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RESEARCH ARTICLE

Sevoflurane Pretreatment Enhances Myocardial Ischemia-Reperfusion Injury Via Activating HIF-1α/iNOS/cGMP to Inhibit Endoplasmic Reticulum Stress

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A B S T R A C T

The objective of this study was to estimate the protective influence of sevoflurane preconditioning on myocardial ischemia-reperfusion (IR) injury. Rat models of myocardial IR and sevoflurane preconditioning were built. The area of myocardial ischemia and infarction were estimated via Evan blue and TTC double staining. The histopathological changes of heart tissues were examined using a hematoxylin & eosin staining kit. TUNEL assay was applied to detect cardiomyocyte apoptosis and calculate the apoptosis rate. The concentrations of ERS related molecules GRP78 and CHOP in the myocardium were estimated via real-time fluorescent quantitative PCR. The GRP78, CHOP, caspase-3, caspase-12, Bax and Bcl-2 concentrations in the myocardium were estimated via western blot. ROS, MDA and SOD were assessed. The concentration of HIF-1 α and its downstream gene inducible nitric oxide synthase (iNOS) and cGMP concentration in myocardial tissue were estimated. Sevoflurane preconditioning lessened the size of myocardial infarction induced via ischemia/reperfusion($P<0.05$), lessened cardiomyocyte apoptosis and oxidative stress ($P<0.05$), enhanced the concentration of HIF-1 α and enhanced the concentration of iNOS and cGMP concentration in myocardium ($P<0.05$). After administration of HIF-1-α proline hydroxylase inhibitor DMOG, the concentration of HIF-1-α enhanced after sevoflurane preconditioning ($P<0.05$), the concentration of iNOS and cGMP enhanced ($P<0.05$). Meanwhile sevoflurane preconditioning protective influence on myocardial injury was also further enhanced ($P<0.05$). Sevoflurane preconditioning protective influence on myocardial injury induced via ischemia/reperfusion may be related to the activation of the HIF-1 α / iNOS/cGMP pathway in myocardial tissue.

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INTRODUCTION

Cardiovascular illness is one of the illnesses with high incidence rates and mortality worldwide (Fan *et al.*, 2023). In clinical practice, cardiac surgery causes myocardial ischemia and leads to cardiac damage, and the myocardium is injured again after the recovery of blood flow during the reperfusion period. This process is called ischemia-reperfusion (I/ R) injury (Zhang *et al.*, 2024). Cardiac I/ R mainly occurs in patients with cardiovascular illnesses such as myocardial infarction, coronary heart illness, stroke, etc. Myocardial ischemia leads to the imbalance of cardiac oxygen supply, which further leads to cardiac tissue dysfunction. Reperfusion after ischemia will restore myocardial blood flow, leading to cardiomyocyte death and increasing the area of myocardial infarction (Zou *et al.*, 2018). Myocardial I/R is the main causes of poor prognosis and survival in patients with cardiovascular illness (Chen *et al.*, 2019). Therefore, exploring the mechanism of cardiac I/R is of great significance in enhancing the survival rate.

The reconstruction of blood perfusion is the most commonly utilized and effective treatment strategy for acute myocardial infarction, but it also causes myocardial

ischemia/reperfusion injury (Yellon *et al.*, 2007). The endoplasmic reticulum (ER) is main place to process proteins and store Ca2+. Ischemia and hypoxia, glucose/nutrient deficiency, ATP depletion, large amounts of free radicals production and Ca2+ homeostasis disruption can cause ER dysfunction and trigger endoplasmic reticulum stress (ERS). It was characterized via the concentration of ERS marker molecules glucose-regulated protein 78 (GRP78) and calreticulin (CRT). Persistent and severe ERS can activate ERS-related apoptotic pathways such as CCAAT/enhancer-binding protein homologous protein (CHOP) and Caspase-12, aggravating I/ R injury (Zhang *et al.*, 2019; Kumari *et al.*, 2021). Many studies indicated ERS is related to pathophysiological process of MIRI and plays a vital role in inducing apoptosis and aggravating inflammation (Zhang *et al.*, 2011; Zhang *et al.*, 2017; Zhu *et al.*, 2018). Rats study showed that the unfolded protein response was activated in the I/R model, and the downstream PERK, IRE1, and ATF6 signalling pathways were all activated (Zhang *et al.*, 2017). ERS is involved in important pathological mechanisms during the development of rat ischaemic heart disease model, and inhibition of ERS may be beneficial to I/R-injured myocardium (Hadj Abdallah *et al.*, 2018; San *et al.*, 2022)

Sevoflurane is a widely utilized anesthetic in the clinic. It has the advantages of rapid induction, rapid and complete awakening, stable hemodynamics, and does not increase the incidence of arrhythmia when combined with catecholamines (Sevoflurane, 2012). Recent studies have found that pretreatment with sevoflurane can enhance brain injury to a certain extent, thereby conducting a neuroprotective role (Wang *et al.*, 2022). Studies have shown that sevoflurane can protect mitochondrial function and reduce liver damage (Liu *et al.*, 2022). However, the specific protective mechanism of sevoflurane in myocardial I/R has not been deeply explored. The activation of hypoxia-inducible factor-1α (HIF-1α) can enhance the concentration of downstream target gene iNOS, affecting the progression of ischemia and hypoxia. Therefore, we propose the following hypothesis: The protective influence of sevoflurane preconditioning on myocardial injury induced via I/ R may be related to the activation of HIF-1 α / iNOS/cGMP pathway in myocardial tissue. This research aimed to explore the role and mechanism of sevoflurane in myocardial I/ R injury by establishing a rat model.

MATERIALS AND METHODS

Experimental animals and subgroups: 80 healthy adult male SD rats, weighing 250g ~300g, were provided via Beijing Weitong Lihua Company. The animals were kept in separate cages (18-26°C, humidity of 40%~70%). The animals were fed with standard feed and sterile water for 1 week. The handling of experimental animals complied with the ethical standards of the Animal Welfare and Ethics Group of the Department of Laboratory Animal Science of Fudan University. They were randomly divided into 4 subgroups by the random number table method: Sham operation subgroup, open chest only without ligation; Ischemia reperfusion subgroup (1% pentobarbital sodium anesthesia operation); Sevoflurane subgroup and sevoflurane pretreatment subgroup. HIF-1-α proline hydroxylase inhibitor DMOG group.

Establishment of rat myocardial ischemia/reperfusion and ischemic postconditioning model: All rats fasted for 12 hours before the operation, drank water freely, and anesthetized rats via intraperitoneal injection of 3% pentobarbital sodium for 50 mg/kg. Following the supine fixation, we performed a precise incision in the middle of the neck, delicately made 3-4 tracheal ring incisions, and expertly inserted the endotracheal tube. We seamlessly connected a small animal ventilator for mechanical ventilation, ensuring optimal oxygen intake at a concentration of 33%.

After endotracheal intubation, the right internal jugular vein and internal carotid artery were filled with a heparin tube and the arterial blood gas index was maintained. Through the left fifth intercostal layer via layer blunt separation and opening the pericardium to expose the heart, the suture line passed through the anterior descending branch of the left coronary artery, stabilized for 30min, and then ligated. The epicardial cyanosis was pale, the electrocardiogram corroborated that the ST segment was elevated, and the high T wave was successful ischemia. After 30min of ischemia, released the ligation line to restore the blood flow of the anterior descending branch of the left coronary artery lasted for 2h. When the electrocardiogram corroborated that the ST segment decreased and the T wave recovered, the myocardial tissue gradually turned red, indicating that the reperfusion was successful (Miyazaki *et al.*, 2011). (Rat myocardial ischemia/reperfusion model with 70% success rate and post-ischemic treatment model with 65% success rate.)

Assessment of cardiac function: The rats were anesthetized again at the end of reperfusion, and a catheter filled with heparin normal saline (50 U/mL) was inserted into the left ventricle through the right carotid artery to evaluate the left ventricular function. The maximum left ventricular pressure ($\pm LVdp/dt_{max}$) and left ventricular end diastolic pressure (LVEDP) were recorded with the PowerLab system (AD Instruments, AUS), Ejection fraction (EF) and Fraction shortening (FS) were assessed. After measuring the cardiac function, blood was collected to separate plasma.

Measurement of myocardial infarction area: After reperfusion, the heart was removed, atrial tissue was removed, and thick sections of 2mm were stained with Tetrazolium chloride (TTC, G3004, Solebo Technology Co., Ltd., China). Incubated at constant temperature in the dark for 25min, washed with PBS buffer, and fixed with 4% formaldehyde for 24h. The infarcted area was stained gray white, and the noninfarcted area was stained brick red. The infarct area was calculated with ImageJ software (National Institutes of Health, Boston, Massachusetts, USA) after the image was taken.

HE and TUNEL staining: The histopathological changes of heart tissues were examined using a hematoxylin& eosin staining kit. All procedures were performed referring to the instructions of the kit. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated in situ flat-end

labeling of dUTP staining. Added 5uL TdT enzyme (D7093, Biyuntian Biotechnology Co. Ltd., Shanghai, China) and fluorescent labeling solution to prepare detection solution. Incubated in the dark at 37°C for 1h and dripped DAPI fluorescent dye for 5min. Rinsed with PBST solution for 5min to remove excess dye, and then rinsed with PBS for 3 times (Lee *et al.,* 2021). The neutral resin film was sealed and finally observed under a laser scanning confocal microscope (FV1200, Olympus Corporation, Tokyo, Japan). The results were expressed as Tunel-positive nuclei/total nuclei × 100%.

Western blot: Took a left ventricular myocardium of rats, cut it into pieces, added protein lysate and phosphatase inhibitor, ultrasonic homogenate at 4°C, centrifuged, collected the supernatant and measured the protein concentration via BCA method. 30μg of myocardial tissue was isolated via SDS-PAGE, and then transferred to NC membrane. 5% BSA (prepared via TBST) closed for 1.5 h. The membrane was incubated with Anti-GRP78 (Catalog: ab21685, Abcam, 1:1000, Cambridgeshire, United Kingdom), Anti-CHOP (Catalog: MA1-250, Invitrogen, 1:1000, California, USA), caspase-3 (Catalog: ab184787, Abcam, 1:1000, Cambridgeshire, United Kingdom), caspase-12 (Catalog: ab255818, Abcam, 1:1000, Cambridgeshire, United Kingdom), HIF-1α (Catalog: ab179483, Abcam, 1:`000, Cambridgeshire, United Kingdom), Anti-iNOS (Catalog: 14-5920-80, Invitrogen, 1:1000, California, USA), β-actin rabbit monoclonal antibody (Catalog: ab181602, Abcam, 1:10000, Cambridgeshire, United Kingdom), Bax (Catalog: ab31564, Abcam, 1:`000, Cambridgeshire, United Kingdom) and Bcl-2 (Catalog: ab215362, Abcam, 1:`000, Cambridgeshire, United Kingdom) at room temperature for 1.5h and washed with TBST (15 min × 3 times). ECL luminescence, color rendering, and SmartView Pro 2000 (UVCI-2100, Major Science, USA) photography were employed (Fu *et al.,* 2022)

The gray value of the band was scanned via ImageJ software, and the ratio of the gray value of the target protein to the gray value of the internal reference protein was utilized as the relative concentration of target protein.

MAD, SOD and Caspase-3 detection: Superoxide dismutase (SOD) kits (C0331, Biyuntian Biotechnology Co. Ltd., Shanghai, China), malondialdehyde (MDA) kits (CS-E01745, Shanghai C-reagent Biotechnology Co. Ltd., Shanghai, China) and Caspase-3 kits (C1115, Biyuntian Biotechnology Co. Ltd., Shanghai, China) were utilized to determine the concentrations of MAD, SOD and Caspase-3, respectively, according to the kits (Wang *et al.,* 2022).

ROS staining: ROS production in tissue homogenates was estimated using 2'-7'-dichlorofluorescein-diacetate (DCFH-DA), and retinas were homogenized in PBS using a glass homogenizer. Samples containing 20μg of protein diluted in PBS were incubated with 5μmol/L DCFH-DA in the dark for 15min. Fluorescence was measured every 15min for 1h using the fluoroenzymeter with excitation (SpectraMax Gemini XPS, Molecular Devices, Sunnyvale, USA) and emission wavelengths of 488, 525 nm, respectively (Kim *et al.,* 2020) and finally observed under a laser scanning confocal microscope (FV1200, Olympus Corporation, Tokyo, Japan).

The cGMP assay method was consistent with previous study of Harloff *et al.* (2022).

Statistical methods: The data were analyzed via SPSS 20.0 (SPSS, Chicago, Illinois, USA) the measurement data were expressed as mean±SD, single factor analysis of variance was utilized for comparison among subgroups, and LSD-t test was utilized for pairwise comparison between two subgroups. The counting data were expressed as a percentage (%). The comparison between the two subgroups was tested via χ^2 test, and the divergence was statistically notable ($P<0.05$).

RESULTS

Cardiac function: Contrasted to the control subgroup, IR rats corroborated a decrease of $\pm LVdp/dt_{max}$ ($P<0.05$). Sevoflurane preconditioning reversed the decrease of $\pm LVdp/dt_{max}$, LVEDP, EF and FS in IR rats contrasted to IR alone ($P<0.05$). Contrast to the control subgroup, sevoflurane preconditioning lessened the LVEDP of IR rats, and there was no divergence in these parameters between the sevoflurane preconditioning and control subgroup ($P>0.05$) (Fig. 1A-E).

Myocardial ischemia and myocardial infarction: Preconditioning lessened the infarct size and cardiomyocyte apoptosis. After myocardial I/R in healthy rats, the myocardial infarction area accounted for about 36% of the total risk area, while the reduction of myocardial infarction area in the sevoflurane preconditioning subgroup accounted for 27.3% of the total risk area. It is suggested that preconditioning may have a protective influence on myocardial ischemia/reperfusion ($P<0.05$) (Fig. 2A). HE staining showed that marked myocardial structure disorders emerged in IR group, which were improved in sevoflurane preconditioning treatment group (Fig. 2B). Contrasted to CON subgroup, the number of apoptotic cells of I/R subgroup enhanced notably ($P<0.05$). Contrasted to I/R subgroup, the number of apoptotic cells of cardiomyocytes in the sevoflurane preconditioning subgroup was notably lower than that in I/R subgroup ($P<0.05$) (Fig. 2C). Sevoflurane pretreatment notably decreased the activity of Caspase-3 and Bax level, increased Bcl-2 level, contrasted to that of I/R subgroup ($P<0.05$) (Fig. 2D-F). In addition, sevoflurane pretreatment up-regulated SOD and down-regulated ROS and MDA ($P<0.05$) (Fig. 2G-I). These results imply that sevoflurane pretreatment can reduce myocardial cell apoptosis and oxidative stress damage caused by ischemia/reperfusion.

Endoplasmic reticulum stress: The upregulation of GRP78 and CHOP is usually utilized as a sign of ERS. The protein concentrations of GRP78, CHOP, caspase-3 and caspase-12 in IR treatment subgroup were higher than control subgroup ($P<0.05$) (Fig. 3A-D). The increase of protein concentration decreased notably after sevoflurane preconditioning ($P<0.05$), and there was no divergence in the concentration of these two proteins between the sevoflurane preconditioning and control subgroup ($P>0.05$).

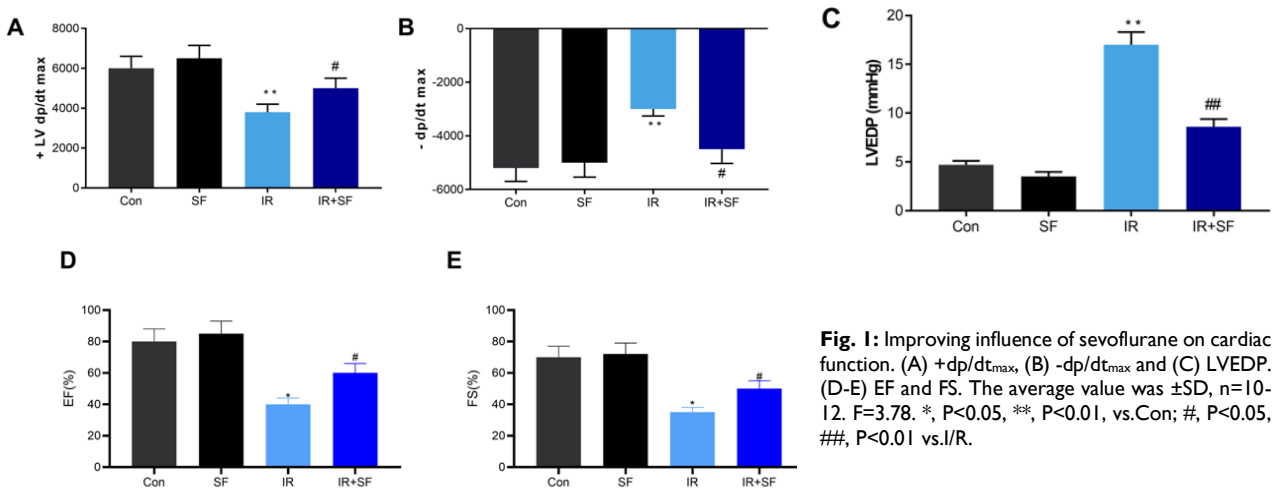

Fig. 1: Improving influence of sevoflurane on cardiac function. (A) +dp/dt$_{max}$, (B) -dp/dt$_{max}$ and (C) LVEDP. (D-E) EF and FS. The average value was ±SD, n=10-12. F=3.78. *, P<0.05, **, P<0.01, vs.Con; #, P<0.05, ##, P<0.01 vs.I/R.

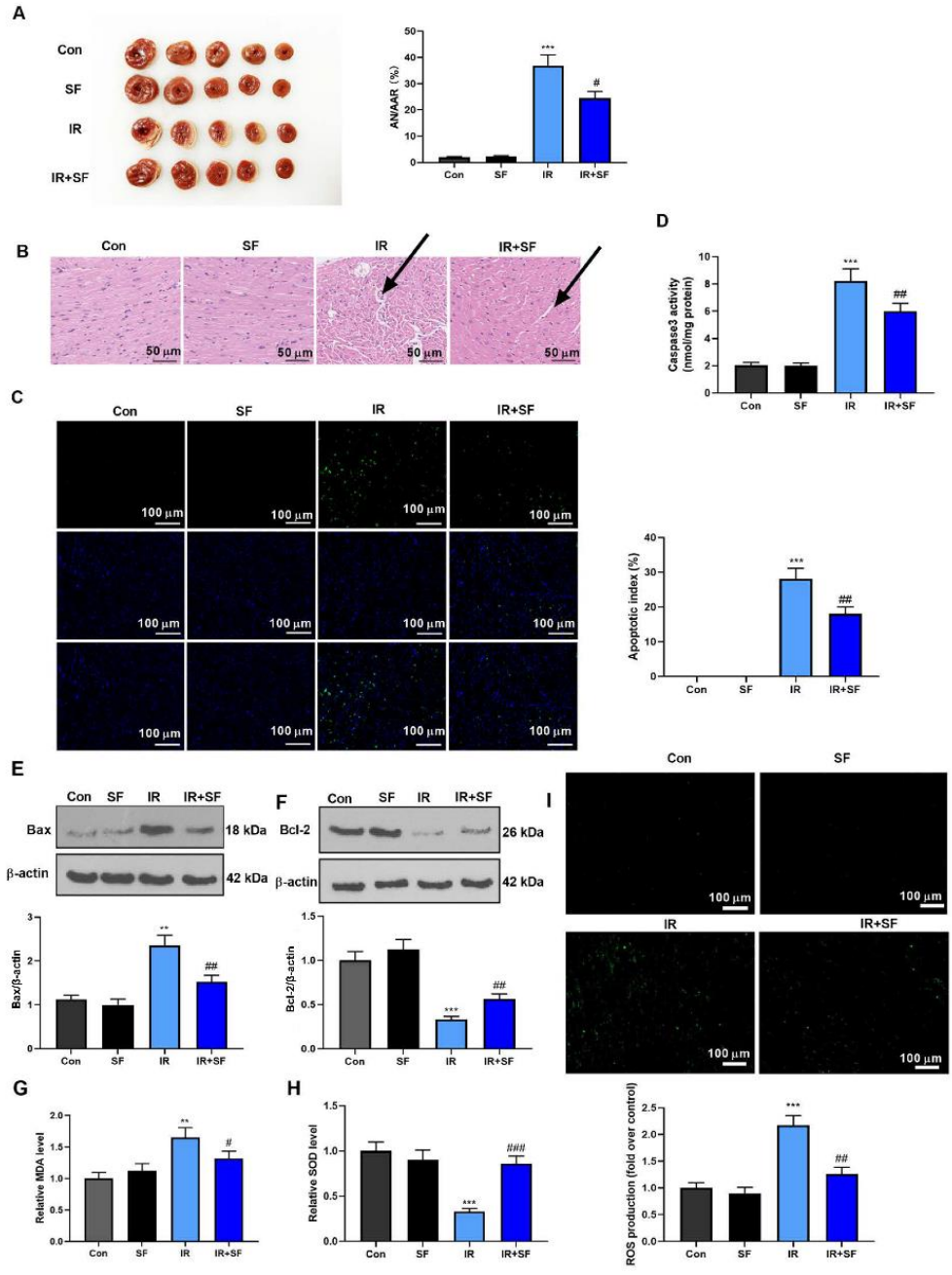

Fig. 2: Sevoflurane preconditioning relieves myocardial ischemia and myocardial infarction. (A) Comparison of myocardial infarct size in each subgroup. The myocardial area of necrosis (AN)/area at risk (AAR) was counted. (B) HE staining was conducted for evaluating the histopathological alterations of myocardial tissues. The pathological changes are indicated by an arrow. HE staining reveals cell swelling and disordered arrangement, as well as a substantial formation of collagen fibers. Scale bar=50 μm. (C) TUNEL staining was applied to assess cardiomyocyte apoptosis index. Scale bar=100 μm. (D) Caspase-3 activity. (E,F) Bax and Bcl-2 levels were assessed by western blot. (G,H) MDA, SOD detection. (I) ROS staining detection.Scale bar=100 μm. n=10-12. F=2.39*, P<0.05, **, *P* < 0.01 vs.Con; #, P<0.05, ##, *P* < 0.01 vs.I/R.

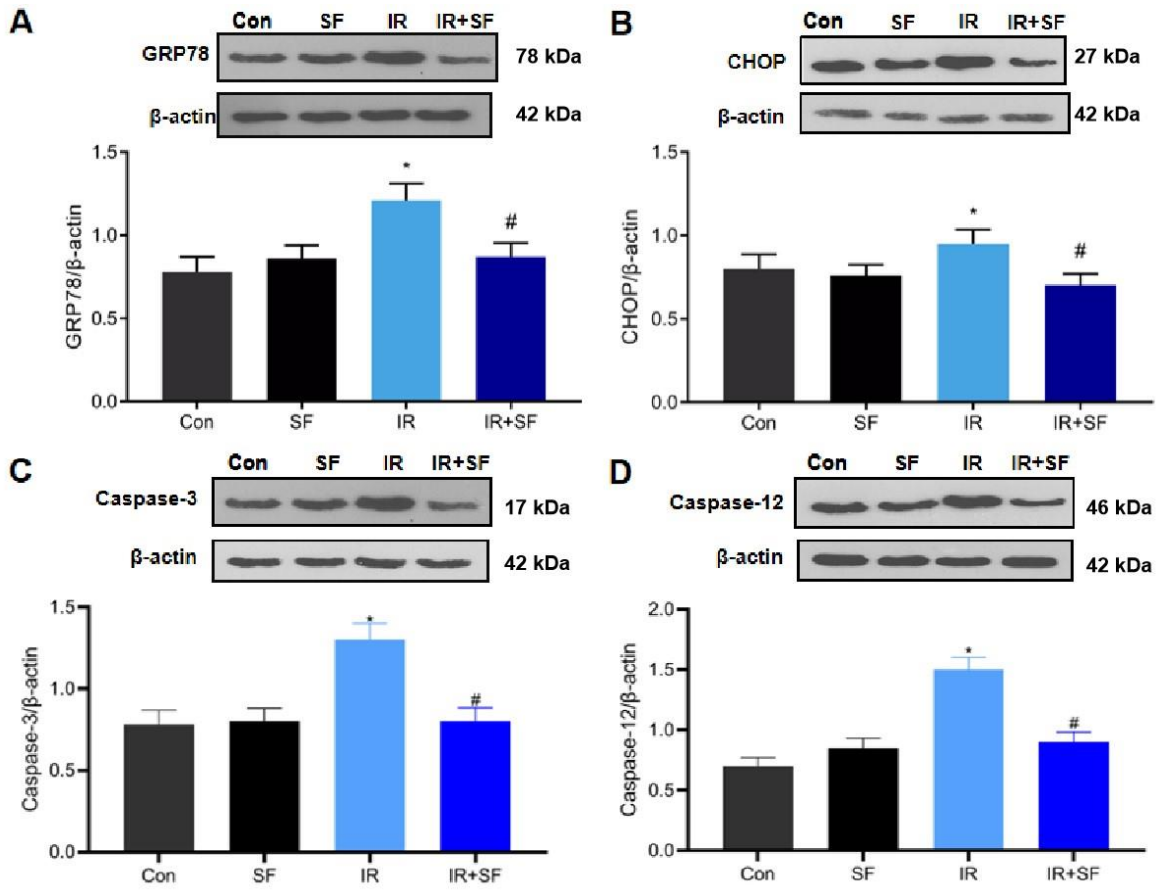

Fig. 3: The protein concentrations of CRT, CRP78, Caspase-12 and CHOP in the myocardium. The relative concentration of (A) GRP78, (B) CHOP, (C) Caspase-3 and (C) Caspase-12 proteins in the myocardial tissue of rats in each subgroup. n=6-7. F=4.01*, P<0.05 vs. Con; #, P<0.05 vs.I/R.

HIF-1 α and iNOS protein: It was corroborated that I/R notably enhanced the protein concentration of HIF-1 α in myocardial tissue. After the ischemic myocardium was pretreated with sevoflurane, the protein concentration of HIF-1 α was further enhanced (P<0.05) (Fig. 4A).

It implies that sevoflurane preconditioning can trigger the concentration of HIF-1 α protein after long-term ischemia. iNOS is one of the most vital downstream regulatory genes of HIF-1 α. It was corroborated that, consistent with the changing trend of HIF-1 α protein concentration, I/R enhanced the mRNA concentration of iNOS in myocardial tissue, while sevoflurane preconditioning further enhanced it (P<0.05) (Fig. 4B). The protein concentration estimated via western blot corroborated the same trend. Under the normoxic condition, HIF-1 α was induced to be degraded via proline hydroxylase (PHD). In this study, DMOG was utilized for inhibiting PHD and enhancing the concentration of HIF-1 α. It was corroborated that contrasted to simple preconditioning, the concentration of iNOS in myocardial tissue enhanced further after administration of DMOG (P<0.05) (Fig. 4C). Contrasted to the I/R subgroup, preconditioning notably enhanced the concentration of cGMP in myocardial tissue, while DMOG enhanced the concentration of HIF-1 α and further enhanced the concentration of cGMP (P<0.05) (Fig. 4D).

DMOG: After pretreatment with sevoflurane, DMOG was given to increase the concentration of HIF-1 α protein in the myocardium to determine whether the HIF-1 α pathway mediated the cardioprotective influence of sevoflurane (P<0.05). It was corroborated that DMOG treatment could block the cardioprotective influence of sevoflurane (± LV dp/dt $_{max}$, LVEDP, Troponin and LDH), and reversed the endoplasmic reticulum stress inhibited by sevoflurane (GRP78, CHOP, Caspase-3 and Caspase-12) (P<0.05) (Fig. 5A-I).

DISCUSSION

Our research found that iNOS and cGMP concentrations enhanced in myocardial tissue pretreated with sevoflurane. In order to further determine whether this change is indeed caused via HIF-1 α and whether it affects the cardioprotective influence of preconditioning, we pretreated with sevoflurane and then given HIF-1 α proline hydroxylase inhibitor DMOG. The concentration of iNOS and the concentration of cGMP in myocardial tissue were further enhanced, while the infarct size and cardiomyocyte apoptosis induced via I/R were notably smaller than those in the simple preconditioning subgroup. It is suggested that the protective influence of preconditioning on myocardial injury may be related to the activation of the HIF-1 α / iNOS/cGMP pathway. As an anesthetic, sevoflurane has the characteristics of stable hemodynamics, a small dosage of muscle relaxant and rapid waking up after an operation (Fang *et al.*, 2021). As a preconditioning or post-treatment of inhaled anesthetic, sevoflurane can effectively inhibit myocardial I/R injury (Zhang *et al.*, 2021; Wu *et al.*, 2021; Niu *et al.*, 2022). In this research, in the sevoflurane

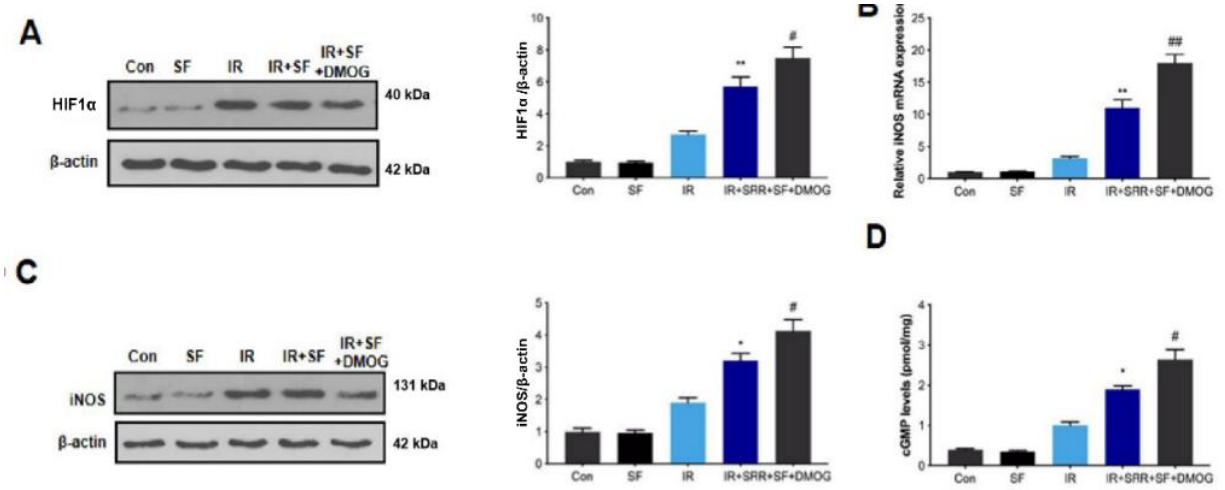

Fig. 4: Influences of sevoflurane preconditioning on HIF-1 α, iNOS protein concentration and cGMP concentration. (A) HIF-1 α protein levels; (B) iNOS protein levels; (C) Relative iNOS mRNA expression; (D)cGMP levels. n=6-7. F=2.55. *，P<0.05 *vs.* I/R；#，P<0.05 *vs.* I/R+SF.

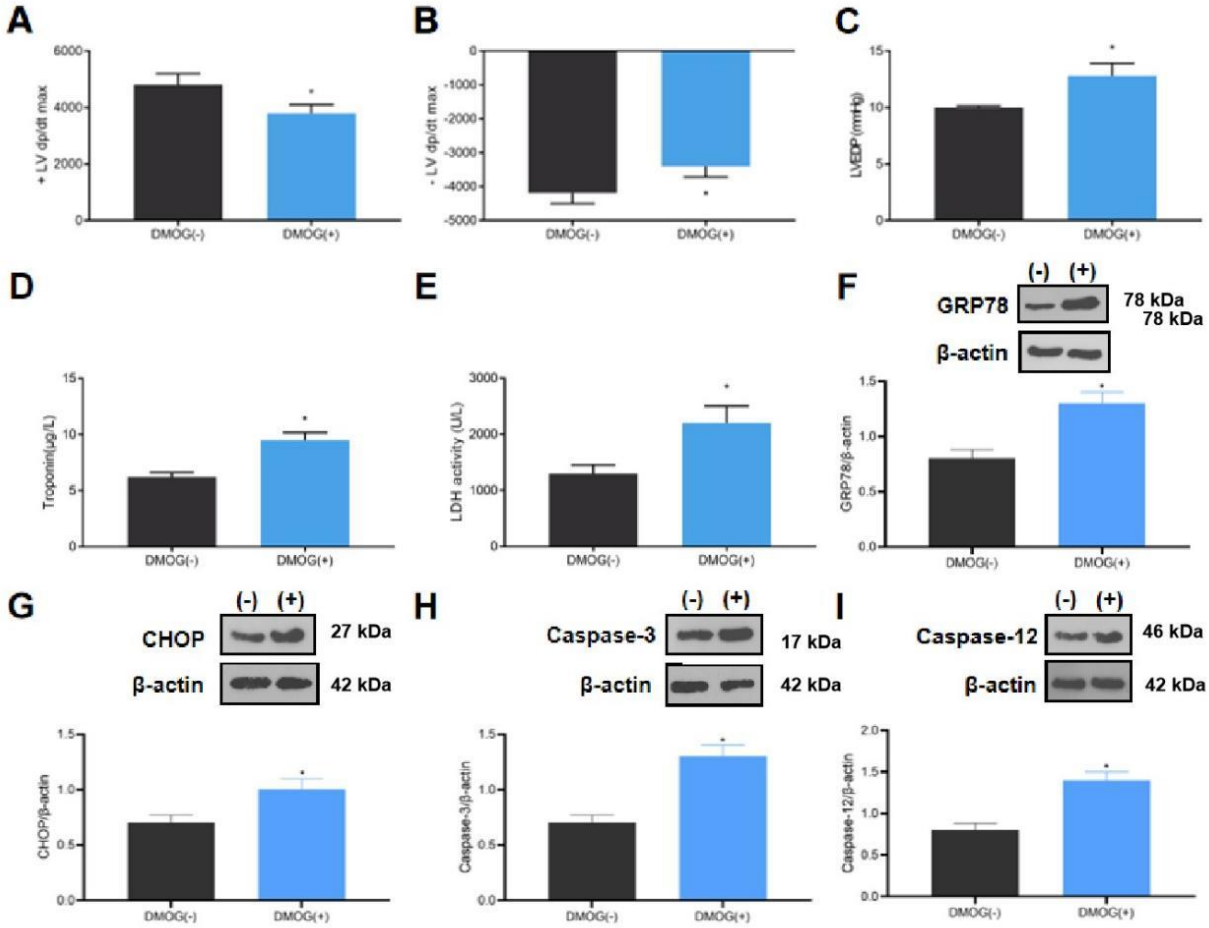

Fig. 5: DMOG blocked the protective influence of sevoflurane on cardiomyocytes. DMOG, HIF-PH inhibitors. (A) +dp/dt$_{max}$; (B)-dp/dt$_{max}$; (C) LVEDP; (D) plasma troponin I concentration, (E) LDH activity, (F) GRP78, (G) CHOP, (H) Caspase-3 and (I) Caspase-12. n=6-7, F=3.35. *, P<0.05 vs. DMOG (-).

preconditioning subgroup, the cardiac function was notably enhanced in I/R rats, myocardial infarct size and LDH release were notably decreased, and plasma inflammatory factors were notably decreased, which further indicated that sevoflurane had a protective influence on myocardial I/R injury. Sevoflurane treatment of brain I/R injury model rats was found to improve the neurological function, reduce the previous findings (Zhao *et al.*, 2017). Rat study have shown that inflammatory response and neuronal apoptosis play important roles in brain I/R injury development (Shi *et al.*, 2018), and reducing the intensity of inflammatory response and inhibiting neuronal apoptosis may be an important target for brain I/R injury diseases treatment.

MIRI refers to the phenomenon that the dysfunction and structural damage of tissue and cells become more serious and even irreversible injury occurs after the restoration of blood perfusion based on myocardial ischemia (Yang *et al.*, 2023). Among the many inducing factors, apoptosis is currently recognized as the main cause. Known pathways to regulate apoptosis include the

mitochondrial, death receptor, and endoplasmic reticulum apoptosis pathways (Cai *et al.,* 2023). The apoptosis pathway of the endoplasmic reticulum has increasingly become a hot spot in apoptosis research. Ischemia, hypoxia and calcium overload caused via MIRI will lead to endoplasmic reticulum homeostasis imbalance and dysfunction. These pathological mechanisms will lead to endoplasmic reticulum stress (endoplasmic reticulum stress, ERS) (Li *et al.,* 2020). ERS has become the target of many drugs in treating MIRI (Wang *et al.,* 2018). GRP78 is a molecular chaperone protein in the endoplasmic reticulum. When ERS occurs, the concentration of GRP78 increases rapidly, so GRP78 is also considered as a significant molecular marker of ERS (Pobre *et al.,* 2020). CRT is a vital Ca^{2+} binding protein in the endoplasmic reticulum, which plays a vital part in maintaining calcium homeostasis and regulating apoptosis. It is another vital stress protein of ER and a vital symbol of ERS (Owusu *et al.,* 2018). The rat MIRI model was established by referring to the methods of previous literature (Zhang *et al.,* 2017).

Recent studies have shown that ERS is involved in the pathophysiological process of MIRI (Zhang *et al.,* 2017; Zhu *et al.,* 2018). ERS means that when the body is stimulated via some stress factors (such as ischemia, hypoxia, calcium disorder, and oxidative stress), it will destroy endoplasmic reticulum homeostasis and accumulate of unfolded or misfolded proteins, which leads to unfolded protein reaction (Marciniak *et al.,* 2022). Most scholars have confirmed through animal experiments that MIRI can cause cerebral ischemic lesions, severe disruption of the BBB, and a significant increase in cerebral oedema in rats, which is one of the most important factors leading to neuronal cell death and the development of brain damage (Liu *et al.,* 2022). ERS is an adaptive response of eukaryotic cells, which helps to restore the homeostasis of endoplasmic reticulum, while overactivation or excessive duration of ERS will activate the apoptosis signal pathway and aggravate the inflammatory response, leading to the fate of cell death. In the MIRI model, on the one hand, due to insufficient oxygen supply, the ability of endoplasmic reticulum folding proteins decreased during myocardial ischemia, resulting in an increase in unfolded proteins (Ren *et al.,* 2021). On the other hand, during the recovery and reperfusion of blood flow, oxidative stress hinders the formation of disulfide bonds of endoplasmic reticulum proteins, resulting in misfolding of proteins, which leads to ERS (Xia *et al.,* 2020). Therefore, the inhibition of ERS can be utilized as a vital target for the treatment of MIRI.

Moderate ERS can restore homeostasis of the internal environment by dealing with abnormal proteins, which is a protective mechanism. However, persistent or excessive ERS will induce apoptosis via activating related factors and pathways, which will adversely affect the body. CHOP belongs to the family of C/EBP transcription factors member and has specific ERS transcription factors. The up-regulation of CHOP concentration is considered to play a vital role in ERS-induced apoptosis (Li *et al.,* 2019). Cysteine proteolytic enzyme (Caspase-12) activation pathway is a unique apoptosis pathway of the endoplasmic reticulum. Under normal physiological conditions, the Caspase-12 protein is usually retained on the outer membrane of the endoplasmic reticulum in the form of an inactive zymogen. Excessive or long-term endoplasmic reticulum stress will activate zymogen, further activate downstream molecules, and eventually lead to apoptosis (Jia *et al.,* 2021). The high concentration of CHOP and Caspase-12 suggests that endoplasmic reticulum stress can no longer maintain the normal morphological function of cells, and cells begin to turn to death or apoptosis. It was corroborated that I/R can induce severe ERS and enhance apoptosis in the myocardium, and sevoflurane preconditioning could effectively inhibit apoptosis induced via excessive endoplasmic reticulum stress through CHOP and Caspase-12 pathway, and then protected the structure and function of ischemic myocardium.

HIF-1 regulate the concentration of various related genes induced via hypoxia (Albanese *et al.,* 2022). It mediates the adaptive response of cells to ischemia and hypoxia by regulating the concentration of nearly 200 genes (Albanese *et al.,* 2020). During ischemia/reperfusion, HIF-1 α reduces cell apoptosis and enhances its proliferation and survival via activating the concentration of some specific target genes, thus alleviating myocardial injury (Loor *et al.,* 2008). This research found that the concentration of iNOS in myocardial tissue pretreated with sevoflurane was notably enhanced, and iNOS, as a downstream regulatory gene of HIF-1 α, does a protective role in a variety of acute and chronic heart illnesses. It has been found that the method of silencing HIF-1 α proline hydroxylase via siRNA can maintain the stability of HIF-1 α and reduce I/R injury, which is considered to be achieved through the iNOS pathway. NOS can catalyze L-arginine to produce NO in vivo, while NO can enhance cGMP production via activating soluble guanylate cyclase (CG), thus activating protein kinase G, which can reduce Ca^{2+} overload in the myocardium, inducing the opening of ATP-sensitive K^{+} channels, reduce release of cytochrome C from mitochondria, reduce cardiomyocyte apoptosis, and do a cardioprotective influence (Dungel *et al.,* 2013; Pereira *et al.,* 2017). First, no specific pharmacological blockers or genetic manipulations were used to block the pathway; second, only animal studies were used to demonstrate that sevoflurane pretreatment has a protective effect on myocardium and that this protection can be achieved through the HIF-1 alpha/iNOS/cGMP pathway, and no clinical studies were performed. Clinical studies were not conducted. Therefore, further clinical studies are necessary to verify this. This study provides a new basis for applying sevoflurane in the perioperative period of cardiac surgery. In conclusion, sevoflurane pretreatment can be protective by attenuating myocardial I/R injury through the HIF-1 alpha/iNOS/cGMP pathway.

Conclusions: To sum up, sevoflurane preconditioning can reduce myocardial I/R injury, and its mechanism may be related to activation of HIF-1 α / iNOS/cGMP pathway, inhibition of excessive endoplasmic reticulum stress and its mediated apoptosis.

Abbreviations: IR: ischemia-reperfusion; iNOS: inducible nitric oxide synthase; ER: Endoplasmic reticulum; ERS: Endoplasmic reticulum stress. GRP78: Glucose-regulated protein 78; CRT: Calreticulin; CCAAT/enhancer-binding protein homologous protein (CHOP); HIF-1α: Hypoxia-inducible factor-1α; ±LVdp/dtmax: Maximum left

ventricular pressure; LVEDP: Left ventricular end diastolic pressure.

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Competing interests: The authors declare that they have no competing interests.

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