The Effects of Anesthetic Preconditioning with Sevoflurane in an Experimental Lung Autotransplant Model in Pigs

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BACKGROUND: Ischemia–reperfusion lung injury is doubly important in thoracic surgery because of the associated ventilation damage to 1 lung. In this study we evaluated the cytoprotective effects of sevoflurane in a pulmonary autotransplant model in pigs.
METHODS: Twenty Large White pigs undergoing pneumonectomy plus lung autotransplant were divided into 2 10-member groups on the basis of the anesthetic received (propofol or sevoflurane). Proinflammatory mediators, oxidative stress, nitric oxide metabolism, and hemodynamic and blood variables were measured at 5 different time points.
RESULTS: There was an increase of oxidative stress markers and proinflammatory mediators in the propofol group, whereas the hemodynamic variables were similar in both groups.
CONCLUSIONS: We demonstrated that sevoflurane decreased the inflammatory response and oxidative stress in a live ischemia–reperfusion lung model. (Anesth Analg 2011;113:742–8)

Ischemia–reperfusion (IR) lung injury is doubly important in thoracic surgery because of the associated ventilation damage to 1 lung. The aim of this study was to evaluate the cytoprotective effects of sevoflurane in a pulmonary autotransplant model in pigs.

Acute inflammatory responses are seen with all forms of lung surgery, and many factors are involved in this process apart from the surgical procedure itself. These factors include changes in the cytokine balance, oxidative stress, the use of one-lung ventilation (OLV), and IR injury.[1] IR-induced lung injury during lung transplantation is a consequence of endothelial and epithelial dysfunction after reperfusion and is characterized by postoperative nonspecific alveolar damage, lung edema, and hypoxemia. This injury could lead to primary graft failure and is considered a significant cause of morbidity and mortality after lung transplantation.

There are several pulmonary surgical procedures during which it is necessary to make complete lung ischemia for a given period of time with the consequent increased risk of IR injury (lung transplantation, arterial sleeve lung resections, pulmonary arterioplasties, living donor lobar lung transplantation, and those cases that require ex situ surgery).[2,3]

Several mediators have been implicated in the pathogenesis of IR lung injury, and their identification determines the magnitude of the damage produced. Several authors have shown that the increase in lung biomarkers is related to postoperative pulmonary complications and poor postoperative outcome.[4,5]

Different methods have been developed to mitigate IR injury with relative success. One of them is anesthetic preconditioning (APC) with volatile anesthetics (sevoflurane, halothane, and isoflurane, among others). It has been shown that sevoflurane reduces IR damage in the myocardium, either through improved coronary flow or ventricle function or by reduced infarct size.[6] The efficacy of APC with volatile anesthetics has also been shown in other organs, including kidney, liver, spinal cord, and brain.[7–10] In the case of the lung, APC with isoflurane and sevoflurane has been shown to attenuate IR damage in isolated rat and rabbit lungs,[11,12] although as far as we know, no in vivo studies have been published.

Therefore, we hypothesized that the inflammatory response of alveolar epithelial cells to IR lung injury may also be altered by pretreatment with volatile anesthetics. The present lung autotransplant model was designed to evaluate the in vivo effects of sevoflurane in IR lung injury, including hemodynamic, gasometric, and biochemical markers.

METHODS

This study was approved by the institution's Research and Animal Experimentation Committee. All experiments were performed according to European and Spanish law regarding the handling and care of experimental animals.

Animal Model and Study Groups

Twenty Large White pigs weighing 30 to 50 kg each were subjected to an orthotopic left lung autotransplantation (left pneumonectomy, ex situ cranial lobectomy, and left caudal lobe reimplantation) with a subsequent 30-minute graft

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reperfusion. Animals were block randomized by random numbers (Microsoft Excel 2003) to receive either propofol in continuous perfusion as anesthetic maintenance throughout the procedure (control group, CON; $n = 10$) or APC with sevoflurane from the anesthetic induction to the beginning of the OLV stage followed by propofol perfusion (sevoflurane group, SEV; $n = 10$).

Anesthesia

Drinking water was allowed ad libitum, but solid food was withheld for 18 hours before each experiment. Premedication was performed with IM ketamine (10 mg/kg; Ketolar, Parke Davis, Pfizer, Dublin, Ireland). Once in the operating room, the animals were placed in a supine position and pulse oxymetry and electrocardiographic monitoring were performed. Anesthesia induction was conducted with propofol (4 mg/kg; Diprivan, AstraZeneca, Macclesfield, Cheshire, UK), fentanyl (3 μg/kg; Fentanest, Kern Pharmaceuticals, Houston, TX), and atracurium (0.6 mg/kg; Tracrium, Glaxo Smith Kline, Brentford, UK). Orotracheal intubation was performed with a 6- to 7-mm cuffed endotracheal tube. Mechanical ventilation with volume-controlled ventilation was used with 5 cm H_2O positive end expiratory pressure and peak pressure <30 cm H_2O throughout the study. A tidal volume approximately 8 mL/kg, a respiratory rate of 12 to 15 respirations per minute (rpm), and an inspiratory:expiratory ratio of 1:2 were chosen to maintain $Paco_2$ in the range of 35 to 40 mm Hg. FiO_2 was maintained at 1 throughout the procedure. Intraoperative crystalloid infusion was maintained at 5 to 6 mL kg^{-1} h^{-1}. Anesthesia was maintained with propofol in continuous infusion (8 to 10 mg kg^{-1} h^{-1}) (CON group) or 3% sevoflurane from the anesthetic induction to the beginning of OLV followed by propofol perfusion (8 to 10 mg kg^{-1} h^{-1}) (SEV group). Supplemental doses of fentanyl and atracurium were used when required.

Surgical Protocol

The surgical technique included preliminary procedures, thoracotomy, left pneumonectomy, back-table cranial lobectomy, caudal lobe reimplantation, and reperfusion.

The animals were scrubbed with betaiodine solution, and all subsequent invasive procedures were performed under aseptic conditions. A surgical tracheotomy was performed, the orotracheal tube was removed, and a 6-mm cuffed tube was inserted into the trachea through the tracheotomy that facilitated the insertion and withdrawal of the tube into and out of the right bronchus during the procedure. A 7F sterile pulmonary artery catheter (Edwards Lifesciences, Irvine, CA) was introduced through the femoral vein, and final position into the right pulmonary artery was confirmed intraoperatively. A 7F sterile femoral artery catheter was used to enable arterial blood pressure monitoring and blood sampling. A suprapubic cystostomy was performed to monitor urine output. After these procedures, a left thoracotomy was performed as described elsewhere.[13] Just before the completion of the pneumonectomy, a bolus of IV heparin (300 IU/kg; Mayne Pharma, Madrid, Spain) was administered to prevent thrombosis in the clamped pulmonary artery. Next, on the back table, the left lung was perfused through the pulmonary artery and veins with University of Wisconsin solution, and a cranial lobectomy was performed to ensure that a thrombus did not form in the graft. The caudal left lobe was then implanted back into the swine, and reperfusion was performed initially in a retrograde direction by unclamping the left atrium. The endobronchial tube was then pulled back into the trachea, which enabled 2-lung ventilation. The left pulmonary artery was then unclamped, and bloodflow was maintained for 30 minutes. At the end of the experiment, the animal was euthanized by a potassium chloride injection while under deep anesthesia.

Measurement and Sampling Time Points

Baseline (B) hemodynamic and arterial blood gas measurements were performed 30 minutes after the thoracotomy with the animal under 2-lung ventilation, but not lung biopsies or blood samples. Hemodynamic arterial gas measurements, blood samples, and lung biopsies were collected at the following time points: prepneumonectomy (PPn) before completing the pneumonectomy and with the animal under OLV; prereperfusion (PRp) before reperfusion and ventilation of the reimplanted left caudal lobe; 10 minutes postreperfusion (Rp10′) of the reimplanted lobe; and 30 minutes postreperfusion (Rp30′) of the reimplanted lobe.

Hemodynamic Measurements

A femoral artery catheter was used to record the systolic, diastolic, and mean arterial blood pressure. We inserted a pulmonary artery catheter via the femoral vein and recorded the pulmonary artery systolic, diastolic, and mean pressures, pulmonary capillary pressure, and central venous pressure. In addition, the cardiac output monitor (Edwards Lifesciences) and thermodilution technique were used at the time points indicated previously (B, PPn, PRp, Rp10′, Rp30′) to record the cardiac index and systolic volume.

Blood Gas Measurements

Arterial blood gas analyses were performed at the previously mentioned time points. In addition, blood gas samples were taken from the femoral arterial catheter and by puncturing the pulmonary vein of the reimplanted lobe at 10 and 30 minutes after reperfusion (Rp10′ and Rp30′).

Blood Biochemical Measurements

Femoral venous blood samples were collected for biochemical determinations at the following time points: PPn, PRp, Rp10′, and Rp30′. The serum nitric oxide (NO) concentration was measured by the Griess reaction[14] (see Appendix, Supplemental Digital Content 1, http://links.lww.com/AA/A296).

Biochemical Studies in Lung Tissue

Lung tissue biopsies were performed for biochemical studies and quantification of lung edema. The first 2 samples of lung tissue (PPn and PRp) were obtained from the cranial lobe (PRp lung sample was taken from the cranial lobe that remained on the back table just before reperfusion of the reimplanted caudal lobe), and the last 2 samples (Rp10′ and Rp30′) from the reimplanted caudal lobe. Every lung sample was divided into 2 parts: one part was stored in polypropylene tubes at −40°C until the quantification of lung edema, and the other part was placed in a cryotube, flash frozen in liquid nitrogen and stored at −80°C until biochemical analysis.

Assessment of lung edema: lung tissue samples were blotted and weighed. After baking in a vacuum oven for 24 hours at 60°C, the tissues were weighed to obtain dry weights. The tissues were again weighed after another 24 hours to verify that complete dehydration had occurred. The data were calculated as the wet weight minus the dry weight divided by the dry weight and used as an indicator of lung edema (as illustrated by an increase in the wet-to-dry weight ratio; mg/mg).

After preparation of tissue homogenates[15] (see Appendix, Supplemental Digital Content 1, http://links.lww.com/AA/A296), oxidative stress, and the levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, endothelial NO synthase (eNOS), neuronal NO synthase (nNOS), and inducible NO synthase (iNOS) were evaluated.

Evaluation of oxidative stress. Lipid hydroperoxides (LPO) indicate the degree of membrane lipid oxidation and were detected using a specific kit for use in lung tissue (K-assay LPO-CC, Kamiya Biochemical Company, Seattle, WA). Malondialdehyde (MDA), an end compound of lipid peroxidation, is a cell damage marker that was detected through the formation of thiobarbituric acid derivatives[16] in lung tissue. The myeloperoxidase (MPO) assay was used to quantitate lung tissue neutrophil accumulation, and it was detected by the modified Bradley method of Bradley et al.[17] (see Appendix, Supplemental Digital Content 1, http://links.lww.com/AA/A296).

Western blot analysis. Western blot analysis was used to measure TNF-α, IL-1β levels, and the expression of nitric oxide synthase (NOS), eNOS, nNOS, and iNOS forms (see Appendix, Supplemental Digital Content 1, http://links.lww.com/AA/A296). Cytokine (TNF-α and IL-1β) levels were semiquantitatively measured by densitometric analysis.

Reproducibility within the assays was evaluated in 3 independent experiments, and each assay was performed with 3 replicates. The overall intra-assay coefficient of variation was calculated to be <5%. Assay-to-assay reproducibility was evaluated in 3 independent experiments; the overall interassay coefficient of variation was calculated to be <6%.

Statistical analysis. The data were expressed as the mean and the SEM (SE). Nonparametric tests were used; the Mann–Whitney *U* test was applied to establish differences between the analyzed groups, and the Wilcoxon test for paired data was used to study the evolution of the intragroup values and to make multiple comparisons between them to avoid type I errors. Statistical significance was considered for $P < 0.05$. The SPSS version 14.0 statistical package (SPSS Inc., Chicago, IL) was used in the statistical analysis.

Table 1. General Variables

Variable	Group	Value
Weight (kg), $P = 0.43$	CON	34.3 ± 13.9
	SEV	37.8 ± 13.4
Duration of OLV (minutes), $P = 0.1$	CON	167.5 ± 73.4
	SEV	183.6 ± 86
Ischemia length (minutes),	CON	96.7 ± 13.8
$P = 0.09$	SEV	91.8 ± 22.5
Peak pressures in OLV (cm H_2O),	CON	25 ± 9
$P = 0.15$	SEV	26 ± 10
Plateau pressures in OLV (cm H_2O),	CON	13 ± 7
$P = 0.09$	SEV	14 ± 6

Data are expressed as the mean ± standard deviation. OLV = one-lung ventilation; SEV = sevoflurane.

RESULTS

There were no differences between the CON and SEV groups in terms of animal weight, lung ischemia time, OLV time, pressure airways during mechanical ventilation, or duration of the entire procedure (Table 1).

Hemodynamics

The hemodynamic variables showed great stability in both groups, the differences between them being limited to heart rate, which increased significantly after reperfusion in the CON group but not in the SEV group. The recorded postreperfusion heart rate was more rapid in the CON group than in the SEV group (Table 2).

Blood Gas Analysis

Arterial oxygenation was similar in both groups during the entire procedure, except for the Rp10′ sample. Also, Pao_2 measured in the pulmonary vein after reperfusion was significantly higher in the SEV group than in the CON group at both 10 and 30 minutes after reperfusion. No other significant differences were observed while comparing systemic arterial and pulmonary arterial Pco_2 and pH throughout the procedure (Table 3).

Pulmonary Edema

Formation of lung edema, as assessed by the wet-to-dry weight ratio of the lung, was decreased only in SEV group

Table 2. Hemodynamics

Hemodynamics	Group	B	PPn	PRp	Rp10′	Rp30′
MBP (mm Hg)	CON	99 ± 4	101 ± 4	90 ± 4	86 ± 4	81 ± 5
	SEV	76 ± 4*	85 ± 6	83 ± 4	74 ± 4	73 ± 5
PAMP (mm Hg)	CON	26 ± 3	28 ± 3	27 ± 2	29 ± 3	29 ± 2
	SEV	20 ± 1*	24 ± 2	24 ± 2	27 ± 2	26 ± 2
HR (bpm)	CON	99 ± 6	94 ± 8	103 ± 9	95 ± 7	93 ± 8
	SEV	107 ± 7	110 ± 5	106 ± 7	102 ± 6	105 ± 8
SV (mL/beat)	CON	58 ± 5	63 ± 8	46 ± 4	54 ± 4	54 ± 6
	SEV	50 ± 4	59 ± 6*	46 ± 5	52 ± 7	49 ± 6
CI ($l \times min^{-1} \times m^{-2}$)	CON	5.2 ± 0.4	5.2 ± 0.5	4.4 ± 0.4	4.8 ± 0.6	5.5 ± 1.1
	SEV	6.6 ± 0.8	7.8 ± 1	5.6 ± 0.6	6 ± 1	5.9 ± 0.8

Data are expressed as the mean ± standard error of the mean. MBP = mean arterial blood pressure; PAMP = pulmonary artery mean pressure; HR = heart rate; bpm = beats per minute; SV = systolic volume; CI = cardiac index; CON = control group; SEV = sevoflurane group; B = basal; PPn = prepneumonectomy; PRp = prereperfusion; Rp10′ = 10 minutes postreperfusion; Rp30′ = 30 minutes postreperfusion.
* $P < 0.05$ SEV vs CON group.

Table 3. Blood Gas Variables

Blood source and variable	Group	B	PPn	PRp	Rp10′	Rp30′
Femoral artery						
PO_2 (mm Hg)	CON	330 ± 55	206 ± 37	302 ± 48	250 ± 48	297 ± 49
	SEV	305 ± 37	169 ± 23	315 ± 42	329 ± 44*	333 ± 49
PCO_2 (mm Hg)	CON	37 ± 2	44 ± 3	42 ± 3	44 ± 4	44 ± 5
	SEV	33 ± 1	49 ± 11	40 ± 2	42 ± 2	40 ± 2
pH	CON	7.52 ± 0.02	7.45 ± 0.03	7.45 ± 0.02	7.44 ± 0.02	7.45 ± 0.03
	SEV	7.56 ± 0.02	7.5 ± 0.03	7.48 ± 0.03	7.44 ± 0.03	7.45 ± 0.03
Pulmonary vein (reimplanted caudal left lobe)						
PO_2 (mm Hg)	CON	—	—	—	278 ± 52	262 ± 41
	SEV	—	—	—	418 ± 29*	391 ± 44*
PCO_2 (mm Hg)	CON	—	—	—	36 ± 4	36 ± 5
	SEV	—	—	—	33 ± 3	32 ± 5
pH	CON	—	—	—	7.54 ± 0.04	7.55 ± 0.05
	SEV	—	—	—	7.52 ± 0.04	7.52 ± 0.06

Data are expressed as the mean ± standard error of the mean. PO_2 = partial pressure of oxygen; PCO_2 = partial pressure of carbon dioxide; CON = control group; SEV = sevoflurane group; B = basal; PPn = prepneumonectomy; PRp = prereperfusion; Rp10′ = 10 minutes postreperfusion; Rp30′ = 30 minutes postreperfusion.
* $P < 0.05$ vs CON group.

Table 4. Lung Edema, Oxidative Stress Metabolites, Myeloperoxidase Activity, and Cytokines

Biochemical and group	PPn	PRp	Rp10′	Rp30′
Lung edema				
CON	4.82 ± 0.21 (4.1–5.6)	4.91 ± 0.48 (4.3–8.0)	4.82 ± 0.35 (3.5–6.5)	4.87 ± 0.01 (4.75–4.9)
SEV vs CON	NS	NS	NS	$P = 0.001$
SEV	4.29 ± 0.22 (3.8–5.7)	4.28 ± 0.28 (4.1–5.0)	4.55 ± 0.29 (3.3–5.4)	3.33 ± 0.2 (2.7–4.5)*†‡
LPO mmol/mg protein				
CON	2.61 ± 0.07 (2.1–3.8)	3.61 ± 0.05 (3.2–4.0)	3.64 ± 0.09 (3.2–4.1)	3.88 ± 0.06 (3.5–4.1)
SEV vs CON	NS	$P = 0.047$	$P = 0.049$	$P = 0.02$
SEV	2.65 ± 0.11 (2.1–2.9)	3.06 ± 0.09 (2.7–3.5)*	2.87 ± 0.14 (2.3–3.3)	3.17 ± 0.11 (2.5–3.8)
MDA (pmol/mg protein)				
CON	3.21 ± 0.13 (2.4–3.8)	3.87 ± 0.079 (3.25–4.8)*	4.99 ± 0.088 (4.7–5.4)*†	5.35 ± 0.093 (5.0–5.7)*
SEV vs CON	NS	NS	$P = 0.044$	$P = 0.04$
SEV	3.12 ± 0.182 (2.4–3.7)	3.52 ± 0.159 (3.3–4.7)*	3.64 ± 0.149 (3.3–4.6)*†	4.47 ± 0.159 (3.6–4.97)*†
MPO (IU/mg protein)				
CON	0.07 ± 0.004 (0.04–0.09)	0.1 ± 0.003 (0.08–0.13)	0.18 ± 0.004 (0.14–0.2)†	0.23 ± 0.005† (0.21–0.28)
SEV vs CON	NS	NS	$P = 0.04$	$P = 0.02$
SEV	0.04 ± 0.003 (0.02–0.06)	0.07 ± 0.007 (0.05–0.1)	0.09 ± 0.006 (0.04–0.12)	0.1 ± 0.007 (0.03–0.12)
IL-1 (arbitrary units)				
CON	1.23 ± 0.00 (1.17–1.26)	1.82 ± 0.04 (1.69–1.93)	1.81 ± 0.05 (1.43–1.98)*	1.85 ± 0.02 (1.74–1.94)*
SEV vs CON	NS	$P = 0.01$	$P = 0.049$	$P = 0.038$
SEV	1.23 ± 0.04 (0.99–1.3)	1.16 ± 0.06 (0.8–1.6)	0.98 ± 0.06 (0.68–1.15)*†	1.06 ± 0.04 (0.92–1.07)
TNF-α (arbitrary units)				
CON	0.70 ± 0.01 (0.64–0.78)	0.84 ± 0.02 (0.7–0.97)*	0.96 ± 0.02 (0.94–1.07)*†	1.19 ± 0.02 (1.12–1.34)*†
SEV vs CON	$P = 0.046$	$P = 0.04$	$P = 0.04$	$P = 0.015$
SEV	0.77 ± 0.01 (0.7–0.81)	0.60 ± 0.01 (0.52–0.71)*	0.63 ± 0.02 (0.51–0.67)*	0.63 ± 0.00 (0.61–0.68)*

Data are expressed as the mean ± standard error of the mean and range of values. LPO = lipid hydroperoxides; MDA = malondialdehyde; MPO = myeloperoxidase; IL-1 = interleukine-1; TNF-α = tumor necrosis factor-α; CON = control group; SEV = sevoflurane group; PPn = prepneumonectomy; PRp = prereperfusion; Rp10′ = 10 minutes postreperfusion; Rp30′ = 30 minutes postreperfusion; NS = nonsignificant.
* $P < 0.05$ in intragroup comparison respect PPn. † $P < 0.05$ in intragroup comparison respect PRp. ‡ $P < 0.05$ in intragroup comparison respect Rp10′.
The Appendix (Supplemental Digital Content 1, http://links.lww.com/AA/A296) contains significant P values for this table.

swines. When we used APC with sevoflurane, lung edema was reduced significantly after 30 minutes of reperfusion in comparison with the CON group (Table 4).

Oxidative Stress in Lung Tissue

MDA was increased progressively in both groups at each time point. The LPO levels were increased in the CON group continuously throughout the experiment, although they decreased after reperfusion and then again increased in the SEV group. However, when animals were submitted to APC, LPO and MDA levels in reperfused lung tissue were significantly lower than were those in the CON group (Table 4).

Lung Tissue Myeloperoxidase

Tissue MPO activity in biopsy specimens from reimplanted lungs increased significantly after reperfusion in the CON group, although this sharp increase was not observed when sevoflurane was administered. Moreover, recorded Rp10′ and Rp30′ MPO activity was higher in the CON group than in the SEV group (Table 4).

Western Blot Analysis of TNF-α and IL_1β

The proinflammatory cytokines IL-1 and TNF-α were significantly higher after IR in control lungs, although this increase was not recorded when APC with sevoflurane took place (Table 4).

Table 5. Nitric Oxide Metabolism

Biochemical and group	PPn	PRp	Rp10′	Rp30′
NO (nmol/mL)				
CON	43.2 ± 3.4 (20–52)	20 ± 1.7 (15.9–32.6)*	26 ± 2.8 (18.3–44.3)*	24.5 ± 2.2 (16–38.7)*
SEV vs CON	NS	$P = 0.035$	$P = 0.00122$	$P = 0.0016$
SEV	38.4 ± 2.3 (29.6–46)	37.9 ± 4.7 (19.2–48.9)	37 ± 3 (23.2–54.5)	41 ± 3.1 (26.8–57)
eNOS (nmol/mL)				
CON	1.66 ± 0.01 (1.53–1.75)	1.32 ± 0.01 (1.24–1.4)*	1.39 ± 0.02 (1.28–1.48)*	1.34 ± 0.01 (1.31–1.39)*
SEV vs CON	NS	$P = 0.01$	$P = 0.02$	$P = 0.01$
SEV	1.55 ± 0.04 (1.2–1.68)	1.56 ± 0.03 (1.44–1.69)*	1.60 ± 0.00 (1.56–1.63)*	1.65 ± 0.01 (1.64–1.7)*
nNOS (nmol/mL)				
CON	1.39 ± 0.04 (1.2–1.7)	1.04 ± 0.05 (0.98–1.4)*	1.24 ± 0.04 (1.04–1.51)*†	1.19 ± 0.04 (1.04–1.36)*
SEV vs CON	NS	$P = 0.017$	$P = 0.038$	$P = 0.02$
SEV	1.47 ± 0.03 (1.3–1.6)	1.49 ± 0.04 (1.2–1.6)	1.43 ± 0.06 (0.98–1.7)	1.44 ± 0.01 (1.34–1.49)
iNOS (nmol/mL)				
CON	1.73 ± 0.02 (1.59–1.87)	1.81 ± 0.06 (1.56–2.23)	1.97 ± 0.03 (1.89–2.28)*†	2.04 ± 0.03 (1.97–2.2)*†
SEV vs CON	NS	$P = 0.045$	$P = 0.01$	$P = 0.01$
SEV	1.89 ± 0.03 (1.97–2.2)	1.77 ± 0.02 (1.63–1.86)	1.55 ± 0.05 (1.46–1.9)*†	1.65 ± 0.02 (1.56–1.76)*†

Data are expressed as the mean ± standard error of the mean. NO = nitric oxide; eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; iNOS = inducible nitric oxide synthase; CON = control group; SEV = sevoflurane group; PPn = prepneumonectomy; PRp = prereperfusion; Rp10′ = 10 minutes postreperfusion; Rp30′ = 30 minutes postreperfusion; NS= nonsignificant.

* $P < 0.05$ in intragroup comparison respect PPn. † $P < 0.05$ in intragroup comparison respect PRp.

The Appendix (Supplemental Digital Content 1, http://links.lww.com/AA/A296) contains significant *P* values for this table.

Serum NO and NOS Expression in Lung Tissue

A striking decrease in serum NO and NOS expression in lung tissue was found pre- and postreperfusion of the implanted lobe in control animal lungs. This decrease was not detected in the SEV group (Table 5).

DISCUSSION

These results show that the administration of sevoflurane before ischemic damage attenuates the deleterious effect of IR on the lung in a pulmonary autotransplant model in pigs. In addition, this approach reduces oxidative stress and the inflammatory response generated during IR. To our knowledge, this is the first study that demonstrates the use of APC with sevoflurane to decrease the inflammatory response and oxidative stress in an in vivo lung transplantation model.

Despite broad experience with volatile anesthetics during mechanic ventilation, its effects on pulmonary inflammation are controversial. In some experimental studies, this approach has been shown to increase the inflammatory lung response during mechanical ventilation,[18–20] although other studies have reported a decrease in the inflammatory response when volatile anesthetics were compared with IV anesthesia.[21] This discrepancy could be based on differences in the experimental models analyzed. Also, it has been demonstrated that the use of volatile anesthetics before lipopolysaccharide-induced lung injury is related to attenuation of the lung inflammatory response and an improved outcome.[22]

The ability of volatile anesthetics to protect against induced acute lung injury has been studied only in an experimental setting. Liu et al. confirmed the protective effects of APC with sevoflurane in isolated rat and rabbit lungs that underwent IR.[11,12] We obtained similar results to Liu et al. in terms of TNF-α and NO metabolism using APC in the lungs of live animals as opposed to the experimental isolated lung model that the previous authors used. However, IR damage is a more complex phenomenon, and as such, it is not possible to rely on its effect on TNF-α as the sole determinant of lung damage. In our study, we measured the effects of sevoflurane on IR damage not only on TNF-α in vivo (not in an isolated lung model) but also on oxidative stress, as reflected by MDA, LPO, and MPO. The proposed mechanism underlying volatile anesthetics' protection of the lung after reperfusion is not yet completely known. There are 2 ways to explain this protection. First, this protection appears to involve a decrease in Na, K-ATPase, and sodium channel activities similar to those found in myocardium.[6] The second is related to hypoxia and microvascular permeability; in a recent study, we observed the ability of sevoflurane to decrease TNF-α-induced microvascular endothelial permeability.[23] During OLV, alveolar hypoxia occurs in the nonventilated lung, which leads to the enhanced expression of adhesion molecules on alveolar epithelial cells with increased neutrophil adherence that enhance endothelial permeability and therefore lung damage, demonstrating that the lower respiratory epithelial compartment might play an important role in inflammatory mechanisms during hypoxia-induced lung injury[24] and altered surface phospholipids and apoprotein biosynthesis by type II alveolar epithelial cells. The effect of OLV hypoxia vasoconstriction reduces ventilation/perfusion mismatch, but it also results in tissue hypoxia. Yin et al. showed congested lung vasculature in an experimental model in pigs after 1 hour of OLV.[25] Therefore, the alveolar alterations and the vasculature findings by Yin et al. support the idea of compartmentalized lung inflammatory reactions.

Oxidative Stress and the Inflammatory Response

OLV has been correlated with oxidative stress, and both in turn have been related to acute lung injury. In our study, there were no differences in the duration of OLV or in airway pressures between the 2 groups; as a result, we eliminated this factor as a source of bias when we compared oxidative stress between the groups. In our study, the only difference between the groups was the administration of sevoflurane for 90 minutes before ischemia.

We recorded an increase in LPO, MDA, and MPO in both groups after reperfusion, although there was evident attenuation of the increase in LPO and therefore of MDA in

the sevoflurane group versus the control group that is attributable only to the cytoprotective effect of sevoflurane.

A lower rate of tissue leukocyte infiltration was observed in the SEV group. Volatile anesthetics inhibit the endothelial adhesion and migration of neutrophils, reducing their activity both in vitro and in vivo,[26,27] and the lower tissue MPO levels in the SEV group probably occurred through this mechanism.

Cytokines are crucial mediators in the bidirectional interaction between leukocytes and endothelial cells. As other authors have found, we observed a reduced inflammatory response in the SEV group, as determined by the progressively lower and descending levels of IL-1 and TNF-α.[4,28]

Nitric Oxide Metabolism

NO exerts both beneficial effects (eNOS or NOS III and nNOS or NOS I) and harmful effects (iNOS). Our results show that NO in the control group decreased drastically after ischemia, whereas the levels remained unchanged throughout the process in the preconditioned group. This may have been due to the decrease in the constitutive forms of NOS observed in the control group after ischemia. A number of studies have reported a reduction in endogenous NO after pulmonary IR in both humans and animals. This discovery could be associated with an increase in iNOS expression that would cause endogenous NO to be rapidly destroyed by reactive oxygen species after reperfusion.[29] Our findings are similar to those reported by other authors who describe increased NO release after preconditioning with sevoflurane in isolated hearts.[30] This observation could represent a cell-protective method based on the maintenance of NO metabolic homeostasis versus other methods such as the administration of exogenous NO in lung transplantation.[31]

Hemodynamics and Oxygenation

Both anesthetic techniques afforded great hemodynamic stability, the differences only being observed in terms of heart rate after reperfusion, with higher values in the CON group. This could be due to the tachycardia generated by an increased amount of toxic metabolites that would recirculate after reperfusion in the CON animals in comparison with the SEV group. Arterial oxygenation was similar between the groups before reperfusion. However, after reperfusion, greater venous oxygenation was observed in swine that received sevoflurane before starting OLV than in the CON group. Oxygenation of reimplanted lobar lung was greater when APC with sevoflurane was used, perhaps indicating better lung function as a consequence of a reduction in the inflammatory response and a decrease in oxidative stress after IR occurred; therefore, there is less interstitial edema that would reduce gas exchange.

Clinical Applicability of the Experimental Model

The experimental model of IR considered in our study was oriented towards clinical usefulness, because there are many published case series on lung tumor resections with cuff bronchoangioplasty.[32,33] This technique has emerged as a valid alternative to pneumonectomy in patients with central tumors and scant respiratory reserve, and also in live donor lobar transplantation,[34] which are characterized by IR damage. Lastly, there are cases of complicated tumors in which the patient does not functionally tolerate pneumonectomy. In such situations, pneumonectomy is followed by ex situ lobectomy and posterior reimplantation of the remaining lobe,[35] with the subsequent lobar reperfusion process. Also, one could surmise that attenuation of lung injury by administering sevoflurane in donor lung cadavers during organ extraction or when ex situ lung surgery is required could be a promising option for clinical application to decrease IR injury in lung recipients. However, clinical studies evaluating the efficacy of this promising pathway are required to improve lung transplantation outcomes.

In conclusion, the results of the present study indicate that sevoflurane exerts an early cell-protective effect against pulmonary IR damage, as evidenced by the decrease in oxidative stress and in the intensity of the inflammatory response. In addition, sevoflurane affords adequate homeostasis of NO metabolism.

DISCLOSURES

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Attestation: Javier Casanova has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
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