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Propofol improves intestinal ischemia-reperfusion injury in rats through NF-κB pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effects of propofol on intestinal ischemia-reperfusion injury in rats through the nuclear factor-kappa B (NF-κB) pathway.

MATERIALS AND METHODS: A total of 24 Sprague-Dawley rats were selected and randomly divided into three groups, including sham operation group, ischemia group and propofol group. Rats in sham operation group were only treated with isolation of superior mesenteric artery, which was clipped for 1 h and reperfused for 2 h in ischemia group. Meanwhile, propofol (60 mg/kg) was injected into the femoral vein 1 h before ischemia in propofol group. TUNEL assay was performed to detect cell apoptosis of intestinal tissues. Real-time quantitative polymerase chain reaction (RT-qPCR) was conducted to measure the expression levels of malondialdehyde (MDA), superoxide dismutase (SOD), myeloperoxidase (MPO), caspase-3 and B-cell lymphoma-2 (Bcl-2) in rats of each group. Western blotting was utilized to detect the protein expression levels of NF-κB pathway related molecules, such as myeloid differential protein-88 (MyD88), v-rel avian reticuloendotheliosis viral oncogene homolog A (RelA) and NF-κB. Furthermore, changes in plasma cytokine levels were determined *via* enzyme-linked immunosorbent assay (ELISA).

RESULTS: The number of apoptotic cells in ischemia group was remarkably higher than that in sham operation group ($p<0.05$). However, it decreased notably in propofol group compared with ischemia group ($p<0.05$). In comparison with sham operation group, significantly up-regulated expression of caspase-3 and down-regulated expression of Bcl-2 were observed in the intestinal tissues of rats in ischemia group ($p<0.05$). Caspase-3 was lowly expressed, while Bcl-2 was highly expressed in the intestinal tissues of rats in propofol group compared with ischemia group ($p<0.05$). In addition, no statistically significant differences were observed in the expression level of SOD among sham operation group, ischemia group and propofol group ($p>0.05$). The expression levels of MDA and MPO were overtly higher in the intestinal tissues of rats in ischemia group than those in sham operation group and propofol group ($p<0.05$). Besides, the protein expression levels of MyD88, RelA and NF-κB in the intestinal tissues of rats in ischemia group were remarkably higher than those in sham operation group and propofol group ($p<0.05$). The activity of the NF-κB pathway in the intestinal tissues of rats in propofol group significantly declined compared with ischemia group ($p<0.05$). Moreover, compared with sham operation group, plasma levels of TNF-α, interleukin (IL)-2, IL-6 and IL-4 increased significantly in rats of ischemia group ($p<0.05$). However, they were markedly lower in propofol group than those in ischemia group ($p<0.05$).

CONCLUSIONS: Propofol protects rats from intestinal ischemia-reperfusion injury through the NF-κB pathway.

Key Words:
Propofol, Intestinal ischemia-reperfusion, NF-κB.

Introduction

Ischemia-reperfusion injury, a common disease in clinical practice, refers to local tissue damage caused by blood reperfusion that mainly occurs after transient or prolonged ischemia of tissues or organs[1,2]. Currently, ischemia-reperfusion injury is more likely to emerge during organ transplantation, cardiopulmonary cerebral resuscitation, various types of shock and post-traumatic blood transfusion[3,4]. Intestinal ischemia-reperfusion on intestinal tissues may cause not only local tissue damage, but also intestinal mucosal necrosis. All of these complications seriously affect the patient's digestive function, which can even result in the situation that intestinal tissues need to be locally removed[5]. Therefore, the exploration of protective substances and specific mechanisms

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of their action in intestinal ischemia-reperfusion injury is conducive to reducing the impact of ischemia-reperfusion on the digestive tract and digestive function.

Propofol, a drug inducing and maintaining anesthesia, exerts good sedative effects when combined with other drugs[6]. Currently, propofol has been proven to play important roles in many diseases, such as choriocarcinoma[7] and hepatocellular carcinoma[8]. It has also been reported that during ischemia-reperfusion, propofol protects tissues through reducing tissue metabolism level[9,10].

Therefore, the aim of this study was to investigate the protective effects of propofol during intestinal ischemia-reperfusion by establishing models of intestinal ischemia-reperfusion in Sprague-Dawley (SD) rats. In addition, we explored the effect of propofol on intestinal ischemia-reperfusion injury in rats through the nuclear factor-kappa B (NF-κB) pathway by detecting cell apoptosis, changes in the protein expression levels of oxidative stress and levels of inflammatory cytokines, as well as changes in expression levels of the NF-κB pathway.

Materials and Methods

Establishment of SD Rat Models

This investigation was approved by the Animal Ethics Committee of Jinan City People's Hospital Animal Center. A total of 24 adult female rats weighing 250-300 g were selected and randomly divided into three groups, including sham operation group (n=8), ischemia group (n=8) and propofol group (n=8). Rats were first intraperitoneally injected with pentobarbital sodium (60 mg/kg) for anesthesia and routinely disinfected with 75% sterilized alcohol. A mid-incision was made to separate the superior mesenteric artery. No treatment was performed for rats in sham operation group, and the wound was covered with sterile gauze moistened with normal saline. After clipping the superior mesenteric artery of rats in ischemia group for 1 h, the vascular clip was released to maintain blood reperfusion for 2 h. Propofol (60 mg/kg; Guangdong Jiabo Pharmaceutical Co., Ltd., NMPN: H20084457, Guangzhou, China) was injected into the femoral vein 1 h before ischemia in rats of propofol group. After the experiment, the rats were sacrificed, and intestinal tissues and blood samples were collected for use.

Detection of Plasma Inflammatory Cytokines

Blood samples were first collected from rats in sham operation group, ischemia group and propofol group. Subsequently, collected samples were placed into a procoagulant tube, followed by centrifugation at 3,000 rpm for 5 min. Next, the upper plasma was taken and placed into a new centrifuge tube. The levels of inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-6 and IL-4 in the plasma of rats in each group were measured *via* enzyme-linked immunosorbent assay (ELISA) kit (BD Biosciences, Franklin Lakes, NJ, USA). Three replicate wells were set for each sample. Optical density at 450 nm was measured by a micro-plate reader (Bio-Rad, Hercules, CA, USA). Finally, cytokine levels in each group were obtained through standard curve conversion.

Cell Apoptosis Detected by Terminal Deoxynucleotidyl Transferase (TdT)-Mediated Deoxyuridine Triphosphate-Biotin Nick End Labeling (TUNEL) Assay

TUNEL Apoptosis Detection Kit (Roche, Basel, Switzerland) was adopted to determine the apoptosis of intestinal tissues in rats of each group. All experimental steps were carried out according to the standard instructions. The simplified operation steps were as follows: Intestinal tissue sections were washed 3 times with phosphate-buffered saline (PBS), with 5 min for each time. TUNEL detection working solution was then prepared using TdT enzyme and fluorescent labeling solution. Next, 50 μL of prepared working solution was added into each sample, followed by incubation at 37°C in the dark for 60 min. Then the samples were washed with PBS for 3 times. Cell apoptosis was finally observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Fluorescence Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

RT-qPCR was conducted to measure the expression levels of malondialdehyde (MDA), superoxide dismutase (SOD), myeloperoxidase (MPO), caspase-3 and B-cell lymphoma-2 (Bcl-2) in each group. Briefly, total ribonucleic acid (RNA) in the intestinal tissues of rats in each group was extracted using TRIzol reagent

(Invitrogen, Carlsbad, CA, USA). The reaction system of PCR was as follows: 1 μL of each primer, 0.5 μL of cDNA, 12.5 μL of SYBR Taq and 10 μL of ddH_2O. PCR was performed under the following conditions: 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 30 s, and 72°C for 5 min. Primers used in this study were as follows: MDA: Forward: (5'→3') 'AACAGAGAGGATTTCGTTTCCG', Reverse: (5'→3') 'TTTGACCTGAGGGTAAGACTTCT'. SOD: Forward: (5'→3') 'GAAGCACGAATGACAGAGGC', Reverse: (5'→3') 'GCTTGGCGGATTAGCTCTTTT'. MPO: Forward: (5'→3') 'GGTGCGGCTCATGTTTACAG', Reverse: (5'→3') 'GATGGCGTCTGATACCACGG'. Caspase-3: Forward: (5'→3') 'ATGGCAGACGATGATCCCTAC', Reverse: (5'→3') 'TGTTGACAGTGGTATTTCTGGTG'. Bcl-2: Forward: (5'→3') 'GGAGGCATGTTCGGTAGTGG', Reverse: (5'→3') 'CCCTGCGTTGGATTTCGTG'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): Forward: (5'→3') 'ATGGCAGACGATGATCCCTAC', Reverse: (5'→3') 'CGGAATCGAAATCCCCTCTGTT'.

Western Blotting (WB)

WB was utilized to detect the expression levels of NF-κB pathway related molecules, myeloid differential protein-88 (MyD88), v-rel avian reticuloendotheliosis viral oncogene homolog A (RelA) and nuclear factor kappa-B (NF-κB), as well as internal reference GAPDH. Firstly, radio immunoprecipitation assay (RIPA) lysis buffer with protease inhibitors was utilized to extract total proteins from the intestinal tissues of rats in each group. After electrophoresis, protein samples were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were blocked with bovine serum albumin (BSA) and incubated with primary antibodies of anti-MyD88, anti-TLR4, anti-RelA, anti-NF-κB and anti-GAPDH at 4°C overnight. On the next day, the membranes were incubated with corresponding secondary antibodies for 1 h. Immunoreactive bands were finally exposed by the chemiluminescent substrate kit.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 23.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Changes in the Number of Apoptotic Cells in Sham Operation Group, Ischemia Group and Propofol Group

Changes in the number of apoptotic cells in sham operation group, ischemia group and propofol group were shown in Figure 1. The results of TUNEL assay displayed that the number of apoptotic cells was remarkably higher in ischemia group than that in sham operation group ($p<0.05$). However, it declined notably in propofol group compared with ischemia group ($p<0.05$).

Expression Levels of Apoptotic Molecules of Intestinal Tissues in Sham Operation Group, Ischemia Group and Propofol Group

Changes in the expression levels of apoptotic molecules (caspase-3 and Bcl-2) of intestinal tissues in sham operation group, ischemia group and propofol group were shown in Figure 2. The results indicated that in comparison with sham operation group, caspase-3 was highly expressed, while Bcl-2 was lowly expressed in ischemia group ($p<0.05$). Meanwhile, caspase-3 was significantly down-regulated, while Bcl-2 was up-regulated in the intestinal tissues of rats in propofol group compared with ischemia group ($p<0.05$).

Expression Levels of Oxidative Stress Proteins in Sham Operation Group, Ischemia Group and Propofol Group

The expression levels of oxidative stress proteins (MDA, SOD and MPO) in sham operation group, ischemia group and propofol group were shown in Figure 3. There was no significant difference in the expression level of SOD among sham operation group, ischemia group and propofol group ($p>0.05$). The expression levels of MDA and MPO were overtly higher in the intestinal tissues of rats in ischemia group in comparison with those in sham operation group and propofol group ($p<0.05$).

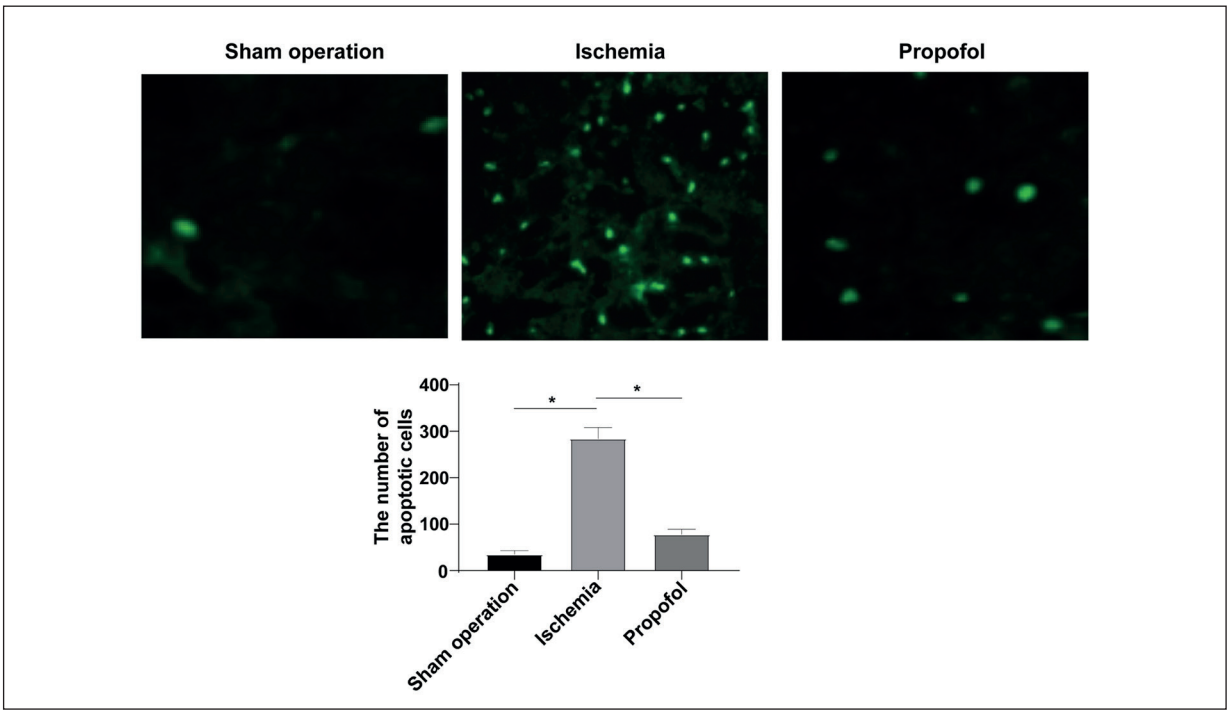

Figure 1. Changes in the number of apoptotic cells in sham operation group, ischemia group and propofol group. (magnification: 400×) (*p<0.05).

Protein Expression Levels of NF-κB Pathway Related Molecules in Sham Operation Group, Ischemia Group and Propofol Group

The protein expression levels of MyD88, RelA and NF-κB in sham operation group, ischemia group and propofol group were shown in Figure 4. The results revealed that the protein expression levels of MyD88, RelA and NF-κB in the intestinal tissues of rats in ischemia group were significantly higher than those in sham operation group and propofol group (p<0.05). However, the

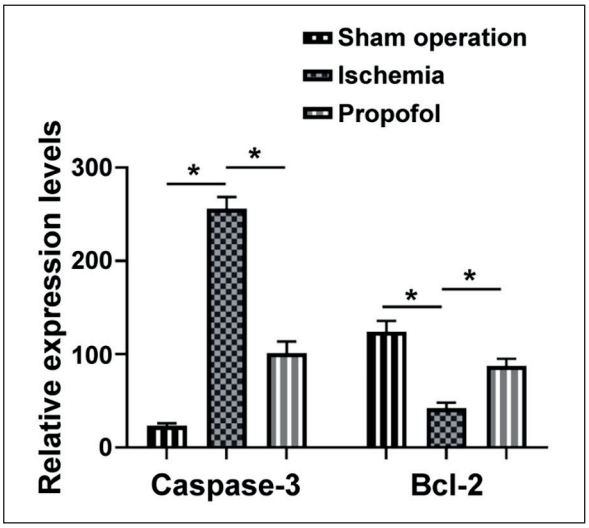

Figure 2. Expression levels of apoptosis-related molecules in sham operation group, ischemia group and propofol group (*p<0.05).

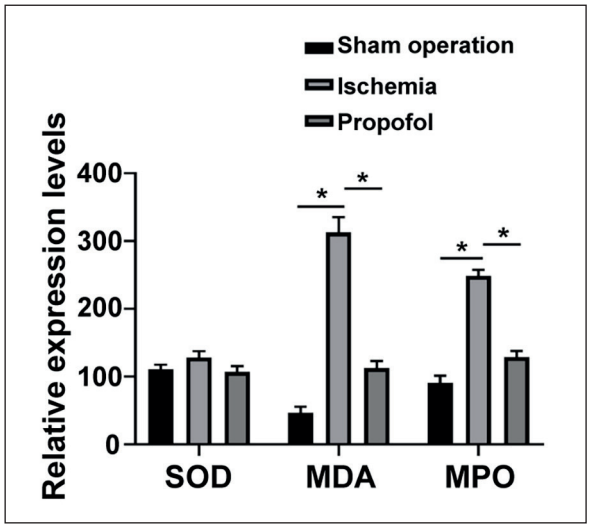

Figure 3. Expression levels of oxidative stress proteins in sham operation group, ischemia group and propofol group (*p<0.05).

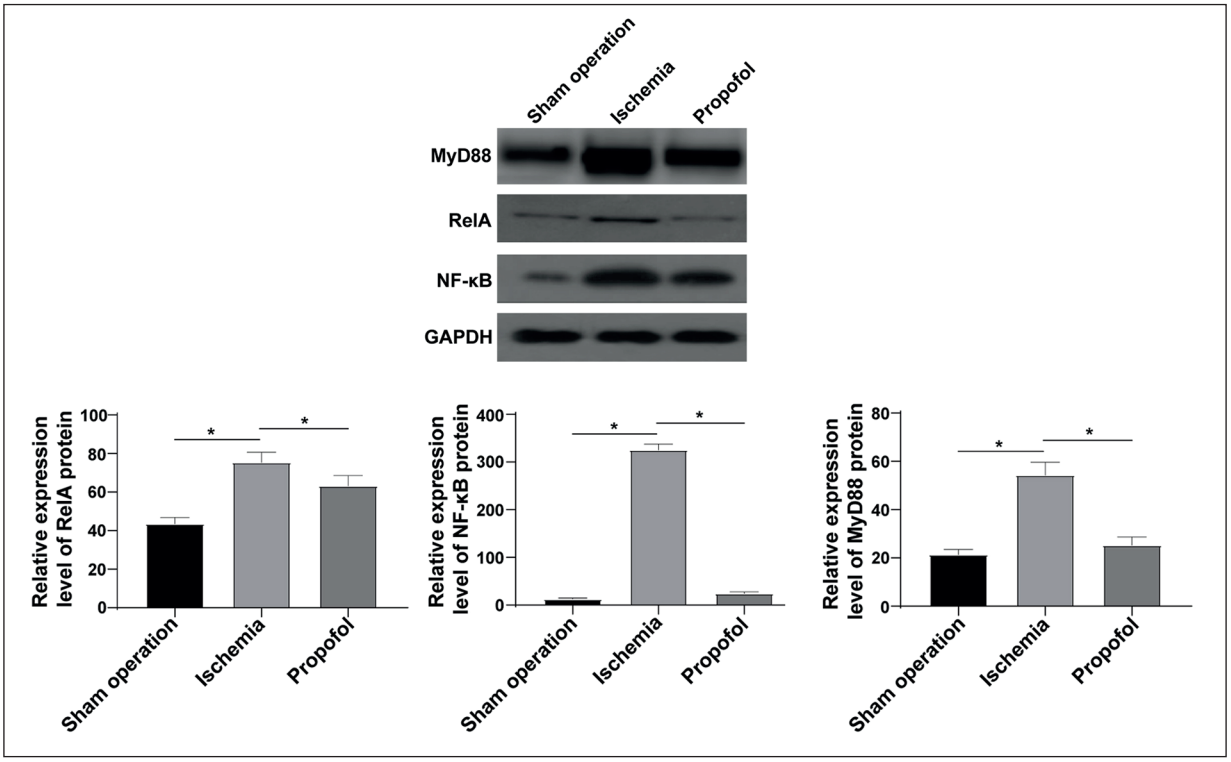

Figure 4. Protein expression levels of NF-κB pathway related molecules in sham operation group, ischemia group and propofol group (*p<0.05).

activity of the NF-κB pathway in the intestinal tissues of rats in propofol group declined significantly compared with ischemia group.

Changes in the Expression Levels of Plasma Inflammatory Cytokines in Sham Operation Group, Ischemia Group and Propofol Group

The changes in the expression levels of plasma inflammatory cytokines (TNF-α, IL-2, IL-6 and IL-4) in sham operation group, ischemia group and propofol group were shown in Table I. The results prompted that compared with sham operation group, plasma levels of TNF-α, IL-2, IL-6 and IL-4 increased significantly in rats of ischemia group (p<0.05). However, they were significantly lower in propofol group than those in ischemia group (p<0.05).

Discussion

Ischemia-reperfusion injury may occur in a variety of clinical diseases. The main injury sites are organs and tissues with high blood demand and oxygen consumption, such as the heart, brain and intestines[11]. Numerous studies have found that the mechanism of ischemia-reperfusion injury is

Table I. Changes in the expression levels of plasma inflammatory cytokines in sham operation group, ischemia group and propofol group.

Group	IL-6 (mg/L)	TNF-α (fmol/mL)	IL-2 (mg/L)	IL-4 (mg/L)
Sham operation group	45.56 ± 3.45	23.15 ± 2.13	78.24 ± 5.11	25.31 ± 3.41
Ischemia group	98.45 ± 5.62[a]	87.35 ± 6.51[a]	111.63 ± 4.81[a]	64.21 ± 4.12[a]
Propofol group	65.83 ± 4.24[b]	45.14 ± 4.53[b]	85.25 ± 6.42[b]	43.86 ± 3.69[b]

Note: The levels of TNF-α, IL-2, IL-6 and IL-4 are significantly increased in ischemia group and relatively declined in propofol group. [a]p<0.05 *vs*. sham operation group, [b]p<0.05 *vs*. ischemia group.

mainly hypoxic damage to local tissues caused by ischemia, accumulation of harmful substances such as lactic acid, and components such as reactive oxygen species and inflammatory cytokines brought by blood during reperfusion[12,13]. Therein, intestines are important members of the digestive tract. Ischemia-reperfusion injury causes local tissue damage, necrosis and dysfunction, which also affects the integrity of intestinal mucosa, intestinal peristalsis, digestion and absorption. This mat eventually leads to a huge impact on the patient's body and mind. Some drugs, such as pentoxifylline, have been confirmed to be able to reduce tissue damage caused by ischemia-reperfusion[14]. Propofol, a commonly utilized anesthetic, is widely applied to relieve the damage due to ischemia-reperfusion of myocardial and brain tissues. Therefore, the exploration of protective effects and specific mechanisms of propofol in the intestinal ischemia-reperfusion injury is conducive to reducing the negative impact of ischemia-reperfusion on intestinal tissues.

In this study, the results demonstrated that the number of apoptotic cells was remarkably higher in ischemia group than that in sham operation group ($p<0.05$). However, it declined notably in propofol group compared with ischemia group ($p<0.05$). In comparison with those in sham operation group, up-regulation of caspase-3 and down-regulation of Bcl-2 were observed in the intestinal tissues of rats in ischemia group ($p<0.05$). However, caspase-3 was lowly expressed and Bcl-2 was highly expressed in the intestinal tissues of rats in propofol group compared with those in ischemia group ($p<0.05$). These results indicated that ischemia-reperfusion might damage the intestinal tissues, eventually leading to increased proportion of apoptotic cells, up-regulated expression levels of apoptosis-related molecules and decreased anti-apoptotic proteins. Moreover, propofol is able to resist the increase of apoptotic cells caused by ischemia-reperfusion to some extent. In addition, there was no significant difference in the expression level of SOD among sham operation group, ischemia group and propofol group ($p>0.05$). The expression levels of MDA and MPO were overtly higher in the intestinal tissues of rats in ischemia group than those in sham operation group and propofol group ($p<0.05$). The above results suggested that ischemia-reperfusion injury to intestinal tissues might be caused by oxidative stress pathway. Meanwhile, propofol could restore the expression of some antioxidant proteins, thereby enhancing the resistance of intestinal tissues against reactive oxygen species. By determining plasma cytokine levels, we found that compared with those in sham operation group, plasma levels of TNF-α, IL-2, IL-6 and IL-4 increased significantly in rats of ischemia group ($p<0.05$). However, they were markedly lower in propofol group than ischemia group ($p<0.05$). This illustrates that ischemia-reperfusion injury to the body is a local response and may activate the systemic inflammatory immune system to amplify the damage effect. However, propofol is capable of resisting this inflammatory response to a certain extent. In summary, propofol resists intestinal ischemia-reperfusion injury and plays a certain protective role.

NF-κB, a powerful complex, plays an important role in the regulation of cellular transcription. It mainly regulates the response of cells to external stimuli[15,16]. NF-κB pathway is associated with the occurrence of multiple diseases, such as degenerative lumbar disc disease[17], hepatocellular carcinoma[18] and breast carcinoma[19]. Thus, it can be seen that the NF-κB pathway plays an important role in the progress of various diseases, including malignant tumors. Ischemia-reperfusion injury is a stressful disease process. It can cause changes in related proteins in the NF-κB pathway to varying degrees, thereby increasing the resistance of local tissues to damage and forming a protective response mechanism of the body against the outside world[20]. During intestinal ischemia-reperfusion, propofol may protect intestinal tissues probably by regulating the expression of related proteins in the NF-κB pathway. The results of this study indicated that protein expression levels of MyD88, RelA and NF-κB in the intestinal tissues of rats were significantly higher in ischemia group than those in sham operation group and propofol group ($p<0.05$). Moreover, the activity of the NF-κB pathway in the intestine tissues of rats in propofol group declined remarkably compared with ischemia group ($p<0.05$).

Conclusions

Altogether, the novelty of this study was that propofol protects against the intestinal ischemia-reperfusion injury in rats to a certain extent, which may be achieved through regulating the NF-κB pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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