intratracheally. Upon injury after 2 h of propofol anesthesia, general anesthesia was continued with either sevoflurane or propofol for 4 h. Arterial blood gases were measured every 2 h. After 6 h of injury, bronchoalveolar lavage was performed and lungs were collected. Total cell count, albumin content, concentrations of the cytokines cytokine-induced neutrophil chemoattractant-1 and monocyte chemoattractant protein-1, and phospholipids were analyzed in bronchoalveolar lavage fluid. Expression of messenger RNA for the two cytokines and for surfactant protein B was determined in lung tissue. Histopathologic examination of the lung was performed.

RESULTS: Significant improvement of the ratio of oxygen tension to inspired oxygen fraction was shown with sevoflurane (mean + or - SD: 243 + or - 94 mmHg [32.4 kPa]) compared with propofol (88 + or - 19 mmHg [11.7 kPa]). Total cell count representing effector cell recruitment as well as albumin content as a measure of lung permeability were significantly decreased in the sevoflurane-lipopolysaccharide group compared with the propofol-lipopolysaccharide group in bronchoalveolar lavage fluid. Expression of the cytokines protein in bronchoalveolar lavage fluid as well as messenger RNA in lung tissue was significantly lower in the sevoflurane-lipopolysaccharide group compared with the propofol-lipopolysaccharide group. CONCLUSIONS: Postconditioning with sevoflurane attenuates lung damage and preserves lung function in an in vivo model of acute lung injury.
Sevoflurane ameliorates gas exchange and attenuates lung damage in experimental lipopolysaccharides-induced lung injury

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SV, RAL, ACL, MS, JS, and MU are research and clinical residents. CB and LR are lab technicians. RCS is a senior researcher. BBS is professor.
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Running title: Pharmacological pulmonary postconditioning

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Summary statement: This study shows for the first time that late postconditioning with a volatile anesthetic, initiated 2 hours after the onset of injury attenuates acute lung injury.

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ABSTRACT

**Background:** Acute lung injury is a common complication in critically ill patients. Several studies suggest that volatile anesthetics have immunomodulating effects. The aim of the present study was to assess a possible postconditioning with sevoflurane in an *in vivo* model of endotoxin-induced lung injury.

**Material and Methods:** Rats were anesthetized, tracheotomized and mechanically ventilated. Lipopolysaccharide (saline as control) was administered intratracheally. Upon injury after two hours of propofol anesthesia, general anesthesia was continued with either sevoflurane or propofol for four hours. Arterial blood gases were measured every two hours. After six hours of injury bronchoalveolar lavage was performed and lungs were collected. Total cell count, albumin content, and concentrations of the cytokines cytokine-induced neutrophil chemoattractant-1, monocyte chemoattractant protein-1 and phospholipids were analysed in bronchoalveolar lavage fluid. Expression of messenger RNA for the two cytokines and for surfactant protein b was determined in lung tissue. Histopathological examination of the lung was performed.

**Results:** A significant improvement of PaO$_2$/FiO$_2$ was shown with sevoflurane (243 ± 94 torr [32.4 kPa]) compared to propofol (88 ± 19 torr [11.7 kPa]) [mean ± SD]. Total cell count as a measure of effector cell recruitment and albumin content as a measure of lung permeability were significantly decreased in the sevoflurane-lipopolysaccharide group compared to the propofol-lipopolysaccharide group in bronchoalveolar lavage fluid. Expression of the cytokines protein in bronchoalveolar lavage fluid as well as messenger RNA in lung tissue was significantly lower in the sevoflurane-lipopolysaccharide group compared to the propofol-lipopolysaccharide group.

**Conclusions:** We conclude that postconditioning with sevoflurane attenuates lung damage and preserves lung function in an *in vivo* model of acute lung injury.
INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are a common finding in today’s intensive care units (ICU) \(^1\). Despite the introduction of new therapeutic approaches, mortality in patients with ARDS could not be improved substantially since its first description \(^2\) and remains high (30-40\%) \(^1,3\). To date, only low tidal volume ventilation has been shown to positively influence mortality in ARDS \(^4\).

Patients on ICUs who need mechanical ventilation due to ALI/ARDS are often sedated using intravenous sedatives like propofol or midazolam \(^5\). Only recently it has become feasible to sedate patients with volatile anesthetics using the Anaesthetic Conserving Device (AnaConDa) (Sedana Medical AB, Sundbyberg, Sweden).

Apart from many direct advantages compared to intravenous drugs \(^6\), volatile anesthetics have been shown to possess antiinflammatory properties \(^7,9\). Furthermore, recent studies suggest that sevoflurane might act as a pre-and postconditioning agent \(^10\) inducing organ protection in models of ALI due to inhibition of the expression of proinflammatory mediators. The knowledge about the immunomodulatory effects of volatile anesthetics mainly originates from ischemia-reperfusion injury studies. Administration of volatile anesthetics prior to ischemia, called anesthetic preconditioning, has been shown to attenuate ischemia-reperfusion induced injury in the heart \(^11,12\), the kidney \(^13\), the lung \(^14,15\) and in the liver \(^9\). While preconditioning seems to be an efficient approach, the possibility of postconditioning would be even more interesting and expand the clinical applicability, as it is not tied to a specific time point. In fact, the administration of volatile anesthetics after the onset of lung injury could be readily applied to many clinical scenarios in the operating room and even later in the intensive care unit.

Based on our previous in vitro data we hypothesized that postconditioning with sevoflurane might attenuate the inflammatory reaction in an in vivo model of endotoxin-induced lung injury.
MATERIAL AND METHODS

Animal preparation

After approval obtained from the local animal care and use committee (Zurich, Switzerland), pathogen-free, male Wistar rats weighing 350 – 500 g (Charles River, Sulzfeld, Germany) were used. The rats were housed in standard cages at 22 ± 1°C temperature under a 12/12-hr light/dark regimen. Food and water were supplied ad libitum.

Rats were anesthetized with intraperitoneal sodium thiopenthal (100 mg/kg; Pentothal, Swissmedic, Ospedalia AG, Hünenberg, Switzerland). For continuous propofol infusion and fluid administration (sodium chloride 10 ml/kg/hour) the tail vein was cannulated with a sterile 22 GA catheter (BD Insyte, Becton Dickinson S.A., Madrid, Spain). A sterile polyethylene catheter for blood sampling and blood pressure monitoring was placed into the left carotid artery (pressure transducer, Spacelabs, Hertford, United Kingdom). The rats were tracheotomized and a sterile metal cannula was inserted into the trachea followed by mechanical ventilation in pressure-controlled modus (Servo Ventilator 300, Maquet, Solna, Sweden). Peak inspiratory pressure (PIP) was 14 cmH₂O with a positive end-expiratory pressure (PEEP) of 3 cmH₂O. The fractional inspired oxygen concentration (FiO₂) was 1.0, I/E ratio was 1:2; respiratory frequency = 30 min⁻¹. Arterial blood samples were analyzed at 0, 2, 4, and 6 hours for PaO₂ and PaCO₂. Body temperature was maintained at 37°C by a warming lamp.

To evaluate the oxygenation capability of the lung over time, the ratio of oxygen tension to inspired oxygen fraction (PaO₂/FiO₂) was calculated at defined time points for each group (0, 2, 4, 6 hours) as well as alveolo-arterial oxygen tension difference with values obtained from Federal Office of Meteorology and Climatology MeteoSwiss.
**Experimental Design**

Rats were randomized into four different groups: 1. propofol-lipopolysaccharide (n=6), 2. propofol-phosphate-buffered saline (PBS) (n=4), 3. sevoflurane-lipopolysaccharide (n=6) 4. sevoflurane-PBS (n=4). Rats in the lipopolysaccharide-groups were intratracheally instilled with 150 μg of *Escherichia coli*-lipopolysaccharide (serotype 055:B5) (Sigma Aldrich, Buchs, Switzerland) in 300 μl PBS. Both control groups (propofol-PBS and sevoflurane-PBS) received 300 μl intratracheally instilled PBS. After the application of either lipopolysaccharide or PBS rats were ventilated as described and propofol was infused intravenously at a dose of 10-20 mg/kg/h to maintain anesthesia. Propofol 97% (Sigma Aldrich, Buchs, Switzerland) was dissolved in a 14% Cremophor® EL (Biochemika Fluka, Buchs, Switzerland) solution to a final concentration of 10 mg/ml. Two hours after the onset of lung injury the anesthetic was changed according to the protocol to either propofol or sevoflurane for subsequent 4 hours (6 hour injury model with 4 hours of postconditioning). Sevoflurane was administered using the AnaConDa-system. The expiratory concentration of sevoflurane was measured with a multigas analyzer (VEO Multigas Monitor, PHASEIN Medical Technologies, Danderyd, Sweden). In all experiments, concentration of sevoflurane was 1-2 Vol. %, respectively 0.5 -1 minimal alveolar concentration (MAC).

**Preparation and analysis of samples**

At the end of the experiment, animals were euthanized. The right heart was flushed with 10 ml PBS, after which a bronchoalveolar lavage was performed (3 x 10 ml PBS, pooled). The collected fluid was centrifuged at 4°C (1.500 x g for 10 min) and aliquots of the supernatant were frozen at -20°C. The cell pellet was re-suspended in 1 ml PBS. After dyeing the cells with trypan blue cells were counted with a Neubauer chamber.
Finally, lungs were shock-frozen in liquid nitrogen and stored at -80°C for isolation of RNA.

**Measurement of lung permeability**

To assess the differences in lung permeability between the study groups total protein and albumin were measured in bronchoalveolar lavage fluid (BALF). Total protein was determined using a Bradford assay (Bio-Rad, Hercules, CA). Albumin levels were assessed using an ELISA assay (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer’s protocol. The detection range for albumin was 7.8-10000 ng ml⁻¹.

**ELISA (Enzyme-linked immunosorbent assay)**

Sandwich ELISAs were performed according to the manufacturer’s protocol assessing the chemokines cytokine-induced neutrophil chemoattractant-1 (CINC-1) (R&D Systems Europe Ltd, Abingdon, United Kingdom) and monocyte chemoattractant protein-1 (MCP-1) (BD Biosciences, San Diego, CA). The detection range for CINC-1 protein was 7.8-1000 pg ml⁻¹ and 62.5-16000 pg ml⁻¹ for MCP-1.

**RNA extraction and real-time PCR for CINC-1 and MCP-1**

Total RNA was isolated from lung tissue using the RNeasy Mini Kit (Qiagen, Basel, Switzerland) according to the manufacturer’s protocol. Tissue was lysed in the provided buffer and subsequently loaded on RNeasy mini spin columns. RNA was eluted with RNase-free water. Total amounts and purity of RNA were determined by absorbance at 260 nm and the 260/280 nm absorbance-ratio, respectively.

Reverse transcription was performed with 0.8 μg total RNA at 20°C for 5 min, 42°C for 30 min and 95°C for 5 min. Random hexanucleotide primers and murine leukaemia virus reverse transcriptase were used for cDNA synthesis.
Real time quantitative TaqMan PCR was performed on a GeneAmp 5700 system (P.E. Applied Biosystems, Waltham, MA). Specific primers (Microsynth, Balgach, Switzerland) and labeled TaqMan probes (Roche Applied Science, Basel, Switzerland) were designed for MCP-1, CINC-1, and 18S. The TaqMan universal PCR Master Mix (Applied Biosystems, Branchburg, N.J.) was used for the assays in a final reaction volume of 15 µl. All primers and probes used in the experiments are presented in table 1. Each experimental PCR run was performed in duplicate with simultaneous assays for controls with no template.

For quantitation of gene expression the comparative Ct method was used as described by Livak et. al. The Ct values of samples (propofol-LPS and sevoflurane-LPS) and controls (propofol-PBS and sevoflurane-PBS) were normalized to the housekeeping gene (18S) and calculated as follows: $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct = \Delta Ct_{\text{samples}} - \Delta Ct_{\text{controls}}$.

**Histopathological Analysis**

For histological examination lungs (previously not flushed in the respiratory compartment) were fixed with 3% paraformaldehyde in PBS and then imbedded in TissueTek (Sakura Finetec Inc., Torrance, CA, USA). A series of microsections (7 µm) of every study group was stained with hematoxylin and eosin. Lung injury was quantified by 3 blinded researchers, using a lung injury score described previously. The lung pathology was assessed by various degrees of edema (0 = no, 1 = mild, 2 = moderate, 3 = severe) and reactive cell infiltration (0 = no, 1 = mild, 2 = moderate, 3 = severe). Adding these two individual scores resulted in the final score ranging from 0 to 6.

**Isolation of Surfactant and Phospholipid Assay**

Surfactant was pelleted by high-speed centrifugation (30,000 g for 45 min at 4°C). The crude pellet was resuspended in 110 µl of 0.9% saline, and total phospholipid content was measured using the method of Stewart. Fifty microliters of sample were added to glass tubes
containing 2 ml of spectroscopic-grade chloroform. Two milliliters of 3.04% (wt/vol) ammonium ferrocyanate and 2.7% (wt/vol) ferric chloride hexahydrate in distilled H₂O were added, and the mixture was vortexed for 1 min. Standards (0 – 100 mg/ml) were prepared with phosphatidylcholine in chloroform. The lower chloroform phase was withdrawn, and absorption was measured at 488 nm with a quartz cuvette.

In vitro Experiments with Rat Pulmonary Artery Endothelial Cells (RPAEC) and Alveolar Epithelial Cells (AEC)

RPAEC cell culture: The cell line, kindly provided by Dr Roscoe Warner PhD, Department of Pathology, University of Michigan, Ann Arbor, MI, was cultured in Dulbecco’s modified Eagle’s Medium (DMEM; Invitrogen AG, Basel, Switzerland), supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% HEPES buffer in an incubator with 5% CO₂. They were grown in uncoated 35 x 10 mm plates (Corning Inc., Corning, NY) to more than 95% confluence. DMEM/10% FBS was replaced by DMEM/1% FBS 24 h before lipopolysaccharide stimulation. RPAEC were stimulated with lipopolysaccharide from Escherichia coli, serotype 055:B5 (Sigma, Buchs, Switzerland) in a concentration of 20 μg/l in DMEM/1% FBS for 6 hours (control group only stimulation with PBS in DMEM/1% FBS instead of lipopolysaccharide).

AEC cell culture: The L2 cell line (CCL 149, American Type Culture Collection, Rockville, MD) was derived through cloning of adult female rat lung of AEC type II origin 22. The cells were cultured and stimulated in the same way as RPAEC.

Hypercapnia: For the incubation time of 6 hours, the following CO₂ concentrations were chosen: 5% (control), 7.5%, and 10%. Supernatants were collected and ELISA’s were performed and expression of CINC-1 and MCP-1 was analyzed.
Incubation with propofol: Control and stimulated RPAEC were exposed to propofol diluted in cremophor EL 14% for 6 hours. Supernatants were collected and ELISA’s were performed and expression of CINC-1 and MCP-1 was analyzed.

For all experiments, cell viability was 95% as determined by measurement of lactatdehydrogenase (CytoTox 96, Non-Radioactive Cytotoxicity Assay, Promega, Madison, WI).

**Statistics**

Values were expressed as mean ± SD.

PaO$_2$/FiO$_2$ ratio and alveolar–arterial oxygen tension difference (PO$_2$ difference) data were tested by analysis of variances for repeated measurements (two-way ANOVA). The interaction testing between group and time from the repeated measures have been performed.

ELISA data were tested by analysis of variances for repeated measurements (one-way ANOVA) with a Tukey-Kramer Multiple post-hoc test. Realtime PCR data were tested using a t-test with two-tailed hypothesis testing. Graphpad Prism4 and GraphPad Instat3 (GraphPad software, La Jolla, CA) were used for statistical analyses. P-values less or equal to 0.05 were considered statistically significant.
RESULTS

Gas Exchange

Intratracheal lipopolysaccharide resulted in a significant decrease of PaO$_2$/FiO$_2$ for both anesthetics (propofol and sevoflurane) compared to the PBS controls after 6 hours of injury (Fig. 1A). Animals in the sevoflurane-lipopolysaccharide group had a significantly higher PaO$_2$/FiO$_2$ (243 ± 94 torr [32.4 kPa]) compared to the propofol-lipopolysaccharide (88 ± 19 torr [11.7 kPa]) group after 6 hours of lipopolysaccharide injury. There were no significant differences between both PBS groups (sevoflurane-PBS, 415 ± 28 torr [55.3 kPa]; propofol-PBS, 433 ± 32 torr [57.7 kPa]) (Fig. 1A). The influence of factor sevoflurane and time was p=0.0169 and p=0.0202, respectively. No significant interaction could be found between sevoflurane and time (p=0.3284).

Accordingly, intratracheal lipopolysaccharide resulted in an increase of the alveolar–arterial oxygen tension difference (PO$_2$ difference) for both anesthetics. The propofol-lipopolysaccharide group had a significantly higher PO$_2$ difference compared to the sevoflurane-lipopolysaccharide group after 6 hours, whereas no differences were found in the PBS groups (Fig. 1B).

PaCO$_2$ levels were higher in both lipopolysaccharide groups compared with PBS groups. PaCO$_2$ was significantly higher in the propofol-lipopolysaccharide group (56.6 ± 8.1 torr [7.5 kPa]) compared with the sevoflurane-lipopolysaccharide group (42.2 ± 7.1 torr [5.6 kPa]) after 6 hours of lipopolysaccharide injury (Tab. 2).

Circulatory Variables

Mean arterial pressure (MAP) decreased in all four study groups during the course of the experiment. There were no significant differences in MAP between the four groups at anytime (Tab. 2).
**BALF Analysis**

The recovery of BALF was comparable in all study groups. 70% of administered fluid was retrieved.

**Total Cell Count**

Total cell count in BALF was determined as a measure of effector cell recruitment. While cells in BALF of PBS animals were identified as alveolar macrophages, 99.5% of the cells in lipopolysaccharide animals were neutrophils. Cell count increased significantly in both lipopolysaccharide groups compared to both control groups. The sevoflurane-lipopolysaccharide group showed a significantly lower total cell number compared to the propofol-lipopolysaccharide group (sevoflurane-LPS, 14.94 ± 5.72 cells/10^6 ml⁻¹; propofol-lipopolysaccharide, 27.18 ± 7.75 cells/10^6 ml⁻¹) (Fig. 2). There were no significant differences between the PBS groups.

**Albumin and Proteins**

Albumin concentration in BALF, reflecting alveolo-capillary permeability, was significantly lower in the sevoflurane-LPS group compared to the propofol-lipopolysaccharide group (sevoflurane-lipopolysaccharide, 4.9 ± 3.8 μg ml⁻¹; propofol-lipopolysaccharide, 10.4 ± 3.5 μg ml⁻¹) (Fig. 3). The alveolar protein content as a measure of accumulation of proteins upon inflammation was significantly higher in the lipopolysaccharide groups compared to the PBS groups. Additionally, a significantly lower protein concentration was found in the sevoflurane-lipopolysaccharide group compared to the propofol-lipopolysaccharide group (sevoflurane-lipopolysaccharide, 1.39 ± 0.51 mg ml⁻¹; propofol-lipopolysaccharide, 2.26 ± 0.32 mg ml⁻¹) (Tab. 2).
Chemokine Analysis

The protein concentration of the chemokines CINC-1 and MCP-1 in BALF was assessed by ELISA. CINC-1 and MCP-1 level increased significantly in both LPS groups compared with both PBS groups. The sevoflurane-lipopolysaccharide group showed significantly lower levels of CINC-1 and MCP-1 compared to the propofol- lipopolysaccharide group (Fig. 4A, 4B). In the sevoflurane-lipopolysaccharide group CINC-1 and MCP-1 expression decreased 29% and 53% respectively compared to the propofol-lipopolysaccharide group.

Lung Tissue Analysis

The expression of mRNA of CINC-1 and MCP-1 was analyzed in total lung tissue by real-time PCR. Values were normalized to 18S and expressed relatively to controls (PBS groups). The mRNA expression in both LPS groups was significantly increased compared to both PBS groups. Again the sevoflurane-lipopolysaccharide group showed significantly lower mRNA levels compared to the propofol-lipopolysaccharide group (Fig. 5A, 5B): in the sevoflurane-lipopolysaccharide group CINC-1 mRNA and MCP-1 mRNA expression decreased 42% and 53% respectively compared to the propofol-lipopolysaccharide group.

Histopathological Analysis

As expected, intratracheal lipopolysaccharide resulted in a pulmonary edema with inflammatory cell recruitment (Fig. 6). Quantification of the injury showed a significant increase of the lung injury score in both lipopolysaccharide groups compared to the PBS groups. However, there was no significant difference between the sevoflurane-lipopolysaccharide group and the propofol- lipopolysaccharide group (Tab. 2).

Alveolar Epithelial Cell Injury
Evalution of SP-B RNA expression in lung tissue revealed a decrease in the expression of SP-B in both lipopolysaccharide groups compared to controls. However, decrease of SP-B in the sevoflurane-lipopolysaccharide animals was less accentuated compared to propofol-lipopolysaccharide animals (Fig. 7).

Furthermore, analysis of the phospholipid content in BAL revealed an increase in the expression of phospholipids in both lipopolysaccharide groups compared to controls. Propofol-lipopolysaccharide showed significantly higher phospholipid levels compared to the sevo-lipopolysaccharide animals (Fig. 8).

**In vitro Experiments with Rat Pulmonary Artery endothelial and Alveolar Epithelial Cells**

As a significant higher PCO$_2$ was observed in the lipopolysaccharide-propofol group after 6 hours of injury, we analysed the possible pro-inflammatory effect of hypercapnia on RPAEC and AEC with or without lipopolysaccharide stimulation. CO$_2$ values of 7.5% or 10% did not seem to have an impact on the inflammatory reaction in RPAEC or AEC compared to 5% CO$_2$ (Fig. 9A and 9B). Similarly, we analyzed the possible pro-inflammatory effects of propofol in 14% cremophor. As AEC are not in direct contact with the anesthetic, we used smaller concentrations of propofol for the *in vitro* approach. No pro-inflammatory effects were shown in non-stimulated RPAEC or AEC. Stimulation with lipopolysaccharide in the presence of propofol resulted in the same increase of CINC-1 and MCP-1 levels as observed in the lipopolysaccharide group (Fig. 9C and 9D).
DISCUSSION

The present study demonstrates that anesthetic postconditioning with sevoflurane improves oxygenation and attenuates lung damage as indicated by less recruitment of effector cells into the respiratory compartment, decreased expression of the proinflammatory mediators CINC-1 and MCP-1, and reduced lung hyperpermeability in an in vivo model of lipopolysaccharide-induced lung injury.

These results corroborate our previous in vitro studies, where we showed a significant reduction of proinflammatory mediators by preconditioning and by postconditioning of alveolar epithelial cells (AEC) with sevoflurane in in vitro models of LPS-induced injury. This is the first in vivo study comparing the postconditioning effects of sevoflurane and propofol in a model of ALI.

First, we focused on the effect of both anesthetics on oxygenation capability of the lung. The significant improvement of the arterial oxygen tension-to-inspired oxygen fraction (PaO2/FiO2) by postconditioning with sevoflurane after 6 hours is most likely due to a less impaired gas exchange compared to propofol sedation. This was also reflected in the calculations of alveolar–arterial oxygen tension difference. As discussed below the reason for this seems to be an attenuation of lung damage after lipopolysaccharide challenge. To our knowledge the amelioration of PaO2 by postconditioning with a volatile anesthetic in an in vivo model of ALI has not yet been described in the literature.

A possible explanation for the deteriorated PaO2/FiO2 ratio could be an inhibition of the hypoxic pulmonary vasoconstriction (HPV) by both anesthetics. Clinical investigations are not conclusive concerning the possible effect of anesthetics on HPV. In animals models, volatile anesthetics seem to inhibit HPV, and increase intrapulmonary shunt fraction or reduce arterial oxygen tension in a dose–response manner, while propofol does not affect HPV. In the clinical scenario, however, in patients undergoing one-lung-ventilation, sevoflurane...
and propofol have been shown to have similar effects on shunt fraction and arterial oxygen tension \(^26,27\). In our model, the impact of the volatile anesthetic-induced inhibition of HPV cannot be excluded.

Second, we the expression of CINC-1 and MCP-1 was studied. These chemoattractants have been shown to possess potent chemotactic activity for neutrophils (CINC-1, MCP-1) and monocytes (MCP-1), and therefore play a significant role in the acute inflammatory response in ALI \(^28-31\). The decrease of CINC-1 and MCP-1 proteins in BAL and of the mRNA in lung tissue by postconditioning with sevoflurane on the molecular level suggests a functional attenuation of inflammation by reduction of effector cell recruitment. In fact, we were able to prove this reduction of effector cells in the BALF (total cell count).

Third, alveolar albumin and protein influx as markers of increased influx of inflammatory proteins and alveolo-capillary leakage, respectively, were evaluated. Lung hyperpermeability causing pulmonary edema is thought to be a main mechanism inducing ARDS \(^5,32\). Again, postconditioning with sevoflurane significantly decreased albumin and protein influx. Recently, it was shown that reduction of lung hyperpermeability protects against lipopolysaccharide-induced lung injury \(^33\). Thus the therapeutic effects of sevoflurane on ALI could be mediated by reduction of lung hyperpermeability.

Fourth, SP-B RNA expression in lung tissue was significantly less decreased upon injury in the sevoflurane compared to the propofol group, indicating a milder degree of injury. SP-B plays a critical role for maintenance of stability of surfactant. As shown in previous experimental approaches expression of SP-B is decreased upon injury, probably as a consequence of destruction of the alveolo-capillary unit with alveolar epithelial type II cells \(^34,35\).

Fifth, lipopolysaccharide-propofol animals showed a significant higher expression of phospholipids in BALF. We hypothesize that increases in phospholipids in the alveolar space could be due to decreases in surfactant clearance by type II cells and the cells resident in the
alveolar space. Summarized, both results regarding SP-B and phospholipids underline a less deteriorated surfactant function by postconditioning with sevoflurane compared to propofol after lipopolysaccharide challenge.

Up to now, several in vivo studies have explored the effects of sevoflurane on lung tissue but with inconsistent results. Takala et al. compared sevoflurane anesthesia with thiopentone anesthesia in a model of ventilated healthy pigs. It was demonstrated that alveolar epithelial cell (AEC) type II cell integrity and ultrastructure remained unchanged after long-term (6 hours) high concentration exposure to sevoflurane (1.5 MAC) 37. Furthermore, a lower gene expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) was detectable in the intact porcine lung tissue after sevoflurane anesthesia 38. On the other hand an increase of pulmonary inflammatory mediators and pulmonary NO3- and NO2- production after sevoflurane anesthesia was revealed by another study using the same model 39. However, this model did not use an ALI model. Additionally, the sevoflurane concentration of 4 Vol. % was rather high compared to our model.

To exclude a negative effect of propofol cremophor on pulmonary cells, we performed in vitro experiments. RPAEC were co-exposed to propofol in concentrations previously reported 40,41. No increased cytotoxicity or enhanced inflammatory response could be observed. Additionally, it has to be mentioned that several studies have pointed out a protective effect of propofol as well 42-44.

Another component, which theoretically could enhance inflammatory injury, is the increased content of CO2 after 6 hours of injury. We discussed this increase as a consequence of injury. In vitro experiments underlined our hypothesis by showing that increased concentrations of CO2 did not interfere with the inflammatory reaction. This is in accordance with the literature, where only CO2 values of 15% or 20% induced an additional injury 45.

Only few reports exist focusing on the postconditioning capabilities of sevoflurane in acute lung injury. In a recent publication, Hofstetter et al. examined the antiinflammatory
effects of sevoflurane in an *in vivo* model of lipopolysaccharide-induced endotoxemia in rats. In this study administration of sevoflurane 15 min after stimulation with lipopolysaccharide resulted in a decrease of TNF-α and IL-1β plasma levels. In contrast to our study, lipopolysaccharide was given intravenously with an early administration of sevoflurane after the injury. In the present study, we were able to show anti-inflammatory effects of sevoflurane even when administered 2 hours after a lipopolysaccharide-stimulation, i.e. with late initiation of postconditioning. This may be of clinical relevance for patients who have already suffered from a trigger event that may result in ALI, or even ARDS in that sevoflurane may beneficially interfere with the further development of the lung injury.

In this study, we focused on the difference between the intravenous anesthetic propofol and the volatile anesthetic sevoflurane. However, it remains questionable if the observed difference would also be found with other intravenous anesthetics. Interestingly, in the cardiac ischemia-reperfusion injury, protection by volatile anesthetics, morphine and propofol is relatively well investigated. It is generally agreed that these agents reduce the myocardial damage caused by ischemia and reperfusion. Other anesthetics, which are often used in clinical practice, such as fentanyl, ketamine, barbiturates and benzodiazepines have been much less studied, and their potential as cardioprotectors is currently unknown. Therefore, general conclusions should not be drawn.

Today, sedation of patient with ALI/ARDS in the ICU is commonly performed using propofol. In the last years, the anti-inflammatory effects of this intravenous anesthetic have been extensively studied in several *in vivo* studies. It has been shown that propofol has anti-inflammatory effects that attenuate cytokine response following endotoxin shock in rats. Several studies suggest that pre-treatment and post-treatment with propofol provides protective effects in endotoxin-induced ALI and lipopolysaccharide-induced shock. However, the anti-inflammatory effects of propofol are thought to be at least in part due to containing EDTA, which is a component of the commercially used propofol formulation.
our study, we used a propofol formulation in 14% Cremophor without EDTA as clinically used propofol would induce hypervolemia in rats due to the low concentration of propofol. This could explain why less antiinflammatory effects in the propofol groups were found. However, a recent clinical trial, comparing the antiinflammatory property of sevoflurane and propofol in patients undergoing thoracic surgery with one-lung ventilation has also shown less inflammatory response in the sevoflurane group, even in the presence of EDTA 53.

Since the AnaConDa was approved for the use in ICU’s, it is now possible to take advantage of the properties of volatile anesthetics like fast induction, fast awakening and easy titration for sedation of postoperative and critically ill patients. Only few studies have assessed the use of volatile anesthetics especially sevoflurane via AnaConDa in ICU patients so far 6,54. Recently, a significantly shorter recovery time and a significantly shorter hospitalization time with a sevoflurane sedation compared to propofol was demonstrated in patients after cardiothoracic surgery 54. Up to now there are no clinical studies regarding the effects of sevoflurane sedation in patients with ALI or ARDS. The results of this in vivo study indicate that a sevoflurane sedation of patients with ALI may be beneficial.

Our study has several limitations. First, as already discussed, we used a special formulation of propofol in 14% cremophor without EDTA, which is not commonly used in the ICU. This could be a reason for the reduced immune response in the propofol group. In addition, findings of this study could be specific to this animal model. However, the lipopolysaccharide injection model has recently been evaluated to promise the most direct clinical relevance considering gram-negative sepsis in which ALI is most common 55,56. Second, our observations are based on a model of a beginning ALI and therefore may not be applicable in already established ARDS. Moreover, we studied the effect of sevoflurane only during a very short period (6 hours) compared to the clinical situation. In addition we administered a FiO₂ of 1.0 in our model which is not commonly used on ICUs except for severe cases of ARDS. To our knowledge nothing is known about any interaction of
hyperoxia and sevoflurane that may influence the antiinflammatory effects of sevoflurane. According to the literature hyperoxia-induced toxic effects on cells appear only after exposure times of more than 12 hours. Despite we cannot exclude that the hyperoxia influences the antiinflammatory effects of sevoflurane in our model.

In spite of these limitations, this study might be of clinical relevance. We could show that in developing ARDS gas exchange deteriorates significantly less by just using sevoflurane as sedative compared to propofol. This property of sevoflurane seems to be mediated due to inhibition of lung inflammation as indicated by lower levels of cytokines and less recruitment of effector cells into the lung tissue. Sedating ICU patients with sevoflurane using the AnaConDa system might therefore be a new promising therapeutic approach for ALI and ARDS. Moreover, the application of sevoflurane can be easily combined with protective ventilation strategies generating further interesting treatment options.

In conclusion, the present study indicates that anesthetic postconditioning with sevoflurane offers beneficial properties compared to propofol in a model of ALI in vivo.
REFERENCES


