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Post-Ischemic Treatment with Sevoflurane: To Reduce Myocardial Ischemic/Reperfusion Injury in Rats by miR-26a-5p/PTEN/PI3K/ Akt Signaling Pathway

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ABSTRACT

The aim of the study was to determine the effect of sevoflurane on myocardial ischemic/reperfusion (I/R) injury in rats and its causative role. The differential expressions of miRNA in the rat myocardial tissue after post-ischemic treatment with sevoflurane were screened by miRNA expression profile chip. The potential targets of miR-26a-5p were predicted by application of Targetscan, microT-CDS, miRtargetLink and miRpathDB database. The luciferin reporter groups were used for verification. We detected the impact of miR-26a-5p expression content on the PTEN/PI3K/Akt pathway via Western blot. We used HE staining for examining how miR-26a-5p expression content affected myocardium morphology of rats in the sevoflurane group, Biochemical experiments for assessing how miR-26a-5p expression content affected myocardial tri-enzymes in the sevoflurane group, as well as Western blot for evaluating how miR-26a-5p expression content affects apoptosis of rats in the sevoflurane group. The impact of miR-26a-5p expression content on levels of oxidative stress indicators of myocardial tissue of rats in the sevoflurane group was detected by ELISA. We found that the inhibition expressed by content of miR-26a-5p in rats after post-ischemic treatment with sevoflurane inhibited the anti-inflammatory effect of post-ischemic treatment with sevoflurane. The post-ischemic treatment with sevoflurane promoted the activity of SOD, CAT as well as GSH-Px, and inhibited MDA and ROS levels, while miR-26a-5p inhibition expression content inhibited the increase in activity of SOD. To conclude, the post-ischemic treatment with sevoflurane is able to inhibit PTEN expression to promote the activation of PI3K/Akt signaling pathway by increase in miR-26a-5p expression level, thereby playing a role in resisting myocardial ischemia/reperfusion damage.

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SZ, SY and KL designed the study. SZ and SY conduced the experiments and analyzed the data. SZ and SY prepared the draft of the manuscript. KL revised the final draft.

Key words
Sevoflurane, miR-26a-5p/PTEN/PI3K/ Akt signaling pathway, Post-ischemic treatment, Myocardial ischemic/ Reperfusion injury

INTRODUCTION

Ischemic heart disease is still the "first killer" that threatens human health worldwide (Lindsey *et al*., 2018). Restoring the blood supply to ischemic myocardium is important to limit injury induced via acute myocardial infarction. Although blood reperfusion restores the oxygen supply of the ischemic myocardium and improves the biological function of the ischemic myocardium, compared with simple ischemia, reperfusion will cause more serious damage to the myocardium, which is called myocardial I/R injury (Bøtker *et al*., 2018). Myocardial I/R injury can lead to irreversible damage to myocardium cells, reduce cell viability and may cause life-threatening consequences. I/R has a significant negative impact on human health, and alleviating I/R damage is still a major challenge for researchers and clinicians. Clinical studies have found that even if volatile anesthetics (such as isoflurane and propofol) are maintained for a short timeframe at the beginning of reperfusion, they can significantly improve heart function after ischemia and reduce the area of myocardial infarction (Tao *et al*., 2009). Similar to post-ischemic adaptation, this phenomenon is called post-anaesthetic adaptation.

Sevoflurane has been widely used in clinical practice due to its stable induction anesthesia performance and pharmacological properties of rapid recovery (Brioni *et al*., 2017). Studies have shown that post-anaesthetic regulation of sevoflurane and other preparations is a good way to decrease I/R-induced myocardium injury, but exact mechanism has not been fully explained. MicroRNA (miRNA), a kind of short non-coding RNA with approximately 22 nucleotides, can bind to the 3'-UTR region of mRNA to have post-transcriptional regulation

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on the expression level of target mRNA. A myriad of studies has pointed out a list of miRNAs such as miR-214 (Yin *et al*., 2019), miR-34b (Pedretti *et al*., 2019) and miR-30e work essentially during the pathological process of ischemic heart disease. Studies have showed that sevoflurane may induce cardioprotective effects by regulating several key miRNA pathways. For example, Xie *et al*. (2017) have pointed out that sevoflurane activates the JAK2/STAT3 signaling pathway by regulating miR-135b-5p level, thereby having the protective impact on I/R-induced myocardial damage. Hereby, we explored the changes in miRNA profile induced by sevoflurane, finding that miR-26a-5p may function pivotally in sevoflurane treating I/R-induced myocardial damage. Our study pointed out that post-ischemic treatment with sevoflurane inhibits PTEN expression to promote the activity of PI3K/Akt pathway by up-regulating miR-26a-5p level, thereby resisting myocardial I/R damage.

MATERIALS AND METHODS

Laboratory animals

In this study, we purchased 40 male SD rats (Sprague-Dawley, weight: 280g-300g, age: 8-10 weeks) from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (production license number: SCXK (Beijing) 2018-004, animal certificate number: No.1100112011035947). All laboratory animals were kept in an SPF-level animal room, with a breeding temperature of 20-26°C (daily temperature difference ≤ 4°C), a breeding environment of 40-70% relative humidity, and artificial lighting alternated with darkness for 12 h.

Animal model construction

In this study, the rat myocardial I/R model was constructed through ligating LAD of rats. The experimental protocol was described in the literature (Koeppen *et al*., 2018), which we briefly described here. We intraperitoneally injected sodium pentobarbital (50 mg/kg) to anesthetize the rats and performed tracheostomy and intubated the trachea of rats. A rodent ventilator was used for mechanical ventilation, the breathing rate of rats was 60 beats/min. We used a heating pad to maintain the temperature of rats at about 37°C. Myocardial ischemia was induced by ligating LAD, and reperfusion was performed by loosening the ligature. The symptoms of myocardial ischemia were cyanotic reaction on the surface of the ventricle with obvious arrhythmia.

In this study, 40 male SD rats were separated into the sham group, the model group, the sevoflurane group as well as the sevoflurane+antagomiR-26a-5p group according to the random number table. In addition to the sham group, rats in the rest groups were subjected to the following experimental procedure, namely, 30 min of ischemia, followed by untying the ligature and reperfusion for 120 min. The rats in the sevoflurane group were exposed to sevoflurane for 15 min at the beginning of reperfusion. Rats in the sevoflurane+antagomiR-26a-5p group were exposed to sevoflurane for 15 min combined with injection of 20mM antagomiR-26a-5p into the tail vein 1 h before ischemia.

miRNA expression profile chip

In order to study the impact of post-ischemic treatment with sevoflurane on miRNA profiles of myocardial tissues, Affymetrix GeneChip miRNA 2.0 arrays (Affymetrix) were used for analysis of miRNA profile, which contained 4560 probe sets of human miRNAs. Among them, a total of 1082 mature miRNAs probe sets were screened. These screened probe sets assured 100% coverage of all mature human miRbase v.15 database. In this study, all steps were performed based on the standardized steps of miRNA 2.0 arrays of Affymetrix. The specific experimental scheme was shown in the literature (Bullard *et al*., 2019).

Biochemical markers detection

After 120 min of reperfusion, blood samples were collected through abdominal aorta to separate plasma. CK (Sigma), LDH (Sigma) as well as C-TnT (Sigma) levels in plasma were determined according to the instructions from manufacturer.

RT-qPCR

In this study, we used RNA extraction kit (BioTeke Corporation, Beijing, China) to extract total RNA from myocardial tissue in rats. The method was to synthesize cDNA using M-MLV-RT (Promega, Wallisellen, Switzerland) kit. The cDNA was amplified using experimental method of qPCR for 45 cycles. The internal reference used in this study was β-actin. Using $2^{-\Delta\Delta Ct}$, we determined relative expression values of relevant miRNA and mRNA. We applied primer sequences in research (Table I).

Western blotting

The myocardial tissue was lysed by RIPA lysis buffer with protease inhibitors, and centrifuged at 14000×g for 30 min in a low-temperature high-speed centrifuge at 4°C, and we took out supernatants. We determined protein content of sample via BCA, and we adjusted them to the same concentration by RIPA lysis buffer. After protein denaturation, each sample was loaded with 20 μg, separated in 8-12% SDS–PAGE (Bio-Rad), and then transferred to PVDF membrane; after being blocked with

5% skim milk for 1 h, one resistance incubation overnight at 4°C was performed, the PVSF membrane was incubated by the peroxidase-coupled secondary resistance for 2 h, the color reaction was performed by the ECL, and the protein was quantified using Image J.

Table I. Primer sequences.

Genes	Primer sequences
miR-26a	Forward: 5'-CAAGTAATCCAGGATAGG-3'
	Reverse: 5'-GAACATGTCTGCGTATCTC-3'
PTEN	Forward: 5'-TGAGTTCCCTCAGCCGTTACCT-3'
	Reverse: 5'-GAGGTTTCCTCTGGTCCTGGTA-3'
IL-1β	Forward: 5'-CCACAGACCTTCCAGGAGAATG-3'
	Reverse: 5'-GTGCAGTTCAGTGATCGTACAGG-3'
IL-5	Forward: 5'-GAGAGTGATTGAGAGTGGACCAC-3'
	Reverse: 5'-CACAACCCTCTGCACCCAGTTT-3'
TNF-α	Forward: 5'-CTCTTCTGCCTGCTGCACTTTG-3'
	Reverse: 5'-ATGGGCTACAGGCTTGTCACTC-3'
ICAM-1	Forward: 5'-AGCGGCTGACGTGTGCAGTAAT-3'
	Reverse: 5'-TCTGAGACCTCTGGCTTCGTCA-3'
VCAM-1	Forward: 5'-GATTCTGTGCCCACAGTAAGGC-3'
	Reverse: 5'-TGGTCACAGAGCCACCTTCTTG-3'
GAPDH	Forward: 5'-GTCTCCTCTGACTTCAACAGCG-3'
	Reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3'

Cell culture

In this study, the H9C2 cell line was from ATCC cell bank in the United States. The cell culture medium is DMEM containing 10% FBS. The culture environment was an incubator with 5% CO2 at 37°C. The miR-26a-5p mimic and miR-NC were purchased from GenePharma. Using 3000 kits of Lipofectamine, we transfected miR-26a-5p mimic into H9C2 cells. After 24h of transfection, the transfection efficiency was determined by RT-qPCR.

Estimation of antioxidant enzyme and inflammatory factor

We homogenized rat myocardial tissue in every group. Using commercial kits to the detection for assay SOD, CAT and GSH- Px, MDA and ROS contents according to the instructions from manufacturer (Beyotime).

Generation of fluorescein reporter groups

In this experiment, to generate WT-type PTEN and mut-type PTEN reporter groups, we amplified and cloned 3'UTR regions of the two types of PTEN into psiCHECK-2 luciferase liposomes (Promega), and then miR-26a-5p mimic or miR-con and Wild-type or mutant liposomes were co-transfected into cells. The activity of luciferase was detected in 48 h after transfection with Dual-Luciferase reporter reagent (Promega).

HE staining

The rats to be tested were sacrificed and their hearts were separated, and they were treated with 4% paraformaldehyde/PBS (pH 7.4) at 4°C for 48h. After washing the tissue with running water, it was dehydrated with 70%, 80% and 95% ethanol and treated with 100% ethanol, and cleared with xylene, and then embedded with paraffin (4 um). Using the HE staining kit (Biyuntian Biotechnology Co., Ltd.) to stain the tissue in accordance with the instructions from manufacturer.

Statistical analysis

In this study, the quantitative indicators were calculated by group means ± standard deviation (SD). The data of each index was analyzed according to the following procedures: Using Levene's to test the homogeneity of variance. If there was no statistical significance ($P>0.05$), analysis of variance (ANOVA) would be used for statistical analysis. If ANOVA had statistical significance ($P\leq0.05$), used LSD for a comprehensive comparison. If variance is disunited ($P\leq0.05$), Kruskal-Wallis test would be adopted. If Kruskal-Wallis test was statistically significant ($P\leq0.05$), Mann-Whitney will be applied to make comparisons between means in pair. The above statistical operations were performed by SPSS 22.0.

RESULTS

Effect of post-ischemic treatment with sevoflurane on the miRNA expression profile of myocardial tissue in rats

First, we used the miRNA expression profile chips to explore the difference in miRNA expression profile induced by post-ischemic treatment with sevoflurane. The screening results show that the miRNA expression profile of myocardial tissue in rats induced by post-ischemic treatment with sevoflurane has significant differences. There is a total of 26 differential expressions of miRNAs in the miRNA expression profile (log2 (Fold Chang)>1 and $P<0.05$), of which 18 were significantly increased and 8 were significantly decreased, as shown in Figure 1A.

To further verify whether the high-throughput screening results were reliable, we confirmed the differences in miRNA expression levels of rats in the sevoflurane group through RT-qPCR. Among them, miR-26a-5p expression level increased significantly ($P<0.05$), as shown in Figure 1B. By taking the intersection of potential targets of miR-26a-5p in four databases of Targetscan, microT-CDS, miRtargetLink and miRpathDB, we obtained a total of 14 potential miR-26a targets (Fig. 1C).

PTEN in cardiomyocytes as a direct miR-26a-5p target

The RT-qPCR experimental results further pointed out that in comparison to IR model group, PTEN mRNA expression level of rat myocardial tissue in the antagomiR-26a-5p group jumped significantly, as shown in (Fig. 2A). In addition, the experimental results of Western blot displayed that in contrast to IR model group, PTEN protein expression level in myocardial tissue of rats in antagomiR-26a-5p group jumped signally, as shown in Figure 2B.

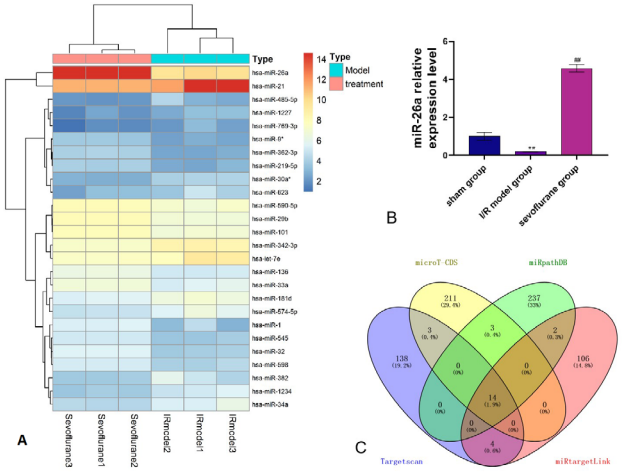

Fig. 1. Post-ischemic treatment with sevoflurane significantly added miR-26a-5p expression level in rat myocardial tissue. **A**: Clustering heatmap for post-ischemic treatment with sevoflurane on miRNA in myocardium of rats (n=3). **B**: The RT-qPCR verified the difference in miRNA expression profile in myocardial tissue of rats after post-ischemic treatment with sevoflurane. **C**: Venn diagram showed the intersection of miR-26a-5p potential targets in databases of Targetscan, microT-CDS, miRtargetLink and miRpathDB.

Figure 2C showed the sequence complementarity between miR-26a-5p and the 3'UTR of WT-type and MUT-type PTEN. The co-transfection experimental results of luciferase reporter plasmids and miR-26a-5p mimic or agomir-con indicated that compared with that in the agomir-con transfection group, the cell luciferin activity used to transfect WT type PTEN-3′UTR and miR-26a-5p mimic was signally reduced (P<0.01) in contrast to agomir-con transfection group, luciferin activity of HGPC cells transfected with MUT type PTEN-3′UTR and miR-12967 mimic had no significant difference. These results all pointed out that PTEN in HGPC cells may be a direct miR-26a-5p target (Fig. 2D).

Post-ischemic treatment with sevoflurane activates PI3K/Akt pathway

Results in existing studies have shown that post-ischemic treatment with sevoflurane significantly up-regulates miR-26a-5p expression level; PTEN serves as a direct miR-26a-5p target. Therefore, post-ischemic treatment with sevoflurane might activate PI3K/Akt signaling pathway by inhibiting PTEN. Compared with that in the IR model group, the PTEN protein level in rat myocardial tissue in sevoflurane group was significantly reduced, while p-PI3K/PI3K and p-AKT/AKT protein levels were signally decreased (P<0.05); in comparison with that in sevoflurane group, PTEN protein expression level in rat myocardial tissue in the antagomiR-26a-5p group increased significantly, and p-PI3K and p-AKT protein expression levels increased significantly (P<0.05), as shown in Figure 3. The results of the study indicated that rats in I/R model, post-ischemic treatment with sevoflurane might activate PI3K/Akt signaling pathway by inhibiting PTEN.

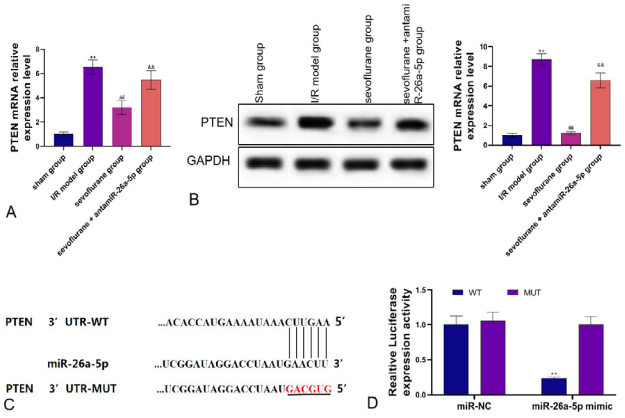

Fig. 2. PTEN as a direct miR-26a-5p target. **A**: Effect of post-ischemic treatment with sevoflurane on PTEN mRNA expression (n=3). **B (left)**: Effect of post-ischemic treatment with sevoflurane on the level of PTEN protein (n=3). **C (right)**: The complementary relationship between miR-26a-5p and the 3'UTR of Wild-type PTEN. **D**: The luciferin reporter groups verified whether miR-26a-5p is a direct PTEN target (n=3). ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

miR-26a-5p inhibition expression level inhibits the myocardial protective effect of post-ischemic treatment with sevoflurane

The results of biochemical detection experiments pointed out that in contrast to that in sham group, myocardial tri-enzymes content in rat myocardial tissue in I/R model

group increased signally (P<0.05). In comparison with that of rats in I/R model group, tri-enzymes content in myocardium of the sevoflurane group was significantly reduced (P<0.05). In addition, in contrast to that of rats in sevoflurane group, miR-26a-5p inhibition expression level significantly increased myocardial tri-enzymes content (P<0.05). The results of study suggested that miR-26a-5p inhibition expression level inhibits the myocardial protective effect induced by post-ischemic treatment with sevoflurane, as shown in Figure 4A.

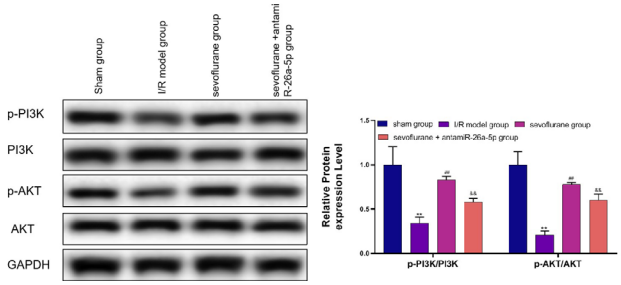

Fig. 3. Post-ischemic treatment with sevoflurane may inhibit PTEN by up-regulating miR-26a-5p to activate the PI3K/Akt pathway. The effect of post-ischemic treatment with sevoflurane on PI3K/Akt signaling pathway (n=3) was detected by Western blot. ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

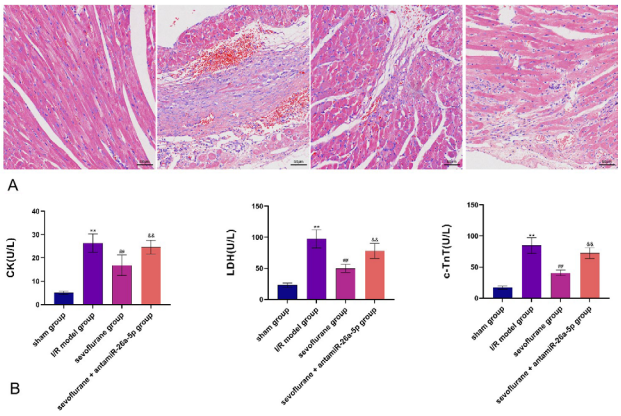

Fig. 4. miR-26a-5p inhibition expression level inhibits the myocardial protective effect of sevoflurane after ischemia. **A**: The effect of miR-26a-5p inhibition expression level on rat myocardial tissue morphology in the sevoflurane group (n = 3). **B**: The impact of miR-26a-5p inhibition expression level on the content of myocardial tri-enzymes in rat myocardial tissue in the sevoflurane group (n = 7). ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

As seen in Figure 4B, the results of HE staining indicated that the size and myocardial cells morphology in the sham group were normal, and the myocardial tissue fibers and nuclei were uniformly stained, and the fibers were neatly arranged and connected tightly with no damage. In I/R model group, sequence of myocardial cells was not orderly, the gap between myocardial cells was obviously widened, the muscle fibers were degenerated and swollen with interstitial edema, a large number of red blood cells leaked out, and neutrophils infiltrated. The myocardium tissue injury of rats in sevoflurane group was improved, but inflammatory cell infiltration and red blood cell leakage were still seen. The myocardial morphology of the rats in the sevoflurane +antagomiR-26a-5p group was signally worse than that in the sevoflurane group.

miR-26a-5p inhibition expression level inhibits the anti-apoptotic effect of the post-ischemic treatment with sevoflurane

The experimental results of Western blot indicated that in contrast to those in sham group, the apoptotic protein Bax/Bcl2, Active-caspase 3, Active-caspase 9 protein expression in rat myocardial tissue in I/R model group increased distinctly (P<0.05). In comparison with those of rats in I/R model group, Bax/Bcl2, Active-caspase 3, and Active-caspase 9 protein expression levels in rat myocardial tissue in sevoflurane group were significantly reduced (P<0.05). In addition, in contrast to that of rats in sevoflurane group, of miR-26a-5p inhibition expression significantly diminished of Bax/Bcl2, Active-caspase 3, Active-caspase 9 protein expressions in rat myocardial tissue (P<0.05) (Fig. 5).

miR-26a-5p inhibition expression content inhibits the anti-oxidative stress effect of post-ischemic treatment with sevoflurane

As seen from Figure 6, in comparison with those in the sham group, activities of SOD, CAT, as well as GSH-Px in rat myocardial tissue in I/R model group were distinctly reduced, while the levels of MDA and ROS were signally increased (P<0.05). The findings of the study showed that I/R model distinctly induced an increase in oxidative stress in rat myocardial tissue. The activities of SOD, CAT, and GSH-Px in rat myocardial tissue in sevoflurane group were significantly increased compared with those in I/R model group, and ROS and MDA levels were signally decreased (P<0.05). The results of the study indicate that post-ischemic treatment with sevoflurane distinctly decreased the oxidative stress level of myocardial tissue of rats induced by IR model. A further study showing in comparison with those in the sevoflurane group, the rat myocardial tissue in the post-ischemic treatment with sevoflurane +antagomiR-26a-5p

group had signally diminished activities of SOD, CAT, and GSH-Px, and ROS and MDA levels increased significantly (P<0.05). Findings in our study indicate that miR-26a-5p inhibition expression level inhibits anti-oxidative stress induced by post-ischemic treatment with sevoflurane.

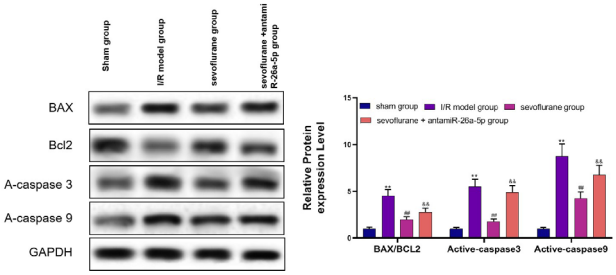

Fig. 5. MiR-26a-5p inhibition expression level inhibits the anti-apoptotic effect induced by post-ischemic treatment with sevoflurane. Western blotting was used to examine the impact of inhibition expression of miR-26a-5p on anti-apoptotic effect induced by post-ischemic treatment with sevoflurane (n = 3). ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

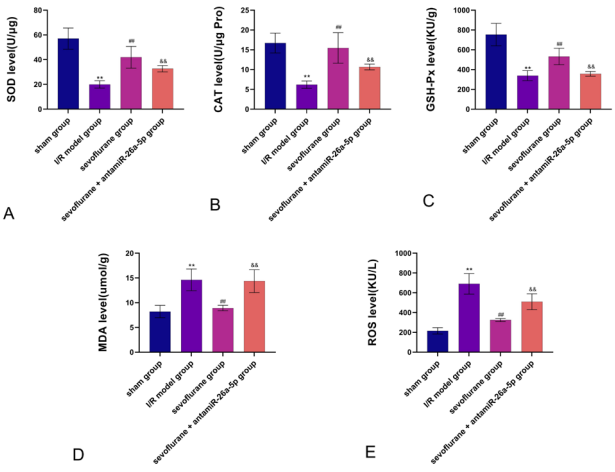

Fig. 6. MiR-26a-5p inhibition expression level inhibits the anti-oxidative stress effect of post-ischemic treatment with sevoflurane. **A**: Influence of antamiR-26a-5p+sevoflurane on the SOD content in myocardial tissue of rats (n = 7). **B**: Influence of antamiR-26a-5p + sevoflurane on CAT content in rat myocardial tissue (n = 7). **C**: Influence of antamiR-26a-5p+sevoflurane on GSH-Px content in rat myocardial tissue (n = 7). **D**: Influence of antamiR-26a-5p + sevoflurane on MDA content in rat myocardial tissue (n = 7). E: Influence of antamiR-26a-5p+sevoflurane on ROS content in rat myocardial tissue (n = 7). ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

miR-26a-5p expression level Inhibition inhibits the anti-inflammatory effects of post-ischemic treatment with sevoflurane

From Figure 7, in contrast to those in sham group, levels of TNF-α, IL-1β, IL-8, ICAM-1 and VCAM-1 mRNA in rat myocardial tissue in the I/R model group increased (P<0.05). Levels of TNF-α, IL-1β, IL-8, ICAM-1, and VCAM-1 in rat myocardial tissue in the sevoflurane group were significantly lower than those in IR model group (P<0.05). Levels of TNF-α, IL-1β, IL-8, ICAM-1 and VCAM-1 in rat myocardial tissues treated with sevoflurane + antagomiR-26a-5p group increased (P<0.05). These findings presented that miR-26a-5p inhibition level inhibited anti-inflammatory effect induced by post-ischemic treatment with sevoflurane.

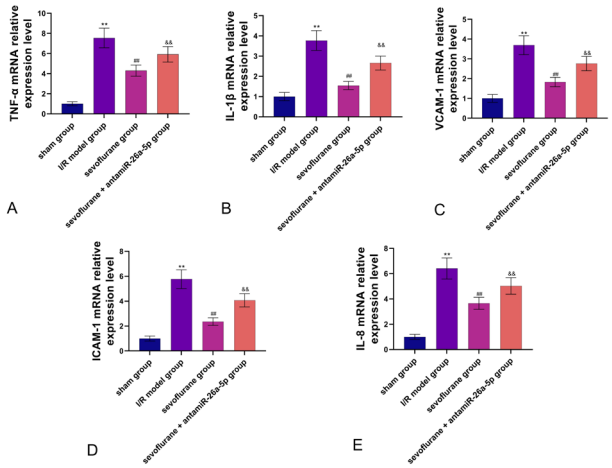

Fig. 7. MiR-26a-5p inhibition expression level inhibits the anti-inflammatory effect of post-ischemia treatment with sevoflurane. **A**: Impact of antamiR-26a-5p+sevoflurane on TNF-α mRNA level in myocardial tissue of rats (n = 7). **B**: Impact of antamiR-26a-5p+sevoflurane on IL-1β mRNA level in myocardial tissue of rats (N = 7). **C**: Impact of antamiR-26a-5p+sevoflurane on IL-8 mRNA level in rat myocardial tissue (n = 7). **D**: Impact of antamiR-26a-5p+sevoflurane on ICAM-1 mRNA level in rat myocardial tissue (n = 7). **E**: Impact of antamiR-26a-5p+sevoflurane on VCAM-1 mRNA level in rat myocardial tissue (n = 7). We adopted RT-qPCR to detect the influences of different treatment methods on TNF-α, IL-1β, IL-8, ICAM-1 and VCAM-1 mRNA levels in rat myocardial tissue (n = 7). ** represents sham group V.S. I/R model group; ## represents I/R model group V.S. sevoflurane group P<0.01; and represents sevoflurane group V.S. sevoflurane+antamiR-26a-5p group P<0.01.

DISCUSSION

Myocardial I/R injury is the main reason leading to

heart dysfunction, myocardial infarction and death and morbidity after cardiac surgery. This study pointed out that in myocardial I/R, miR-26a-5p level is relative to existence of PTEN/PI3K/Akt pathway, and miR-26a-5p may act vitally in mediating myocardial protection of post-ischemic treatment with sevoflurane. We found that post-ischemic treatment with sevoflurane up-regulated miR-26a-5p expression level, thereby inhibiting PTEN expression and activating the PI3K/Akt pathway to prevent myocardial I/R damage.

First of all, our research results confirmed that sevoflurane was capable of alleviating myocardial I/R injury with myocardial protective function. Our research results also pointed out that the myocardial morphology of rats after post-ischemic treatment with sevoflurane was significantly improved and the levels of tri-enzymes as the myocardial injury indicators (LDH, C-TnT, CK) were significantly down regulated.

In addition, post-ischemic treatment with sevoflurane inhibited the increase in apoptosis level of myocardial tissue induced by I/R injury, and reduced Bax/Bcl2, active-caspase 3, and active-caspase 9 protein levels. Apoptosis is the autonomous orderly death of cells under certain physiological or pathological conditions, which is regulated by genes. Its morphology features membrane blistering, cell atrophy, nuclear pyknosis, and chromatin condensation. The significant activation of caspase pathway is a molecular trait of cell apoptosis. Apoptosis plays an essential role in I/R-induced myocardium injury. Knockout of a series of myocardial apoptosis genes in mice can significantly inhibit I/R-induced myocardial injury. For example, compared with that of wild-type mice, the mice knocked out Fas and TRAF1in the I/R model group had a smaller area of I/R-induced myocardial infarction (Jeremias *et al*., 2000; Zhang *et al*., 2013). In addition, in comparison with that of wild type mice, the area of I/R-induced myocardial infarction was smaller in mice that were knocked out bax or treated with small molecule inhibitors of Omi/Htr2 (Hochhauser *et al*., 2003, 2007). Consistent with our findings, in I/R animal models, the post-ischemic treatment with sevoflurane could significantly diminish myocardial tissue apoptosis induced by I/R injury (Qiao *et al*., 2019).

Subsequently, the post-ischemic treatment with sevoflurane reduced pro-inflammatory factors expression, including TNF-α, IL-1β, IL-8, ICAM-1, and VCAM-1, indicating that post-ischemic treatment with sevoflurane signally diminished the activation of myocardial I/R-induced inflammation. When myocardial I/R is injured, phospholipids of cell membrane are degraded, and arachidonic acid metabolites such as leukotrienes increase, attracting a large number of white blood cells into the injured tissue. Activated inflammatory cells could release inflammatory substances, including a large amount of reactive oxygen species, various proteolytic enzymes and cytokines, etc., resulting in cytotoxicity, damage to cell structure and its dysfunction. IL-1β could promote vascular endothelial cells to express ICAM-1, VCAM-1, and leukocytes to express CD11b/CD18 and other adhesion molecules, thereby enhancing the adhesion of leukocytes and vascular endothelial cells, which is conducive to leukocyte inflammatory exudation (Monnerat *et al*., 2016). In addition, IL-1 can stimulate endothelial cells and neutrophils to produce NO, oxygen free radicals, etc., causing oxidative stress damage. As an inflammatory cascade amplifier, TNF-α can activate and chemoattract monocytes, macrophages, neutrophils, vascular endothelium cells, etc., induces and promotes the secretion and release of inflammatory medications such as IL-1 and IL-8 by these cells, initiates inflammatory cascade reaction, and then amplify the inflammatory responses (Ma *et al*., 2016). The function of IL8 as an important chemokine has been recognized, it can transfer neutrophil adhesion molecules from intracellular to the cell surface, and interacts with endothelial cell surface adhesion molecules ICAM-1 to strengthen the force of neutrophils cells and endothelial cells, making them migrate to the inflammatory lesions (Flori *et al*., 2019).

Finally, post-ischemic treatment with sevoflurane promoted the activation of SOD, CAT, and GSH-Px, inhibiting MDA and ROS contents. It was pointed out that post-ischemic treatment with sevoflurane significantly reduced the oxidative stress level of myocardial tissue in rats induced by I/R. It was currently believed that increased reactive oxygen acts critically in occurrence and progress of myocardial I/R injury. Oxygen free radicals were generated explosively in the first few min of reperfusion, which could cause membrane lipid peroxidation and protein dysfunction. Lipid peroxidation of mitochondrial membrane can inhibit oxidative phosphorylation and reduce the production of ATP, lipid peroxidation of cell membrane increases its permeability, leading to electrolyte imbalance in myocardial cells, in addition, ROS can also directly damage muscle fiber proteins, such as the sulfhydryl oxidation of contractile proteins, which makes it less sensitive to Ca2+ and inhibits myocardial contractility (Li *et al*., 2016). SOD, GSH-Px and CAT can inhibit ROS during myocardial ischemia/reperfusion by degrading superoxide and hydroxyl free radicals, respectively.

In this study, our results showed that post-ischemic treatment with sevoflurane inhibits I/R-induced myocardial injury by inhibiting oxidative stress, reducing myocardial tissue inflammation level, and inhibiting myocardial tissue and cardiac muscle tissue apoptosis.

In order to further explore at the molecular level, the mechanism of post-ischemic treatment with sevoflurane inhibiting I/R-induced myocardial injury, we screened the differentially expressed miRNA which was significantly changed during the process of post-ischemic treatment with sevoflurane through miRNA profile chips and RT-qPCR. The experimental findings indicated that miR-26a-5p level in rat myocardial tissue in sevoflurane group jump signally. The experimental results of luciferin reporter groups pointed out that PTEN was the direct miR-26a-5p target. Analysis on pathway indicated that post-ischemic treatment with sevoflurane inhibited PTEN expression by up-regulating miR-26a-5p, ultimately activating the PI3K/Akt pathway. Consistent with our findings, it was pointed out that sevoflurane ischemic post-treatment can protect against I/R-induced myocardial damage by activation of the PI3K/AKT/mTOR signaling pathway (Zhang *et al*., 2014). In our research, miR-26a-5p functions as a protective factor in myocardial I/R, which could inhibit PTEN expression at the levels of mRNA and protein to activate PI3K/AKT pathway. Consistent with our results, it was also found that overexpression of miR-26a-5p in C57BL/6 mice could significantly reduce the area of myocardial infarction in mice by activating PTEN/PI3K/AKT and inhibit myocardial tissue apoptosis (Xing *et al*., 2020).

In conclusion, our findings pointed out that post-ischemic treatment with sevoflurane could up-regulate miR-26a-5p level and inhibit PTEN expression to promote the activation of PI3K/Akt pathway, thereby exerting functions in resisting I/R injury.

Statement of conflict of interest

The authors have declared no conflict of interest.

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