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SCIENTIFIC ARTICLE

The effect of anesthetic preconditioning with sevoflurane on intracellular signal-transduction pathways and apoptosis, in a lung autotransplant experimental model☆

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KEYWORDS
Lung transplantation;
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Abstract
Background: Anesthetic pre-conditioning attenuates inflammatory response during ischemia-reperfusion lung injury. The molecular mechanisms to explain it are not fully understood. The aim of our investigation was to analyze the molecular mechanism that explain the anti-inflammatory effects of anesthetic pre-conditioning with sevoflurane focusing on its effects on MAPKs (mitogen-activated protein kinases), NF-κB (nuclear factor kappa beta) pathways, and apoptosis in an experimental lung autotransplant model.
Methods: Twenty large white pigs undergoing pneumonectomy plus lung autotransplant were divided into two 10-member groups on the basis of the anesthetic received (propofol or sevoflurane). Anesthetic pre-conditioning group received sevoflurane 3% after anesthesia induction and it stopped when one-lung ventilation get started. Control group did not receive sevoflurane in any moment during the whole study period. Intracellular signal-transduction pathways (MAPK family), transcription factor (NF-κB), and apoptosis (caspases 3 and 9) were analyzed during experiment.

☆ The study was made in the experimental laboratory of Hospital General Universitario Gregorio Marañón and Biochemistry Department Medicine Faculty of Universidad Complutense de Madrid, Madrid, Spain.

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Results: Pigs that received anesthetic pre-conditioning with sevoflurane have shown significant lower values of MAPK-p38, MAPK-P-p38, JNK (c-Jun N-terminal kinases), NF-κB p50 intranuclear, and caspases ($p < 0.05$) than pigs anesthetized with intravenous propofol.
Conclusions: Lung protection of anesthetic pre-conditioning with sevoflurane during experimental lung autotransplant is, at least, partially associated with MAPKs and NF κB pathways attenuation, and antiapoptotic effects.

PALAVRAS-CHAVE
Transplante pulmonar;
Sevoflurano;
Inflamação;
Apoptose

O efeito do pré-condicionamento anestésico com sevoflurano sobre as vias de transdução de sinal intracelular e a apoptose, em modelo experimental de autotransplante pulmonar

Resumo
Justificativa: O pré-condicionamento anestésico atenua a resposta inflamatória durante a lesão de isquemia-reperfusão do pulmão. Os mecanismos moleculares para explicá-lo não são totalmente compreendidos. O objetivo de nossa investigação foi analisar o mecanismo molecular que explica os efeitos anti-inflamatórios do pré-condicionamento anestésico com sevoflurano, enfocando seus efeitos sobre as proteínas quinases ativadas por mitógenos (MAPKs), o fator nuclear kappa beta (NF-κB) e a apoptose em modelo experimental de autotransplante pulmonar.
Métodos: Vinte porcos Large White submetidos à pneumonectomia e autoimplante de pulmão foram divididos em dois grupos de 10 membros com base no anestésico recebido (propofol ou sevoflurano). O grupo de pré-condicionamento anestésico recebeu sevoflurano a 3% após a indução da anestesia, que foi descontinuado quando a ventilação monopulmonar foi iniciada. O grupo controle não recebeu sevoflurano em nenhum momento durante todo o período do estudo. As vias de transdução de sinal intracelular (família MAPK), o fator de transcrição (NF-κB) e a apoptose (caspases 3 e 9) foram analisados durante o experimento.
Resultados: Os suínos que receberam pré-condicionamento anestésico com sevoflurano apresentaram valores mais baixos de MAPK-p38, MAPK-P-p38, c-Jun N-terminal quinases (JNK), NF-κB p50 intranuclear e caspases ($p < 0,05$) que os suínos anestesiados com propofol intravenoso.
Conclusões: A proteção pulmonar do pré-condicionamento anestésico com sevoflurano durante o autotransplante pulmonar experimental está, pelo menos, parcialmente associada à atenuação das vias de MAPKs e NF κB e aos efeitos antiapoptóticos.

Introduction

Ischemia reperfusion injury (IRI) is present in a great variety of clinical situations but it is more easily recognizable in solid organs' transplants. In the lung, there are different mechanisms involved in the ischemia reperfusion-induced lung injury (IRLI), like lung transplant, one-lung ventilation, cardiac surgery with extracorporeal circuit, etc. IRLI is characterized by unspecified alveolar damage with permeability alterations, pulmonary edema, and secondary hypoxemia.[1] After lung transplantation, IRLI represents a significant obstacle for a patient's optimal outcome since it is the principal cause of primary graft failure, which correlates with an enhanced morbid–mortality level after transplantation. In the past years, many studies have examined different treatment protocols shooting to mitigate the IRI. It has been demonstrated that halogenated anesthetic agents have a potential utility in providing pre-conditioning to the organ against ischemia, which is termed anesthetic pre-conditioning (APC). Several studies have demonstrated their efficacy in a variety of organs such as the liver, kidney, heart, spinal cord, brain, and lung.[2–7]

Previously, our group has tested the anti-inflammatory effects of APC on IRLI in the same lung auto-transplant model.[7] However, the molecular mechanisms through which APC with sevoflurane protects against IRLI are not completely clear yet. The intracellular signaling systems (signal transduction pathways) can reversibly modify the activity of many inflammatory proteins. The mitogen activated protein kinases (MAPKs) family of proteins is intracellular signal-transduction pathways that have been shown to play an important role in the development of IR injury. Several studies have shown that IR can activate the MAPKs, protecting the cells from the depletion of oxygen and

modulating apoptosis.[8–10] It has been shown that the MAPK pathway provides protective effects against hypoxia in cardiomyocytes and is involved in preserving the cell from any process with oxygen deprivation, which leads to reduced cellular apoptosis.[9,10] The following three main subtypes of MAPKs have been implicated in inflammatory responses: extracellular-signal regulated kinase (ERK), MAPK p-38, and c-Jun N-terminal kinase (JNK). Thus, the facts that MAPKs have been implicated in the cellular signaling of numerous inflammatory processes have led MAPKs to become the focus of analysis of numerous studies in recent years. These studies have altered the direction of current research initiatives that have traditionally examined the role of pro-inflammatory cytokines in IRI. Furthermore, cellular signaling pathways control gene expression by modifying the activity of the so-called ''transcription factors''. Nuclear factor-κB (NF-κB) is an inducible transcription factor essential to regulate the expression of many genes involved in the inflammatory response and in the apoptosis secondary to IRLI. In a miocardic IR model, it has been observed that the advantageous effects of APC with sevofluorane derive from the diminution of the activation of NF-κB during the reperfusion.[11]

We hypothesized that sevoflurane anti-inflammatory effects provide protection against IRLI through different molecular mechanisms including pathways that regulate intracellular signaling systems (mainly MAPKs, NF-κB, and apoptosis). To test this hypothesis, we used blood samples from animals of one previous study conducted by our research team.[7] The aim of the present study was to analyze the molecular mechanisms that explain the protective effects of APC with sevoflurane in an experimental lung autotransplant model.

Material and methods

This study was granted approval by the Institution's Research and Animal Experimentation Committee. All experiments adhered to European and Spanish legislation regarding the handling and care of experimental animals.

Animal model and study groups

Twenty large white pigs weighing between 30 and 50 kg were subjected to an orthotopic left lung autotransplantation (which included a left pneumonectomy, ex situ cranial lobectomy, and left caudal lobe reimplantation) that was followed by 30 min of graft reperfusion. Animals were block randomized by random numbers (Microsoft Excel 2003) to receive either propofol delivered via continuous perfusion as anesthetic maintenance throughout the procedure (control group—CON, $n = 10$) or anesthetic preconditioning with sevoflurane from the anesthetic induction to the beginning of the one lung ventilation (OLV) stage followed by propofol perfusion (sevoflurane group—SEVO, $n = 10$).

Anesthesia

Large white pigs were allowed drinking water ad libitum, but solid food was withheld for 18 h before each experiment. Premedication was performed with intramuscular ketamine 10 $mg.kg^{-1}$ (Ketolar, Parker Davis). Once in the operating room, the animals were placed in a supine position, and pulseoximetry and electrocardiographic monitoring were performed. A femoral artery catheter was inserted to record mean arterial pressure (MAP). Also, we introduced a Swan-Ganz through femoral vein and registered the pulmonary artery mean pressure (PAMP). In addition, the cardiac output monitor (Edwards Lifesciences) and thermodilution technique were used to measure cardiac index.

Anesthesia induction was carried out with propofol (4 $mg.kg^{-1}$; Diprivan, Fresenius K), fentanyl (3 $\mu g.kg^{-1}$; Fentanest, Kern Pharma), and atracurium (0.6 $mg.kg^{-1}$; Tracrium, Glaxo Smith Kline). Orotracheal intubation was performed with a 6–7 mm cuffed endotracheal tube. Volume-controlled mechanical ventilation was used with a 5 cm H_2O positive end expiratory pressure (PEEP) and a peak pressure <30 cm H_2O during the entire study duration. A tidal volume of up 8 $mL.kg^{-1}$, respiratory rate of 12–15 rpm, and inspiratory/expiratory ratio of 1:2 were chosen to maintain the $PaCO_2$ in the range of 35–40 mmHg. FiO_2 was maintained at 1 throughout the procedure. Intraoperative crystalloid infusion was maintained at 5–6 $mL.kg^{-1}.h^{-1}$. Anesthesia was maintained with propofol under continuous perfusion (8–10 $mg.kg^{-1}.h^{-1}$) (CON group) or 3% sevoflurane from the beginning of anesthetic induction to the beginning of OLV followed by propofol perfusion (8–10 $mg.kg^{-1}.h^{-1}$) (SEVO group). Supplemental doses of fentanyl and atracurium were used when required.

Surgical protocol

The surgical techniques included preliminary procedures, thoracotomy, left pneumonectomy, back-table cranial lobectomy, caudal lobe reimplantation, and reperfusion. This procedure has been extensively described previously as a model to be carried out on pigs in order to study the early stages of IRLI.

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. This facilitated the insertion and withdrawal of the tube into and out of the right bronchus during the procedure. A 7F pulmonary artery catheter (Edwards Lifesciences) was introduced through the femoral vein and its final position in the right pulmonary artery intraoperatively was confirmed. A 7F femoral artery catheter was used to enable blood pressure monitoring and blood sampling. A suprapubic cystostomy was performed to monitor urine output.

After these preliminary procedures, the swine was placed in a right lateral position, and a left thoracotomy was carried out by means of a fourth or fifth rib resection. To perform the left pneumonectomy, pulmonary artery, cranial vein, caudal vein, and main left bronchus were dissected progressively. Two-lung ventilation was maintained in all swine for a 90 min

period. Afterwards, the pulmonary vessels were dissected, the main left bronchus was sectioned, and the endotracheal tube was placed into the right bronchus. Just after the pneumonectomy was completed, a bolus of intravenous heparin (300 IU.kg^{-1}; MaynePharma Spain) was administered to prevent thrombosis in the clamped pulmonary artery. Next, on the back table, left lung is perfused through the pulmonary artery and veins with University of Wisconsin solution and a cranial lobectomy carried out. The caudal left lobe was then implanted back into the swine and a bronchus to bronchus anastomosis was performed, as well as a pulmonary artery to pulmonary artery anastomosis and an inferior vein to left atrium anastomosis. Graft reperfusion was performed initially in a retrograde direction by unclamping the left atrium and then the endobronchial tube was pulled back into the trachea enabling two-lung ventilation. The left pulmonary artery was then unclamped, and blood flow was maintained for 30 min. At the end of the experiment, the animal was euthanized through potassium chloride injection under deep anesthesia.

Measurement and sampling time points

Blood samples and lung biopsies were collected at the following time points: pre-pneumonectomy (PPn)—before completing pneumonectomy and with the animal under OLV; pre-reperfusion (PRp)—before reperfusion and ventilation of the reimplanted left caudal lobe; 10 min post-reperfusion (Rp + 10′)—10 min after the reperfusion of the reimplanted lobe; 30 min post-reperfusion (Rp + 30′)—30 min after the reperfusion of the reimplanted lobe.

Biochemical studies in lung tissue

Lung tissue biopsies were performed for biochemical studies and quantification of lung edema. The first two 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 two last (Rp + 10′ and Rp + 30′) were taken from the reimplanted caudal lobe. Every lung sample was divided into the following two parts: one was kept frozen in polypropylene tubes at −40 °C until quantification of lung edema; the other was placed in a cryotube, flash-frozen in liquid nitrogen, and stored at −80 °C until biochemical analysis.

Preparation of tissue homogenates

Lung tissue was quickly dissected and frozen with dry ice. Frozen lung samples were weighed and transferred to 50 mL polypropylene tubes (Falcon; Becton Dickinson, Lincoln Park, NJ) containing lysis buffer (4 °C) at a ratio of 10 mL buffer. 1 g^{-1} of wet tissue lysis buffer consisted of 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma Chemical Company) and 1 μg.mL^{-1} pepstatin A (Sigma Chemical Company), aprotinin (Sigma Chemical Company), and leupeptin (Sigma Chemical Company) in 1 × phosphate buffered (pH 7.2) saline solution (Biofluids, Rockville, MD) containing 0.05% sodium azide (Sigma Chemical Company). Samples were homogenized for 30 s with an electrical homogenizer (Polytron; Brinkmann Instruments, Westminster, NY) at maximum speed, and the tubes were immediately frozen in liquid nitrogen. The samples were homogenized three times for optimal processing. The homogenates were later thawed in a 37 °C water bath and centrifuged at 119 000*g* (1 h at 4 °C) to separate cellular organelles. The supernatants were frozen at −80 °C to allow the formation of macromolecular aggregates. After thawing at 4 °C, the aggregates were pelleted at 3000*g* (4 °C), and the final homogenate volume was measured with a graduated pipette.

Mitogen-activated protein kinases (MAPKs) p-38, JNK, and ERK

The levels were determined in lung tissue though an ELISA tests using specific commercial kits (Oncogene Science, Cambridge, MA).

Evaluation of oxidative stress

Lipid hydroperoxides (LPO) reflected the degree of membrane lipid oxidation and was determined using a specific kit for application in lung tissue (K-assay LPO-CC, Kamiya Biochemical Company, USA).

Inflammatory mediators measured by western blot analysis in lung tissue

Hemoxygenase 1 (HO-1) and hemoxygenasa-2 (HO-2), nuclear and cytoplasmatic NF-κB, and IkB. The results were determined by western blot using specific antibodies for anti-hemoxigenase I and anti-hemoxigenase II, anti-NF-κB p-50, anti-NF-κB p-52, and anti-NF-κB p-65 (Chemicon International, Inc.). After washing of the membranes, proteins were visualized by chemiluminescence (ECL system, Amersham, Oakville, Ontario), and these measurements were recorded in arbitrary units.

Caspases

The levels of caspases 3 and 9 were determined in lung tissue though an ELISA test using specific commercial kits (respectively Sigma, St Louis Missouri and Bionova, Spain).

Statistical analysis

All data are expressed as the mean and the standard error of the mean (SE). Nonparametric tests were used for all analyses. Accordingly, the Mann–Whitney *U*-test was applied to establish differences between the analyzed groups. In addition, the Wilcoxon test for paired data was used to study the evolution of the intra-group values. To compare the activity of caspases 3 and 9, the Wilcoxon test was replaced with the Kruskal–Wallis test. Statistical significance was considered for $p < 0.05$. The SPSS version 14.0 statistical package (SPSS Inc., Chicago, USA) was used throughout.

Results

Animals of CON and SEVO groups were similar in terms of weight (34.3 ± 14 vs. 37.8 ± 13, $p = 0.43$), lung ischemia length (97 ± 14 vs. 92 ± 22.5 min, $p = 0.09$), and OLV length

Table 1 Hemodynamic, respiratory, and gasometric values.

	Group	BAS	PPn	PRp	Rp 10 min	Rp 30 min
MAP (mmHg)	CON	99 (4)	101 (4)	90 (4)	86 (4)	81 (5)
	SEVO	76 (4)[a]	85 (6)	83 (4)	74 (4)	73 (5)
PAMP (mmHg)	CON	26 (3)	28 (3)	27 (2)	29 (3)	29 (2)
	SEVO	20 (1)[a]	24 (2)	24 (2)	27 (2)	26 (2)
HR (beat min)	CON	99 (6)	94 (8)	103 (9)	95 (7)	93 (8)
	SEVO	107 (7)	110 (5)	106 (7)	102 (6)	105 (8)
CI ($L\,min^{-1}.m^{-2}$)	CON	5.2 (0.4)	5.2 (0.5)	4.4 (0.4)	48 (0.6)	5.5 (1.1)
	SEVO	6.6 (0.8)	7.8 (1)	5.6 (0.6)	6 (1)	5.9 (0.8)
PaO_2 (mmHg)	CON	330 (55)	206 (37)	302 (48)	250 (48)	297 (49)
	SEVO	305 (37)	169 (23)	315 (42)	329 (44)[a]	333 (49)
$PvpO_2$ (mmHg)	CON	–	–	–	278 (52)	262 (41)
	SEVO	–	–	–	418 (29)[a]	391 (44)[a]
$PaCO_2$ (mmHg)	CON	37 (2)	44 (3)	42 (3)	44 (4)	44 (5)
	SEVO	33 (1)	49 (11)	40 (2)	42 (2)	40 (2)
$PvpCO_2$ (mmHg)	CON	–	–	–	36 (4)	36 (5)
	SEVO	–	–	–	33 (3)	32 (5)
PPAW (cmH_2O)	CON	20 (2)	26 (2)	25 (2)	22 (2)	21 (1)
	SEVO	19 (2)	27 (2)	26 (2)	22 (1)	22 (1)
PplatAW (cm H_2O)	CON	13 (1)	16 (1)	16 (1)	15 (1)	15 (1)
	SEVO	12 (1)	15 (1)	15 (1)	14 (1)	14 (1)

Data are expressed as the mean (standard error of the mean).
MAP, mean arterial pressure; PAMP, pulmonary artery mean pressure; HR, heart rate; CI, cardiac index; PaO_2, arterial pressure of oxygen; $PvpO_2$, pulmonary venous pressure of oxygen; $PaCO_2$, arterial pressure of carbon dioxide; $PvpCO_2$, pulmonary venous pressure of carbon dioxide; PPAW, peak pressure airway; PplatAW, plateau pressure airway; CON, control group; SEVO, sevoflurane group; BAS, basal; PPn, prepneumonectomy; PRp, prereperfusion; Rp10 min, 10 min postreperfusion; Rp30 min, 30 min postreperfusion.
[a] $p = 0.05$ SEVO vs. CON group.

(167 ± 73 vs. 184 ± 86 min, $p = 0.1$). Hemodynamic, ventilatory, and gasometric data are shown in Table 1.

MAPKs behavior

Pneumonectomy induced a significant increase of MAPK p-38 and its phosphorylated isoform (MAPK-P p-38) in both groups of animals with no differences between them. After reperfusion levels of MAPK p-38 and MAPK-P p-38 decreased in both groups, but the values obtained Rp + 30′ were significantly lower ($p < 0.05$) in the SEVO group compared with the CON group. JNK-P values were significantly lower in the CON group in all measurements, except in the PPn ($p < 0.05$). SEVO group showed significantly lower levels of ERK-P in the two samples taken before reperfusion ($p < 0.05$). But reperfusion induced an increase of this protein in the two groups analyzed and after reperfusion values were similar between the two groups (Fig. 1) (Table 2).

NF-κB behavior

Ischemia caused an increase in levels of nuclear and cytoplasmic NF-κB p-50 and of nuclear p-65 in both groups. After reperfusion, an increase in nuclear NF-κB p-50 and p-65 was observed in both groups, whereas NF-κB p-52 was increased only in the CON group. After reperfusion only in the CON group, the levels of IKB and cytoplasmic NF-κB p-50 were decreased and the levels of nuclear NF-κB p-65 were increased. Comparing the two groups, we found that the values of NF-κB p-50 were significantly lower in the CON group than in the group SEVO (Fig. 2) (Table 3).

Caspases behavior

Values of caspase 3 obtained in the SEVO group were significantly lower than those obtained in the CON group ($p < 0.05$) only before reperfusion, whereas values of caspase 9 were significantly lower in the SEVO group than in the CON group ($p < 0.05$) before and after reperfusion (Fig. 2).

Ischemia-reperfusion-induced lung injury (IRLI), oxidative stress, and inflammation

After pneumonectomy, before and after reperfusion, LPO levels are increased being significantly higher in the CON group compared with the SEVO group ($p < 0.05$) (Fig. 3). No statistically significant differences between groups were observed analyzing HO-1 and HO-2 levels.

Discussion

In this study, we have shown that the beneficial effects of APC with sevoflurane are correlated with the attenuation of the activity of the intracellular signal-transduction pathways that regulate inflammation and apoptosis.

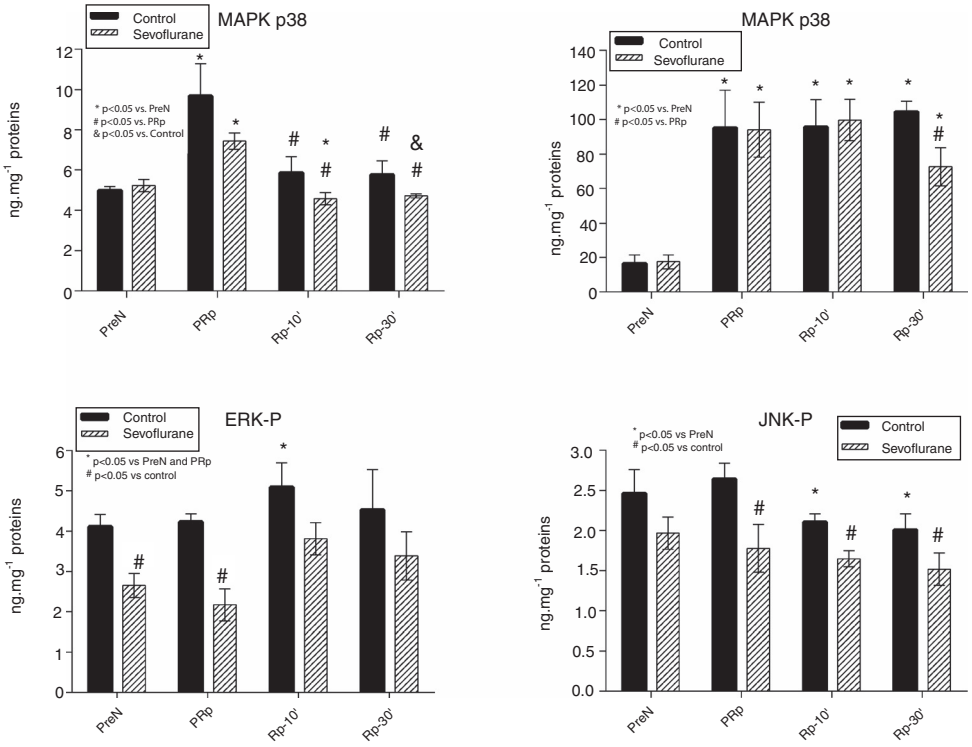

Figure 1 Values of MAPK family during study. Data are expressed as the mean ± standard error of the mean. MAPK p-38, mitogen-activated protein kinases p-38; JNK, c-Jun N-terminal kinase; ERK, extracellular-signal-regulated kinase; —P, phosphorylated isoform; CON, control group; SEV, sevoflurane group; PreN, prepneumonectomy; PRp, prereperfusion; Rp-10; 10 min postreperfusion; Rp-30; 30 min postreperfusion.

Table 2 Values of MAPK family during study.

	Grupo	PreN	PRp	Rp-10′	Rp-30′
MAPK p-38 ng.mg^{-1} protein	CON	4.99 ± 0.2	9.68 ± 1.6[b,c]	5.86 ± 0.8[c]	5.76 ± 0.7[c]
	SEVO	5.23 ± 0.3	7.44 ± 0.4[b]	4.58 ± 0.3[b,c]	4.72 ± 0.1[a,c]
MAPK-P p-38 ng.mg^{-1} protein	CON	16.5 ± 5	95.1 ± 22[b]	95.6 ± 16[b]	104.5 ± 6[b]
	SEVO	17.5 ± 4	94.1 ± 16[b]	99.7 ± 12[b]	72.6 ± 11[a,b]
JNK-P ng.mg^{-1} protein	CON	2.46 ± 0.3	2.64 ± 0.2	2.11 ± 0.1[c]	2.01 ± 0.2[c]
	SEVO	1.97 ± 0.2	1.78 ± 0.3[a]	1.65 ± 0.1[a]	1.52 ± 0.2[a]
ERK-P ng.mg^{-1} protein	CON	4.12 ± 0.3	4.24 ± 0.2	5.09 ± 0.6	4.53 ± 0.1
	SEVO	2.65 ± 0.3[a]	2.17 ± 0.4[a]	3.82 ± 0.4[b,c]	3.39 ± 0.6

Data are expressed as the mean ± standard error of the mean.
MAPK p-38, mitogen-activated protein kinases p-38; JNK, c-Jun N-terminal kinase; ERK, extracellular-signal-regulated kinase; —P, phosphorylated isoform; CON, control group; SEVO, sevoflurane group; PreN, prepneumonectomy; PRp, prereperfusion; Rp-10; 10 min postreperfusion; Rp-30; 30 min postreperfusion.
[a] $p < 0.05$ SEVO vs. CON.
[b] $p < 0.05$ vs. PreN.
[c] $p < 0.05$ vs. PRp.

Inflammation

When IRLI occurs, there is an increase in the inflammatory parameters and oxidative stress markers due to an imbalance between the production of reactive oxygen species and antioxidant mechanisms. This leads to an increase in neutrophils and macrophages in the alveoli, permeability alterations of alveolus-capillary membrane, alveolus-interstitial edema, and cell damage affecting pneumocytes with increased necrosis and apoptosis.[12] The important role of MAPK and NF-κB pathways in the upregulation of various inflammatory mediators and their relation with apoptosis during pro-inflammatory states are well known.[13,14] IRLI development is associated with the activation of pro-inflammatory intracellular signaling cascades in the lung parenchyma cells, which amplify the inflammatory response. Activation of MAPK during reperfusion contributes to acute inflammatory responses

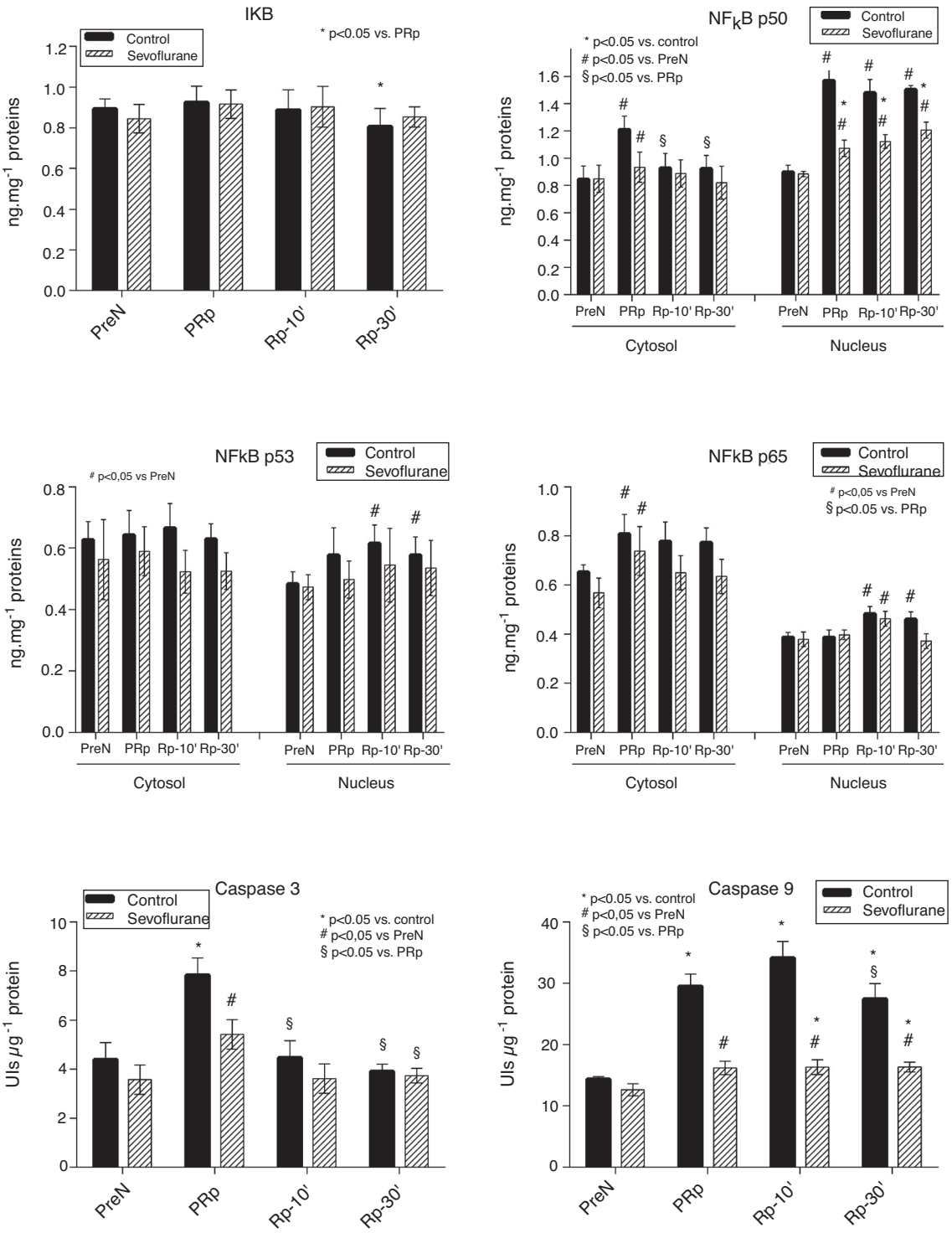

Figure 2 Values of NF-κB (cytosol and nucleus) and caspases. Data are expressed as the mean ± standard error of the mean. NF-κB, nuclear factor-κB; PreN, prepneumonectomy; PRp, prereperfusion; Rp-10, 10 min postreperfusion; Rp-30, 30 min postreperfusion.

and apoptosis of lung grafts.[15–17] Moreover, several investigations have demonstrated that pharmacological inhibition of MAPK p-38 or JNK can be useful tool to decrease IRLI in the early period of lung graft reperfusion,[18–20] and similar results are observed when ischemic postconditioning inactivates p-38 MAPK.[21] Several authors have demonstrated that inhalatory APC attenuates MAPK p-38 activation during ischemia reperfusion, and it has been considered as a critical mechanism to explain the protective effects of APC.[22,23] However, the role of sevoflurane APC on MAPK has not been investigated in lung transplantation. In a recent study using an in vitro model of rat lung tissue, sevoflurane decreased TNFα-induced permeability changes in endothelial cells from the pulmonary microvasculature and this decrease in

Table 3 Pathway nuclear factor-κB.

	Group	Mean ± SE PreN	PRp	Rp-10′	Rp-30′
NF-κB P50_Cit	CON	0.843 ± 0.10	1.209 ± 0.10[b]	0.926 ± 0.11[‡]	0.921 ± 0.10[‡]
	SEVO	0.849 ± 0.10	0.934 ± 0.11[b]	0.887 ± 0.10	0.820 ± 0.12
NF-κB P50_NUC	CON	0.898 ± 0.05	1.569 ± 0.11[b]	1.479 ± 0.10[b]	1.503 ± 0.03[b]
	SEVO	0.884 ± 0.02	1.073 ± 0.06[a,b]	1.123 ± 0.05[a,b]	1.206 ± 0.06[a,b]
NF-κB P52_CIT	CON	0.627 ± 0.06	0.643 ± 0.08	0.666 ± 0.08	0.630 ± 0.05
	SEVO	0.563 ± 0.13	0.590 ± 0.08	0.523 ± 0.07	0.525 ± 0.06
NF-κB P52_NUC	CON	0.483 ± 0.04	0.577 ± 0.09	0.616 ± 0.06[b]	0.577 ± 0.06[b]
	SEVO	0.473 ± 0.04	0.498 ± 0.06	0.545 ± 0.12	0.535 ± 0.09
NF-κB P65_CIT	CON	0.652 ± 0.03	0.808 ± 0.08[b]	0.777 ± 0.08	0.773 ± 0.06
	SEVO	0.568 ± 0.06	0.738 ± 0.10[b]	0.650 ± 0.07	0.635 ± 0.07
NF-κB P65_NUC	CON	0.387 ± 0.02	0.387 ± 0.03	0.483 ± 0.03[b,c]	0.460 ± 0.03[b,c]
	SEVO	0.379 ± 0.03	0.397 ± 0.02	0.463 ± 0.03[b]	0.372 ± 0.03
IkB	CON	0.893 ± 0.05	0.925 ± 0.08	0.888 ± 0.10	0.806 ± 0.09[c]
	SEVO	0.845 ± 0.07	0.917 ± 0.07	0.904 ± 0.10	0.854 ± 0.05

Data are expressed as the mean ± standard error of the mean.
NF-κB, nuclear factor-κB; IkB, inhibitor of NF-κB proteins; CIT, cytosol; NUC, nuclear; CON, control group; SEV, sevoflurane group; PreN, prepneumonectomy; PRp, prereperfusion; Rp-10; 10 min postreperfusion; Rp-30; 30 min postreperfusion.
[a] $p < 0.05$ SEVO vs. CON.
[b] $p < 0.05$ vs. PreN.
[c] $p < 0.05$ vs. PRp.

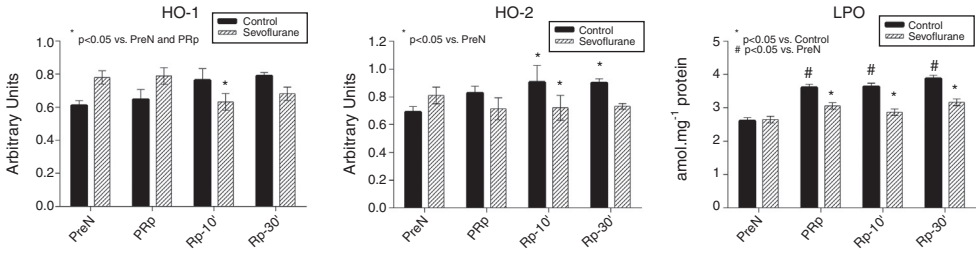

Figure 3 Biomarkers of oxidative stress during study. Data are expressed as the mean ± standard error of the mean. LPO, lipid hydroperoxides; HO, hemoxigenase; PreN, prepneumonectomy; PRp, prereperfusion; Rp-10; 10 min postreperfusion; Rp-30; 30 min postreperfusion.

permeability was correlated with a decrease in the MAPK-P p-38 levels.[24] In our work, we observed a reduced activation of the MAPK p-38 active form and JNK-P, during reperfusion in the SEVO group which supports the idea that sevoflurane APC could inhibit this pro-inflammatory pathway in the lung. Recently, in a rat lung transplantation model, Ohsumi et al. showed similar findings in a sevoflurane preconditioned group, with less inflammatory response and significantly inhibition of apoptotic cells.[25]

At the moment, the precise mechanism by which sevoflurane provides these protective effects is well known in the heart and brain,[26] but is less understood on the lung. Furthermore, there is scientific evidence that the protective effects of sevoflurane APC are related with opening (activation) of mitochondrial K_{ATP} channel.[27,28] The MAPK p-38 and ERK have a crucial role in the intracellular signaling involved in K_{ATP} channel opening.[29,30] Therefore, with the results of our study with less activation of MAPK p-38 and ERK in sevoflurane group, we hypothesized that, at least a large proportion of the lung protective effects of sevoflurane APC was due to the inhibition of MAPK family during I/R and subsequent activation of these K_{ATP} channels, similar to the mechanisms involved in brain and heart.[26]

Another mechanism to explain lung protection that provided sevoflurane administration before ischemia would be related with NF-κB activity. When NF-κB is activated (inflammation or ischemia), it enters the nucleus and regulates the expression of inflammatory cytokines inducing a cascade of inflammatory mediators. For that reason, a downregulation of this molecule is desirable. It has been described that MAPK family activation, in particular MAPK p-38, have numerous direct and indirect interactions enhancing NF-κB expression.[31,32] We observed a general postreperfusion increase in nuclear NF-κB subunits, even if this increase was not always significant. Sevoflurane APC was able to moderate the activation only in NF-κB nuclear subunits, which suggests that its protective effect could act through this way too. Our results are consistent with those published by Mikrou et al. in rat lungs in a liver ischemia/reperfusion injury model[33] and showed the importance of signaling pathways involving NF-κB subunits and MAPKs in the regulation

of the inflammatory response secondary to IRLI and confirm anti-inflammatory effects of sevoflurane APC.

Another one of the consequences of IRLI is the increase in the oxidative stress, which perpetuates and enlarges the inflammatory response.[34] In our study, we observed an attenuation of oxidative stress-induced cell damage in the group that received sevoflurane, which was evidenced by lower LPO levels in the SEVO group. That is, there was less damage of the cellular membranes by reactive oxygen species during the reperfusion due to sevoflurane APC. On the other hand, we did not observe any significative changes in the expression of HO-1, which is related with anti-oxidative, anti-apoptotic, and anti-inflammatory effects.[35] So, in accordance with our results, it seems that the protective effects of sevoflurane APC are not related with the expression of HO-1. However, other authors found that the protective mechanism of IRI, in a lung ischemia reperfusion model in rats, was due in part to the action of HO-1.[36] These differences could be attributed in part to the methodology of the studies, since these authors establish a hypercapnic acidosis environment that does not occur in our model. Hypercapnic acidosis could be the trigger to release HO-1 activity.

Apoptosis

Previously, the role of apoptosis in the pathogenesis of IRI is well recognized. It is believed that apoptosis may be decisive in the development of lung lesions that appear as a result of IRLI.[37,38] Several authors have noted that there is a significant percentage of transplanted human lung's cells suffering apoptosis in the early stages of graft reperfusion peaking at 2 h.[39,40] This means that the measures aimed to reduce apoptosis during IRLI should be initiated as soon as possible, so that the APC could result particularly helpful. To our knowledge, there are no studies which have investigated the effect of APC with sevoflurane on apoptosis in IRLI. We have found a slight increase in apoptosis during ischemia, getting to be much more evident during reperfusion. The group receiving sevoflurane showed less activation of apoptosis before graft reperfusion, as assessed by caspase 3 and 9 decreased levels of SEV group. Our findings are similar to those watched by Wang et al. in an experimental endotoxin model of acute lung injury in rats.[41] Animal and human studies have shown that the inhalational anesthesia without the presence of I/R is related to an increase in apoptotic cell activity.[42] In addition, it has been described a pro-apoptotic effect of sevoflurane in the lung of mechanically ventilated patients.[43] By contrast, when sevoflurane was administered in preconditioning or in post-conditioning, it proved anti-apoptotic effects in the brain and heart.[44,45] These contrasting results regarding the effect of sevoflurane may be explained by the presence of different concentrations of inflammatory and anti-inflammatory factors. Such that when ischemia predominates (and inflammation with it), such as in lung transplantation, may result more evident the anti-apoptotic effects of sevoflurane. The mechanism by which sevoflurane exerts protective anti-apoptotic effects in the lung during IRLI is not well known. It has been suggested that a decrease in anti-inflammatory response could be linked with its ability to attenuate apoptotic response in the presence of IR. Several investigations have demonstrated that activation of MAPK p-38 or NF-κB and caspase-3 could be responsible for these pro-apoptotic responses.[41,46–48] An attenuation of expression of them related with sevoflurane APC can explain anti-apoptotic effects seen in our investigation. In conclusion, lung protection of APC with sevoflurane during experimental lung auto-transplant is at least partially associated with MAPKs and NF-κB pathways attenuation, and anti-apoptotic effects.

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Conflicts of interest

The authors declare no conflicts of interest.

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