The Heart Surgery Forum 2022-4721
25 (3), 2022 [Epub May 2022]
doi: 10.1532/hsf.4721

Online address: http://journal.hsforum.com

Sevoflurane Postconditioning Inhibits Pulmonary Apoptosis via PI3K/AKT in Dog Cardiopulmonary Bypass Model

Junli Luo,[1] Dongdong Li,[2] Song Chen,[1] Hong Zhang,[1] Huijun Cai[1]

[1]Department of Anesthesiology, Affiliated Hospital of Zunyi Medical University, China;
[2]Department of Pain Treatment, Weifang Hospital of Traditional Chinese Medicine, China

ABSTRACT

Aims: The study aimed to investigate the protective effects and regulatory mechanism of sevoflurane postconditioning (SPC) in pulmonary apoptosis induced by cardiopulmonary bypass (CPB).

Methods: Twenty-four healthy dogs were divided into a control (C group), ischemia/reperfusion (I/R group), sevoflurane postconditioning (S group), and wortmannin group (S+W group). At 10 min after the establishment of CPB, the left pulmonary artery was blocked. When the pulmonary artery was reopened, 2% sevoflurane was administered. Wortmannin was delivered 10 min before the pulmonary artery was open. Before thoracotomy was implemented (T1), when the artery was reopened (T2) and 2 h after CPB (T3), blood and the inferior lobe of the left lung were isolated and subjected to gas analysis, pathological examination, western blot, and TUNEL staining.

Results: No obvious changes were observed in the C group throughout the experiment. The conditions of all treated groups progressively deteriorated, and no difference could be found except in the number of apoptotic cells of T3 between the S+W and I/R groups. At T2, the treated groups showed similar conditions. At T3, the lung function and structure of the S group were improved in I/R and S+W groups. The S group showed the highest p-Akt expression, the lowest cleaved-caspase 3 expression, and apoptotic cell percentage.

Conclusions: Ischemia-reperfusion of the lung during CPB reduces lung function and injures the pulmonary structure via inducing lung apoptosis. Sevoflurane postconditioning preserves lung function and structure by alleviating apoptosis via activation of PI3K/Akt.

Received March 17, 2022; accepted March 31, 2022.

Correspondence: Huijun Cai and Hong Zhang, Department of Anesthesiology, Affiliated Hospital of Zunyi Medical University, No.149 Dalian Road, Huichuan District, Zunyi City, 563099, Guizhou Province, China, Telephone +86-18685630890 and 86-18985653899 (e-mail: caihuijun2021@126.com and zhanghong12021@126.com).

INTRODUCTION

Every year, more than one million patients worldwide undergo cardiac surgery [Denault 2010]. Cardiopulmonary bypass (CPB) facilitates the repair of cardiac lesions in adults and children. Pulmonary dysfunction is the most frequent complication in the early postoperative phase following cardiac surgery involving extracorporeal circulation, which greatly influences the outcome of patients [Kiessling 2014]. In addition, it was found that standard CPB and cardioplegic arrest-initiated apoptosis in pneumocytes and the cell loss might be responsible for severe organ dysfunction [Klass 2007; Yu 2013; Fischer 2000; Van Putte 2005]. Therefore, the inhibition of apoptosis of pneumocytes is beneficial to protect against lung injury induced by CPB. Nowadays, sevoflurane preconditioning as an antiapoptosis agent exerts a protective effect against ischemia-reperfusion damage in heart, brain, and liver [Rodríguez 2015; Wang 2016; Wang 2010; Li 2014]. Furthermore, sevoflurane postconditioning (SPC) was found to exert a protective effect in different models [Inamura 2010; Jeon 2013; Meybohm 2011]. However, it was unknown whether sevoflurane postconditioning exerts a protective effect on CPB-induced lung injury. Therefore, this study was designed to explore the effect of sevoflurane postconditioning on lung injury induced by CPB and whether antiapoptosis was involved in the protective mechanisms.

The PI3K/AKT pathway plays a key role in normal cellular functions including proliferation, adhesion, migration, invasion, energy metabolism, protein synthesis, and prosurvial. It has been found that the PI3K associated signaling pathway is involved in the cardioprotective pathway against ischemia/reperfusion (I/R) injury [Zhang 2014; Wei 2015; Yao 2014]. Moreover, researchers found that sevoflurane preconditioning is associated with its properties of antiapoptosis via activation of the PI3K/Akt pathway [Zhang 2015; Liu 2015]. Accordingly, we hypothesize that the PI3K/Akt pathway activation is involved in lung-protective signaling induced by sevoflurane postconditioning and that this activation inhibits apoptosis.

MATERIALS AND METHODS

Animals and experimental design: All animal studies were performed in accordance with Zunyi Medical College's policy for the care and use of animals for scientific purposes

and approved by the institutional ethics committee. Twenty-four dogs (10–15 kg) were purchased from the Experimental Animal Center of Zunyi Medical College. In this study, to avoid the influence of other organs-lung crosstalk [White 2012; Zhou 2015], a CPB model with a blocked lung artery was used. These dogs were randomly assigned to the control group (C group), ischemia-reperfusion group (I/R group), sevoflurane postconditioning group (S group), and wortmannin group (S+W group) ($N = 6$). The dogs were anesthetized with 2.5% sodium pentobarbital (intraperitoneal injection, 25 mg/kg) and monitored with electrocardiogram and oxygen saturation of tongue mucosa. Then double-lumen endotracheal intubation was carried out. The animals were placed on a ventilator in the volume control mode. Initial ventilatory settings included a fraction of inspiration O2 (FIO2) of 90%, the delivered tidal volume measured at the endotracheal tube of 12–15 ml/kg, and the respiratory rate (RR) of 16 times per min. The mean arterial pressure (MAP) and central venous pressure (CVP) were monitored by a UT4000 Fpro type multifunctional monitor (Jinkewei, Shenzhen, China) by placing femoral arterial and femoral venous catheters. Intermittent boluses of sodium pentobarbital were administered for maintenance of anesthesia. After a median sternotomy, the pericardium was incised. Following administration of heparin (3 mg/kg), cannulation was accomplished by placing an arterial cannula in the ascending aorta and a venous cannula in the right atrium. Each dog was placed on conventional nonpulsatile CPB at a flow of 100–120 ml·kg–1·min–1. Ten min later, the left pulmonary artery was separated and blocked. Meanwhile, the right single-lung ventilation was executed. After 60 min, the blocked artery was opened followed by 30 min CPB. During CPB, MAP was maintained 50–80 mmHg, each animal was cooled to a nasopharyngeal temperature of 32-34°C, and the results of arterial blood gas analysis were used to adjust pH value and electrolyte concentration. After CPB, each dog received vasoactive drugs to stabilize blood pressure for 2 h. In the C group, the left pulmonary artery was not clamped. In the S group, 2% sevoflurane was administered for 30 min starting at the beginning of reperfusion. In S+W group, wortmannin was infused (0.16mg·kg-1) for 10 min before the left pulmonary artery was reopened, the other treatment was the same in the S group.

Specimen collection: Before median sternotomy was completed (T1), at the onset of opening the left pulmonary artery (T2) and 2 h after CPB (T3), the blood was taken from the femoral artery and the left inferior lobe was taken and portioned into two parts. One was saved in liquid nitrogen for further determination. Another was soaked in 10% formaldehyde. The blood was used for the calculation of blood gas analysis, respiratory index (RI), and oxygenation index (OI).

Respiration parameters: Respiratory index (RI) and oxygenation index (OI) were determined with the arterial blood using a gas analyzer. RI and OI were calculated using the following formula: OI= PaO2/FiO2; RI=P(A-a)O2/PaO2[PA-aDO2 = FiO2 × 713–PaCO2 – PaO2 (Fio2, fraction of inspiration oxygen; PaCO2, partial pressure of CO2 in arterial blood; PaO2, partial pressure of O2 in arterial blood)].

Histological examination: To evaluate the morphological severity, the tissues were fixed in 10% formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E).

Western blotting analysis: The tissue was homogenized with protein lysis buffer. The homogenate was centrifuged (15 000g, 4°C) for 15 min. The supernatants were assayed for protein concentration using a Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China) and stored at –80°C. All protein samples were electrophoresed on sodium dodecyl sulfate (SDS)–polyacrylamide gels (60 µg protein/sample was loaded for each immunoblot lane) and transferred to polyvinylidene fluoride membranes. Membranes were blocked in phosphate buffered saline (PBS) buffer containing 5% powdered milk for 1.5-2 h, washed, and incubated with primary antibody overnight at 4°C [p-Akt: 1:700 (Cell Signaling Technology, USA); cleaved-caspase3: 1:600 (Abcam, UK); β-actin: 1:800(Bioss, China)]. After incubation, samples were washed five times with PBST (phosphate buffered saline with 0.1% Tween 20) and incubated with near-infrared-tag monoclonal antibody (1:6000) for 1.5 h. Membranes were imaged using a LiCor Odyssey scanner (LiCor, Lincoln, NE, USA) and values were obtained with Odyssey 3.0 analytical software (LiCor, Lincoln, NE, USA).

TUNEL staining: The occurrence of apoptosis was assessed using TUNEL assay. In brief, the sections were treated with 20 µg/ml proteinase K for 15-30 min at room temperature and at 37 C for 15 min. Subsequently, the sections were washed with PBS. After the addition of 50 µl TUNEL reaction mixture, the sections were incubated for 60 min at 37°C, rinsed with PBS, and visualized using converter-POD for 30 min at 37°C in a dark place. Then, the sections were washed with PBS and incubated with 50–100 µL 0.05 % 3,3'-diaminobenzidine (DAB) as a chromogen for 10 min at room temperature. Under the light microscope, images were then obtained at 400 × magnification following the random selection of five non-overlapping regions. The number of apoptotic cells and total cells were counted, and then the cell apoptosis rate was determined by the following equation: cell apoptosis rate = the number of apoptotic cells/total cells × 100%.

Statistical analysis: Statistical analyses were carried out using SPSS 17.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). Data were presented as mean±SD. Comparisons between groups for statistical significance were performed with Student's t test and analysis of variance. Comparisons of data in the same group were performed with repeated measures analysis of variance (ANOVA), and Mauchly's test of sphericity was used (the epsilon correction was used when necessary). $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics: No significant differences were observed among the four dog groups with regard to body weight, body area surface, CPB time, after CPB time, the lowest nose temperature, and volume of liquid ($P > 0.05$). (Table 1)

Sevoflurane postconditioning improves lung function following I/R. Table 2 shows the changes in pulmonary function parameters. (Table 2) There was no difference among the four groups at T1 ($P > 0.05$). In the C group, signs of reduced lung function could not be found ($P > 0.05$). As the CPB went on, the lung function in the I/R, S, and S+W groups deteriorated indicated by growing OI and decreasing RI. At T2, no difference could be found among I/R, S, and S+W groups ($P > 0.05$). However, at T3, the lung function of the S group was better than both the I/R and S+W groups ($P < 0.05$). There was no significant difference between the I/R group and the S+W group ($P > 0.05$). These results show that sevoflurane postconditioning protects the lung function and the effect could be inhibited by wortmannin.

Sevoflurane postconditioning alleviates pulmonary ultrastructural disorder: H&E staining results showed that the alveolar structure at T1 was complete and had no abnormal changes, such as effusions of inflammatory cells and widened pulmonary interstitial space in all groups. At T2, no significant changes could be found in the C group. (Figure 1) In the other three groups (I/R, S, and S+W groups), some alveolar walls broke and some alveolar intervals widened, few inflammation cells and angiotelectasis could be found. At 2 h after CPB, in the C group, the alveolar walls were still smooth and intact, rare inflammation cells could be found; in I/R and S+W groups, the most serious and obvious changes were found, including ruptured walls, collapsed alveolar cavity with lots of red cells and inflammation cells; in S group, parts of walls ruptured with few inflammation cells and red cells. These results indicated that during CPB, ischemia-reperfusion was one of the main reasons of pulmonary damage and sevoflurane postconditioning could preserve lung structure and the protective effect could be abolished by the inhibitor of PI3K.

Sevoflurane postconditioning reduces the apoptotic index of lung: At T1, the rate of apoptosis in lung tissues was not significantly different among all groups ($P > 0.05$). (Figure 2) In the C group, no difference could be found at all three time points. In the other three groups, the number of apoptotic cells steadily increased with time after CPB was processed. At T2, the rate of apoptosis in the I/R, S, and S+E groups was no different ($P > 0.05$). At T3, the number of TUNEL-positive cells was significantly higher in the I/R group compared with the other three groups ($P < 0.05$), sevoflurane postconditioning reduced the number significantly and PI3K inhibitor alleviated but did not abolish the effect of sevoflurane.

The expression of cleaved caspase 3, one of the important markers of apoptosis, was detected via Western blot. (Figure 3) At T1, there was no significant difference among all the groups ($P > 0.05$). As the extension of CPB, the expression of cleaved caspase 3 in the C group was no different, the levels of cleaved-caspase 3 increased continually in the other three

Table 1. The general statistics of each group during CPB ($N = 6$)

Variables	Control group	I/R group	S group	S+W group	*P*-values
Body weight (kg)	12.4±1.14	12.08±1.53	11.75±1.33	11.92±1.16	$P > 0.05$
Body surface area (m^2)	0.58±0.04	0.57±0.05	0.56±0.04	0.56±0.04	$P > 0.05$
CPB time (min)	104.83±1.47	105.67±2.88	106.17±3.31	105.50±2.74	$P > 0.05$
After CPB time (min)	123.83±1.72	124.50±1.87	124.50±1.64	124.00±2.00	$P > 0.05$
Lowest nose temperature (°C)	31.68±0.41	31.83±0.50	31.76±0.48	31.51±0.43	$P > 0.05$
Volume of liquid (ml)	359.67±8.40	350.00±7.07	356. 00±5.89	354.17±10.68	$P > 0.05$

Data were presented as mean ± SD. $P > 0.05$ for comparison of four dog groups

Table 2. Pulmonary function parameters

	RI			OI		
Group	T1	T2	T3	T1	T2	T3
C	0.40±017	0.44±0.19	0.56±0.24	450.67±25.70	438.17±23.59	431.50±19.81
I/R	0.41±0.22	1.37±0.25 *‡	3.72±0.64*†‡	456.50±22.69	373.33±21.98*‡	226.67±24.76*†‡
S	0.49±0.17	1.24±0.14 *‡	2.03±0.29 *†‡§	466.33±20.12	376.33±17.64*‡	325.33±19.02*†‡§
S+W	0.44±0.22	1.28±0.21*‡	3.23±0.52*†‡&	448.50±22.58	369.00±14.10*‡	250.67±41.82*†‡&

Data were expressed with mean ± SD. *$P < 0.05$, compared with T1 in the same group; †$P < 0.05$, compared with T2 in the same group; ‡$P < 0.05$, compared with the C group at the same time point ; §$P < 0.05$ compared with the I/R group at the same time point; &$P < 0.05$, compared with the S group at the same time point.

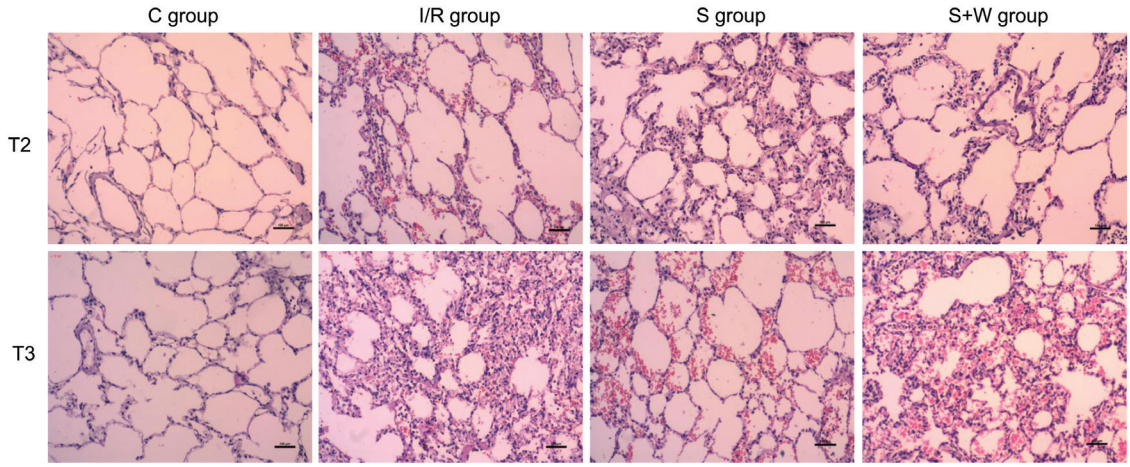

Figure 1. Pathological examination of lung in different groups using H&E staining. At T2, no significant changes could be found in the C group, whereas the other three groups showed broken alveolar walls and some extent of inflammation and angiotelectasis. At T3, no significant changes could be found in the C group; I/R and S+W groups showed serious inflammation. Ruptured walls collapsed alveolar cavity with numerous red cells and inflammation cells were observed. In contrast, the S group showed medium extent of inflammation, suggesting the protective effects of sevoflurane postconditioning.

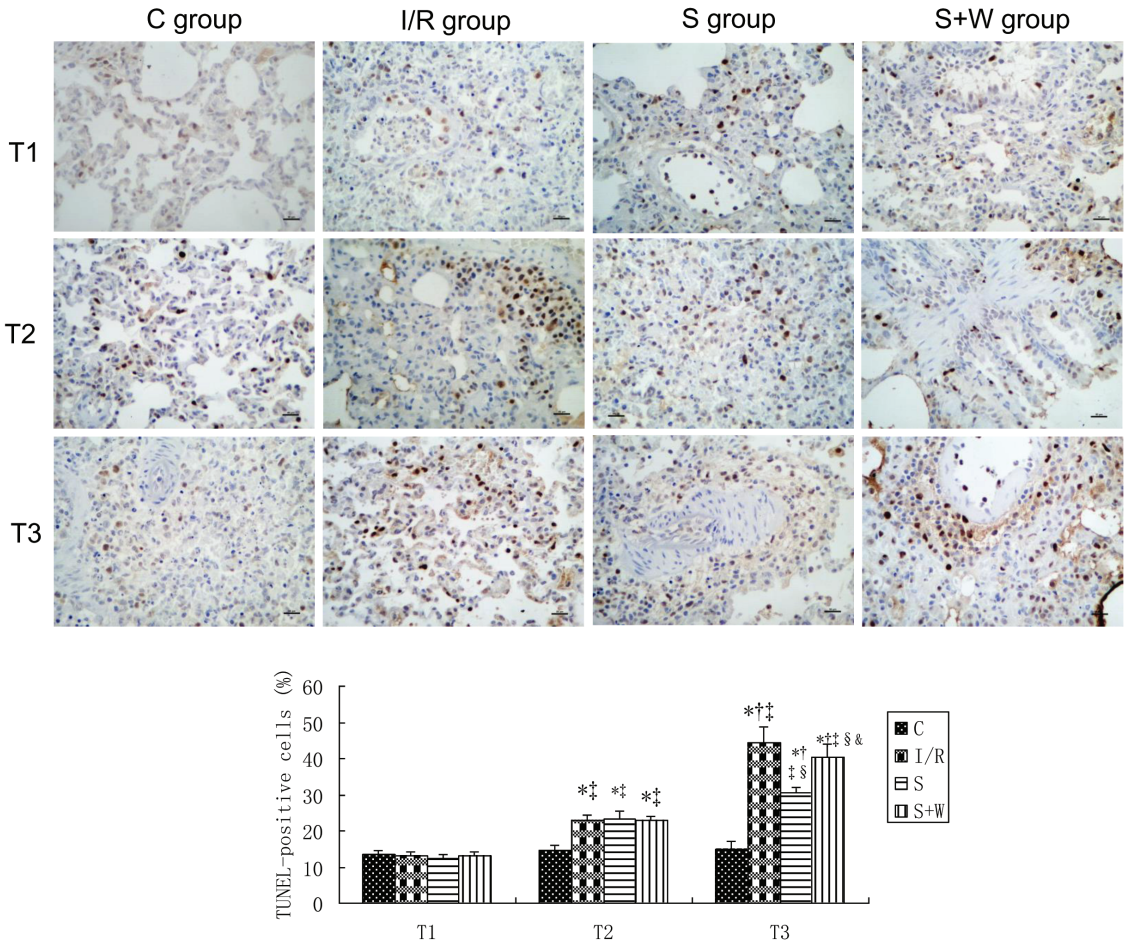

Figure 2. Sevoflurane postconditioning reduces the apoptotic index of lung
Representative images and quantification of TUNEL stainings at T1, T2, and T3. Data were expressed with mean ± SD. *$P < 0.05$, compared with T1 in the same group; †$P < 0.05$, compared with T2 in the same group; ‡$P < 0.05$, compared with the C group at the same time point; §$P < 0.05$ compared with the I/R group at the same time point; &$P < 0.05$, compared with the S group at the same time point.

groups. At T2, the expression was no different among I/R, S, and S+W groups ($P > 0.05$). At T3, the expression of cleaved-caspase 3 of the I/R group was significantly higher compared with the S group ($P < 0.05$), however, there was no difference between the S+W group and I/R group ($P > 0.05$).

Sevoflurane postconditioning alleviates the CPB-indued apoptosis in the lung activating the PI3K/AKT pathway: The PI3K/AKT signaling pathway is a key intracellular signal transduction pathway regulating apoptosis. The expression of p-AKT was examined by Western blot. As shown in Figure 4, there was no difference among all groups at T1 ($P > 0.05$). (Figure 4) As the extension of CPB, the difference could not be found in the C group, the levels of p-AKT gradually increased in the other three groups. At T2, there was no difference among the I/R, S, and S+W groups ($P > 0.05$). At T3, the expression of p-AKT in the S group was significantly increased compared with the I/R and S+W groups ($P < 0.05$), there was no difference between the I/R and S+W groups ($P > 0.05$). These results show that the anti-apoptosis effect of sevoflurane postconditioning is mediated by simulating the PI3K/AKT pathway.

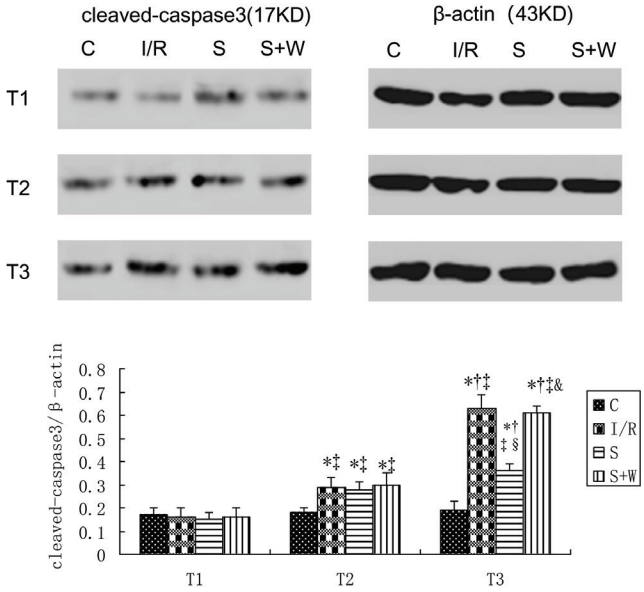

Figure 3. Sevoflurane postconditioning reduces expression of cleaved-caspase3 in lung. Expression of cleaved-caspase3 in lung tissue was detected by western blot. Data were expressed with mean ± SD. *P < 0.05, compared with T1 in the same group; †P < 0.05, compared with T2 in the same group; ‡P < 0.05, compared with the C group at the same time point; §P < 0.05, compared with the I/R group at the same time point; &P < 0.05, compared with the S group at the same time point.

DISCUSSION

In the present study, 2.0% sevoflurane was administered for 30 min since the onset of reperfusion following ischemia. We found that SPC significantly improved lung function and lung structure and decreased the apoptotic level. The novel finding of this study is that SPC increases the phosphorylation of AKT. Administration of wortmannin before SPC abolished the protective effect against lung injury. Our study demonstrated a direct link between PI3K/AKT pathway activation and the attenuation of lung apoptosis induced by SPC.

Most studies of SPC are on the heart and brain and indicate that SPC is an effective method to reduce I/R injury [Lee 2015; Li 2013; Zhang 2015]. Few studies found that SPC was effective against lung injury induced by lipopolysaccharide [Zhao 2014; Steurer 2009]. The results of this study indicate that SPC exerts a protective effect against lung injury during CPB. Therefore, these studies support the idea that SPC serves as a protective strategy.

In this study, the expression of p-Akt increased during the ischemic period in all groups except the C group. This result is similar to Li et al. [Li 2015]. In their study, the expression of p-Akt in the IRI group was higher than in the control group, but with no significant difference. We think the difference between our studies was caused by different models. In their study, the pulmonary hilum was occluded with a non-crushing microvascular clamp without the CPB process. Therefore, the damage was not as harmful as our model. However, our results are similar to the findings of Zhang and R that ischemia leads to activation of PI3K/AKT [Inamura 2010; Li 2012]. So, we believe that the active AKT is an important endogenous protective mechanism. Nowadays, it was found that sevoflurane exerts protective effects against cerebral and intestinal ischemia-reperfusion damage via the PI3K/Akt pathway [Zhang 2015; Liu 2015; Ye 2015]. The same result could be found in this study. It could be seen that sevoflurane postconditioning preserved lung function and alleviated changes of structure, which were abolished by wortmannin.

Researchers found that antiopoptosis was involved in the protective mechanisms of sevoflurane [Inamura 2010; Zhang 2015]. In addition, PI3K/Akt is an important signal transduction pathway that involves many cellular physiological activities. There is consensus that activation of Akt, which is known to be pro-survival signaling, plays an important role in myocardial protection [Tsai 2015]. So, we think the apoptosis effect of SPC was associated with PI3K/Akt signal pathway. The notion was verified by the results of this study. A recent study indicated that the level of miR-320 was positively associated with RI, but negatively correlated with OI [Yang 2015]. Furthermore, it was found that miR-320 can promote apoptosis [Ren 2009]. In this study, RI and OI in the C group were with no difference in the extension of CPB. However, in the other three groups that underwent IR, both greatly changed. The changes in lung function were similar to cleaved-caspase-3 which is the key enzyme required in the mitochondria-caused apoptosis. Combing with the results of TUNEL staining, it can be concluded that IR is one of the main reasons for pulmonary apoptosis during CPB. This conclusion is accordant with previous research [Yang 2015; Slottosch 2014]. In this study, all apoptosis-related indexes were relieved by SPC and all were reversed by wortmannin. So, this study provided the direct information to support our notion that antiapoptosis via the PI3K/Akt pathway against

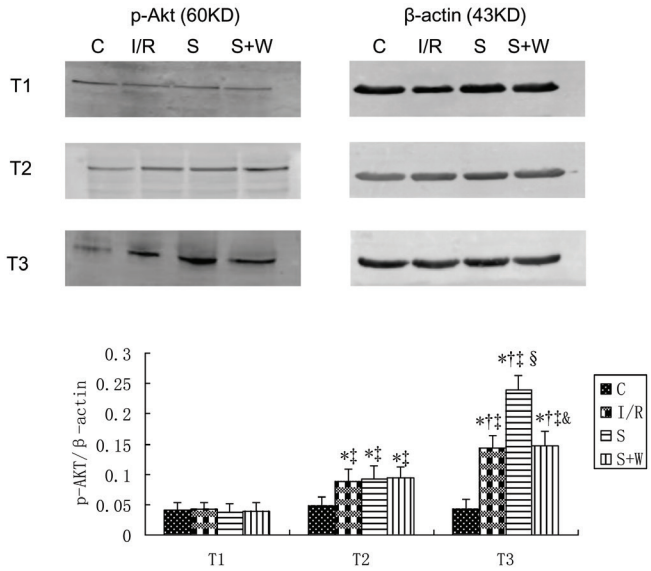

Figure 4. Sevoflurane postconditioning increases the levels of p-AKT. At T1, there was no difference among all groups ($P > 0.05$). As the extension of CPB, difference could not be found in the C group, the levels of p-AKT gradually increased in the other three groups. At T2, there was no difference among the I/R, S and S+W groups ($P > 0.05$). At T3, the expression of p-AKT in the S group was significantly increased. Comparing with the I/R and S+W groups ($P < 0.05$), there was no difference between the I/R and S+W groups ($P > 0.05$). Data were expressed with mean ± SD. *$P < 0.05$, compared with T1 in the same group; †$P < 0.05$, compared with T2 in the same group; ‡$P < 0.05$, compared with C group at the same time point ; §$P < 0.05$, compared with I/R group at the same time point; &$P < 0.05$, compared with the S group at the same time point.

pulmonary injury induced by IR is involved in the protective mechanisms of SPC during CPB. Furthermore, the apoptotic cells of S+W were less than the I/R group, indicating that the antiapoptosis effect of SPC could be totally reversed by PI3K inhibitor. Therefore, we inferred that other mechanisms of antiapoptosis were involved in SPC.

CONCLUSION

In conclusion, 2% sevoflurane delivered at the onset of reperfusion for 30 min is beneficial to those who receive CPB treatment exerting protective effect against pulmonary apoptosis. This study provides direct data for the notion that the pI3K/Akt pathway plays an important role in the antiapoptosis induced by sevoflurane postconditioning on lung injury caused by CPB.

ACKNOWLEDGEMENT

Funding: The study was funded by the Graduate Student Fund of Zunyi Medical University Yuan Zi (2015) No. 39 and Qian LH [2015] No. 7488.

REFERENCES

Denault AY, Deschamps A, Couture P. 2010. Intraoperative hemodynamic instability during and after separation from cardiopulmonary bypass. Semin Cardiothorac Vasc Anesth. 14(3):165-82.

Fischer S, Cassivi SD, Xavier AM, Cardella JA, Cutz E, Xavier AM, et al. 2000. Cell death in human lung transplantation: Apoptosis induction in human lungs during ischemia and after transplantation. Ann Surg. 231:424–431.

Inamura Y, Miyamae M, Sugioka S, Domae N, Kotani J. 2010. Sevoflurane postconditioning prevents activation of caspase 3 and 9 through antiapoptotic signaling after myocardial ischemia-reperfusion. J Anesth. 24(2):215-24.

Jeon YT, Hwang JW, Lim YJ, Kim AN, Park HP. 2013. A combination of sevoflurane postconditioning and albumin increases Bcl-2 expression after transient global cerebral ischemia compared with either sevoflurane postconditioning or albumin alone. J Neurosurg Anesthesiol. 25(1):43-50.

Kiessling AH, Guo FW, Gökdemir Y, Thudt M, Reyher C, Scherer M, et al. 2014. The influence of selective pulmonary perfusion on the inflammatory response and clinical outcome of patients with chronic obstructive pulmonary disease undergoing cardiopulmonary bypass. Interact Cardiovasc Thorac Surg. 18(6):732-9.

Klass O, Fischer UM, Antonyan A, Bosse M, Fischer JH, et al. 2007. Pneumocyte apoptosis induction during cardiopulmonary bypass: effective prevention by radical scavenging using N-acetylcysteine. J Invest Surg. 20: 349–356.

Lee H, Park YH, Jeon YT, Hwang JW, Lim YJ, Kim E, et al. 2015. Sevoflurane post-conditioning increases nuclear factor erythroid 2-related factor and haemoxygenase-1 expression via protein kinase C pathway in a rat model of transient global cerebral ischaemia. Br J Anaesth. 114(2):307-18.

Li D, Huang B, Liu J, Li L, Li X. 2013. Decreased brain K(ATP) channel contributes to exacerbating ischemic brain injury and the failure of neuroprotection by sevoflurane post-conditioning in diabetic rats. PLoS One. 26;8(8):e73334.

Li J, Zhou J, Zhang D, Song D, She J, Bai C. 2015. Bone marrow-derived mesenchymal stem cells enhance autophagy via PI3K/AKT signalling to reduce the severity of ischaemia/reperfusion-induced lung injury. J Cell Mol Med. 19(10):2341-51.

Li R, Xu X, Chen C, Yu X, Edin ML, Degraff LM, et al. 2012. Cytochrome P450 2J2 is protective against global cerebral ischemia in transgenic mice. Prostaglandins Other Lipid Mediat. 2012, 99(3-4):68-78.

Li X, Luo P, Wang F, Yang Q, Li Y, Zhao M, et al. 2014. Inhibition of N-myc downstream-regulated gene-2 is involed in an astrocyte-specific neuroproction induced by sevoflurane preconditioning. Anesthesiology. 121(3):549-62.

Liu C, Shen Z, Liu Y, Peng J, Miao L, Zeng W, Li Y. 2015. Sevoflurane protects against intestinal ischemia-reperfusion injury partly by phosphatidylinositol 3 kinases/Akt pathway in rats. Surgery. 157(5):924-33.

Meybohm P, Gruenewald M, Albrecht M, Müller C, Zitta K, Foesel N, et al. 2011. Pharmacological postconditioning with sevoflurane after cardiopulmonary resuscitation reduces myocardial dysfunction. Crit Care. 15(5): R241.

Ren XP, Wu J, Wang X, Sartor MA, Qian J, Jones K, et al. 2009. MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. Circulation.

119(17):2357-66.

Rodríguez A, Taurà P, García Domingo MI, Herrero E, Camps J, Forcada P, et al. 2015. Hepatic cytoprotective effect of ischemic and anesthetic preconditioning before liver resection when using intermittent vascular inflow occlusion: a randomized clinical trial. Surgery. 157(2):249-59.

Slottosch I, Liakopoulos O, Kuhn E, Deppe A, Lopez-Pastorini A, Schwarz D, et al. 2014. Controlled lung reperfusion to reduce pulmonary ischaemia/reperfusion injury after cardiopulmonary bypass in a porcine model.Interact Cardiovasc Thorac Surg. 19(6):962-70.

Steurer M, Schläpfer M, Steurer M, Z'graggen BR, Booy C, Reyes L, et al. 2009. The volatile anaesthetic sevoflurane attenuates lipopolysaccharide-induced injury in alveolar macrophages. Clin Exp Immunol.155(2):224-30.

Tsai HJ, Huang SS, Tsou MT, Wang HT, Chiu JH. 2015. Role of Opioid Receptors Signaling in Remote Electrostimulation - Induced Protection against Ischemia/Reperfusion Injury in Rat Hearts. PLoS One. 10(10):e0138108.

Van Putte BP, Kesecioglu J, Hendriks JM, Persy VP, van Marck E, van Schil P, et al. 2005. Cellular infiltrates and injury evaluation in a rat model of warm pulmonary ischemia-reperfusion. Crit Care. 9: R1–R8.

Wang C, Xie H, Liu X, Qin Q, Wu X, Liu H, et al. 2010. Role of nuclear factor-κB in volatile anaesthetic preconditioning with sevoflurane during myocardial ischaemia/reperfusion. Eur J Anaesthesiol. 27(8):747-56.

Wang H, Shi H, Yu Q, Chen J, Zhang F, Gao Y. 2016. Sevoflurane Preconditioning Confers Neuroprotection via Anti-apoptosis Effects. Acta Neurochir Suppl. 121:55-61.

Wei K, Liu L, Xie F, Hao X, Luo J, Min S. 2015. Nerve growth factor protects the ischemic heart via attenuation of the endoplasmic reticulum stress induced apoptosis by activation of phosphatidylinositol 3-kinase. Int J Med Sci. 12(1):83-91.

White LE, Cui Y, Shelak CM, Lie ML, Hassoun HT. 2012. Lung endothelial cell apoptosis during ischemic acute kidney injury. Shock. 38(3):320-7.

Yang K, Gao B, Wei W, Li Z, Pan L, Zhang J, et al. 2015. Changed profile of microRNAs in acute lung injury induced by cardio-pulmonary bypass and its mechanism involved with SIRT1. Int J Clin Exp Pathol. 8(2):1104-15.

Yao H, Han X, Han X. 2014. The cardioprotection of the insulin-mediated PI3K/Akt/mTOR signaling pathway. Am J Cardiovasc Drugs. 14(6):433-42.

Ye Z, Xia P, Cheng ZG, Guo Q. 2015. Neuroprotection induced by sevoflurane-delayed post-conditioning is attributable to increased phosphorylation of mitochondrial GSK-3β through the PI3K/Akt survival pathway. J Neurol Sci. 348(1-2):216-25.

Yu Y, Gao M, Li H, Zhang F, Gu C. 2013. Pulmonary artery perfusion with anti-tumor necrosis factor alpha antibody reduces cardiopulmonary bypass-induced inflammatory lung injury in a rabbit model. PLoS One. 8(12): e83236.

Zhang J, Wang C, Yu S, Luo Z, Chen Y, Liu Q. 2014. Sevoflurane Postconditioning Protects Rat Hearts against Ischemia-Reperfusion Injury via the Activation of PI3K/AKT/mTOR Signaling. Sci Rep. 4:7317.

Zhang L, Zhao X, Jiang X. 2015. Sevoflurane Post-conditioning Protects Primary Rat Cortical Neurons Against Oxygen-Glucose Deprivation/Resuscitation: Roles of Extracellular Signal-Regulated Kinase 1/2 and Bid, Bim, Puma. Neurochem Res. 40(8):1609-19.

Zhang LM, Zhao XC, Sun WB, Li R, Jiang XJ. 2015. Sevoflurane post-conditioning protects primary rat cortical neurons against oxygen-glucose deprivation/resuscitation via down-regulation in mitochondrial apoptosis axis of Bid, Bim, Puma-Bax and Bak mediated by Erk1/2. J Neurol Sci. 357(1-2):80-7.

Zhang Y, Tian SY, Li YW, Zhang L, Yu JB, Li J, et al. 2015. Sevoflurane preconditioning improving cerebral focal ischemia-reperfusion damage in a rat model viaPI3K/Akt signaling pathway. Gene. 569(1):60-5.

Zhao S, Wu J, Zhang L, Ai Y. 2014. Postconditioning with sevoflurane induces heme oxygenase-1 expression via the PI3K/Akt pathway in lipopolysaccharide-induced acute lung injury. Mol Med Rep. Jun;9(6):2435-40.

Zhou L, Zhao D, An H, Zhang H, Jiang C, Yang B. 2015. Melatonin prevents lung injury induced by hepatic ischemia-reperfusion through anti-inflammatory and anti-apoptosis effects. Int Immunopharmacol. pii: S1567-5769(15)30146-6.