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Research Article
Protective Effect of Sevoflurane Post-Conditioning on Ischemia-Reperfusion Injury in Elderly Rats

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Abstract

Background and Objective: Sevoflurane post-treatment (SPostC) and HIF-MIF pathway might be associated with cardiac protection. The mechanism underlying HIF-MIF pathway-mediated SPostC in elderly rats with myocardial ischemia/reperfusion (I/R) injury. **Materials and Methods:** Healthy SD rats were randomly divided into the following groups (n = 15 per group): Control fake surgical group (sham group), ischemia/reperfusion injury group (I/R group), SPostC group, HIF-1α excited group (DMOG group) and HIF blocker group (2 ME2 group). The TUNEL method, CCK-8 method, colorimetric method and the Western blot method were used in the experiments. **Results:** The inter-group difference was significant for cell viability, ATP, SOD and MDA oxidative stress among the groups (all $p<0.05$). The cell viability and ATP increased with the positive intervention of HIF and decreased with the negative intervention of HIF. With the positive intervention of HIF, the SOD level increased and the MDA level decreased. With the negative intervention of HIF, the SOD level declined and the MDA level increased. The expression of HIF-1α, MIF and p-AMPKα protein in the SPostC group was more highly expressed than the sham, I/R and 2ME2 groups, but lower than the DMOG group (all $p<0.05$). **Conclusion:** The SPostC has a certain protective effect on rat tissue cells with a myocardial I/R injury, which might be regulated by the HIF-MIF signal shaft.

Key words: Cardiomyocyte, ischemia/reperfusion, protein expression, sevoflurane post-treatment, cardiac protection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Myocardial ischemia has received growing scholarly and clinical attention at home and abroad due to its poor prognosis and high mortality rate[1]. Myocardial ischemia irreversibly impairs cardiac function with ischemia-reperfusion injury (I/RI) as a common complication, which is also a primary reason for the poor prognosis in patients with ischemic heart disease[1]. The interventional therapy addressing I/RI is mainly intended to lessen cardiomyocyte injury[1]. Sevoflurane is a commonly used anesthetic that has found extensive clinical applications because of its fast-acting feature, quick awakening after surgery, low circulatory and respiratory burden and low blood-gas partition coefficient[2,3]. Sevoflurane not only has a fast, intense and long-lasting local analgesic effect with an adequate safety profile[2], but also protects the cardiac function by regulating the inflammatory reaction and reducing the injury of cardiomyocytes[3]. Sevoflurane pre- or post-conditioning has been found to effectively reduce myocardial I/RI[4-6]. However, sevoflurane pre-conditioning has a strict timing requirement, thus it must be implemented before the ischemic event, which narrows the application of sevoflurane pre-conditioning. In contrast, sevoflurane post conditioning (SPostC) can be used in many application scenarios, however, we still know little about the mechanism of the myocardial protective effect facilitated by SPostC.

The SPostC might be associated with improvement in mitochondrial respiratory function after upregulation of Hypoxia-Inducible Factor-1α (HIF-1α) and giant macrophage Movement Inhibitory Factor (MIF) expression to protect cardiac function[7,8]. One study analyzed the correlation between the expression of HIF-1α, MIF and p-AMPKα protein and the protective effect of SPostC for myocardial I/RI[8]. It was reported that upregulation of the Hypoxia-Inducible Factor-Migration Inhibitory Factor (HIF-MIF) pathway is closely related to myocardial I/RI[9]. Another experiment found that reperfusion arrhythmias can be reduced by SPostC without affecting the severity of myocardial stunning[10]. In these previous reports, SPostC plays a great role in cardiac protection[7-10]. However, in the older subjects in other reports, the protective effect appears to be diminished or failed[11,12].

Therefore, the present study focused on the *in vivo* myocardial protective effect of SPostC in a myocardial I/RI model in elderly rats. Attempts were done to validate whether the myocardial protective effect of SPostC was weakened in elderly rats and provide the theoretical basis for addressing the key targets in myocardial I/RI in the elderly.

MATERIALS AND METHODS

Study area: This study was proceeded at the Laboratory of Xinjiang Medical University between March and July, 2021.

Laboratory animals: The clean-grade healthy Sprague Dawley rats (n = 75) were all males, each weighing 450-550 g and 22-24 months old. Ethical approval for laboratory animals was obtained from the First Affiliated Hospital of Xinjiang Medical University. The animals were managed per the guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health (1996 version). The success criteria for modeling were as follows: Cyanosis and whitening of the myocardial tissues and relevant ST segment changes on ECG after ligation of the left anterior descending coronary artery (LAD)[13]. Cyanosis of the ischemic myocardium disappeared while the congestion reaction was observed after loosening of the LAD ligation. Failure criteria for modeling were as follows: (1) Premature ventricular contractions, ventricular tachycardia, or even ventricular fibrillation were frequent after loosening of LAD ligation, (2) Ventricular fibrillation occurred repeatedly after the blood supply was restored by loosening the LAD ligation and (3) The LAD could not be accessed or had abnormal orientation. The rats were fed from June 2018 to June, 2020. The article number of the laboratory animals was 1B1325-KIT.

This study was approved by the Institutional Animal Ethical Committee of our hospital (No. K202106-07). All the procedures during the experiments were implemented according to the national principles of protection and use of laboratory animals.

Method for building the animal model: The random number table was used to divide the rats into the following five groups, with 15 rats in each group: Sham operation group, the rats only received a thoracotomy, with a piece of silk thread placed around LAD but untied, I/R group, the LAD was ligated for 40 min, then the blood supply was restored for 2 hrs by loosening the ligation, SPostC group, the LAD was ligated for 40 min and just before the blood supply was restored, 2.4% sevoflurane was administered by inhalation for 15 min using a ventilator, followed by loosening the ligation to restore the blood supply for 105 min, HIF-1α agonist group (DMOG group), before LAD ligation, DMOG (40 mg kg^{-1}) was administered by intraperitoneal injection, the LAD ligation lasted for 40 min and 2.4% sevoflurane was administered by inhalation for 15 min using a ventilator, then the ligation was

loosened to restore the blood supply for 105 min, HIF blocker group (2ME2 group), 2ME2 (15 mg kg^{-1}) was administered intraperitoneal, then the LAD was ligated for 40 min and just before the blood supply was restored, 2.4% sevoflurane was administered by inhalation for 15 min using a ventilator, then the ligation was loosened to restore the blood supply for 105 min and SPostC group, SPostC was performed at a concentration of 2.4%, the sevoflurane concentration was monitored using a gas detector to ensure that it was maintained at 1.0 MAC, then sevoflurane was administered continuously for 15 min by inhalation, the evaporator was turned off, the oxygen flow rate was adjusted to 1 L min^{-1} and the reperfusion continued for 105 min.

Anesthetic procedure: The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg kg^{-1}), with intermittent boluses to maintain the desired depth of anesthesia. A 16-gauge line was placed trans-orally to deliver mechanical ventilation under the following parameters: VT5-6 mL, RR 60-80 beats/min, FiO_2, 50% and PEEP, 0 cm H_2O. The body temperature was maintained at approximately 37°C with continuous ECG monitoring. A thoracotomy was performed in the third and fourth intercostal spaces on the left side to open the pericardium and expose the heart. At the originating site of the coronary artery between the left auricle and the pulmonary conus, a 7-0 silk thread was placed horizontally along the lowest margin of the left auricle to ligate the LAD for 40 min. The ligation was loosened by cutting the thread. The anesthesia was deepened as appropriate before harvesting the heart tissues. The rats were sacrificed by injecting 10% potassium chloride into the heart chambers.

Outcome indicators

Infarct area: After reaching the endpoint of reperfusion, the LAD was ligated *in situ* using the above-mentioned silk thread. As 2-3 mL of 3% evan blue were injected via the jugular vein to localize the non-ischemic and ischemic myocardial tissues. The non-ischemic myocardial tissues stained blue, while the area at risk did not stain. Ten percent KCl (2 mL kg^{-1}) was injected via the jugular vein after blue staining. It was confirmed that the heart ceased to beat in the diastolic phase. The heart was harvested after a thoracotomy, with excess fluid squeezed out. The heart was washed clean, wrapped in a preservative film and stored at -20°C for 2 hrs. The left and right atria and the right ventricle were sampled and the left ventricle was longitudinally cut open from the non-infarct area. The infarct ventricular muscles were sectioned into five slices in parallel with the atrioventricular groove, with each slice measuring 1-2 mm in thickness. The tissues were added with 1.0% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, St. Louis, Missouri, USA) in phosphate buffer (pH adjusted to 7.4) and placed in a water bath of 37°C for 15 min in the dark. The satisfactorily-stained, flake-like myocardial tissues were fixed in 10% neutral formalin overnight and arranged in a sequence. Photographs were taken using a digital camera. The infarct area did not stain. The area at risk was stained red. The sum of the red- and non-stained areas was the infarct area. The infarct volume ratio was expressed as the ratio of the infarct area (non-stained area) to the weight of the total myocardial tissues of the left ventricle (stained and non-stained myocardial slices from different groups, n = 6 for each group, Fig. 1).

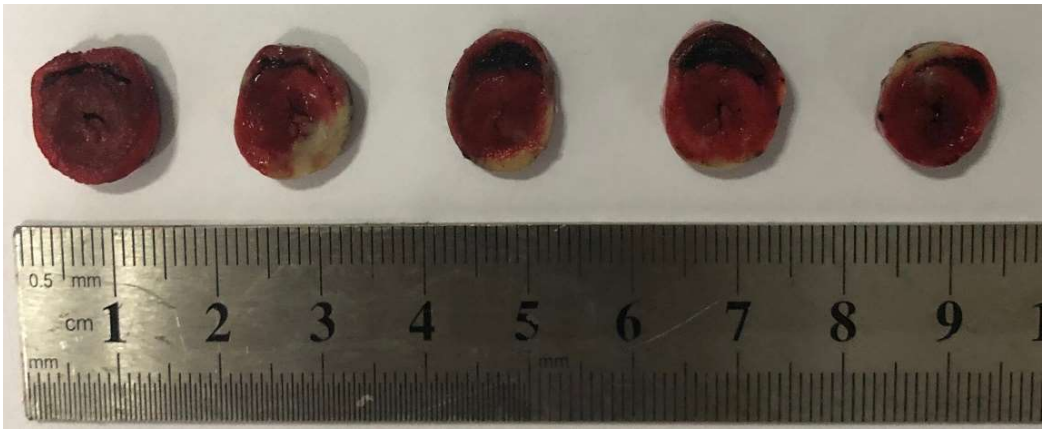

Fig. 1: EVAN stained and non-stained myocardial tissues
Non-stained area represents the infarct area

Detection of cardiomyocyte apoptosis: Myocardial tissues were harvested from the left ventricular area at risk and fixed in 10% neutral formalin overnight. The tissues were then subjected to dehydration through an ethanol gradient concentration, paraffin embedding and sectioning. The tissue specimens were subjected to TUNEL staining. Detection was performed for the negative control group using the reagents supplied in the TUNEL kit (Roche, USA). Brown fine particles (positive) were observed in the nuclei of apoptotic cells. Under an optical microscope, four positive fields of view were randomly chosen for each sample in each group. Photographs were taken and filed. The percentage of apoptotic cells-to-all cells in each field of view was estimated using LeicaQwinPlusV3 software (Leica, Germany) (n = 4 for each group).

Determination of the oxidative stress level and expression of relevant proteins: Left ventricular myocardial tissues (0.5 g) were added with 10 times the volume of pre-cooled normal saline. The tissues were centrifuged at 4°C at 12,000 r min^{-1} for 10 min. The supernatant was collected and stored at -80°C before use. The superoxide dismutase (SOD) (Jianglaibio, Shanghai, China) activity was determined using the xanthine oxidase method and the malondialdehyde (MDA) (Jianglaibio, Shanghai, China) content was determined using thiobarbituric acid colorimetry. All procedures were undertaken in strict accordance with the kit instructions. The CCK-8 assay was performed to determine cell viability and the level of adenine nucleoside triphosphate. Ventricular myocardial tissues (0.5 g) were obtained and added with a complete medium to prepare a single-cell suspension at 5×10^4 cells mL^{-1}. The cells were inoculated into 96-well plates and incubated at 37 in a 5% CO_2 incubator for 24 hrs. The cells were divided into different groups after the cells grew to 80% confluence. Background wells were set up and there were five replicates for each group (n = 5). The cell supernatant was pipetted and 100 μL of the 10% CCK-8 solution was added to each well. After incubation at 37°C for 2 hrs, the optical density (OD) value was measured at 450 nm and the adenosine triphosphate (ATP) activity was determined. Twenty milligrams of myocardial tissues were added with 200 μL of the lysis buffer. Adequate homogenization was performed using a glass homogenizer to ensure that the homogenate was properly lysed. Then, the tissues were further centrifuged at 12,000 r min^{-1} at 4 for 5 min. The supernatant was collected for subsequent determination. All procedures were performed according to the kit instructions (Jianglaibio, Shanghai, China). HIF-1α, MIF, p-AMPKα and AMPKα were electrotransferred using the semi-dry method, the sandwich successively consisted of filter paper, gel, membrane and filter paper. The sandwich wetted with the electrotransferred buffer, was directly placed between the positive and negative electrodes of the electrotransferred buffer. The gel was placed closest to the negative electrode and the membrane closest to the positive electrode. The electrotransferred buffer used for the semi-dry method consisted of the following: 48 mM Tris, 39 mM glycine, 0.04% SDS and 20% methanol. The electrotransfer was conducted at 25 V for 30 min. Before that, the polyvinylidene difluoride membrane was soaked in methanol for 2 min. The nitrocellulose (NC) membrane was soaked in the electrotransferred buffer for 10-20 min, then incubated in the cool electrotransferred buffer for 5 min. The proteins in the NC and polyvinylidene difluoride membranes were detected after Ponceau S staining. The membranes were sealed and incubated with antibodies. The gels were visualized using enhanced chemiluminescence detection and scanned.

Instruments and reagents: The following reagents were used for the TUNEL assay: Paraffin wax (China Petroleum and Chemical Corporation, China), formaldehyde, xylene and absolute ethanol (Shanghai Sinopharm Group Co., Ltd., Shanghai, China), concentrated DAB substrate (Shanghai Long Island Biotech. Co., Ltd., Shanghai, China), neutral resin (Shanghai Long Island Biotech. Co., Ltd.) and a TUNEL assay kit (Roche). The following instruments were used for the TUNEL assay: Upright microscope (CX41, Olympus, Japan), thermostatic incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China), paraffin slicing machine (SQ2125, Leica, Hubei, China) and tablet spreading machine (PPTHK-21B, Leica). The reagents used for Western blot analysis were as follows: RIPA lysis buffer (R0020, Solarbio, Beijing, China), BCA Protein Assay Kit (PICPI23223, Therma), TEMED (T8090, Solarbio), 10% ammonium persulfate (A1030, Solarbio), 0% SDS (S1010, Solarbio), Tris-HCl buffer (pH = 6.8, T1020, Solarbio), Tris-HCl buffer (pH = 8.8, T1010, Solarbio), 4* protein sample loading buffer (P1015, Solarbio), pre-stained protein marker (SM1811, Fermentas), NC membrane (HATF00010, Millipore), skimmed milk powder (D8340, Solarbio), PBS buffer (P1010, Solarbio), Tween-20 (T8220, Solarbio) and enhanced chemiluminescence buffer (WBKLS0100, Millipore). Instruments used for Western blot analysis: electrophoresis apparatus (Mini Protean 3 Cell, Bio-Rad), electrotransfer unit (TE77XP, Hoefer), microplate reader (MK3, Thermo Fisher Scientific, Finland), Pipetman® Pipette Kit (Gilson, Germany) and a water bath (HI1210, Leica).

Statistical analysis: Each variable underwent statistical analysis using SPSS 16.0. Normal distribution and homogeneity of variance were evaluated using one-way ANOVA and a *post hoc* Test was conducted using the Student-Newman-Keuls method, based on at least three independent experiments. The enumeration data were analyzed using the chi-square test, with p-values less than 0.05 indicating statistical significance.

RESULTS

Comparison of infarct volume ratio across the groups: In the sham group, no infarction was observed. The infarct volume ratio did not change significantly among the SPostC, I/R, DMOG and 2ME2 groups, Moreover, the infarct volume ratio did not change considerably after positive or negative intervention with HIF (p>0.05, Table 1).

Comparison of cardiomyocyte apoptosis index across the groups: The cardiomyocyte apoptosis index of the SPostC group was slightly lower than the I/R group, but the difference between the two groups was insignificant (p>0.05). The cardiomyocyte apoptosis index of the SPostC group was not significantly different from the DMOG and 2ME2 groups (p>0.05). The cardiomyocyte apoptosis index did not change considerably after positive or negative intervention with HIF (p>0.05, Table 1 and Fig. 2).

Table 1: Comparison of outcome indicators among the groups

Outcome indicators	Sham group N = 15	I/R group N = 15	SPostC group N = 15	DMOG group N = 15	2ME2 group N = 15	F-value	p-value
Infarct volume ratio (n, %)	0.87±0.05	28.65±3.79	28.33±3.44	28.24±3.51	28.67±3.84	1.025	>0.05
Cardiomyocyte apoptosis index	3.96±1.06	27.67±1.85	25.43±1.83	25.33±1.82	25.29±1.79	0.514	>0.05
Cell viability μmol g^{-1} prot	1.26±0.03	0.88±0.03	1.13±0.02	1.21±0.03	0.91±0.03	7.512	<0.05
ATP concentration μmol g^{-1} prot	309.54±66.89	75.97±12.26	199.96±31.84	268.89±44.55	99.32±15.32	10.254	<0.05
SOD level (U/mg/mmol/g)	143.28±10.91	103.05±7.02	126.99±8.93	135.24±9.23	108.12±6.24	15.474	<0.05
MDA level (U/mg/mmol/g)	3.18±0.75	8.89±1.21	4.86±0.81	4.04±0.77	6.94±0.78	5.422	<0.05
HIF-1α	1.07±0.11	1.18±0.48	1.49±0.17	1.89±0.29	0.88±0.08	7.147	<0.05
MIF	0.23±0.01	0.39±0.04	0.62±0.14	0.83±0.17	0.42±0.06	5.741	<0.05
AMPKα	0.55±0.03	0.55±0.02	0.53±0.06	0.54±0.06	0.49±0.06	1.054	>0.05
p-AMPKα	0.31±0.07	0.41±0.06	0.67±0.13	0.77±0.16	0.39±0.06	4.451	<0.05

Mean±SD

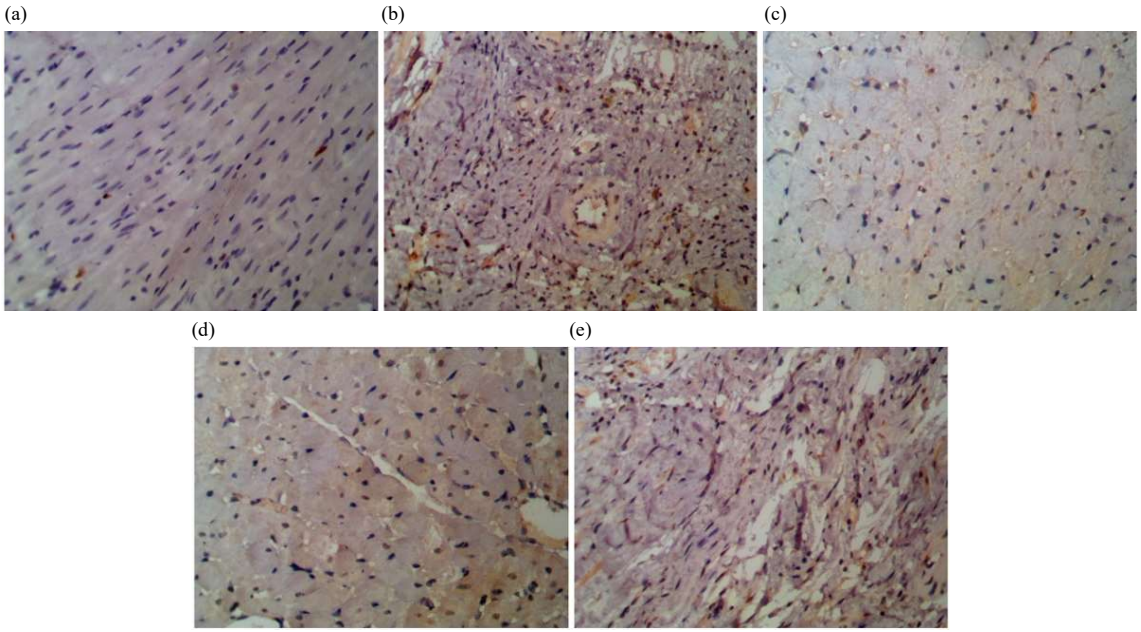

Fig. 2(a-e): Apoptosis histochemistry experiments in different groups, (a) Sham group, (b) I/R group, (c) SPostC group, (d) DMOG group and (e) 2ME2 group

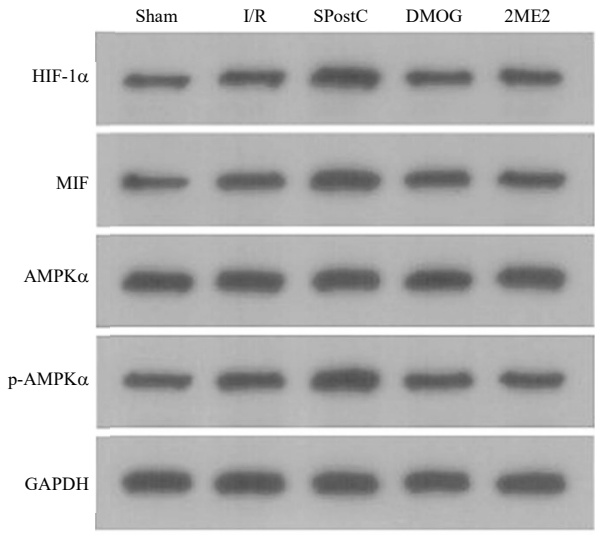

Fig. 3: Western blotting image of HIF-1α, MIF, AMPKα and p-AMPKα in the Sham, I/R, SPostC, DMOG and 2ME2 groups

Comparison of cell viability and ATP concentration across the groups: The I/R group showed a significant decrease in cell viability and ATP concentration compared to the sham group ($p<0.05$, Table 1). The cell viability and ATP concentration of the SPostC group were significantly higher than the I/R and 2ME2 groups, but significantly lower than the DMOG group (both $p<0.05$, Table 1). The cell viability and ATP concentration increased along with the positive intervention by HIF ($p<0.05$, Table 1), however, these two indicators decreased along with the negative intervention by HIF ($p<0.05$, Table 1).

Comparison of oxidative stress levels across the groups: Among the indicators of oxidative stress, the MDA content of the SPostC group was significantly lower than the 2ME2 and I/R groups ($p<0.05$) and significantly higher than the DMOG group ($p<0.05$). The SOD level of the SPostC group was considerably higher than the 2ME2 and I/R groups ($p<0.05$), but dramatically lower than the DMOG group ($p<0.05$). The SOD level increased and the MDA level decreased ($p<0.05$) along with the positive intervention by HIF ($p<0.05$), opposite results were obtained with the negative intervention by HIF ($p<0.05$). The sham group showed the lowest level of MDA and the highest level of SDA as compared to the other groups ($p<0.05$) (Table 1).

Comparison of the expressions of relevant proteins across the groups: The HIF-1α, MIF and p-AMPKα were upregulated in the SPostC group compared with the I/R and 2ME2 groups ($p<0.05$). However, these proteins were significantly downregulated in the SPostC group compared with the DMOG group (all $p<0.05$). The five groups did not differ significantly concerning AMPKα expression (all $p>0.05$) (Table 1 and Fig. 3).

DISCUSSION

The inter-group difference was significant for cell viability, ATP, SOD and MDA oxidative stress among the groups. The cell viability, ATP, SOD and MDA level changed significantly with the intervention of HIF. The expression of HIF-1α, MIF and p-Ampkα protein in the SPostC group was more highly expressed than the sham, I/R and 2ME2 groups, but lower than the DMOG group.

Cardiomyocyte apoptosis is a main pathogenic factor that leads to myocardial I/RI[14]. In the present study, the I/RI model was created with 75 elderly healthy clean-grade SD rats. The I/RI group revealed a significant reduction in cardiomyocyte viability and a dramatic increase in cardiomyocyte apoptosis than the sham group, which indicated the success of I/RI modeling[14].

Coronary contractions following ischemia can be minimized by regulating the arterial purinergic system and ATP levels[15]. The expression of HIF-1α is closely related to cardiac myocytes in rats with an I/R injury[16]. The HIF-1α activates cardioprotective signaling pathways and downstream protective genes, which supports the use of HIF-1α as a therapeutic target[17]. The MIF activates AMPKα in ischemic preconditioning-mediated cardioprotection under ischemic stress[18]. The enhancement of the AMPKα pathway has a significant role in cardioprotection according to previous reports[15,18,19]. These findings were indicative of the important role of HIF-1α, MIF and AMPKα in the myocardial protective effect against I/RI in rats. Compared with the sham group, the ATP concentration decreased significantly in the I/R group, the relevant proteins (HIF-1α, MIF and p-AMPKα) were downregulated and the cardiomyocyte apoptosis index increased markedly ($p<0.05$). These observations suggested pronounced cardiomyocyte injury in rats with I/RI[15-19]. The HIF-1α might mediate the SPostC-induced myocardial protective effect in rats. A recent study involving H9c2 cardiomyocytes showed that the HIF-1α/MIF/AMPK signaling pathway has an important role in SPostC against H/R injury[8]. SPostC cardioprotection is restored by upregulating HIF-1α and MIF protein expression under hyperglycemia[8]. Current study showed upregulated expression of HIF-1α, MIF and p-AMPKα protein in the SPostC group than the I/R and 2ME2 groups, but downregulated expression in the DMOG group ($p<0.05$). We thus concluded that SPostC may potentially reduce cardiomyocyte injury in patients with I/RI and maintain cardiomyocyte integrity.

The HIF-1α is a key target involved in the endogenous mechanism underlying the myocardial protective effect[16,17]. In hypoxic cardiomyocytes, HIF-1α upregulation effectively reduced mitochondrial reactive oxygen species (ROS), thereby lessening cardiomyocyte injury in patients[16,17]. This study showed that as HIF-1α was upregulated, the infarct area decreased, but not in a dramatic manner, while cardiomyocyte viability increased significantly along with the positive intervention by HIF-1α. This finding might be the result of HIF-1α upregulation activating VEGF expression and hence, the angiogenesis signaling, which further promotes cell viability[16,17]. The MIF is a multipotent key cytokine that plays a very important role in immune regulation and physiological processes[18,20,21]. On the one hand, MIF inhibits inflammatory cell infiltration, thus maintaining normal endothelial function and reducing infarct area and cell apoptosis. On the other hand, MIF also causes damage in infectious and autoimmune diseases[20,21]. In the rat model of I/RI receiving SPostC, both HIF-1α and MIF were significantly upregulated and the expression of the two was positively correlated. We further analyzed the expression of relevant proteins (HIF-1α, MIF and p-AMPKα) and found that SPostC exerted a protective effect in the rat model of I/RI, mainly by upregulating the HIF-MIF pathway. To further verify the assumed correlation, the DMOG and 2ME2 were experimented separately. When HIF-1α expression was inhibited, the protective effect of SPostC disappeared. By contrast, a positive intervention resulted in an improvement of the protective effect of SPostC, accompanied by simultaneous changes in MIF and p-AMPKα expressions. Therefore, we inferred that the HIF-MIF signaling pathway is crucial for the protective effect of SPostC in rats.

The limitation of this study was that this was only an animal model experiment, which needs validation of *in vitro* experiments and clinical studies.

CONCLUSION

Sevoflurane post-conditioning exerted a specific myocardial protective effect against I/RI in elderly rats. According to the results of the experiments in this study, the cell viability, ATP, SOD and MDA levels were regulated with the intervention of HIF. The expression of HIF-1α, MIF and P-AMPKα protein in the SPostC group was more highly expressed than the sham, I/R and 2ME2 groups, but lower than the DMOG group. The myocardial protective effect was deducted against I/RI in elderly rats by sevoflurane post-conditioning and may be possibly regulated via the HIF-MIF pathway. This study elucidated the mechanism of SPostC mediated by the HIF-MIF pathway on myocardial ischemia/reperfusion in elderly mice and laid a theoretical foundation for the myocardial protection of elderly patients with SPostC during the perioperative period. These results need further *in vitro* experiments and clinical studies to validate.

SIGNIFICANCE STATEMENT

The SPostC is an important means to combat myocardial I/RI, but the specific molecular regulation mechanism has not been fully elucidated. The HIF-1α and MIF play important roles in myocardial protection by regulating oxidative stress and reducing myocardial apoptosis. This study established a rat myocardial I/R model of model with the SPostC intervention and observed the effect of MIF through the regulation of HIF, so as to verify the key target effect of MIF in HIF-1α-mediated sevoflurane post-treatment on aging myocardium in the animal model. The results found that sevoflurane post-conditioning exerted a specific myocardial protective effect against I/RI in elderly rats, possibly by regulating the HIF-MIF pathway.

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