

Protective effect of remifentanyl on myocardial ischemia-reperfusion injury through Fas apoptosis signaling pathway

Z.-JU. OU¹, Z.-JI. OU², H.-B. ZHOU¹, C.-S. XU¹, D.-Z. ZHANG¹, G. WANG¹

¹Department of Anesthesia, Beijing Jishuitan Hospital, Beijing, China

²Department of Emergency, Qingdao Eighth People's Hospital, Qingdao, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the protective effect of remifentanyl (RFT) on myocardial ischemia-reperfusion (IR) injury through Fas apoptosis signaling pathway.

MATERIALS AND METHODS: A total of 36 Sprague-Dawley (SD) rats were randomly divided into 3 groups, including the sham operation (Sham) group, IR model (IR) group and RFT pretreatment (RFT) group, with 12 rats in each group. Myocardial tissues of rats in each group were collected. Hematoxylin and eosin (H&E) staining was used to examine the pathological differences of the myocardium in the three groups. The levels of lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD) and malondialdehyde (MDA) in the serum of rats in each group were detected by enzyme-linked immunosorbent assay (ELISA). Meanwhile, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was adopted to detect the apoptosis level of myocardial cells in each group. Furthermore, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Western blotting were applied to measure the mRNA and protein expression levels of Fas and its pathway indexes, respectively.

RESULTS: Compared with the Sham group, LDH and CK activities and MDA level in the IR group were significantly increased, whereas the level of SOD was remarkably decreased ($p<0.05$). Compared with the IR group, RFT pretreatment could significantly reduce the release of LDH and CK-muscle/brain (CK-MB), increase SOD level and decrease MDA level ($p<0.05$). TUNEL results manifested that the apoptosis rate of myocardial cells in the IR group was markedly increased than that of the Sham group ($p<0.05$). Meanwhile, the apoptosis rate of myocardial cells in the RFT group was notably decreased when compared with that of the IR group ($p<0.05$). ELISA results demonstrated that the levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) proteins in the RFT group were significantly lower than those of the IR group ($p<0.05$). RT-PCR and Western blotting results indicated that the expressions

of Fas, Fas ligand (FasL), and Fas-associated protein with death domain (FADD) in IR and RFT groups were significantly higher than those of the Sham group ($p<0.05$). However, RFT pretreatment could markedly reduce the levels of Fas, FasL, and FADD ($p<0.05$).

CONCLUSIONS: RFT can reduce the apoptosis of myocardial cells as well as IR-induced oxidative stress and inflammation by inhibiting the Fas/FasL signal transduction pathway.

Key Words:

RFT, Fas signaling pathway, Apoptosis, Myocardial ischemia-reperfusion injury.

Introduction

Ischemic reperfusion (IR) injury can be attributed to many factors, such as oxygen free radical release, continuous lipid peroxidation, cell apoptosis or necrosis and inflammatory cytokines and micro-vessels injury^{1,2}. Although the blood flow returns to normal after reperfusion, IR injury still triggers several stress signal processes. This may eventually lead to different degrees of myocardial injury. Apoptosis is programmed cell death, which plays an important role in myocardial cell death after IR³. According to the results of Anversa's quantification of myocardial cell death patterns in rats, 86% and 14% of myocardial cell death are based on apoptosis and necrosis after 2 h of continuous ischemia, respectively⁴. Therefore, the molecular mechanism of IR cardiomyocyte apoptosis is a hot topic at present.

Fas/Fas ligand (FasL) signaling pathway was first proved to play an important role in cell apoptosis⁵. Fas is a 45 kDa membrane receptor protein (type I membrane protein) that exists on the cell surface. It is known that Fas belongs to a member of the tumor necrosis factor (TNF) receptor family⁶. FasL is a type II membrane protein with a mo-

lecular weight of about 40 kDa, which also belongs to the TNF family. It is mainly expressed in activated T lymphocytes, cluster of differentiation 4 (CD4)⁺ T cells and CD8⁺ T cells⁷. Under the control of complex signal pathways, Fas binding to FasL can initiate apoptotic signal transduction to induce apoptosis. This includes a series of steps. First, ligands induce trimerization of receptors, thus forming an apoptosis-inducing complex on the cell membrane. It includes Fas-associated protein with death domain (FADD). Once Fas binds to FasL, it can initiate lethal signal transduction through Fas molecules, eventually leading to cell death⁸.

Remifentanyl (RFT) is a fentanyl derivative, which has unique pharmacokinetic characteristics. RFT can recover quickly and predictably, and is widely applied in cardiac anesthesia⁹. With characteristics similar to some anesthetics, RFT has also been found to have a cardioprotective effect on IR. However, little research has focused on the mechanism of RFT-induced cardiac protection. This study aimed to explore the protective mechanism of RFT on IR injury in SD rats, thus providing a new direction for IR treatment.

Materials and Methods

Animal Model

A total of 36 Sprague-Dawley (SD) rats were randomly divided into the Sham group, IR group and RFT group, with 12 rats in each group. Rats were first anesthetized with 2% isoflurane by inhalation. Subsequently, all rats were placed on a heating pad in a supine position to maintain normal body temperature. Rats in the Sham group received thoracic opening and closing operation. Meanwhile, anesthetized rats were intubated and connected to the ventilator for intercostal thoracotomy, followed by closure immediately. In the IR group, the heart was exposed and the left anterior descending branch (LAD) of the coronary artery was placed under the line of sight. Then, the AD of coronary artery was separated or ligated directly with hemostatic forceps to cause ischemic myocardial infarction below the ligation site. The success of ligation was marked by a significant elevation of the S-T segment of the electrocardiogram and the darkening of the myocardium below the ligature line. After 1 h, hemostatic forceps were released, and hemoperfusion resumed. The chest was clean up and closed through squeezing. However, rats in the RFT group received intravenous instillation of RFT (50 mg/mL) for 5 min

before the operation. This investigation was approved by the Animal Ethics Committee of the Beijing Jishuitan Hospital Animal Center.

Detection of Serum Indicators

After 120 min of reperfusion, 4 mL blood (about) was collected from the abdominal aorta of rats in each group, followed by centrifugation at 3000 r/min and 4°C for 15 min. The supernatant was stored in a refrigerator at -20°C for standby application. The serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), creatine kinase (CK), lactate dehydrogenase (LDH), TNF- α (TNF- α), interleukin-6 (IL-6), and other biochemical indexes were determined. The specific operation was strictly in accordance with the instructions of the kit (Solarbio, Beijing, China).

Detection of Apoptosis via Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL)

After dewaxing at 60°C for 20 min, xylene was used for dewaxing twice for 5-10 min. Hydration was performed with gradient ethanol from high concentration to low concentration, so that the subsequent binding reaction was sufficient and uniform. Protease K was selected for incubation, and cell permeability was conducted for 10-30 min. TUNEL reaction solution (Sigma-Aldrich, St. Louis, MO, USA) was used for incubation at 37°C for 1 h. The color development reaction was carried out with 3,3'-diaminobenzidine for about 10 min. Meanwhile, the background color was controlled under the mirror for a maximum of 30 min. Finally, Phosphate-Buffered Saline (PBS) was applied for thorough washing. The results were observed under a fluorescence microscope.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Rat myocardial tissue was first ground by liquid nitrogen grinding method. 1 mL TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was then added to extract total ribonucleic acid (RNA). Next, the liquid was aspirated, transferred to a 2.5 mL Eppendorf (EP) tube and placed on ice for 15 min. 200 μ L chloroforms were added for centrifugation at 12000 g and 4°C for 15 min. The upper liquid was carefully transferred to a new EP tube. 200 μ L isopropyl alcohol reagent was added, followed by shaking for several times and incubated on ice for 10 min. Subsequently, the samples were centrifuged at 12000 g and 4°C for 15 min. The supernatant was discarded, and 2 mL 75%

Table I. RT-PCR primer sequences.

Gene name	Primer sequence	Size (bp)
Fas	F: 5'-3'CTGCATCATGATGGCCAATTCTGC3 R: 5'-3'ATGACACTAAGTCAAGTTAAAGGC	391
FasL	F: 5'-3'CAGCCCATGAATTACCCATGT R: 5'-3'ATTGTGTTGTGGTCCTTCTTCT	228
FADD	F: 5'-3'GCTGGCTCGTCAGCTCAAA R: 5'-3'ACTGTTGCGTTCTCCTTCTCT	132
GAPDH	F: 5'-3'AGGTCGGTGTGAACGGATTG R: 5'-3'GGGGTCGTTGATGGCAACA	95

ethanol was added to wash RNAs. Then, centrifugation was conducted at 12000 g and 4°C for 10 min, and the liquid was discarded. RNA precipitate was desiccated at room temperature. Finally, RNA precipitate was dissolved by adding a proper amount of RNA-free water. The concentration of extracted RNA was measured under a spectrophotometer. According to the experimental procedure provided by TaKaRa (Article No.: 2690A) (Otsu, Shiga, Japan), 1 µg RNA was taken for RT. Complementary deoxyribonucleic acids (cDNAs) obtained were stored at -20°C for subsequent use. The messenger RNA (mRNA) level of each index was measured in strict accordance with All-in-One quantitative PCR (qPCR) Mix kit. The formula for calculating the relative expression level of each mRNA was $2^{-\Delta Ct}$, in which $\Delta Ct = Ct$ (target gene) – Ct [glyceraldehyde 3-phosphate dehydrogenase (GAPDH)]. The corresponding primer sequences were shown in Table I.

Western Blotting

Rat myocardial tissue was first sheared and placed in a 2.5 mL EP tube. The mixture of 150 µL radio-immunoprecipitation assay buffer and protease phosphorylation inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO, USA) was added. Then, the EP tube was placed in a tissue homogenizer to homogenize for 10 min. Subsequently, the EP tube was taken out and ultrasonically pulverized with an ultrasonic instrument (40 A, 3 s/time, 3 times). After that, centrifugation was carried out at 12000 g and 4°C for 15 min, and the upper liquid was collected to obtain tissue protein. The concentration of protein was measured using the bicinchoninic acid assay kit (Beyotime, Shanghai, China). After denaturation of total protein, it was separated by 10% acrylamide gel and transferred onto 0.22 µm nitrocellulose membranes (Millipore, Billerica, MA, USA) for 1.5 h. After sealing with 5% skim milk for 1 h, the membranes were incubated overnight with primary monoclonal

antibodies of Fas (1:1000), FasL (1:2000), FADD (1:1000), and GAPDH (1:2000). On the next day, the membranes were incubated with anti-mouse or anti-rabbit immunoglobulin G antibodies for 1 h. Immunoreactive bands were detected by the enhanced chemiluminescence (ECL) system (Bio-Rad, Hercules, CA, USA). The relative level of the target protein was calculated by the ratio of the gray value of the target protein to that of the corresponding internal reference band.

Statistical Analysis

GraphPad Prism software (Version 5.01; La Jolla, CA, USA) was used for all statistical analysis. Measurement data were expressed by mean standard ± standard deviation. Independent *t*-test was performed to compare the difference between the two groups. One-way analysis of variance was adopted to compare the difference among the groups, followed by Post-Hoc Test (Least Significant Difference). $p < 0.05$ was considered statistically significant.

Results

Myocardial Morphology of Rats in the Three Groups Via H&E Staining

As shown in Figure 1, myocardial tissue and myofibrils in the Sham group were regular, with normal transverse striation structure and normal cell morphology. In the IR group, myocardial cells in the infarction area showed cytoplasmic condensation, fatty degeneration, nuclear condensation and even fragmentation, with inflammatory cell infiltration in local areas. Compared with those of the IR group, myocardial cells in the RFT group had significant transverse striation structure. Meanwhile, a few nuclei were condensed, and inflammatory cells were infiltrated locally. These results suggested that IR modeling was successful, and RFT pretreatment could protect the IR-induced injury.

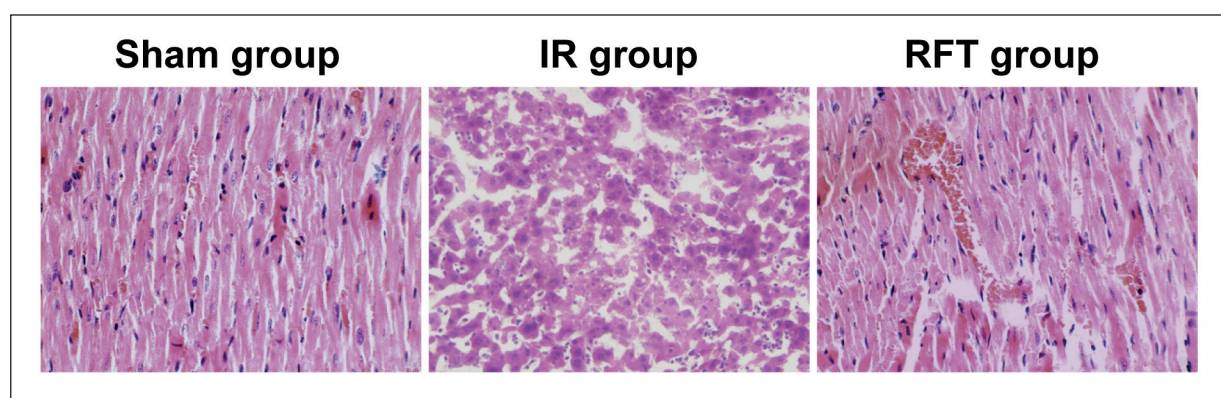


Figure 1. Morphological differences of myocardial cells in rats of each group. Note: Myocardial tissue in the Sham group had normal transverse striation structure and normal cell morphology. In the IR group, cells showed cytoplasmic condensation, fatty degeneration and nuclear condensation, with inflammatory cell infiltration in local areas. In the RFT group, a few nuclei were condensed, and inflammatory cells infiltrated locally.

Effects of RFT on Serum Indexes of IR Rats

Compared with the Sham group, the activities of LDH and CK and the level of MDA in the IR group were markedly increased, whereas the level of SOD was evidently decreased ($p<0.05$). Compared with the IR group, RFT pretreatment could significantly reduce the release of LDH and CK-muscle/brain (CK-MB), increase the level of SOD and decrease the level of MDA ($p<0.05$). This indicated that RFT had a certain protective effect on IR (Table II).

Cell Apoptosis Via TUNEL

Compared with the Sham group, the apoptosis rate of myocardial cells in the IR group was notably increased ($p<0.05$). The apoptosis rate of myocardial cells in the RFT group was significantly decreased than that of the IR group ($p<0.05$). This indicated that RFT pretreatment could markedly reduce the apoptotic level of myocardial cells (Figure 2).

Effects of RFT on Inflammatory Factors by Enzyme-Linked Immunosorbent Assay (ELISA)

The serum sample of rats in each group was collected, and the levels of inflammatory factors

were detected by ELISA. The results revealed that the serum levels of TNF- α and IL-6 proteins in each group were significantly increased when compared with the Sham group ($p<0.05$). However, compared with the IR group, the levels of TNF- α and IL-6 proteins in the RFT group were notably decreased ($p<0.05$) (Figure 3).

The mRNA Expression Level of Apoptosis Indexes Via RT-PCR

The mRNA expression levels of Fas, FasL, and FADD were detected by RT-PCR. Compared with the Sham group, the mRNA expressions of Fas, FasL, and FADD in the IR and RFT groups were markedly increased ($p<0.05$). Compared with the IR group, RFT could significantly decrease the mRNA levels of Fas, FasL, and FADD, and the differences were statistically significant ($p<0.05$) (Figure 4).

The Expression Level of Apoptotic Proteins Via Western Blotting

Western blotting was further used to detect the protein expression level of apoptosis indexes in cardiomyocytes of rats in each group. The results manifested that, compared with the Sham

Table II. Expression levels of serum indexes in each group of rats ($\bar{x}\pm s$).

Group	LDH (U·L ⁻¹)	CK (U·L ⁻¹)	MDA (μ mol/L)	SOD (μ mol/L)
Sham group	4124.5 $\pm$ 405.5	214.1 $\pm$ 82.4	35.5 $\pm$ 10.8	85.6 $\pm$ 12.4
IR group	8224.8 $\pm$ 780.6 [#]	524.2 $\pm$ 124.7 [#]	76.8 $\pm$ 13.4 [#]	26.7 $\pm$ 8.8
RFT group	6234.4 $\pm$ 572.4	350.1 $\pm$ 104.6 [*]	52.7 $\pm$ 16.8 [*]	59.5 $\pm$ 14.0 [*]

Note: [#] $p<0.05$ vs. Sham group and ^{*} $p<0.05$ vs. IR group.

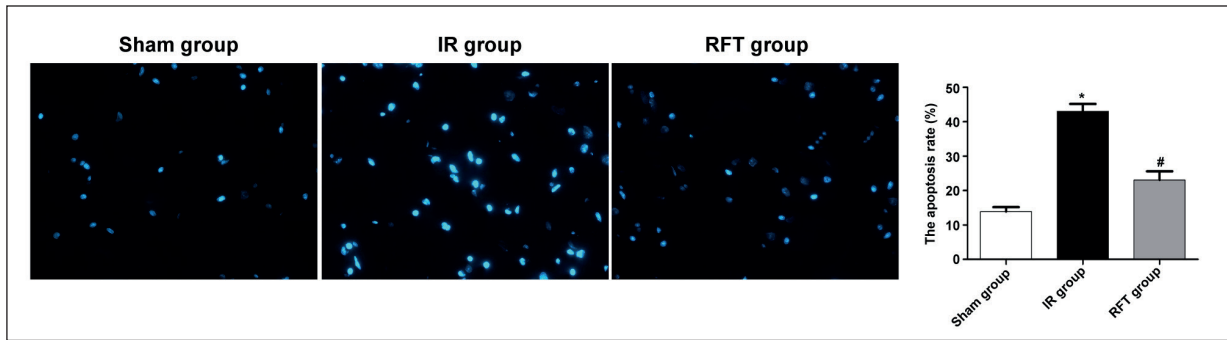


Figure 2. Effect of RFT on the apoptosis of myocardial cells in IR rats. Note: TUNEL staining was used to detect the apoptosis rate of cells. # $p < 0.05$ vs. Sham group and * $p < 0.05$ vs. IR group.

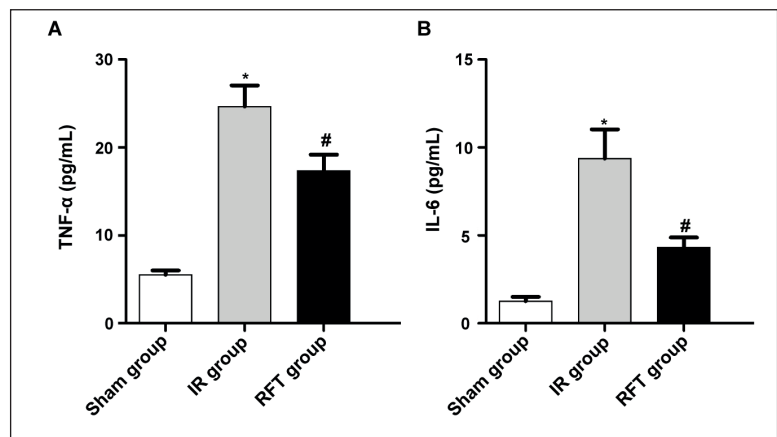


Figure 3. Effect of RFT on the expression of inflammatory factors in RI rats. Note: # $p < 0.05$ vs. Sham group and * $p < 0.05$ vs. IR group.

group, the protein expressions of Fas, FasL, and FADD in rats of the IR group and RFT group were significantly increased ($p < 0.05$). However,

RFT pretreatment significantly decreased the protein expression of apoptosis indexes in rats when compared with the IR group ($p < 0.05$). The above results indicated that RFT had an anti-apoptosis effect on myocardial cells in IR rats (Figure 5).

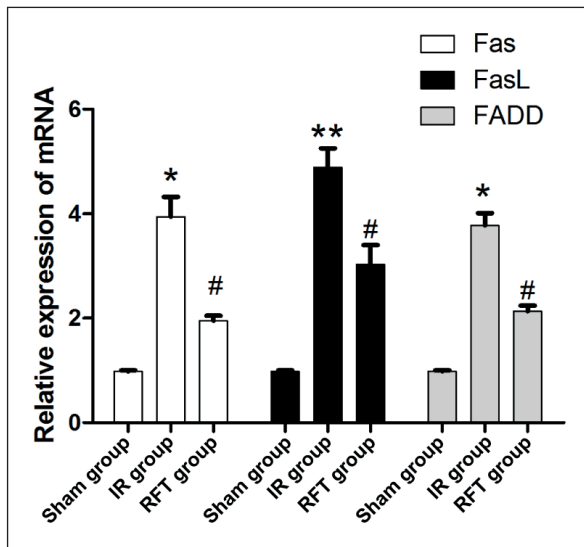


Figure 4. Effects of RFT on apoptosis indexes and Fas expression in RI rats. Note: # $p < 0.05$ vs. Sham group and * $p < 0.05$ vs. IR group.

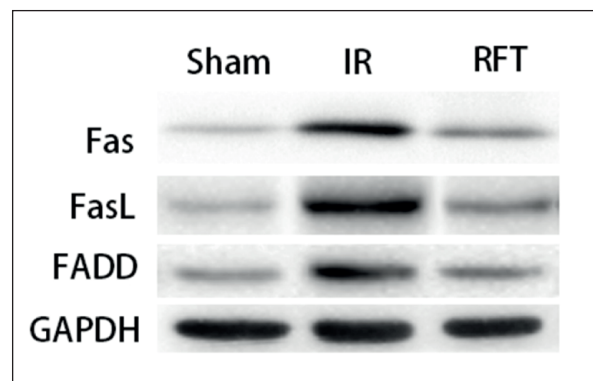


Figure 5. Effect of RFT on myocardial cell apoptosis in RI rats. Note: Fas, FasL and FADD expressions in rats of the IR group and RFT group were evidently increased, while RFT pretreatment significantly decreased the expression of apoptosis indexes.

Discussion

Apoptosis is closely related to IR-induced myocardial injury, which is also considered to be one of the crucial mechanisms of myocardial infarction in rats¹⁰. To alleviate IR injury, ischemic preconditioning is used as an effective treatment. This protective treatment may be mechanical (transient ischemia and reperfusion) or drug-based therapy¹¹. Ischemic preconditioning was first proposed as a possible treatment strategy in 1986, when it was proved to reduce infarction size of rat hearts by 75%¹². Subsequently, ischemic preconditioning was tested. It has been proved to alleviate ischemia-reperfusion in organs, including lung, kidney, liver, brain, spinal cord and intestine¹³. RFT is a synthetic opioid analgesic with ultrashort action time. Due to the reason that RFT is rapidly degraded by esterases in tissues and blood, it takes effect for only one minute. Meanwhile, the half-life (3-5 minutes) of RFT is relatively short. These unique pharmacokinetic characteristics make it an easy-to-manage pre-adaptation agent¹⁴. However, the protection mechanism of IR in RFT has not been fully clarified.

Previous studies have demonstrated that there is a good correlation between positive expression of Fas in cardiomyocytes and cardiomyocyte apoptosis. Some researches have indicated that some inflammatory cells infiltrated in and around IR myocardial infarction area are activated to express FasL. Meanwhile, inflammatory cells expressing FasL are likely to interact with neighboring myocardial cells expressing Fas. This may eventually induce myocardial cell apoptosis¹⁵. In addition, studies have revealed that the mRNA and protein expressions of Fas genes are increased after myocardial ischemia and reperfusion. The peak expressions of Fas gene appears at 24 h and 72 h after reperfusion, respectively, prior to the peak of myocardial cell apoptosis, which is 72 h after reperfusion¹⁶. Pathological reports have shown that there are myocardial necrosis foci of different sizes in myocardial tissues after IR, with more inflammatory cell infiltration around them. Cells with positive expression of Fas are mainly myocardial cells around the myocardial infarction area. However, cells with positive expression of FasL are mostly inflammatory cells infiltrating in or around the myocardial infarction area¹⁷.

In the current work, it was found that RFT pretreatment could effectively combat myocardial cell apoptosis caused by IR. After RFT treatment, myocardial cell injury was significantly improved. Meanwhile, local oxidative stress and inflammatory reaction were markedly reduced, mainly

manifested as remarkably up-regulated SOD and down-regulated MDA. It could be observed microscopically *via* H&E staining that myocardial cells in the IR group showed cytoplasmic condensation, fatty degeneration, nuclear condensation, even fragmentation and dissolution, and local inflammatory cell infiltration. However, myocardial cell injury in the RFT group was significantly reduced when compared with that of the IR group, with significant transverse striation structure, few nuclei condensation and local inflammatory cell infiltration. RFT is used for patients by means of bolus or continuous intravenous infusion. Sheng et al¹⁸ have shown that both pretreatment and continuous infusion can reduce myocardial infarction to a similar extent. Most importantly, mechanical ischemic preconditioning of the heart can increase the expression of apoptosis proteins, Fas and FasL. Fas can activate Caspase 3 by binding to its membrane expression ligand FasL^{19,20}. It can be found that RFT plays its role through proteins involved in the anti-apoptotic survival pathway. In this work, Fas and FasL were weakly positive in the Sham group, whereas their levels in isolated rat myocardium were remarkably increased in the IR model. However, in the RFT group, a significant inhibition on Fas and FasL expressions was observed. Moreover, TUNEL assay indicated that the level of apoptosis in the RFT group was significantly lower than that of the RI model group. These results indicated that RFT played a role in myocardial protection by resisting the apoptosis of cardiomyocytes in IR rats.

Conclusions

We found that RFT had a protective effect on IR myocardial cell injury in rats. The possible underlying mechanism was to inhibit the Fas/FasL signal transduction pathway, thereby inhibiting myocardial cell apoptosis and reducing oxidative stress level and inflammatory response after IR..

Conflict of Interests

The authors declare that they have no conflict of interest.

References

- 1) IBANEZ B, HEUSCH G, OVIZE M, VAN DE WERF F. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol* 2015; 65: 1454-1471.

- 2) HU YQ, CHEN W, YAN MH, LAI JJ, TANG N, WU L. Ischemic preconditioning protects brain from ischemia/reperfusion injury by attenuating endoplasmic reticulum stress-induced apoptosis through PERK pathway. *Eur Rev Med Pharmacol Sci* 2017; 21: 5736-5744.
- 3) CATTELAN A, CELOOTTO G, BOVA S, ALBIERO M, KUPUSAMY M, DE MARTIN S, SEMPLICINI A, FADINI GP, DE KREUTZENBERG SV, AVOGARO A. NAD(+)-dependent SIRT1 deactivation has a key role on ischemia-reperfusion-induced apoptosis. *Vascul Pharmacol* 2015; 70: 35-44.
- 4) ANVERSA P, CHENG W, LIU Y, LERI A, REDAELLI G, KAJSTURA J. Apoptosis and myocardial infarction. *Basic Res Cardiol* 1998; 93 Suppl 3: 8-12.
- 5) ZHANG JF, SHI LL, ZHANG L, ZHAO ZH, LIANG F, XU X, ZHAO LY, YANG PB, ZHANG JS, TIAN YF. MicroRNA-25 negatively regulates cerebral ischemia/reperfusion injury-induced cell apoptosis through Fas/FasL pathway. *J Mol Neurosci* 2016; 58: 507-516.
- 6) WANG Y, MU Y, ZHOU X, JI H, GAO X, CAI WW, GUAN Q, XU T. SIRT2-mediated FOXO3a deacetylation drives its nuclear translocation triggering FasL-induced cell apoptosis during renal ischemia reperfusion. *Apoptosis* 2017; 22: 519-530.
- 7) CHEN B, WU Z, XU J, XU Y. Calreticulin binds to Fas ligand and inhibits neuronal cell apoptosis induced by ischemia-reperfusion injury. *Biomed Res Int* 2015; 2015: 895284.
- 8) LIU Y, LIU X, WANG L, DU Y, CHEN Z, CHEN H, GUO J, WENG X, WANG X, WANG M, WANG Z. Effects of apigenin on the expression levels of B-cell lymphoma-2, Fas and Fas ligand in renal ischemia-reperfusion injury in rats. *Exp Ther Med* 2017; 14: 5345-5354.
- 9) SHEN JT, LI YS, XIA ZQ, WEN SH, YAO X, YANG WJ, LI C, LIU KX. Remifentanyl preconditioning protects the small intestine against ischemia/reperfusion injury via intestinal delta- and mu-opioid receptors. *Surgery* 2016; 159: 548-559.
- 10) MORCIANO G, GIORGI C, BONORA M, PUNZETTI S, PAVASINI R, WIECKOWSKI MR, CAMPO G, PINTON P. Molecular identity of the mitochondrial permeability transition pore and its role in ischemia-reperfusion injury. *J Mol Cell Cardiol* 2015; 78: 142-153.
- 11) LI J, WANG F, XIA Y, DAI W, CHEN K, LI S, LIU T, ZHENG Y, WANG J, LU W, ZHOU Y, YIN Q, LU J, ZHOU Y, GUO C. Astaxanthin pretreatment attenuates hepatic ischemia reperfusion-induced apoptosis and autophagy via the ROS/MAPK pathway in mice. *Mar Drugs* 2015; 13: 3368-3387.
- 12) GRISHAM MB, HERNANDEZ LA, GRANGER DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am J Physiol* 1986; 251: G567-G574.
- 13) JAESCHKE H, FARHOOD A, SMITH CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. *FASEB J* 1990; 4: 3355-3359.
- 14) LIN PT, CHEN WH, ZHENG H, LAI ZM, ZHANG LC. Involvement of AQP 1 in the cardio-protective effect of remifentanyl post-conditioning in ischemia/reperfusion rats. *Int J Clin Exp Med* 2015; 8: 12736-12745.
- 15) FILEP JG. Attenuation of lung ischemia-reperfusion injury: silencing the Fas gene. *Crit Care Med* 2016; 44: 1619-1620.
- 16) ZHENG M, SONG D, LUO Z, LU Z, WU Y, WANG W. Effect of puerarin on expression of Fas/FasL mRNA in pulmonary injury induced by ischemia-reperfusion in rabbits. *Nat Prod Commun* 2015; 10: 253-256.
- 17) LIU Y, LIU X, WANG L, DU Y, CHEN Z, CHEN H, GUO J, WENG X, WANG X, WANG M, WANG Z. Effects of apigenin on the expression levels of B-cell lymphoma-2, Fas and Fas ligand in renal ischemia-reperfusion injury in rats. *Exp Ther Med* 2017; 14: 5345-5354.
- 18) SHENG M, ZHANG G, WANG J, YANG Q, ZHAO H, CHENG X, XU Z. Remifentanyl induces cardio protection against ischemia/reperfusion injury by inhibiting endoplasmic reticulum stress through the maintenance of Zinc homeostasis. *Anesth Analg* 2018; 127: 267-276.
- 19) LIU Z, CHEN JM, HUANG H, KUZNICKI M, ZHENG S, SUN W, QUAN N, WANG L, YANG H, GUO HM, LI J, ZHUANG J, ZHU P. The protective effect of trimetazidine on myocardial ischemia/reperfusion injury through activating AMPK and ERK signaling pathway. *Metabolism* 2016; 65: 122-130.
- 20) ZHAO J, DUAN S, ZHOU J, SUN R, ZHANG L, WANG D. Mild hypothermia reduces expression of Fas/FasL and MMP-3 after cerebral ischemia-reperfusion in rats. *Iran J Basic Med Sci* 2014; 17: 454-459.