Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of propofol (PPF) on myocardial ischemia-reperfusion injury (MI-RI) by inhibiting the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, and to explore the possible underlying mechanism.

MATERIALS AND METHODS: A total of 60 Sprague-Dawley (SD) rats were randomly divided into 5 groups, including the Sham group (n=12), the MIRI model group (n=12), the PPF pretreatment group (n=12), the RG81640-CH (RG) pretreatment group (n=12) and the PPF+RG pretreatment group (n=12). The hemodynamic parameters of rats in each group were measured. Serum samples were collected from rats in each group. Meanwhile, the levels of lactate dehydrogenase (LDH), creatine kinase-muscle/brain (CK-MB), nicotinamide adenine dinucleotide+ (NAD+) and inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemotactic protein (MCP), were detected by enzyme-linked immunosorbent assay (ELISA). Myocardial infarction area of rats in each group was detected via 2,3,5-triphenyl tetrazolium chloride (TTC) staining. Moreover, the JAK/STAT pathway, as well as apoptosis indexes in myocardial cells of rats, were detected via Western blotting.

RESULTS: Compared with the Sham group, the contents of LDH, CK-MB, NAD+ and inflammatory factors, as well as the area of myocardial infarction were significantly increased in the MIRI group (p<0.05). In terms of hemodynamic parameters, the left ventricular end-diastolic pressure (LVEDP) was significantly increased in the MIRI group. However, heart rate (HR), left ventricular developed pressure (LVDP) and maximal rate of the increase/decrease of left ventricular pressure (±dp/dtmax) were remarkably increased (p<0.05). No significant differences in each index were found between the PPF + RG group and the MIRI group (p>0.05). Western blotting revealed that the protein level of B-cell lymphoma-2 (Bcl-2) was remarkably increased, while the activity of Caspase-3 was decreased in the PPF group when compared with the MIRI group (p<0.05). In addition, the protein expression levels of JAK1, STAT1 and STAT3 in the PPF group were significantly decreased than those of the MIRI group (p<0.05). However, completely opposite trends were found in the RG group.

CONCLUSIONS: PPF reduces the release of inflammatory factors and alleviates tissue damage caused by myocardial apoptosis in MIRI rats by inhibiting the activation of the JAK/STAT pathway. Our findings indicate that PPF has a certain myocardial protective effect on MIRI.

Key Words: Propofol (PPF), JAK/STAT, Myocardial ischemia-reperfusion, Apoptosis.

Introduction

Myocardial ischemia-reperfusion injury (MIRI) is one of the major causes of death and disability in the world nowadays. The main mechanism of MIRI is that the decline of blood supply to heart leads to myocardial ischemia and hypoxia. This may also affect the normal energy metabolism of the heart, thereby producing clinical symptoms such as angina and arrhythmia. In thrombolytic therapy and percutaneous coronary intervention, timely and effective reperfusion therapy is the choice to reduce acute myocardial ischemic injury and limit the size of myocardial infarction. However, the reperfusion process can result in myocardial death, namely myocardial reperfusion injury. In addition, there have been no effective therapeutic methods yet.
Propofol (PPF), also known as 2,6-diisopropylphenol, is a rapid- and short-acting intravenous anesthetic. PPF has been widely applied in sedation after cardiac surgery and coronary artery surgery in the intensive care unit (ICU), which also has a good pharmacokinetic feature of rapid recovery after withdrawal\(^1\). Studies have found that PPF has various non-anesthetic effects, such as organ protective effect that can scavenge free radicals \textit{in vitro}\(^4\). In addition, PPF also inhibits transmembrane calcium currents in ventricular myocytes, thereby potentially helping to reduce the severity of MIRI\(^5\). Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is a stress response mechanism that delivers signals from the cell surface to the nucleus, thus regulating gene expression. Latest researches have found that the JAK/STAT pathway can be quickly activated by myocardial ischemia-reperfusion\(^6\). However, the direct effects of PPF and JAK/STAT signaling pathway on MIRI have not been reported yet. Therefore, the aim of this study was to investigate the effect of PPF on IRI.

In this work, the effects of single and combined pretreatment with PPF on apoptosis of MIRI were detected, and the possible underlying mechanism was explored. Our investigation might provide a new theoretical and experimental basis for the prevention and treatment of MIRI.

**Materials and Methods**

**Establishment of a MIRI Model in Rats**

A total of 60 Sprague-Dawley (SD) rats were randomly divided into 5 groups, including the Sham group (n=12), the MIRI model group (n=12), the PPF pretreatment group (n=12), the RG81640-CH (RG) pretreatment group (n=12) and the PPF+RG pretreatment group (n=12). This study was approved by the Animal Ethics Committee of Capital Medical University Animal Center.

Establishment of a MIRI model: SD rats were anesthetized \textit{via} intraperitoneal injection of 10% chloral hydrate (330 mg·kg\(^{-1}\)) and fixed in supine position. After shaving the hair and disinfection, the neck was cut, and the right common carotid artery was separated for standby application. The trachea was exposed and cut. The tracheal cannula was placed and connected to the rodent ventilator for positive pressure ventilation (respiratory frequency: 60-70 times/min). The electrode was subcutaneously inserted into the four limbs and connected to the BL-420S biological functional experiment system. Meanwhile, the II-lead electrocardiogram was continuously recorded. The skin was cut longitudinally at the left border of the sternum, and the chest was opened between the 4\(^{\text{th}}\) and 5\(^{\text{th}}\) intercostal spaces layer by layer. The pericardium was cut to fully expose the heart. 5-0 nondestructive silk thread was inserted through the surface layer of the heart (about 1.5 mm deep) at 2 mm below the left atrial appendage. Subsequently, the rubber band was used as the bottom, followed by ligation of the left anterior descending coronary artery for 30 min of myocardial ischemia. Then the ligature was loosened for 6 h of reperfusion. The signs of successful establishment of the MIRI model were as follows: paleness or cyanosis of local myocardial tissues in ischemia, ST-segment elevation or T-wave towering in electrocardiogram; reddening of myocardial tissues in the ischemic region in reperfusion, and decline in the significant ST-segment elevation by more than 50%.

30 min before ligation, PPF (2 mg/kg, Selleck Chemical, Houston, TX, USA) and/or JAK/STAT agonist RG81640-CH (5 mg/kg, Sino Biological) was injected into the jugular vein of rats. 6 h after reperfusion, a 1.4f catheter tip micro-manometer (Aria, Millar Instruments, Houston, TX, USA) was inserted into the left ventricle through the right carotid artery for hemodynamic measurement. The first-order derivative of left ventricular pressure (dp/dt\(_{\text{max}}\)) was analyzed using the PowerLab SP software (AD Instruments, Shanghai, China). Echocardiography (15 MHz) was performed. Meanwhile, left ventricular contraction fraction, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, etc., were calculated.

**Determination of Myocardial Infarction Area**

The left anterior descending artery was blocked, and 1 mL 1.0% Evans blue (Sigma-Aldrich, St. Louis, MO, USA) was injected into the jugular vein to delineate non-ischemic tissues. Then the heart was resected, washed with phosphate-buffered saline (PBS), and sliced into four-layer transverse sections. Subsequently, the sections were stained with 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) for 5 min, and the area of myocardial infarction was determined. Left ventricle and infarction areas were measured using ImageJ software. The area of infarction was expressed as the percentage of infarction area in the left ventricle region.
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Detection of Biochemical Indexes
At 120 min after reperfusion, about 4 mL of blood was collected from the abdominal aorta of rats in each group. After centrifugation at 3000 rpm, 4°C for 15 min, the supernatant was collected and cryopreserved in a refrigerator at -20°C. The serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), creatine kinase (CK), CK-MB, catalase (CAT), glutathione peroxidase (GSH-Px), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were measured in strict accordance with the instructions of the relative kit.

Western Blotting
A total of 40 μg protein samples were loaded for electrophoresis and were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% skim milk at room temperature for 1 h, the membranes were incubated with primary antibodies of JAK1 (1:1 000, Proteintech, Rosemont, IL, USA), STAT1, STAT3, Bcl-2 and Caspase-3 (1:1000, Abcam, Cambridge, MA, USA) at 4°C overnight. After washing with Tris-Buffered Saline with Tween-20 (TBST), the membranes were incubated with the corresponding horseradish peroxidase-labeled secondary antibodies (1:5000, Beyotime Biotechnology Co., Ltd., Shanghai, China) at room temperature for 2 h. Immunoreactive bands were visualized by the enhanced chemiluminescence method (Bio-Rad, Hercules, CA, USA), and gray-scale analysis was performed by the gel analyzer. The relative amount of target protein = gray value target protein / gray value internal reference band

Statistical Analysis
Statistical Product and Service Solutions 13.5 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Measurement data were expressed as (x±s). One-way analysis of variance was used for comparing the differences among groups, followed by Post-Hoc Test (Least Significant Difference). p<0.05 was considered statistically significant.

Results
Expression Levels of LDH, CK-MB, and NAD+ in Each Group
The contents of LDH, CK-MB and NAD+, as well as the area of myocardial infarction were found significantly increased in the MIRI group when compared with the Sham group (p<0.05). They were significantly decreased in the PPF group when compared with the MIRI group. Meanwhile, they were also remarkably increased in the RG group when compared with the MIRI group (p<0.05). After pretreatment with PPF and RG, no statistically significant differences were found in biochemical indexes and the area of myocardial infarction between the PPF+RG group and the MIRI group (p>0.05). These results indicated that PPF could inhibit MIRI and the effect of JAK/STAT agonist RG on the above indexes (Table I).

Comparisons of Oxidative Stress Indexes Among Groups
Compared with the MIRI group, PPF significantly reduced the content of serum MDA, whereas increased the content of serum SOD in MIRI rats (p<0.05). Meanwhile, the serum content of MDA was significantly increased, while the serum level of SOD was markedly decreased in the RG group when compared with the MIRI group (p<0.05). Such a trend could significantly be reversed by PPF+RG, suggesting that PPF had the anti-oxidative stress ability (Figure 1).

Influence of PPF Treatment on Left Ventricular Hemodynamics in Rats
Results revealed that at 60 min after reperfusion, LVEDP was significantly increased, while

| Table I. LDH, CK-MB and NAD+ expression levels in each group of rats (x±s). |
|------------------|------------------|------------------|------------------|------------------|
| Group            | LDH (U•L⁻¹, n=12)| CK-MB (U•L⁻¹, n=12)| NAD+ (nmol/g, n=12)| Area of myocardial infarction (%) |
| Sham group       | 4245.7±312.4     | 462.5±106.5       | 76.5±12.5         | 0                |
| MIRI group       | 5167.2±354.2     | 685.9±133.8       | 95.2±16.5         | 25.2±8.5         |
| PPF group        | 4568.5±256.4     | 537.1±152.6       | 82.9±15.5         | 15.2±6.8         |
| RG group         | 5951.6±634.8     | 723.8±158.4       | 106.8±19.5        | 31.5±11.2        |
| PPF+RG group     | 5098.6±268.1     | 581.5±164.5       | 98.6±11.5         | 26.9±12.0        |

Note: *p<0.05 vs. Sham group, *p<0.05 vs. MIRI group.
HR, LVDP and ±dp/dt max were remarkably decreased in other groups when compared with the Sham group (p<0.05). Compared with the MIRI group, LVEDP was declined, while HR, LVDP and ±dp/dt max were remarkably increased in the PPF group (p<0.05). Opposite trends were found between the RG group and the PPF group. No statistically significant difference in each index was found between the PPF+RG group and the MIRI group (p>0.05) (Table II).

**Expressions of Serum Inflammatory Factors in Rats**

Compared with the Sham group, the contents of serum TNF-α, IL-6 and monocyte chemotactic protein (MCP) in other groups were significantly increased (p<0.05). However, they were markedly decreased in the PPF group when compared with the MIRI group (p<0.05). Meanwhile, the content of these serum inflammatory factors in the RG group was increased when compared with the MIRI group (p<0.05). However, there were no statistically significant differences between the PPF+RG group and the MIRI group (p>0.05) (Figure 2).

**Expression Levels of Apoptosis Proteins in Rats**

Compared with the Sham group, Western blot results indicated that the protein expression of Bcl-2 was remarkably decreased, while Caspase-3 was significantly increased in the MIRI group. The protein expression of Bcl-2 was remarkably increased, while the expression of the Caspase-3 protein was remarkably decreased in the PPF group when compared with the MIRI group. Moreover, pro-apoptotic protein in the RG group was remarkably increased when compared with the MIRI group. Moreover, the expression of the Bcl-2 protein was remarkably increased, while the expression of Caspase-3 protein was significantly decreased in the PPF+RG group. These results suggested that PPF had an anti-apoptosis effect on myocardial cells in MIRI rats (Figure 3).

**Figure 1.** Influences of PPF on SOD/MDA in MIRI rats. Note: #p<0.05 vs. Sham group, *p<0.05 vs. MIRI group.

**Table II.** Left ventricular hemodynamic indexes in each group of rats (x ±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>HR/min-1</th>
<th>LVDP/kPa</th>
<th>±dp/dtmax/kPa</th>
<th>±dp/dtmax/kPa</th>
<th>LVEDP/kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham group</td>
<td>246.5±15.8</td>
<td>11.5±0.8</td>
<td>325.6±28.5</td>
<td>256.4±15.0</td>
<td>1.06±0.08</td>
</tr>
<tr>
<td>MIRI group</td>
<td>175.0±16.5a</td>
<td>6.2±0.5a</td>
<td>195.2±22.2a</td>
<td>145.3±16.8a</td>
<td>4.58±0.71a</td>
</tr>
<tr>
<td>PPF group</td>
<td>192.1±19.7'</td>
<td>7.8±0.8'</td>
<td>239.1±18.4'</td>
<td>183.4±19.5'</td>
<td>3.21±0.28'</td>
</tr>
<tr>
<td>RG group</td>
<td>144.2±23.5'</td>
<td>4.9±0.7'</td>
<td>177.2±16.6'</td>
<td>116.5±22.5'</td>
<td>6.37±0.67'</td>
</tr>
<tr>
<td>PPF+RG group</td>
<td>168.9±20.7</td>
<td>5.8±0.9</td>
<td>186.5±14.5</td>
<td>138.6±20.8</td>
<td>4.28±0.57</td>
</tr>
</tbody>
</table>

Note: *p<0.05 vs. Sham group, *p<0.05 vs. MIRI group.
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JAK/STAT Pathway Detected via Western Blotting
The JAK/STAT pathway was significantly activated in the MIRI group when compared with the Sham group. PPF pretreatment could significantly inhibit the protein expression levels of JAK and STAT. Compared with the MIRI group, RG pretreatment significantly increased the protein expression levels of JAK and STAT. Moreover, the protein expression levels of JAK and STAT were inhibited by the combination of JAK/STAT agonist RG and PPF (Figure 4). The above results indicated that the protective effect of PPF on MIRI in rats might be related to the inhibition of the JAK/STAT signaling pathway.

Discussion
In the progression of MIRI, a large number of complex signaling pathways are initiated by myocardial cells. They either mediate adaptive stress-induced protective response or lead to the death of myocardial cells by activating cell death pathways. Cardiomyocyte death is the main result of MIRI, and such irreparable injury has been considered one of the major causes of heart failure. The apoptosis of myocardial cells depends on the balance between pro-apoptotic factors and anti-apoptotic factors. Currently, more evidence has demonstrated that the anti-apoptotic therapeutic intervention can alleviate MIRI-induced heart injury. According to new reports, the JAK/STAT signaling pathway plays an important role in the process of ischemic preconditioning. Particularly, transient myocardial ischemia/reperfusion activates JAK1 and JAK2, thereby activating STAT1 and STAT3. Relative levels of activated STAT1 or STAT3 may determine the balance between the death and survival of myocardial cells after MIRI. For example, it has been proved that STAT1 induces apoptosis, while STAT3 protects myocardial cells after MIRI. Multiple effects of STAT1 and STAT3 are related to the direct binding to DNA as well as transcriptional activation of target genes. In addition, STAT1 can enhance the functional activity of the pro-apoptotic transcription factor p53. Some studies have indicated that the JAK/STAT pathway is also activated in the complete heart. Blocker AG-490 inhibits the phosphorylation of STAT1 and STAT3, which also blocks the cardio-protective effect of preconditioning. Besides, the classical pre-treatment effect is inhibited in STAT3-deficient mice. Therefore, the STAT1 or STAT3 pathway is a potential therapeutic target for the prevention of ischemic heart disease.

Figure 2. Influence of PPF on the expressions of inflammatory factors in MIRI rats. Note: *p<0.05 vs. Sham group, †p<0.05 vs. MIRI group.

Figure 3. Influence of PPF on myocardial apoptosis in MIRI rats. Note: *p<0.05 vs. Sham group, †p<0.05 vs. MIRI group.
In this investigation, a rapid- and short-acting intravenous anesthetic, PPF, was studied. We also explored its protective mechanism in MIRI rats. JAK/STAT agonist RG81640-CH was used as a control. The rats were first pretreated with PPF and RG alone or together. After blockage of the left anterior descending coronary artery for 30 min, the MIRI model was successfully established through 6 h of reperfusion. Results found that the content of LDH, CK-MB, and NAD^+ in the PPF group was significantly declined when compared with the MIRI group. However, it was significantly increased in the RG group than that of the MIRI group. Studies have demonstrated that oxygen free radicals are produced inside and outside myocardial and endothelial cells in the MIRI model, which can lead to lipid peroxidation in cell membrane, Ca^{2+} overload in cells and Ca^{2+} accumulation in myocardial cells. This may eventually result in mechanical dysfunction and metabolic changes in isolated perfused heart\(^7\). Our investigation revealed that PPF could significantly reduce the content of serum MDA and inflammatory factors, whereas increasing the content of serum SOD in MIRI rats. Moreover, PPF could significantly reduce infarction area, increase the Bcl-2 level and decrease Caspase-3 activity. Completely opposite trends were found in rats of the RG group. In addition, PPF pretreatment significantly reduced the protein expression levels of JAK1, STAT1 and STAT3, indicating that PPF exerted a protective effect on MIRI by inhibiting the JAK/STAT pathway. Si et al\(^19\) have found that pretreatment with JAK inhibitor can reduce the phosphorylation of STAT3 and enhance apoptosis after MIRI.

In this study, the protective effect of PPF on MIRI was demonstrated, which may provide a new strategy for the treatment of myocardial ischemia-reperfusion injury.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Conclusions

We found that PPF reduced the release of inflammatory factors and alleviated tissue damage caused by myocardial apoptosis in MIRI rats by inhibiting the activation of the JAK/STAT pathway. Our study indicated that PPF had a myocardial protective effect on MIRI.

References

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