

Sevoflurane Ameliorates Gas Exchange and Attenuates Lung Damage in Experimental Lipopolysaccharide-induced Lung Injury

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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 ± SD: 243 ± 94 mmHg [32.4 kPa]) compared with propofol (88 ± 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.**

ACUTE lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common findings in today's intensive care units (ICUs).[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 in ICUs who need mechanical ventilation because of ALI/ARDS are often sedated using intravenous sedatives such as propofol or midazolam.[5] Only recently it has become feasible to sedate patients with volatile anesthetics using the Anesthetic Conserving Device (AnaConDa; Sedana Medical AB, Sundbyberg, Sweden).

Apart from many direct advantages compared with intravenous drugs,[6] volatile anesthetics have been shown to possess antiinflammatory properties.[7–9] Furthermore, recent studies suggest that sevoflurane might act as a preconditioning 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 before ischemia, called *anesthetic preconditioning*, has been shown to attenuate ischemia–reperfusion-induced injury in the heart,[11,12] kidney,[13] lung,[14,15] and liver.[9] Although preconditioning seems to be an efficient approach, the possibility of postconditioning would be even more interesting and expand the clinical applicability, because 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 ICU.

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.

Materials and Methods

Animal Preparation

After approval was 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 under a 12/12-h light–dark regimen. Food and water were supplied *ad libitum*.

Rats were anesthetized with intraperitoneal sodium thiopental (100 mg/kg; Pentothal, Ospedalia AG, Hünen-

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berg, Switzerland). For continuous propofol infusion and fluid administration (10 ml · kg^{-1} · h^{-1} sodium chloride), the tail vein was cannulated with a sterile 22-gauge 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 mode (Servo Ventilator 300; Maquet, Solna, Sweden). Peak inspiratory pressure was 14 cm H_2O, with a positive end-expiratory pressure of 3 cm H_2O. The fractional inspired oxygen concentration (F_{IO_2}) was 1.0, inspiratory:expiratory ratio was 1:2, and respiratory frequency was 30 min^{-1}. Arterial blood samples were analyzed at 0, 2, 4, and 6 h for arterial oxygen tension (Pa_{O_2}) and arterial carbon dioxide tension (Pa_{CO_2}). 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 (Pa_{O_2}/F_{IO_2}) was calculated at defined time points for each group (0, 2, 4, and 6 h), as well as alveoloarterial oxygen tension difference with values obtained from the Federal Office of Meteorology and Climatology MeteoSwiss (Zurich, Switzerland).

Experimental Design

Rats were randomly assigned to four different groups: (1) propofol–lipopolysaccharide (n = 6), (2) propofol–phosphate-buffered saline (PBS) (n = 4), (3) sevoflurane–lipopolysaccharide (n = 6), and (4) sevoflurane–PBS (n = 4). Rats in the lipopolysaccharide groups were intratracheally instilled with 150 μg *Escherichia coli*–lipopolysaccharide (serotype 055:B5; Sigma Aldrich, Buchs, Switzerland) in 300 μl PBS.[16] 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^{-1} · h^{-1} 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.[17] Two hours after the onset of lung injury, the anesthetic was changed according to the protocol to either propofol or sevoflurane for a subsequent 4 h (6-h injury model with 4 h 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, the concentration of sevoflurane was 1–2 vol% (0.5–1 minimum alveolar concentration, respectively).

Preparation and Analysis of Samples

At the end of the experiment, animals were killed. The right heart was flushed with 10 ml PBS, after which a bronchoalveolar lavage was performed (3 × 10 ml PBS, pooled). The collected fluid was centrifuged at 4°C (1,500*g* for 10 min), and aliquots of the supernatant were frozen at −20°C. The cell pellet was resuspended in 1 ml PBS. After the cells were dyed with trypan blue, they 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 enzyme-linked immunosorbent assay (ELISA; Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's protocol. The detection range for albumin was 7.8–10,000 ng/ml.

ELISA

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 was 7.8–1,000 pg/ml for CINC-1 protein and 62.5–16,000 pg/ml 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 leukemia virus reverse transcriptase were used for complementary DNA synthesis.

Real-time quantitative TaqMan polymerase chain reaction (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, NJ) was used for the assays in a final reaction

Table 1. Primers and Probes Used for the Real-time Quantitative TaqMan PCR*

Gene	Primer Sequence	Length of Amplicon, nt
CINC-1		
Up	5′ CAC ACT CCA ACA GAG CAC CA 3′	120
Down	5′ TGA CAG CGC AGC TCA TTG 3′	
Probe 49	5′ CAG CCA CC 3′	
MCP-1		
Up	5′ AGC ATC CAC GTG CTG TCT C 3′	78
Down	5′ GAT CAT CTT GCC AGT GAA TGA GT 3′	
Probe 62	5′ ACC TGC TG 3′	
SP-B		
Up	5′ TCT GCA ATG CTT CCA AAC C 3′	65
Down	5′ GGT CCT TTG GTA CAG GTT GC 3′	
Probe 116	5′ CCA GGC TC 3′	
18S		
Up	5′ GGA GCC TGA GAA ACG GCT A 3′	64
Down	5′ TCG GGA GTG GGT AAT TTG C 3′	
Probe 74	5′ GGC AGC AG 3′	

* P.E. Applied Biosystems, Waltham, MA.

18S = housekeeping gene; CINC-1 = cytokine-induced neutrophil chemoattractant-1; MCP-1 = monocyte chemoattractant protein-1; nt = nucleotides; PCR = polymerase chain reaction; SP-B = surfactant protein B.

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 C_t method was used as described by Livak *et al.*[18] The C_t values of samples (propofol– lipopolysaccharide and sevoflurane–lipopolysaccharide) and controls (propofol–PBS and sevoflurane–PBS) were normalized to the housekeeping gene (18S) and calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t = \Delta C_{t,samples} - \Delta C_{t,controls}$.

Histopathologic Analysis

For histologic 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). A series of microsections (7 μm) of every study group was stained with hematoxylin and eosin. Lung injury was quantified by three blinded researchers, using a lung injury score described previously.[19,20] 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 saline, 0.9%, and total phospholipid content was measured using the method of Stewart.[21] Fifty microliters of sample was added to glass tubes containing 2 ml spectroscopic-grade chloroform. Two milliliters ammonium ferrocyanate, 3.04% (wt/vol), and ferric chloride hexahydrate, 2.7% (wt/vol), in distilled H_2O was 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 RPAECs and AECs*

Rat Pulmonary Artery Endothelial Cell Culture. The cell line, kindly provided by Roscoe Warner, Ph.D. (Research Assistant Professor, Department of Pathology, University of Michigan, Ann Arbor, Michigan), was cultured in Dulbecco modified Eagle 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% CO_2. They were grown in uncoated 35 × 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. Rat pulmonary artery endothelial cells (RPAECs) 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 h (control group only stimulation with PBS in DMEM–1% FBS instead of lipopolysaccharide).

Alveolar Epithelial Cell Culture. The L2 cell line (CCL 149; American Type Culture Collection, Rockville, MD) was derived through cloning of adult female rat lung of alveolar epithelial cell (AEC) type II origin.[22] The cells were cultured and stimulated in the same way as RPAECs.

Hypercapnia. For the incubation time of 6 h, the following carbon dioxide concentrations were chosen: 5% (control), 7.5%, and 10%. After the incubation supernatants were collected, ELISAs were performed and expression of CINC-1 and MCP-1 was analyzed.

Incubation with Propofol. Control and stimulated RPAECs and AECs were exposed to propofol diluted in 14% Cremophor EL for 6 h. After the incubation, supernatants were collected, ELISAs were performed, and expression of CINC-1 and MCP-1 was analyzed.

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

Statistical Analysis

Values were expressed as mean ± SD.

The ratio of oxygen tension to inspired oxygen fraction and alveoloarterial oxygen tension difference (Po_2 difference) data were tested by analysis of variances for repeated measurements (two-way analysis of variance). The interaction testing between group and time from the repeated measures has been performed. ELISA data were tested by analysis of variance for repeated measurements (one-way analysis of variance) with a Tukey–Kramer multiple *post hoc* test. Real-time PCR data were tested using a *t* test with

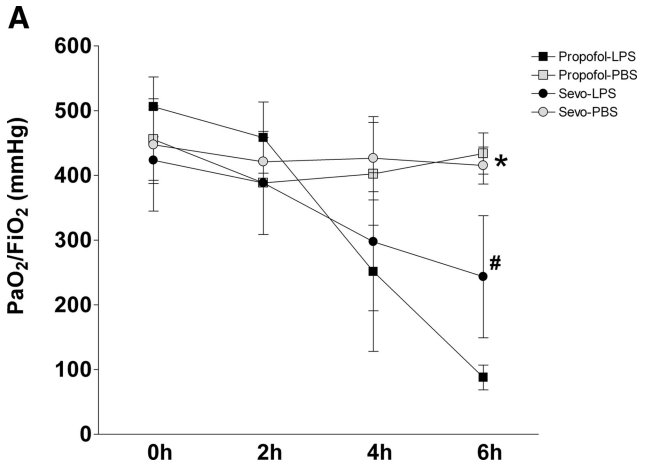

Fig. 1. Determination of ratio of oxygen tension to inspired oxygen fraction (Pao_2/Fio_2). Pao_2/Fio_2 was determined in the four study groups after 0, 2, 4, and 6 h: propofol–lipopolysaccharide (LPS) (*closed squares*), propofol–phosphate-buffered saline (PBS) (*open squares*), sevoflurane (Sevo)–LPS (*closed circles*), and Sevo–PBS (*open circles*). # $P < 0.05$ Sevo–LPS *versus* propofol–LPS. * $P < 0.05$, both PBS groups *versus* both LPS groups. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

two-tailed hypothesis testing. GraphPad Prism4 and GraphPad Instat3 (GraphPad Software, La Jolla, CA) were used for statistical analyses. *P* values of 0.05 or less 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 with the PBS controls after 6 h of injury (fig. 1). Animals in the sevoflurane–lipopolysaccharide group had a significantly higher Pao_2/Fio_2 (243 ± 94 mmHg [32.4 kPa]) compared with the propofol–lipopolysaccharide group (88 ± 19 mmHg [11.7 kPa]) after 6 h of lipopolysaccharide injury. There were no significant differences between the two PBS groups (sevoflurane–PBS, 415 ± 28 mmHg [55.3 kPa]; propofol–PBS, 433 ± 32 mmHg [57.7 kPa]; fig. 1). 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 alveoloarterial oxygen tension difference (Po_2 difference) for both anesthetics. The propofol–lipopolysaccharide group had a significantly higher Po_2 difference compared with the sevoflurane–lipopolysaccharide group after 6 h, whereas no differences were found in the PBS groups (data not shown).

Arterial carbon dioxide tension levels were higher in both lipopolysaccharide groups compared with the PBS groups. $Paco_2$ was significantly higher in the propofol–lipopolysaccharide group (56.6 ± 8.1 mmHg [7.5 kPa]) compared with the sevoflurane–lipopolysaccharide group (42.2 ± 7.1 mmHg [5.6 kPa]) after 6 h of lipopolysaccharide injury (table 2).

Circulatory Variables

Mean arterial pressure decreased in all four study groups during the course of the experiment. There were no significant differences in mean arterial pressure between the four groups at any time (table 2).

BALF Analysis

The recovery of BALF was comparable in all study groups. Seventy percent of administered fluid was retrieved.

Table 2. $Paco_2$, MAP, Protein in BAL, and Lung Injury Score

	Propofol–LPS	Propofol–PBS	Sevoflurane–LPS	Sevoflurane–PBS
$Paco_2$, mmHg				
0 h	33.9 ± 7.9	28.1 ± 2.2	38 ± 15.5	30.4 ± 5.1
2 h	40.8 ± 8.4	39.1 ± 16.3	34.5 ± 10.9	30 ± 6.5
4 h	44.9 ± 9	35.9 ± 1.2	37.3 ± 16.4	44.6 ± 21.8
6 h	56.6 ± 8.1	27.5 ± 9.5	42.2 ± 7.1*	42.4 ± 15.9
MAP, mmHg				
0 h	126 ± 16	140 ± 26	140 ± 18	150 ± 8
2 h	112 ± 15	120 ± 29	117 ± 15	114 ± 15
4 h	108 ± 27	103 ± 24	113 ± 27	106 ± 28
6 h	83 ± 27	95 ± 24	105 ± 24	114 ± 23
Protein BAL, mg/ml	2.26 ± 0.32	0.76 ± 0.07	1.39 ± 0.51†*	0.91 ± 0.23
Lung injury score	3.17 ± 1.33‡	0.83 ± 0.75	2.17 ± 1.72†	1.5 ± 0.75

Data are mean ± SD. Partial arterial carbon dioxide tension ($Paco_2$) levels were determined in the four study groups after 0, 2, 4, and 6 hours. Mean arterial pressure (MAP) was determined in the four study groups after 0, 2, 4, and 6 h. Values were not statistically significant at any time.

* $P < 0.05$ *vs.* propofol–lipopolysaccharide (LPS). Protein concentration in bronchoalveolar lavage (BAL) fluid: † $P < 0.01$ *vs.* propofol–LPS; ‡ $P < 0.001$ *vs.* propofol–phosphate-buffered saline (PBS). Lung injury score: † $P < 0.001$ *vs.* sevoflurane–PBS and propofol–PBS; ‡ $P < 0.001$ *vs.* sevoflurane–PBS and propofol–PBS.

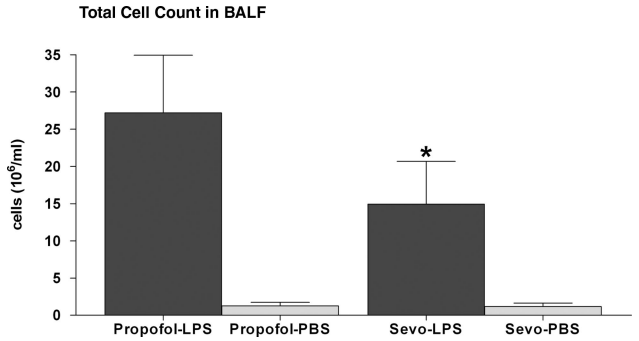

Fig. 2. Evaluation of total cell count in bronchoalveolar lavage fluid. Bronchoalveolar lavage fluid was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). Trypan staining was performed with the solid part of bronchoalveolar lavage. * $P < 0.05$ *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

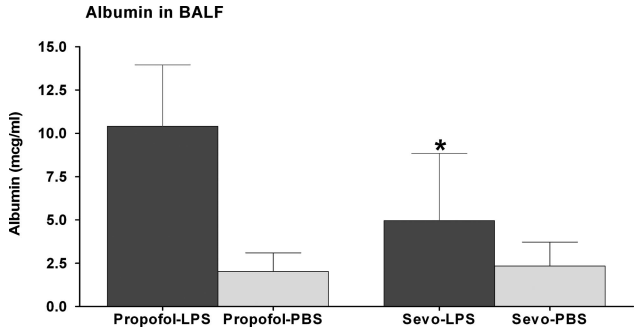

Fig. 3. Measurement of lung hyperpermeability. Bronchoalveolar lavage fluid was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). Albumin enzyme-linked immunosorbent assay was performed with supernatants. * $P < 0.05$ *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

Total Cell Count

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

Albumin and Proteins

Albumin concentration in BALF, reflecting alveolocapillary permeability, was significantly lower in the sevoflurane-LPS group compared with the propofol–lipopolysaccharide group (sevoflurane–lipopolysaccharide, 4.9 ± 3.8 μg/ml; propofol–lipopolysaccharide, 10.4 ± 3.5 μg/ml; fig. 3). The alveolar protein content as a measure of accumulation of proteins upon inflammation was significantly higher in the lipopolysaccharide groups compared with the PBS groups. In addition, a significantly lower protein concentration was found in the sevoflurane–lipopolysaccharide group compared with the propofol–lipopolysaccharide group (sevoflurane–lipopolysaccharide, 1.39 ± 0.51 mg/ml; propofol–lipopolysaccharide, 2.26 ± 0.32 mg/ml; table 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 lipopolysaccharide groups compared with both PBS groups. The sevoflurane–lipopolysaccharide group showed significantly lower levels of CINC-1 and MCP-1 compared with the propofol–lipopolysaccharide group (figs. 4A and B). In the sevoflurane–lipopolysaccharide group, CINC-1 and MCP-1 expression decreased 29% and 53%, respectively, compared with the propofol–lipopolysaccharide group.

Lung Tissue Analysis

The expression of messenger RNA (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 lipopolysaccharide groups was significantly increased compared with both PBS groups. Again, the sevoflurane–lipopolysaccharide group showed significantly lower mRNA levels compared with the propofol-lipopolysaccharide group (figs. 5A and B): in the sevoflurane–lipopolysaccharide group, CINC-1 mRNA and MCP-1 mRNA expression decreased by 42% and 53%, respectively, compared with the propofol–lipopolysaccharide group.

Histopathologic 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 with the PBS groups. However, there was no significant difference between the sevoflurane–lipopolysaccharide group and the propofol–lipopolysaccharide group (table 2).

AEC Injury

Evaluation of surfactant protein B (SP-B) RNA expression in lung tissue revealed a decrease in the expression of SP-B

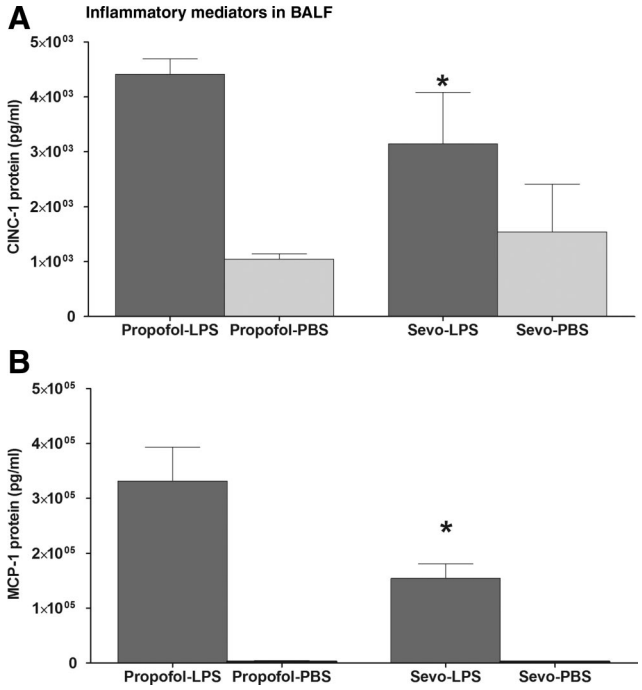

Fig. 4. Evaluation of cytokine-induced neutrophil chemoattractant-1 (CINC-1; *A*) and monocyte chemoattractant protein-1 (MCP-1; *B*) protein expression in bronchoalveolar lavage fluid. Bronchoalveolar lavage fluid was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). CINC-1 and MCP-1 enzyme-linked immunosorbent assays were performed with supernatants. * *P* < 0.05 *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo-PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

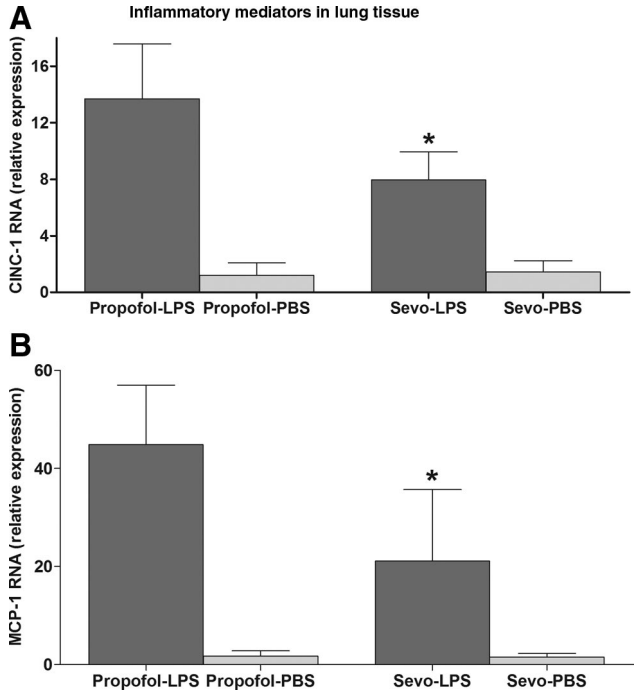

Fig. 5. Evaluation of cytokine-induced neutrophil chemoattractant-1 (CINC-1; *A*) and monocyte chemoattractant protein-1 (MCP-1; *B*) messenger RNA expression in lung tissue. Lung tissue was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). CINC-1– and MCP-1–specific real-time polymerase chain reactions were performed on random transcribed complementary DNA. * *P* < 0.05 *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with Sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

in both lipopolysaccharide groups compared with controls. However, decrease of SP-B in the sevoflurane–lipopolysaccharide animals was less accentuated compared with propofol–lipopolysaccharide animals (fig. 7).

Furthermore, analysis of the phospholipid content in bronchoalveolar lavage revealed an increase in the expression of phospholipids in both lipopolysaccharide groups compared with controls. The propofol–lipopolysaccharide animals showed significantly higher phospholipid levels compared with the sevoflurane–lipopolysaccharide animals (fig. 8).

In Vitro *Experiments with RPAECs and AECs*

Because a significantly higher P_{CO_2} was observed in the lipopolysaccharide–propofol group after 6 h of injury, we analyzed the possible proinflammatory effect of hypercapnia on RPAECs and AECs with or without lipopolysaccharide stimulation. Carbon dioxide values of 7.5% or 10% did not seem to have an impact on the inflammatory reaction in RPAECs or AECs compared with 5% CO_2 (figs. 9A and B). Similarly, we analyzed the possible proinflammatory effects of propofol in 14% Cremophor. Because AECs are not in direct contact with the anesthetic, we used smaller concentrations of propofol for the *in vitro* approach. No proinflammatory effects were shown in nonstimulated RPAECs or AECs. 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 (figs. 9C and D).

Discussion

The current 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, decreases expression of the proinflammatory mediators CINC-1 and MCP-1, and reduces 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[7] and by postconditioning[10] of AECs with sevoflurane in *in vitro*

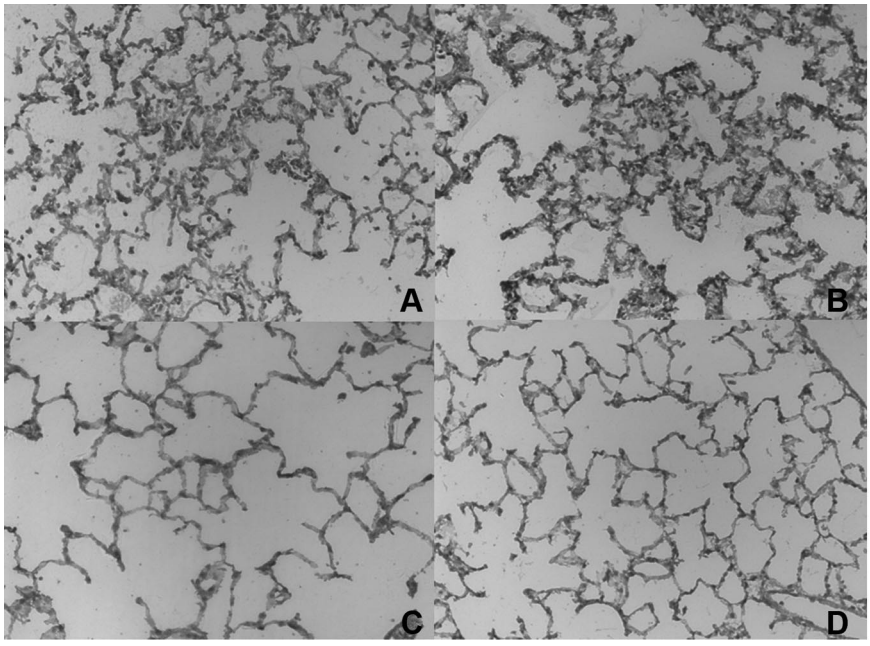

Fig. 6. Micrographs of representative lung section. (*A*) Propofol–lipopolysaccharide (LPS), (*B*) sevoflurane (Sevo)–LPS, (*C*) propofol–phosphate-buffered saline (PBS), and (*D*) Sevo–PBS. Hematoxylin and eosin staining, original magnification ×20. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h.

models of lipopolysaccharide-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 Pao_2/Fio_2 by postconditioning with sevoflurane after 6 h is most likely due to a less impaired gas exchange compared with propofol sedation. This was also reflected in the calculations of alveoloarterial 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 Pao_2 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 Pao_2/Fio_2 ratio could be an inhibition of the hypoxic pulmonary vasoconstriction (HPV) by both anesthetics. Clinical investigations are not conclusive regarding 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,[15,23,24] whereas propofol does not affect HPV.[25] 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

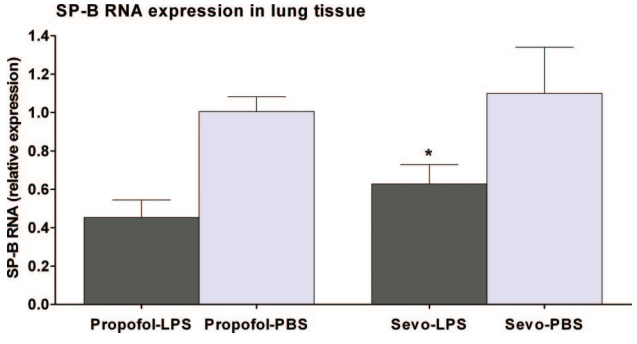

Fig. 7. Evaluation of surfactant protein B (SP-B) messenger RNA expression in lung tissue. Lung tissue was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). SP-B–specific real-time polymerase chain reaction was performed on random transcribed complementary DNA. * $P < 0.05$ *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

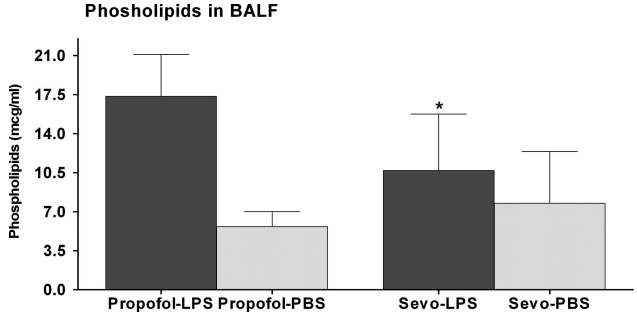

Fig. 8. Evaluation of phospholipids in bronchoalveolar lavage fluid. Bronchoalveolar lavage fluid was collected after 6 h in the four study groups (propofol–lipopolysaccharide [LPS], propofol–phosphate-buffered saline [PBS], sevoflurane [Sevo]–LPS, and Sevo–PBS). Phospholipid assay was performed with supernatants. * $P < 0.05$ *versus* propofol–LPS. Propofol–LPS, propofol–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 6 h. Sevo–LPS, Sevo–PBS: Instillation of LPS or PBS intratracheally, followed by ventilation and sedation with propofol for 2 h and with sevoflurane for the following 4 h. Values are mean ± SD from n = 6 (LPS) and n = 4 (PBS) experiments.

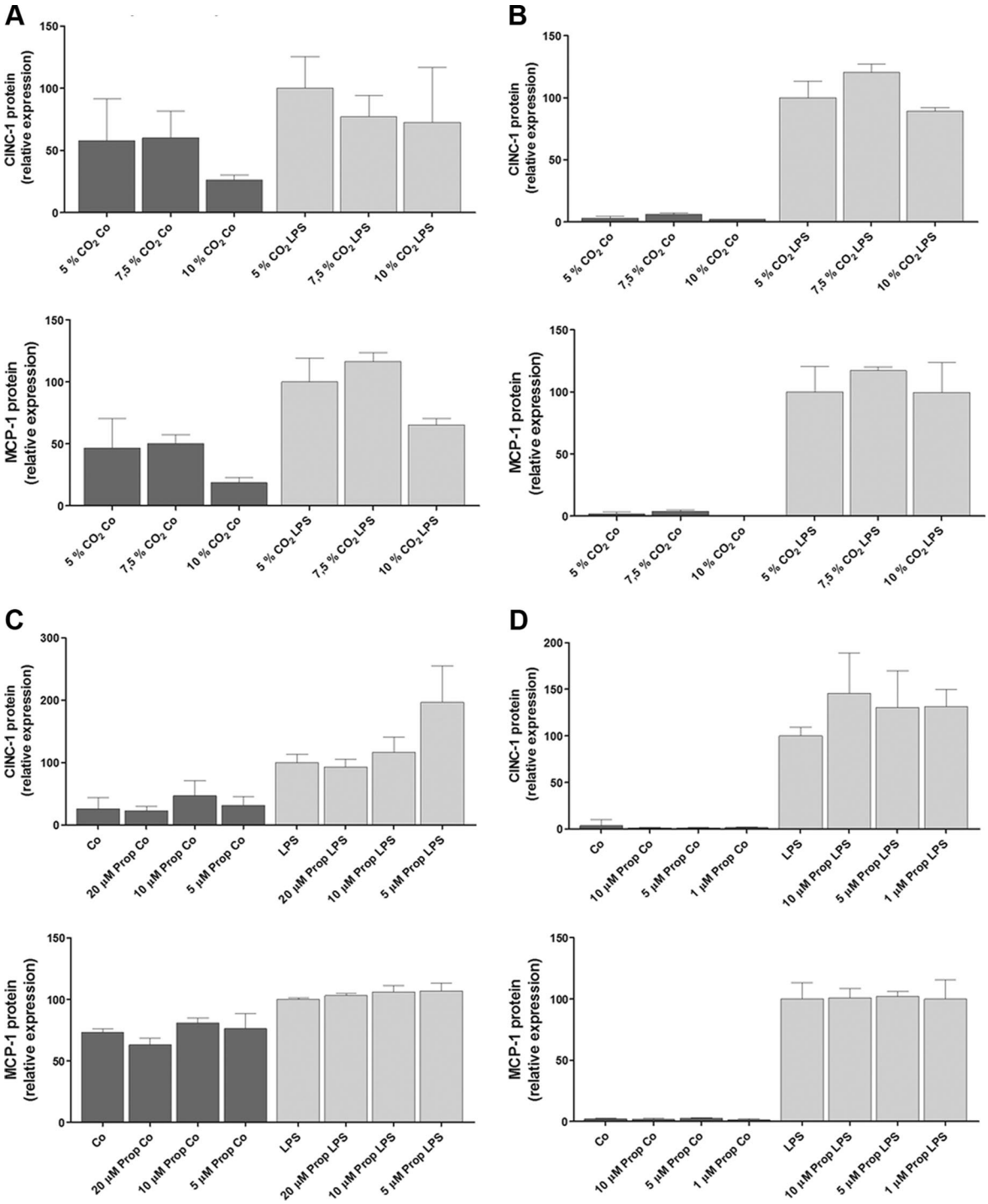

Fig. 9. (*A*) Evaluation of cytokine-induced neutrophil chemoattractant-1 (CINC-1) and monocyte chemoattractant protein-1 (MCP-1) expression in rat pulmonary artery endothelial cells (RPAECs) in hypercapnia. RPAECs were incubated with carbon dioxide (CO_2) at concentrations of 5, 7.5, and 10% for 6 h after stimulation with lipopolysaccharide (LPS). Co = control without LPS stimulation. Supernatants were collected. Enzyme-linked immunosorbent assays of CINC-1 and MCP-1 were performed. (*B*) Evaluation of CINC-1 and MCP-1 expression in alveolar epithelial cells (AECs) in hypercapnia. AECs were incubated with CO_2 at concentrations of 5, 7.5, and 10% for 6 h after stimulation with LPS. Supernatants were collected. Enzyme-linked immunosorbent assays of CINC-1 and MCP-1 were performed. (*C*) Evaluation of CINC-1 and MCP-1 expression in RPAECs after incubation with propofol. RPAECs were incubated with propofol at concentrations of 20, 10, and 5 μM for 6 h after stimulation with LPS. Supernatants were collected. Enzyme-linked immunosorbent assays of CINC-1 and MCP-1 were performed. (*D*) Evaluation of CINC-1 and MCP-1 expression in AECs after incubation with propofol. AECs were incubated with propofol at concentrations of 10, 5, and 1 μM for 6 h after stimulation with LPS. Supernatants were collected. Enzyme-linked immunosorbent assays of CINC-1 and MCP-1 were performed. Values are mean ± SD from 3 experiments.

model, the impact of the volatile anesthetic–induced inhibition of HPV cannot be excluded.

Second, the expression of CINC-1 and MCP-1 was studied. These chemoattractants have been shown to possess potent chemotactic activity for neutrophils (CINC-1 and 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 bronchoalveolar lavage 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 alveolocapillary 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] Therefore, 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 lipopolysaccharide injury in the sevoflurane group compared with 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 alveolocapillary unit with alveolar epithelial type II cells.[34,35]

Fifth, lipopolysaccharide–propofol animals showed a significantly 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 with propofol after lipopolysaccharide challenge.[36]

Up to now, several *in vivo* studies have explored the effects of sevoflurane on lung tissue but with inconsistent results. Takala *et al.*[37] compared sevoflurane anesthesia with thiopentone anesthesia in a model of ventilated healthy pigs. It was demonstrated that AEC type II cell integrity and ultrastructure remained unchanged after long-term (6-h) high-concentration exposure to sevoflurane (1.5 minimum alveolar concentration). Furthermore, a lower gene expression of tumor necrosis factor-α and interleukin-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 NO_3 and NO_2 production after sevoflurane anesthesia was revealed by another study using the same model.[39] However, this study was not based on an ALI model. In addition, the sevoflurane concentration of 4 vol% was rather high compared with our model.

To exclude a proinflammatory effect of propofol dissolved in Cremophor on pulmonary cells, we performed *in vitro* experiments. RPAECs were coexposed to propofol in concentrations previously reported.[40,41] No increased cytotoxicity or enhanced inflammatory response could be observed. In addition, it should 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 carbon dioxide after 6 h of injury. We discussed this increase as a consequence of injury. *In vitro* experiments underlined our hypothesis by showing that increased concentrations of carbon dioxide did not interfere with the inflammatory reaction. This is in accord with the literature, where only carbon dioxide values of 15% or 20% induced an additional injury.[45]

Few reports exist focusing on the postconditioning capabilities of sevoflurane in acute lung injury. In a recent publication, Hofstetter *et al.*[46] 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 tumor necrosis factor-α and interleukin-1β plasma levels. In contrast to our study, lipopolysaccharide was given intravenously with an early administration of sevoflurane after the injury. In the current study, we were able to show antiinflammatory effects of sevoflurane even when administered 2 h after a lipopolysaccharide stimulation, *i.e.*, with late initiation of postconditioning. This may be of clinical relevance for patients who have already experienced 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 whether the observed difference would also be found with other intravenous anesthetics. Interestingly, in cardiac ischemia–reperfusion injury, protection by volatile anesthetics, morphine, and propofol is relatively well investigated.[47] 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 patients with ALI/ARDS in the ICU is commonly performed using propofol. In the last years, the antiinflammatory effects of this intravenous anesthetic have been extensively studied in several *in vivo* studies. It has been shown that propofol has antiinflammatory effects that attenuate cytokine response after endotoxin shock in rats.[48,49] Several studies suggest that pretreatment and posttreatment with propofol provides protective effects in endotoxin-induced ALI[19,50] and lipopolysaccharide-induced shock.[51] However, the antiinflammatory 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.[52] In our study, we used a propofol formulation in 14% Cremophor without EDTA as clinically used propofol would induce hypervolemia in rats because of the low concentration of propofol. This could explain why fewer 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 ICUs, it is now possible to take advantage of the properties of volatile anesthetics, such as fast induction, fast awakening, and easy titration, for sedation of postoperative and critically ill patients. 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 sevoflurane sedation compared with propofol was demonstrated in patients after cardiothoracic surgery.[54] Up to now, there have been no clinical studies regarding the effects of sevoflurane sedation in patients with ALI or ARDS. The results of this *in vivo* study indicate that 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 h) compared with the clinical situation. In addition, we administered an F_{IO_2} of 1.0 in our model, which is not commonly used in 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 h.[57] However, we cannot exclude that hyperoxia influences the antiinflammatory effects of sevoflurane in our model.

Despite 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 a sedative compared with propofol. This property of sevoflurane seems to be mediated by 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 promising new 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 current study indicates that anesthetic postconditioning with sevoflurane offers beneficial properties compared with propofol in a model of ALI *in vivo*.

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