European Review for Medical and Pharmacological Sciences 2020; 24: 3915-3922

Remifentanil improves myocardial ischemia-reperfusion injury in rats through inhibiting IL-18 signaling pathway

X.-Q. NI, Z.-Y. HU

Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Abstract. – OBJECTIVE: To explore the protective effect of remifentanil against myocardial ischemia-reperfusion injury (MIRI) in rats and its mechanism.

MATERIALS AND METHODS: The rat models of IRI were established and randomly divided into 1) sham-operation group (S group), 2) IRI rat model group (M group), 3) low-dose remifentanil group (R-L group), 4) moderate-dose remifentanil group (R-M group), and 5) high-dose remifentanil group (R-H group). The rats in R-L group, R-M group, and R-H group were administrated with remifentanil at 0.4 μg/kg/min, 2 μg/kg/min, and 10 μg/kg/min, respectively. The activity of creatine kinase-MB (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in myocardial cells was detected using the automatic biochemical analyzer, and the apoptosis rate of myocardial cells was detected *via* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Moreover, the messenger ribonucleic acid (mRNA) and protein levels of related cytokines in myocardial cells were determined through quantitative Polymerase Chain Reaction (qPCR) and Western blotting, and the content of interleukin-1β (IL-1Symbol) and IL-18 in peripheral blood was detected *via* enzyme-linked immunosorbent assay (ELISA).

RESULTS: Remifentanil at different concentrations could protect myocardium from IRI, and remifentanil at 2 μg/kg/min and 10 μg/kg/min could significantly down-regulate the myocardial enzyme indexes in IRI myocardial cells ($p<0.01$). Besides, remifentanil reduced the mRNA expressions of IL-18, INF-γ, TNF-β, and IL-1β ($p<0.01$), significantly decreased the protein expression of IL-18, and raised the protein expression of IL-18BP, thereby improving myocardial pathological damage.

CONCLUSIONS: The protective mechanism of remifentanil on the myocardium of MIRI rats may be related to the inhibition on the IL-18 signaling pathway.

Key Words:
Remifentanil, IL-18 signaling pathway, Myocardial ischemia-reperfusion.

Introduction

The morbidity rate of acute myocardial infarction (AMI) is extremely high among coronary heart diseases. In the United States, there is 1 new case of AMI about every 44 s, as well as about 900,000 new or recurrent cases every year[1]. At present, MI is mainly treated with the rapid recovery of blood perfusion in the ischemic region in clinic[2]. However, the tissue injury will be further aggravated after recanalization in the case of long-term myocardial ischemia and hypoxia. Moreover, far from alleviating the myocardial injury, recanalization may cause myocarditis and apoptosis, aggravating myocardial injury, which is the so-called ischemia-reperfusion injury (IRI)[3].

IRI is closely related to oxidative stress and inflammatory factors[4,5]. As a pro-inflammatory factor, interleukin-18 (IL-18) is a member of the IL-1 gene family, which can be produced by a variety of cells, such as neutrophils and T cells[6]. IL-18 is able to improve the myocardial injury induced by ischemia-reperfusion[7]. There is a relatively high affinity between IL-18 binding protein (IL-18BP) and IL-18[8], and a large amount of IL-18BP binds to IL-18 to exert a protective effect against renal injury caused by hepatic IRI. In the lipopolysaccharide-induced acute lung injury model, it was found that remifentanil can reduce the concentration of tumor necrosis factor-α (TNF-α), thereby exerting an anti-inflammatory effect[9]. Sheng et al[10] found that remifentanil can improve the myocardial injury induced by ischemia-reperfusion. In addition, another study[11] has also confirmed that remifentanil can reduce the lactate dehydrogenase (LDH) activity and apoptosis of myocardial cells, thereby relieving IRI.

Therefore, the IRI model was established in this laboratory, and the protective mechanism of remifentanil on myocardium of IRI rats was explored from the IL-18 signaling pathway as an entry point.

Corresponding Author: Zhengyang Hu, MM; e-mail: 383836979@qq.com

Materials and Methods

Laboratory Animals

A total of 60 specific pathogen-free male Sprague-Dawley (SD) rats weighing 200-220 g were purchased from the Laboratory Animal Center of Wenzhou Medical University. This investigation was approved by the Animal Ethics Committee of Wenzhou Medical University Animal Center.

Drugs

Remifentanil was purchased from Shanghai Qitai Biotechnology Co., Ltd. (Shanghai, China) [CAS No.: 138402-11-6, purity ≥98%].

Reagents and Instruments

The detection kits of related myocardial enzyme indexes creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), and LDH were purchased from Shanghai YS Industrial Co., Ltd. (Shanghai, China); the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) apoptosis kit from Shanghai KeyGEN Biotech Co., Ltd. (Shanghai, China); the quantitative Polymerase Chain Reaction (qPCR) reagents from TOYOBO (Tokyo, Japan), the rabbit anti-IL-18 (ab191860), IL-18BP (ab52914) and β-actin (ab179467) antibodies used in Western blotting from Abcam (Cambridge, MA, USA), and horseradish peroxidase (HRP)-coupled secondary antibodies from Proteintech (Wuhan, China).

Multi-lead electrophysiological recorder ABD Serotec (Kidlington, Oxford, UK), SmartChem full-automatic biochemical analyzer (Alliance, France), Olympus IX-71 binocular fluorescence microscope (Olympus, Tokyo, Japan), SpectraMax Paradigm multifunctional microplate reader (Novus, Littleton, CO, USA), and StepOne Plus real-time fluorescence qPCR instrument (Bio-Rad, Hercules, CA, USA).

Grouping and Drug Administration

The above 60 male SD rats were randomly divided into 1) sham-operation group (S group, n=12), 2) IRI rat model group (M group, n=12), 3) low-dose remifentanil group (R-L group, n=12), 4) moderate-dose remifentanil group (R-M group, n=12), and 5) high-dose remifentanil group (R-H group, n=12). The rats in R-L group, R-M group, and R-H group were administrated with an equal amount of remifentanil at 0.4 μg/kg/min, 2 μg/kg/min, and 10 μg/kg/min *via* gavage, respectively, while those in S group and M group were gavaged with an equal amount of normal saline, once a day for 7 consecutive days.

Establishment of Rat Model of MIRI

In this experiment, according to the IRI modeling method, the proximal left anterior descending coronary artery was ligated for 30 min. Only operation was performed without ligation for simple myocardial ischemia in S group, while the slipknot was released after 30 min for reperfusion for 2 h in the other four groups.

Detection of Effects of Remifentanil on Activity of Serum Myocardial Enzymes CK-MB, AST, and LDH in IRI Rats Using Full-Automatic Chemical Analyzer

The serum samples in each group were collected and placed in the heparin tube at room temperature for 20 min, followed by centrifugation at 3000 rpm for 20 min. Then, the supernatant was collected to detect the concentration of CK-MB, AST, and LDH using the full-automatic biochemical analyzer.

Detection of Myocardial Apoptosis Using TUNEL Assay

After the rats were sacrificed, the heart tissues were taken and fixed with 4% paraformaldehyde overnight. On the next day, the tissues were taken out, dehydrated with ethanol at different concentrations, transparentized with xylene I and xylene II, and embedded into paraffin blocks. Then, the blocks were sliced into 4 μm-thick sections using the microtome, spread flat, and dried on the slide. The sections were placed in an oven at 60°C for 3 h and then taken out, followed by deparaffinization and hydration. DNase-free proteinase K (20 μg/mL) was dropwise added onto the sections for incubation at room temperature for 20-30 min, and then proteinase K was washed away with phosphate-buffered saline (PBS). 50 μL of TUNEL assay solution was dropwise added onto the sections for reaction in a dark place at 37°C for 40-60 min. Then, the sections were washed with PBS, sealed, and observed microscopically, and the apoptotic myocardial cells were counted in each group.

Detection of Messenger Ribonucleic Acid (mRNA) Expression Levels of IL-18, IL-1β, TNF-α, and INF-γ in Myocardial Cells Via QPCR

The total RNA was extracted from myocardial tissues using the spin-column in each group, and transcribed into complementary deoxyribonucleic acids (cDNAs) according to the instructions of the

ReverTra Ace qPCR RT Kit. Then, 20 μL of reaction system was prepared using the synthesized cDNA according to the instructions of the SYBR™ Green Real-Time PCR Master Mix, followed by real-time qPCR using the StepOnePlus (Thermo Fisher Scientific, Waltham, MA, USA) instrument. The thermal cycle conditions are as follows: 95°C for 60 s, 95°C for 15 s, –60°C for 15 s, –72°C for 45 s (40×). With β-actin as an internal reference gene, the relative expression levels of target genes in each group were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table I.

Western Blotting

The left ventricular myocardial tissues were lysed with radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China), and centrifuged at 12,000 rpm and 4°C for 10 min. The total protein was collected and the protein concentration in each group was quantified using bicinchoninic acid (BCA; Solarbio Biotechnology, Beijing, China). Then, 5 μL of proteins were loaded and separated *via* 10% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE). The target band was cut according to the marker, transferred onto a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) for 90 min, sealed with 5% skim milk or 5% albumin from bovine serum (BSA) prepared by Tris-Buffered Saline with 0.1% Tween-20 (TBST) for 2 h, and incubated with IL-18 and IL-18BP primary antibodies and internal reference antibody β-actin (1:1000) at 4°C overnight. After the membrane was washed with TBST for 3 times (10 min/time), the protein was incubated again with horseradish peroxidase (HRP)-coupled secondary antibodies (1:5000) at room temperature for 2 h. Next, the membrane was washed again with TBST for 3 times (10 min/time). Finally, the protein band was detected using enhanced chemiluminescence (ECL) solution (Beijing 4A Biotech Co., Ltd., Beijing, China), and the optical density (OD) was detected using the Image-Pro Plus 6.0 (Media Cybernetics, Silver Springs, MD, USA) image analyzer.

Detection of Content of IL-18 and IL-1β in Peripheral Blood Through ELISA

The blood samples in each group were collected, coagulated at room temperature, and centrifuged for 10 min. The serum was taken, and the standards were prepared according to the instructions of the ELISA kit. The reaction was terminated with stop buffer. Then, the OD values of IL-18 and IL-1β in each group were detected using the microplate reader, and the standard curves were plotted, based on which the concentration of IL-18 and IL-1β in each group was determined.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 (SPSS, Chicago, IL, USA) software was used for data analysis. The measurement data were expressed as mean ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) was performed for the comparison among groups. $p<0.05$ suggested the statistically significant difference.

Results

Effects of Remifentanil on Activity of Serum Myocardial Enzymes CK-MB, AST, and LDH in IRI rats

Compared with that in S group, the activity of serum myocardial enzymes CK-MB, AST and LDH in M group was significantly increased ($p<0.001$), indicating that the IRI model was established successfully in this experiment. Compared with that in group M, the activity of myocardial enzymes CK-MB, AST, and LDH declined after intervention in IRI rats with remifentanil at different concentrations, and it was down-regulated more significantly by moderate- and high-concentration remifentanil ($p<0.01$ or $p<0.001$), indicating that moderate- and high-concentration remifentanil can effectively alleviate MIRI (Table II).

Table I. qPCR primer sequences.

Target gene	Forward	Reverse
IL-18	5'-GCCTCTATTTGAAGATATGACTGA-3'	5'- GAGATAGTACAGCCATACCTCTA-3'
TNF-α	5'-GGCTTTCGGAACTCACTGGA-3'	5'-GGGAACAGTCTGGGAAGCTC-3'
IFN-γ	5'-AAGACAACCAGGCCATCAGCAA-3'	5'-GAACTTGGCGATGCTCATGAATGC-3'
β-actin	5'-TGACAGGATGCAGAAGGAGA-3'	5'-TAGAGCCACCAATCCACACA-3'

Table II. Effects of remifentanil on serum myocardial enzymes in IRI rats (χ±s).

Group	CK-MB (U/L)	AST (U/L)	LDH (U/L)
S	896.33±227.80	24.87±10.83	988.57±181.70
M	1992.83±367.30***	53.57±17.84***	1711.83±158.28***
R-L	1639.24±223.97$^{\Delta\Delta}$	46.87±13.19	1594.84±230.35
R-M	1494.84±190.45$^{\Delta\Delta\Delta}$	35.03±12.67$^{\Delta\Delta}$	1392.67±245.21$^{\Delta\Delta\Delta}$
R-H	1041.50±183.24$^{\Delta\Delta\Delta}$	23.88±14.88$^{\Delta\Delta\Delta}$	1011.15±179.49$^{\Delta\Delta\Delta}$

***p<0.001 *vs.* S group, $^{\Delta\Delta}p$<0.01, $^{\Delta\Delta\Delta}p$<0.001 *vs.* M group

Effect of Remifentanil on Myocardial Apoptosis Rate in IRI Rats

In TUNEL assay, the nucleus stained green indicated the apoptotic myocardial cells. Compared with that in S group, the TUNEL-positive rate of cells in IRI rats was significantly increased in M group (p<0.001). Compared with that in M group, the TUNEL-positive rate of cells in IRI rats declined after intervention with remifentanil at different concentrations, showing a statistically significant difference (p<0.01 or p<0.001). The above results suggest that remifentanil can protect myocardial cells in IRI rats from apoptosis to some extent (Table III, Figure 1).

Effects of Remifentanil on mRNA levels of IL-18, IL-1β, TNF-α, and INF-γ in IRI Rats

The changes in the relative mRNA expression levels of IL-18, IL-1β, TNF-α, and INF-γ were studied using qPCR. As shown in Figure 2, the mRNA expression levels of IL-18, IL-1β, TNF-α, and INF-γ were evidently increased in M group compared with those in S group (p<0.001), indicating that the IL-18 inflammatory pathway is activated after myocardial ischemia-reperfusion. Compared with those in M group, the mRNA expression levels of IL-18, IL-1β, TNF-α, and INF-γ declined in a dose-dependent manner after intervention in IRI rats with remifentanil at different concentrations, while they had significant differences in R-H group compared with those in M group (p<0.001). The above results further demonstrate that remifentanil can inhibit the inflammatory effect caused by myocardial ischemia-reperfusion.

Table III. Myocardial apoptosis in IRI rats detected using TUNEL assay (χ±s).

Group	Apoptosis rate (%)
S	3/17±1.17
M	53.83±7.73***
R-L	38.67±6.44$^{\Delta\Delta}$
R-M	18.17±6.11$^{\Delta\Delta\Delta}$
R-H	10.33±3.27$^{\Delta\Delta\Delta}$

***p<0.001 *vs.* S group, $^{\Delta\Delta}p$<0.01, $^{\Delta\Delta\Delta}p$<0.001 *vs.* M group.

Effects of Remifentanil on Protein Expression Levels of IL-18 and IL-18BP in IRI Rats

Western blotting was performed to study the abnormalities of IL-18 signal transduction in myocardium of IRI rats. As shown in Figure 3, the IL-18 expression was increased and the IL-18BP expression was evidently down-regulated in M group compared with those in S group. After treatment with remifentanil, the IL-18 expression was decreased and the IL-18BP expression was increased in myocardium of IRI rats. It further demonstrates that remifentanil can suppress the activation of IL-18 signaling pathway in myocardium of IRI rats.

Effects of Remifentanil on Content of IL-18 and IL-1β in Peripheral Blood of IRI Rats

The content of IL-18 and IL-1β in peripheral blood of IRI rats was analyzed using ELISA. It was found that compared with S group, M group had relatively higher content of IL-18 and IL-1β, while the content of IL-18 and IL-1β remarkably declined after treatment with remifentanil in IRI rats (Figure 4).

Discussion

Ischemic cardiomyopathy is a disease with a high mortality rate, and its commonly-used clinical therapeutic method is coronary recanalization[12]. However, the tissue injury will be further aggravated after recanalization in the case of long-term myocardial ischemia and hypoxia. Moreover, far from alleviating the myocardial injury,

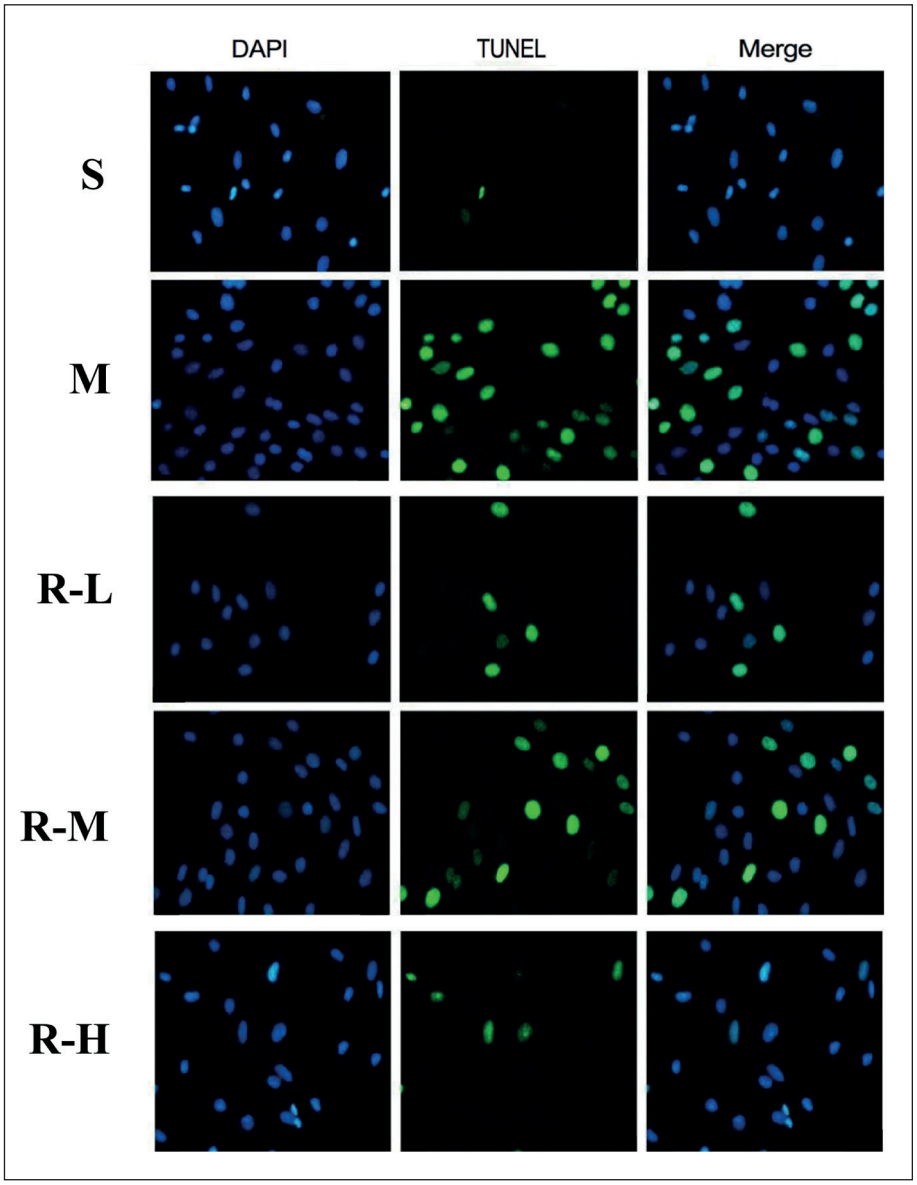

Figure 1. Effect of remifentanil on myocardial apoptosis rate in IRI rats (400 X).

recanalization may cause myocarditis and apoptosis, aggravating myocardial injury, which is the so-called ischemia-reperfusion injury (IRI)[3].

Remifentanil is a kind of analgesic drug with a strong analgesic effect, about 250 times that of morphine[13]. In the lipopolysaccharide-induced acute lung injury model, it was found that remifentanil can reduce the concentration of TNF-α, thereby exerting an anti-inflammatory effect[9]. Moreover, remifentanil has a protective effect on the uterus, small intestine, and liver during IRI[14-16]. Sun et al[17] established the rat model of IRI and injected remifentanil *via* caudal vein, and it was found that remifentanil can lower the CK-MB concentration and reduce the MI area, thereby protecting the myocardium in the rat model of IRI. Dou et al[11] showed that remifentanil reduces the LDH activity and apoptosis of myocardial cells, thereby improving IRI, which may be related to the δ receptor-mediated PI3K/AKT pathway. Besides, Kim et al[18] found that remifentanil can reduce the MI area after IRI, and such a cardioprotective effect is related to the decrease of expressions of pro-apoptotic proteins. In coronary artery bypass surgery with extracorporeal circulation, remifentanil infused at 0.5 μg/kg/min for 30 min can lower the expressions of CK-MB and cTnI[19]. In this experiment, the activity of serum myocardial enzymes CK-MB, AST, and LDH in M group was significantly increased compared with that in S group, indicating that the

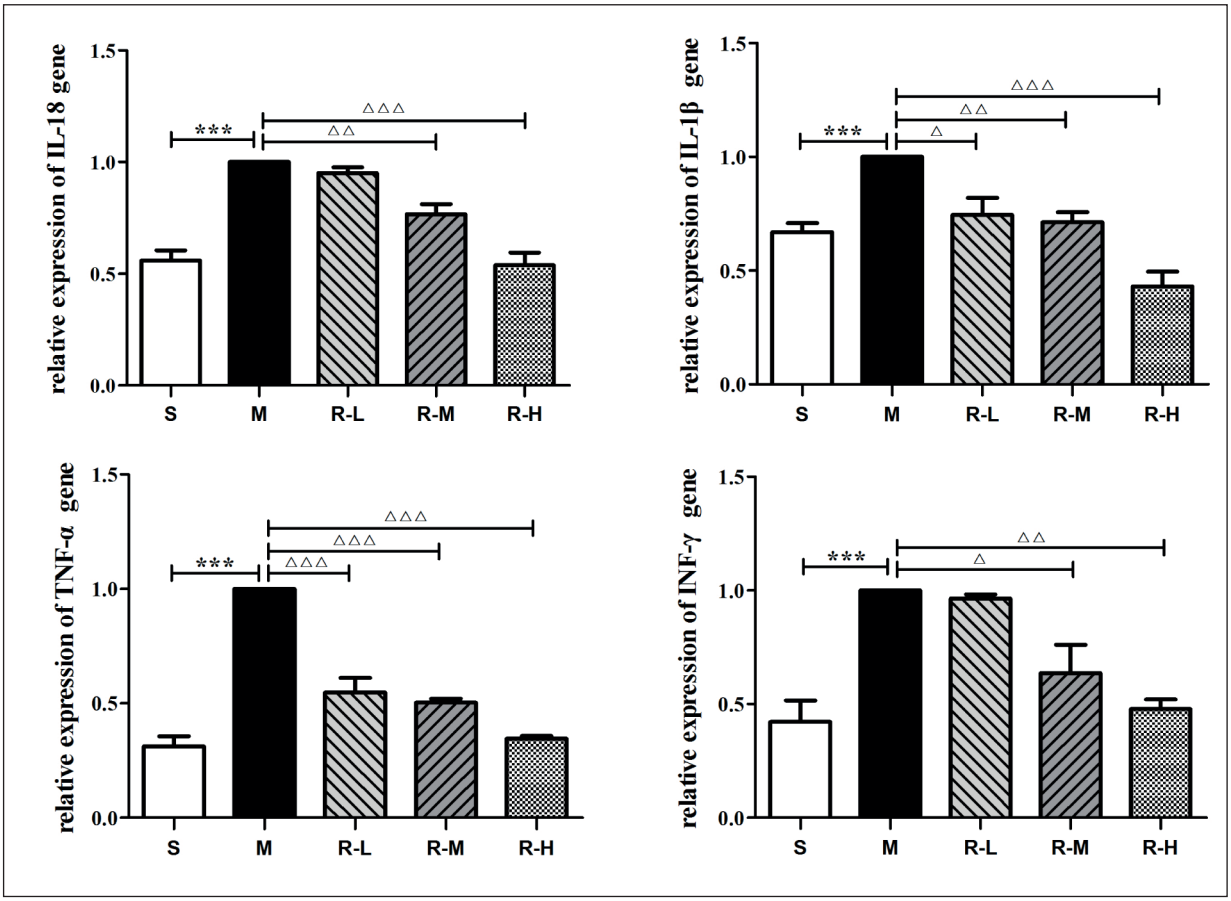

Figure 2. Effects of remifentanil on mRNA levels of IL-18, IL-1β, TNF-α, and INF-γ in IRI rats (χ±s). ***p<0.001 *vs.* S group, $^{\Delta}p$<0.05, $^{\Delta\Delta}p$<0.01, $^{\Delta\Delta\Delta}p$<0.001 *vs.* M group.

IRI model was established successfully. Compared with that in group M, the activity of myocardial enzymes CK-MB, AST, and LDH declined after intervention with low-, moderate-, and high-concentration remifentanil, and it was down-regulated more significantly by moderate- and high-concentration remifentanil, which suggests that remifentanil can improve IRI. In addition, compared with that in S group, the TUNEL-positive rate of cells was significantly increased in M group. After intervention with low-, moderate-, and high-concentration remifentanil, the TUNEL-positive rate of cells significantly declined, demonstrating that remifentanil can reduce the expression of apoptosis protein and exert a protective effect on the heart.

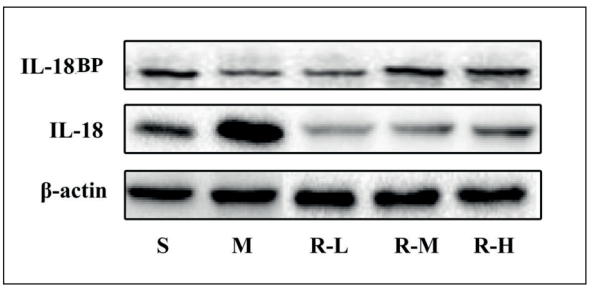

Figure 3. Effects of remifentanil on protein expression levels of IL-18 and IL-18BP in IRI rats (χ±s).

It has been confirmed that the mechanism of IRI is associated with a variety of factors, such as calcium overload, mitochondrial damage, and inflammation[20-23]. Hydrogen sulfide has a protective effect on IRI in rats, which has a certain correlation with the decrease of such inflammatory factors as IL-18[24]. IL-18 is a pro-inflammatory factor produced by a variety of cells, such as neutrophils[6]. IL-18BP, also known as IL-18 binding protein, can bind to IL-18 to block the production of IFN-γ, and IL-18 can also induce the production of TNF-α, IL-1β, etc[25,26]. Mallat et al[6] found that the expressions of IL-18 and IL-18Rα rise, and the IL-18BP expression declines

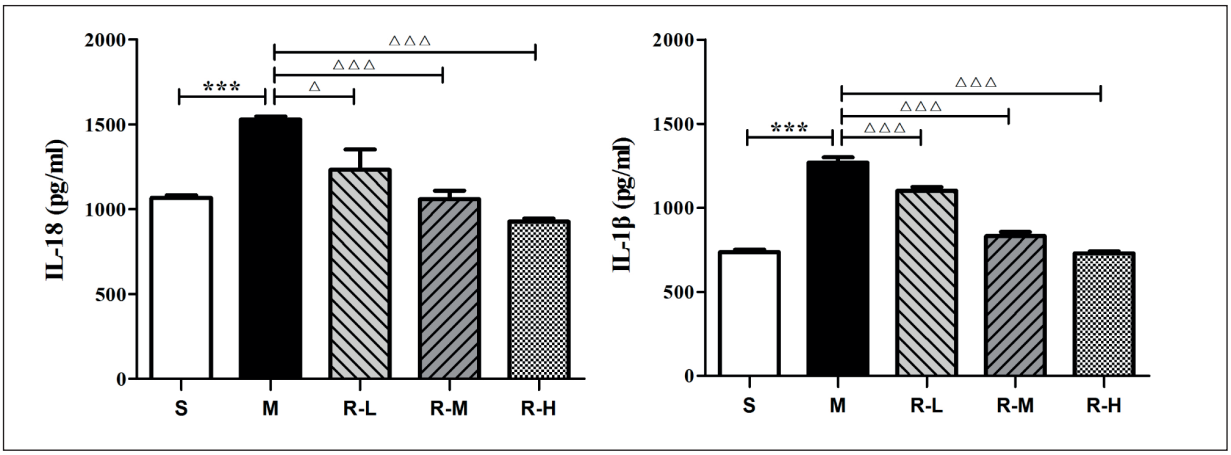

Figure 4. Effects of remifentanil on content of IL-18 and IL-1β in peripheral blood of IRI rats ($\bar{\chi}$±s). ***p<0.001 *vs.* S group, $^{\Delta}p$<0.05, $^{\Delta\Delta\Delta}p$<0.001 *vs.* M group.

in myocardium of heart failure patients compared with those in normal people. Also, they observed through immunohistochemistry that besides endothelial cells and macrophages, IL-18 is also expressed in myocardial cells infiltrated by inflammatory cells. Pomerantz et al[7] established the IRI model using the isolated atrial muscles, and found that the mRNA expression level of IL-18 rises and the myocardial systolic function declines, but the expression of IL-18 in myocardial cells is decreased and the myocardial systolic function is improved after IL-18BP is used[7]. Moreover, the mouse model of hepatic IRI was established and treated with remifentanil by researchers, and the results manifested that remifentanil can protect the liver of mice from IRI by up-regulating IL-18BP[27]. In this study, compared with those in S group, the mRNA expression levels of IL-18, IL-1β, TNF-α, and INF-γ were evidently increased, the protein expression of IL-18 was increased, the protein expression of IL-18BP was down-regulated, and the content of IL-18 and IL-1β in peripheral blood was evidently increased in M group. After intervention with low-, moderate-, and high-concentration remifentanil, the mRNA expression levels of IL-18, IL-1β, TNF-α, and INF-γ declined, the content of IL-18 and IL-1β in peripheral blood was evidently decreased, the protein expression of IL-18 was down-regulated, and the protein expression of IL-18BP was increased. The above findings demonstrate that remifentanil can reduce the production of IL-1β, TNF-α, and INF-γ by inhibiting the IL-18 signaling pathway.

Conclusions

In summary, remifentanil can improve myocardial IRI by inhibiting the IL-18 signaling pathway, which provides a new idea for the clinical treatment of IRI.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Xi H, Yu RH, Wang N, Chen XZ, Zhang WC, Hong T. Serum potassium levels and mortality of patients with acute myocardial infarction: a systematic review and meta-analysis of cohort studies. Eur J Prev Cardiol 2019; 26: 145-156.
2) Heusch G, Gersh BJ. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. Eur Heart J 2017; 38: 774-784.
3) 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.
4) Wang J, De-Qiong X, Hong DQ, Zhang QQ, Zhang J. Attenuation of myocardial ischemia reperfusion injury by geniposide preconditioning in diabetic rats. Curr Res Transl Med 2019; 67: 35-40.
5) Li J, Hu HP, Li Y, Shao W, Zhang JZ, Wang LM. Influences of remifentanil on myocardial ischemia-reperfusion injury and the expressions of

Bax and Bcl-2 in rats. Eur Rev Med Pharmacol Sci 2018; 22: 8951-8960.

6) Mallat Z, Heymes C, Corbaz A, Logeart D, Alouani S, Cohen-Solal A, Seidler T, Hasenfuss G, Chvatchko Y, Shah AM, Tedgui A. Evidence for altered interleukin 18 (IL)-18 pathway in human heart failure. FASEB J 2004; 18: 1752-1754.

7) Pomerantz BJ, Reznikov LL, Harken AH, Dinarello CA. Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta. Proc Natl Acad Sci U S A 2001; 98: 2871-2876.

8) Gonul Y, Ozsoy M, Kocak A, Ozkececi ZT, Karavelioglu A, Bozkurt MF, Cartilli O, Keles I, Kocak H, Celik S. Antioxidant, antiapoptotic and inflammatory effects of interleukin-18 binding protein on kidney damage induced by hepatic ischemia reperfusion. Am J Med Sci 2016; 351: 607-615.

9) Zhang Y, Du Z, Zhou Q, Wang Y, Li J. Remifentanil attenuates lipopolysaccharide-induced acute lung injury by downregulating the NF-kappaB signaling pathway. Inflammation 2014; 37: 1654-1660.

10) Sheng M, Zhang G, Wang J, Yang Q, Zhao H, Cheng X, Xu Z. Remifentanil induces cardio protection against ischemia/reperfusion injury by inhibiting endoplasmic reticulum stress through the maintenance of zinc homeostasis. Anesth Analg 2018; 127: 267-276.

11) Dou MY, Wu H, Zhu HJ, Jin SY, Zhang Y, He SF. Remifentanil preconditioning protects rat cardiomyocytes against hypoxia-reoxygenation injury via delta-opioid receptor mediated activation of PI3K/Akt and ERK pathways. Eur J Pharmacol 2016; 789: 395-401.

12) Moran AE, Forouzanfar MH, Roth GA, Mensah GA, Ezzati M, Murray CJ, Naghavi M. Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study. Circulation 2014; 129: 1483-1492.

13) Porter-Stransky KA, Bentzley BS, Aston-Jones G. Individual differences in orexin-I receptor modulation of motivation for the opioid remifentanil. Addict Biol 2017; 22: 303-317.

14) Yu CK, Li YH, Wong GT, Wong TM, Irwin MG. Remifentanil preconditioning confers delayed cardioprotection in the rat. Br J Anaesth 2007; 99: 632-638.

15) Atalay YO, Aktas S, Sahin S, Kucukodaci Z, Ozakpinar OB. Remifentanil protects uterus against ischemia-reperfusion injury in rats. Acta Cir Bras 2015; 30: 756-761.

16) Shen JT, Li YS, Xia ZQ, Wen SH, Yao X, Yang WJ, Li C, Liu KX. Remifentanil preconditioning protects the small intestine against ischemia/reperfusion injury via intestinal delta- and mu-opioid receptors. Surgery 2016; 159: 548-559.

17) Sun HT, Xue FS, Liu KP, Sun L, Xu YC, Liao X, Yang QY, Zhang YM. [Effect of remifentanil preconditioning on myocardial ischemia-reperfusion injury]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2009; 31: 612-615.

18) Kim HS, Cho JE, Hong SW, Kim SO, Shim JK, Kwak YL. Remifentanil protects myocardium through activation of anti-apoptotic pathways of survival in ischemia-reperfused rat heart. Physiol Res 2010; 59: 347-356.

19) Wong GT, Huang Z, Ji S, Irwin MG. Remifentanil reduces the release of biochemical markers of myocardial damage after coronary artery bypass surgery: a randomized trial. J Cardiothorac Vasc Anesth 2010; 24: 790-796.

20) Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord E, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa A, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 2014; 515: 431-435.

21) Yang CF. Clinical manifestations and basic mechanisms of myocardial ischemia/reperfusion injury. Ci Ji Yi Xue Za Zhi 2018; 30: 209-215.

22) Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annu Rev Pharmacol Toxicol 2017; 57: 535-565.

23) Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol 2014; 11: 255-265.

24) Zhang Y, Peng L, Yu X. [Protective effect of hydrogen sulfide on rats with myocardial ischemia/reperfusion injury and its mechanism]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2015; 31: 316-320.

25) Puren AJ, Razeghi P, Fantuzzi G, Dinarello CA. Interleukin-18 enhances lipopolysaccharide-induced interferon-gamma production in human whole blood cultures. J Infect Dis 1998; 178: 1830-1834.

26) Dinarello CA. IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999; 103: 11-24.

27) Liu X, Yang H, Liu Y, Jiao Y, Yang L, Wang X, Yu W, Su D, Tian J. Remifentanil upregulates hepatic IL-18 binding protein (IL-18BP) expression through transcriptional control. Lab Invest 2018; 98: 1588-1599.