

Original Research Article

Effect of sevoflurane post-conditioning on apoptosis and the expressions of Bcl-2 and Bax in lung tissue of cardiopulmonary bypass dogs

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Abstract

Purpose: To investigate the effects of sevoflurane post-conditioning on the expressions of Bcl-2 and Bax in lung tissues of cardiopulmonary bypass (CPB) dogs.

Methods: Twelve healthy hybrid mongrels were divided into control (C) and sevoflurane post-conditioning (S) groups. All dogs were subjected to thoracotomy in order to establish CPB. Ischemia-reperfusion was conducted in the left lung of both groups. Thereafter, sevoflurane post-conditioning was given to group S. Femoral artery blood specimens were obtained prior to CPB (T_1), as the left pulmonary artery was opened (T_2), and 2 h after CPB (T_3), for blood gas analysis. Respiration index (RI), oxygenation index (OI), and dynamic lung compliance (Cd) were calculated.

Results: When T_1 was compared with T_2 and T_3 , their OI and Cd were significantly decreased in both groups, while RI showed the opposite trend ($p < 0.05$). Values of OI and Cd at T_3 in group S were increased significantly, relative to group C, while RI decreased ($p < 0.05$). There were less lung tissue inflammation and structural disorder at T_3 in Group S than in group C. Protein expressions and positive integral of Bcl-2 and Bax, and apoptosis at T_2 and T_3 in dog lung tissues of both groups were higher than those at T_1 ($p < 0.05$).

Conclusion: Post-conditioning with sevoflurane is lung-protective in CPB dogs. The underlying mechanism may be based on the promotion of Bcl-2 expression and inhibition of Bax expression, thereby reducing apoptosis in dog lung tissue. Further investigations to determine its suitability for clinical applications in humans are, however, required.

Keywords: Sevoflurane, Cardiopulmonary bypass, Bcl-2, Bax, Apoptosis, Pulmonary ischemia-reperfusion injury

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INTRODUCTION

It is widely known that most patients undergoing cardiac surgery have varying degrees of lung injury after CPB, which have serious adverse

effects on patients' postoperative recovery. Therefore, measures for CPB lung protection have been studied. In clinical practice, the application of pre-conditioning is limited because it is difficult to predict the timing of ischemia.

Therefore, the concept of ischemic post-conditioning was proposed. The timing of pharmacological post-conditioning refers to the short time before reperfusion. It is more suitable for clinical application. Pharmacological post-conditioning is administered with drugs within a few minutes before the start of reperfusion. In this way, possible mechanical damage to the blood vessels during IPC can be avoided [1].

Sevoflurane is a frequently used inhalation anesthetic in clinical practice, because it has a good anesthetic effect, and it also protects tissues and organs. Studies on lung ischemia-reperfusion injury have suggested that the use of sevoflurane for lung protection during anesthetic pre-conditioning in lung autotransplant surgery is, to some extent, linked with anti-apoptotic effect [2]. However, it is not clear whether sevoflurane post-conditioning can reduce lung tissue injury during CPB. Moreover, the effects of sevoflurane post-conditioning on expressions of Bcl-2 and Bax, and apoptosis in lung tissue of cardiopulmonary bypass dogs have not been clarified. Thus, there are no relevant reports on these areas.

This study was aimed at investigating the effects of sevoflurane post-conditioning on lung injury, levels of Bcl-2 and Bax, and apoptotic lesions in lung tissues of cardiopulmonary bypass dogs. It is expected to serve as an extensive theoretical foundation for the clinical application of sevoflurane.

EXPERIMENTAL

Animals and grouping

Twelve healthy mongrel dogs of both sexes, weighing 10 - 15 kg, were assigned to two groups, each with 6 dogs. In the control group (C), ischemia-reperfusion was conducted in the left lung after they were subjected to thoracotomy to establish CPB. In the sevoflurane post-conditioning group (group S), ischemia-reperfusion was conducted in the left lung after they were subjected to thoracotomy to establish CPB, and post-conditioning sevoflurane was given immediately after opening the left pulmonary artery. The rest of the steps were the same as those for group C. The study received approval from the Ethics Committee of Affiliated Hospital of Zunyi Medical University (approval no. KLL-2021-051). All procedures were carried out in accordance with the principles of laboratory animal care [3].

Preparations

Membrane oxygenator and CPB tubes for infants

were pre-filled with lactic Ringer's solution and hydroxyethyl starch. Then, a WEL-1000HA artificial cardiopulmonary machine (Huikang Medical Equipment Co. Ltd., Tianjin) was connected for use. The dogs received anesthesia with 2.5 % sodium pentobarbital *via* intraperitoneal injection, and were then fixed and mechanically ventilated with a double-lumen bronchial tube at tidal volume of 12 - 15 mL/kg and respiratory rate 16 breaths/min (I: E = 1: 2). The ECG and SpO₂ of tongue mucosa were monitored. Femoral arteriovenous incisions for an intravenous line were performed for both groups and arterial blood pressure and central venous pressure were continuously monitored. Changes in vital signs were closely monitored during the operation, and pentobarbital sodium was added intermittently to ensure the dogs remained anesthetized.

Procedures

After the anesthesia and monitoring were conducted and the vital signs of the dogs remained relatively stable, the chest of each dog was opened in the middle of the sternum and the pericardium was cut open to expose the heart and the right and left pulmonary arteries. Heparin was injected intravenously at a dose of 0.003 g/kg. After the activated clotting time (ACT) of whole blood was monitored for more than 300 sec, the subclavian artery and the right atrium were intubated. The left pulmonary artery was separated after connecting the CPB tube. The CPB began after ACT > 480 sec, and the left pulmonary artery was blocked for 10 min. The right lung ventilation was performed and the respiratory indexes were adjusted (tidal volume of 8 - 10 mL/kg, and respiratory rate of 20 - 22 beats/min) as in a previous study. After 60 min of blocking, the left pulmonary artery was opened and the mechanical ventilation for both lungs was simultaneously restored. After 30 min of CPB, ischemia-reperfusion was conducted in the left lung of the CPB dogs. Heparin was neutralized with protamine sulfate and epinephrine (0.01 - 0.3 µg/kg/min) and dopamine (2 - 10 µg/kg/min) which were injected intravenously. The experiment was completed after a blood recycle was maintained for 2 h *via* the femoral vein using a cell saver. Normothermic CPB was used in the experiment. The perfusion flow rate was kept at 100 - 120 ml/kg/min, with mean arterial pressure at 50 - 80 mmHg. The acid-base and the electrolyte balance were maintained through arterial blood gas analysis.

In group S, sevoflurane was inhaled through an oxygenator continuously for 30 min when the left pulmonary artery was opened, and the end-

expiratory concentration of sevoflurane was maintained at 2 % by adjusting the gas flow through the air-oxygen blender. The remaining steps were the same as those for group C.

Specimen collection and assessment of parameters

Blood from femoral artery was taken before CPB (T_1), as soon as left pulmonary artery was opened (T_2), and 2 h after CPB (T_3) for blood gas with an i-stat blood gas analyzer. Values of RI, OI, and Cd were computed with data from blood gas analysis, using Eqs 1 – 3, respectively.

$$RI = \frac{PaO_2 - aO_2}{PaO_2} \dots\dots\dots (1)$$

$$OI = \frac{PaO_2}{FiO_2} \dots\dots\dots (2)$$

$$Cd = TV/PAP-PE-EP \dots\dots\dots (3)$$

where $P(A-a)O_2 = (Pa - P_{H_2O}) \times (FiO_2 - PaO_2 - PaCO_2)$; Pa refers to standard atmospheric pressure, P_{H_2O} is standard saturated water vapor pressure, and FiO_2 (%) is a fraction of inspiration O_2 . Pa refers to standard atmospheric pressure, P_{H_2O} to standard saturated water vapor pressure, FiO_2 (%) to fraction of inspired O_2 . While TV refers to Tidal volume, PAP is peak airway pressure, PE is positive end and EP is expiratory pressure.

At the same time, lung tissue was taken and preserved in formalin solution, and pathological sections were made using H & E staining. The sections were examined under light microscopy for histopathological changes. Some of the lung tissues were stained with hematoxylin and made into immunohistochemical sections. Lung tissue levels of Bcl-2 and Bax in the dogs were determined with immunohistochemistry, while apoptosis in the lung tissues was determined with TUNEL assay. Pathological sections were observed under light microscopy, and the expressions of Bcl-2, Bax, and apoptotic cells were considered positive when brownish-yellow granules were observed in the cytoplasm and cell membrane. A comprehensive score was given according to the proportion of positive cells in the lung tissue and the depth of staining, based on 100 cells from each of 5 fields chosen without bias, making a total of 500 cells. The percentage of positive cells was calculated.

When the percentages of the positive cells were < 10 %, within 11 - 25 %, > 25 - 50 %, and > 50 %, the scores were recorded as 0, 1, 2 and 3, respectively. Scores were also given according to the depth of staining of the tissues, with 0 for

colorless, 1 for light yellow, 2 for tan, and 3 for yellowish-brown. The final score for each sample was the product of the score for percentage of the positive cells and the score for the staining depth [4]. A higher score represented a more severe degree of apoptosis. Protein levels of Bcl-2 and Bax were determined with the method of Western blotting.

Lung tissue (100 mg) frozen in liquid nitrogen was fully shredded and placed in a cold lysis solution containing 1 % PMSF. After lysis for 30 min, the lysate was clarified by centrifugation, and the protein content was determined using BCA procedure. Then, SDS-PAGE and western blot were performed. Analysis was conducted after scanning the images and the integral optical density (IOD) was determined. The IOD ratio of the 2 proteins were obtained relative to that of β -actin protein which was loading control.

Statistics

The SPSS 17.0 software was used for statistical analysis. All data are expressed as mean \pm standard deviation (SD). One-way ANOVA or t -test for group design data was used for comparison between two groups, while ANOVA for repeated measurement data or t -test of self-paired samples was used for intra-group comparison. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Respiratory function

There were no significant differences in IO, RI, and Cd between the two groups before CPB, but there were markedly lower OI and Cd at T_2 and T_3 than at T_1 , while RI had the opposite trend ($p < 0.05$). There were no significant differences in respiratory parameters between the two groups at T_1 and T_2 . However, OI and Cd were markedly higher at T_3 in group S than in group C, while RI was significantly lower than that of group C ($p < 0.05$; Table 1).

Lung tissue pathology

Sections of lung tissue showed that the structures of lung tissue in the two groups before CPB were normal. After the opening of the left pulmonary artery, the lung tissues of both groups were clear, while there were many inflammatory cells and red blood cells in the alveolar cavity, and the alveolar interstitium was slightly broadened. The lung tissues of both groups were disorganized 2 h after CPB.

Table 1: Changes in respiratory parameters in the two groups at different time points (mean \pm SD, n = 6)

Group	OI			I			Cd		
	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
C	481.07 \pm 28.39	392.43 \pm 25.64 ^a	262.33 \pm 53.36 ^{ab}	0.36 \pm 0.09	0.73 \pm 0.11 ^a	1.64 \pm 0.42 ^{ab}	162.25 \pm 7.29	103.38 \pm 4.54 ^a	58.79 \pm 5.14 ^{ab}
S	511.73 \pm 40.47	425.10 \pm 24.59 ^a	336.72 \pm 24.11 ^{abc}	0.40 \pm 0.04	0.70 \pm 0.03 ^a	1.16 \pm 0.14 ^{abc}	154.55 \pm 11.89	104.60 \pm 9.44 ^a	67.48 \pm 6.73 ^{abc}

^a $P < 0.05$, vs T₁; ^b $p < 0.05$, vs T₂; ^c $p < 0.05$, vs group C

Table 2: Comparison of Bcl-2/Bax scores and Bcl-2/Bax ratio between the two groups at different time points (mean \pm SD, n = 6)

Group	Bcl-2			Bax			Bcl-2/ Bax		
	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
C	1.31 \pm 0.37	3.27 \pm 0.16 ^a	4.35 \pm 0.31 ^{ab}	1.17 \pm 0.33	3.64 \pm 0.16 ^a	6.37 \pm 0.36 ^{ab}	0.94 \pm 0.24 ^a	0.91 \pm 0.19 ^a	0.96 \pm 0.18 ^{ab}
S	1.44 \pm 0.21	3.19 \pm 0.22 ^a	5.57 \pm 0.38 ^{abc}	1.24 \pm 0.29	3.77 \pm 0.22 ^a	4.93 \pm 0.28 ^{abc}	0.97 \pm 0.17 ^a	0.95 \pm 0.11 ^a	1.31 \pm 0.32 ^{abc}

When compared with T₁, ^a $p < 0.05$; ^b $p < 0.05$, vs T₂; ^c $p < 0.05$, vs group C

The lung tissue of group C showed massive alveolar cavity collapse and alveolar wall rupture, while the lung tissue of group S showed partial alveolar cavity collapse and alveolar wall rupture. The inflammation was milder in group S than in group C.

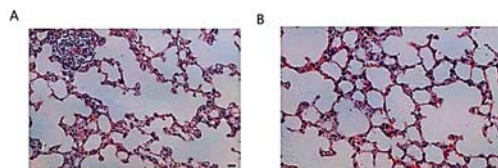


Figure 1: Sections of lung tissue. (A) H & E staining of group C at T₃ (×200). (B) H & E staining of group S at T₃ (×200)

Immunohistochemical scores

The positive scores of Bcl-2 and Bax expressions in the lung tissues of the dogs in the two groups before CPB had no significant difference ($p > 0.05$). When compared with the scores at T₁, the positive scores of Bcl-2 and Bax expressions in the two groups gradually increased over time. The positive score of Bcl-2 expression for group S was markedly higher for group S than for group C at T₃. However, an opposite trend was seen in the positive score of Bax expression. There was markedly higher Bcl-2/Bax ratio in group S than in group C (Figure 2 and Table 2).

Apoptosis

The positive score of apoptosis was comparable in the 2 groups prior to CPB. Compared with that at T₁, the positive scores of apoptotic cells in the two groups gradually increased over time ($p < 0.05$). Comparison between both groups showed markedly lower positive score of apoptotic cells in group S at T₃ than in group C (Figure 3 and Table 3).

Bax and Bcl-2 protein expression

Table 4 and Figure 4 show that before CPB, the expressions of Bcl-2 and Bax proteins were similar in both groups ($p > 0.05$). Protein levels of Bcl-2 and Bax in the dogs in the two groups gradually increased over-time, when compared

with values at T₁ ($P < 0.05$). Comparison between groups showed that Bcl-2 protein was markedly upregulated in the dogs in group S, relative to group C, at T₃. There was higher Bcl-2/Bax ratio in group S than in group C ($p < 0.05$).

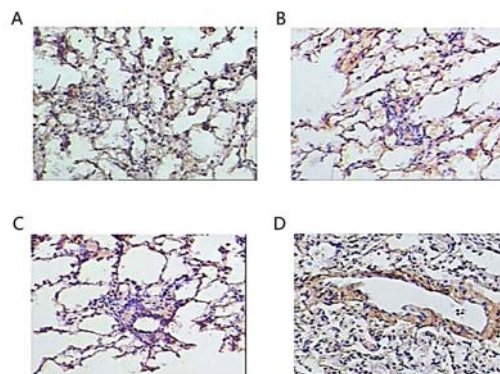


Figure 2: Immunohistochemical sections. (A) Bcl-2 expression in group C at T₃ (×200). (B) Bcl-2 expression in group S at T₃ (×200). (C) Bax expression in group C at T₃ (×200). (D) Bax expression in group S at T₃ (×200)

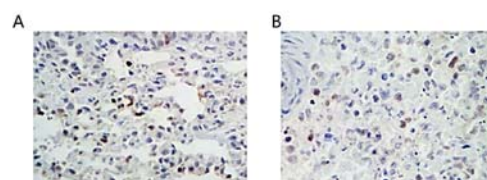


Figure 3: Apoptosis index in the lung tissue of the two groups. (A) TUNEL result for group C at T₃ (×400). (B) TUNEL result for group S at T₃ (×400)

DISCUSSION

Lung lesion associated with CPB is amongst the common side effects of cardiac surgery, which could lead to acute respiratory failure in severe cases, with postoperative mortality as high as 50 – 70 % [5]. Sevoflurane is now widely used in anesthesia for cardiac surgery and pediatric surgery because it takes a shorter time for induction of general anesthesia. Moreover, the level of anesthesia could be altered rapidly when sevoflurane is used to maintain anesthesia.

Table 3: Comparison of lung tissue apoptosis index between the two groups at different time points (mean ± SD, n = 6)

Group	Apoptosis index		
	T ₁	T ₂	T ₃
C	1.67±0.33	3.16±0.84 ^a	8.33±0.77 ^{ab}
S	1.83±0.29	3.43±0.57 ^a	6.67±0.81 ^{abc}

^a $P < 0.05$, vs T₁; ^b $p < 0.05$, vs T₂; ^c $p < 0.05$, vs group C

Table 4: Protein levels of Bcl-2 and Bax in the dogs of the two groups at different time points (mean \pm SD, n = 6)

Group	Bcl-2			Bax			Bcl-2/Bax		
	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
C	0.35 \pm 0.37	0.46 \pm 0.16 ^a	0.62 \pm 0.31 ^{ab}	0.33 \pm 0.33	0.58 \pm 0.16 ^a	0.83 \pm 0.36 ^{ab}	1.04 \pm 0.24 ^a	0.81 \pm 0.19 ^a	0.72 \pm 0.18 ^{ab}
S	0.29 \pm 0.21	0.47 \pm 0.22 ^a	0.69 \pm 0.38 ^{abc}	0.31 \pm 0.29	0.54 \pm 0.22 ^a	0.75 \pm 0.28 ^{abc}	0.95 \pm 0.17 ^a	0.88 \pm 0.11 ^a	0.94 \pm 0.32 ^{abc}

When compared with T₁, ^a*p* < 0.05; when compared with T₂, ^b*p* < 0.05; when compared with group C, ^c*p* < 0.05

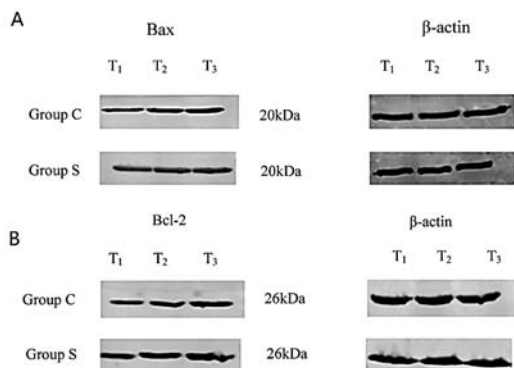


Figure 4: Proteins of Bax and BCL-2 in lung tissues of the dogs in the two groups. (A) Western blots of Bax and β-actin (20 kDa). (B) Western blots of Bcl-2 and β-actin (26 kDa)

Besides, it has less intensive depressive effects on circulation, and it protects organs. Studies have shown that sevoflurane reduced inflammation and oxidative stress in the lung tissue, and reduced lung ischemia-reperfusion injury [6]. In addition, sevoflurane pre-conditioning and post-conditioning reduced lung injury induced by IRI in rat lung transplantation models because it reduced inflammation, and it exerted anti-apoptosis effect [7]. At the same time, studies have revealed that sevoflurane treatment reduced lung tissue permeability, thereby reducing lung injury caused by ischemia-reperfusion [8]. Respiratory index (RI) and oxygenation index (OI) as well as pulmonary dynamic compliance (Cd) are commonly used as indicators of lung function. Respiratory index (RI) indicates the pulmonary ventilation function, with normal values at 0.10 - 0.37. The lower the RI, the better the pulmonary function. Oxygenation index (OI) reflects the function of pulmonary oxygenation and ventilation, and the normal value is within a range of 400 - 500 mmHg. When the OI is < 300 mmHg, it indicates respiratory dysfunction. Besides, OI is negatively correlated with RI. In addition, Cd reflects lung function. The higher the Cd, the better the lung function, and *vice versa*. The results in this study showed that OI and Cd of the two groups decreased gradually, while RI increased over time after CPB. After 30 min of sevoflurane post-conditioning, the left pulmonary artery was immediately opened and it was found that OI and Cd were markedly higher at T₃ in group S than in

group C, while RI had an opposite trend. This indicates that sevoflurane post-conditioning improved pulmonary ventilation and air exchange function after CPB, and reduced lung tissue destruction, inflammation, and lung injury caused by CPB. Apoptosis is crucial for maintaining stability in tissue structure and balance between cell proliferation and cell death. Studies have found that apoptosis directly leads to decreases in the numbers of alveolar epithelial cells and alveolar type II cells: in particular, it affected trans-differentiation process, destroyed the structure of alveoli, and caused pulmonary ischemia-reperfusion injury [9]. The main causes of apoptosis in the lung tissue during CPB are as follows: (1) massive release of oxygen free radicals, calcium overload, and impaired cellular energy metabolism due to mitochondrial damage during ischemia-reperfusion; (2) systemic inflammatory response syndrome from CPB, and the release of large amounts of inflammatory factors. Studies suggest that sevoflurane pre-conditioning inhibited hypoxia-reoxygenation-induced apoptosis in cardiomyocytes [10]. Studies by Zhang *et al* [11] found that the expression of Bcl-2 and Bcl-xL decreased significantly if there was an ischemia-reperfusion injury in the lung. Studies by Cooke *et al* [12] showed that overexpression of human Bcl-2 transfected with adenovirus in rats inhibited apoptosis after lung transplantation. Studies have reported that sevoflurane attenuated lung injury after CPB by reducing the levels of NF-κB and TNF-α [13]. Members of the BCL-2 family play key roles in apoptosis, including pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2. Changes in the cell after receiving the stimulation signal depend on the ratio of Bcl-2/Bax [14]. When Bcl-2 is overexpressed and Bcl-2 binds Bax, the excess Bcl-2 forms a Bcl-2/Bcl-2 dimer, thereby inhibiting apoptosis. When Bax is overexpressed, the excess Bax forms a Bax/Bax dimer which promotes apoptosis [15]. Studies in rats have shown that sevoflurane post-conditioning reduced apoptosis by increasing Bcl-2 expression while decreasing Bax expression, thereby altering the Bcl-2/Bax ratio and reducing transient global cerebral ischemia [16].

The results of this experiment showed that the expression of Bcl-2 protein in the lung tissue of the dogs was markedly up-regulated in group S,

relative to group C at T3 after sevoflurane post-conditioning. In contrast, apoptosis index and Bax protein expression in the lung tissue of the dogs in group S were significantly lower than those in dogs in group C. This suggests that sevoflurane post-conditioning increased the expression of anti-apoptosis protein Bcl-2 and decreased the expression of pro-apoptosis protein Bax, thereby reducing the apoptosis of lung tissue cells.

CONCLUSION

Sevoflurane post-conditioning exerts a protective effect on the lungs of CPB dogs due to its effect of reducing apoptosis by facilitating the expression of Bcl-2 in the lung tissues of CPB dogs, while suppressing the expression of Bax. Further investigations to determine the suitability of sevoflurane in lung protection during cardiopulmonary bypass in humans will be required.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contributions of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Song Chen and Hong Zhang conceived and designed the study, and drafted the manuscript. Song Chen, Junli Luo, Xuejiao Dou, Lu You and Dong Li collected, analyzed and interpreted the experimental data. SC, JL and HZ revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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REFERENCES

- Ramirez DA, Upchurch W. Pharmacological Conditioning Reduces Damage From Ischemia-Reperfusion Injury in Porcine Skeletal Muscle and Ex-Vivo Functional Hearts. *The J Heart Lung Transplant* 2020; 4(39): 357.
- Garutti I, Gonzalez-Moraga F, Sanchez-Pedrosa G, Casanova J, Martin-Piñero B, Rancan L, Simón C, Vara E. The effect of anesthetic preconditioning with sevoflurane on intracellular signal-transduction pathways and apoptosis, in a lung autotransplant experimental model]. *Braz J Anesthesiol* 2019; 69(1): 48-57.
- World Health Organization. Principles of laboratory animal care. *WHO Chron* 1985; 39: 51-56.
- Zhang L, Gao L, Li Y, Lin G, Shao Y, Ji K, Yu H, Hu J, Kalvakolanu DV, Kopecko DJ, Zhao X, Xu DQ. Effects of plasmid-based Stat3-specific short hairpin RNA and GRIM-19 on PC-3M tumor cell growth. *Clin Cancer Res* 2008; 14(2): 559-568.
- Qiu W, Zheng L, Gu H, Chen D, Chen Y. Comparison between adult and infant lung injury in a rabbit ischemia-reperfusion model. *J Thorac Cardiovasc Surg* 2008; 136(2): 352-359.
- Casanova J, Garutti I, Simon C, Giraldez A, Martin B, Gonzalez G, Azcarate L, Garcia C, Vara E. The effects of anesthetic preconditioning with sevoflurane in an experimental lung autotransplant model in pigs. *Anesth Analg* 2011; 113(4): 742-748.
- Ohsumi A, Marseu K, Slinger P, McRae K, Kim H, Guan Z, Hwang DM, Liu M, Keshavjee S, Cypel M. *Trop J Pharm Res*, July 2022; 21(7): 1389

- Sevoflurane Attenuates Ischemia-Reperfusion Injury in a Rat Lung Transplantation Model. *Ann Thorac Surg* 2017; 103(5): 1578-1586.
8. Li XH, Liu ZH, Ma HB, Li Y, Zhao H, Che JB, Liu WC, Shi GN. Effect of sevoflurane on tissue permeability of lung ischemia-reperfusion injury in rats. *Asian Pac J Trop Med* 2014; 7(4): 276-279.
 9. Fang X, Wang L, Shi L, Chen C, Wang Q, Bai C, Wang X. Protective effects of keratinocyte growth factor-2 on ischemia-reperfusion-induced lung injury in rats. *Am J Respir Cell Mol Biol* 2014; 50(6): 1156-1165.
 10. Liu ZY, Hu SP, Ji QR, Yang HB, Zhou DH, Wu FF. Sevoflurane pretreatment inhibits the myocardial apoptosis caused by hypoxia reoxygenation through AMPK pathway: An experimental study. *Asian Pac J Trop Med* 2017; 10(2): 148-151.
 11. Cooke DT, Hoyt EG, Robbins RC. Overexpression of human Bcl-2 in syngeneic rat donor lungs preserves posttransplant function and reduces intragraft caspase activity and interleukin-1beta production. *Transplantation* 2005; 79(7): 762-767.
 12. Ravagnan L, Roumier T, Kroemer G. Mitochondria, the killer organelles and their weapons. *J Cell Physiol* 2002; 192(2): 131-137.
 13. Zhang Y, Li D, Luo J, Chen S, Dou X, Han M, Zhang H. Pharmacological postconditioning with sevoflurane activates PI3K/AKT signaling and attenuates cardiopulmonary bypass-induced lung injury in dog. *Life Sci* 2017; 173: 68-72.
 14. Ghate NB, Hazra B, Sarkar R, Mandal N. In vitro anticancer activity of *Spondias pinnata* bark on human lung and breast carcinoma. *Cytotechnol* 2014; 66(2): 209-218.
 15. Lamb HM, Hardwick JM. Unlatched BAX pairs for death. *Cell* 2013; 152(3): 383-384.
 16. Kim HC, Kim E, Bae JI, Lee KH, Jeon YT, Hwang JW, Lim YJ, Min SW, Park HP. Sevoflurane Postconditioning Reduces Apoptosis by Activating the JAK-STAT Pathway After Transient Global Cerebral Ischemia in Rats. *J Neurosurg Anesthesiol* 2017; 29(1): 37-45.