

Effect of sevoflurane on hepatic ischemia-reperfusion injury in rats via JAK2-STAT3 pathway

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Abstract. – OBJECTIVE: To investigate the effect of sevoflurane on hepatic ischemia-reperfusion injury in rats via janus kinase 2/signal transducer and activator of transcription 3 (JAK2-STAT3) pathway.

MATERIALS AND METHODS: Forty healthy male Sprague-Dawley (SD) rats were randomly divided into sham group (n=10), model group (HIRI group, n=10), sevoflurane intervention group (SF group, n=10), and sevoflurane combined with AG490 intervention group (AG490 group, n=10). Liver and serum samples were collected after reperfusion for 6 h. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), interleukin (IL) -1 β , IL-6 and tumor necrosis factor (TNF) - α were detected by enzyme-linked immunosorbent assay (ELISA). JAK2, STAT3, p-JAK2 and p-STAT3 were detected by Western-blot. The expression level of cobalt quenching technique was used to detect the permeability of mitochondrial membrane permeability transition pore (mPTP).

RESULTS: The levels of ALT, AST, AKP, IL-1 β , IL-6 and TNF- α in HIRI group were higher than those in sham group ($p < 0.05$), those in SF group were lower than those in HIRI group ($p < 0.05$), while those in AG490 group were higher than those in SF group ($p < 0.05$). The levels of JAK2 protein, pJAK2 protein, STAT3 protein and P STAT3 protein in HIRI group were lower than those in sham group ($p < 0.05$); the levels of each protein in SF group were higher than those in HIRI group ($p < 0.05$), while those in AG490 group were lower than those in SF group ($p < 0.05$). The open degree of HIRI group was higher than that of sham group ($p < 0.05$); SF group was lower than that of HIRI group, and AG490 group was higher than that of SF group ($p < 0.05$).

CONCLUSIONS: Sevoflurane can significantly improve HIRI and reduce hepatic immune inflammation in rats. The mechanism may be related to activating JAK2-STAT3 pathway and inhibiting the overopening of mPTP.

Key Words:

Sevoflurane, Hepatic ischemia-reperfusion injury, Janu kinase signal transducer, Transcription activator,

Mitochondrial membrane permeability transporter, Immune inflammation.

Introduction

Ischemia reperfusion injury refers to the exacerbation of functional metabolic disorder and structural failure in human beings and animals caused by hemorrhage after ischemia reperfusion, which should have lead a recovery of the function of organs and tissues¹. During the hepatic intervention, hepatic ischemia-reperfusion injury (HIRI) is very likely to occur. HIRI may in a short time cause acute immuno-inflammatory responses, hepatocellular injury and hepatic dysfunction, and in serious cases, organ failure may also occur. The pathogenesis is unclear yet and possibly related to calcium overload, immune inflammation, complement activation and autophagy and other reasons^{1,2}. Given that the HIRI is associated with patients' survival and prognosis, it is quite important to discover its pathogenesis and find effective therapeutics. It was reported in many studies that the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2-STAT3) signal pathway is connected with ischemia reperfusion injuries in cardiac muscle, brain, kidney and other tissues³⁻⁵. Reports related to the expression of JAK2-STAT3 in HIRI, however, are few. Li et al⁶ found miRNA-17 may promote the autophagy and thus the progression of HIRI through STAT3 expression inhibition. Sevoflurane is clinically a common inhalational anesthetic. Numerous previous studies have confirmed that sevoflurane can improve the condition of ischemia reperfusion injury with the mechanism possibly related to the open and close of mitochondrial membrane permeability transition pore (mPTP). Our study aimed to find a new pathogenesis of HIRI evaluating the changes of JAK2-STAT3 pathway in rats with HIRI after sevoflurane treatment and

through the investigation of the role it plays in the open and close of mPTP.

Materials and Methods

Experimental Animals

40 healthy male Sprague Dawley (SD) rats at the weight of 220-300g (provided by Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were selected. Rats were placed into cages with a light/dark cycle of 12 h/12 h and a temperature of $22\pm 2^{\circ}\text{C}$. All of the four rats in each cage were given sufficient water and specific pathogen free (SPF) fodder and protected from noise. The experiment started 2 weeks later after the rats had adapted to the surroundings. Ethical Approval was granted by the First Affiliated Hospital of Henan University.

Methods

Establishment of HIRI Model in Rats

The rats were anaesthetized with 20% urethane (1 g/kg) and fixed on the operating floor in overhead position. An incision of about 2 cm was created at the median of the abdomen. The perihepatic ligament was dissociated and the first porta hepatis and hepatic pedicle exposed. Vascular clamps were being placed to close the portal veins of the left and middle period of the liver and branches of hepatic arteries to make approximately a 70% liver ischemia. In the meantime, blood in the right period and caudate lobe flowed freely. Blood stasis in portal veins and the stomach and intestine was released. Liver ischemia is marked by the grayed and softened liver. The clamps were taken down after 1 h-blood occlusion to regain hepatic blood supply. The recovery of the ruddy liver showed a successful reperfusion and model establishment^{7,8}.

Experimental Grouping and Treatments

1) Sham-operated group (the sham group, $n=10$): the rats in this group were treated with anesthesia, laparotomy, perihepatic ligament dissociation and the first porta hepatis exposure successively only. No blood supply blocked. Pure oxygen was only given for breath during the procedure. 2) HIRI group ($n=10$) served as the model group. Pure oxygen was only given for breath during the procedure. 3) Sevoflurane intervention group (the SF group, $n=10$): on the basis of HIRI group, before the hepatic blood flow occlusion, the rats were placed

into a closed container with an end attached to the anesthesia machine and the other end attached to the anesthetic gases detector. A sevoflurane evaporator (Weifang Delkang Medical Technology Co. Ltd., Weifang, China) conveys sevoflurane (produced by the Shanghai Bodun Biochemical Industry Co. Ltd., Art. No. URS1612540, Shanghai, China) at the speed of 2 L/min for 30 min rat inhalation. 4) JAK2-STAT3 signal pathway blocker AG490 group (the AG490 group, $n=10$): on the basis of the SF group, the rats were given AG490 (produced by the Shanghai Tuhe Company, Art. No. A125648 Shanghai, China) by $3\text{ }\mu\text{g/g}$. The interferential methods are the same as the SF group.

Liver Morphological Observation

6 h after reperfusion, the rest of 5 rats in each group were immobilized with 10% formalin, paraffin embedded, sectioned, dewaxed, and dyed by HE stain. Pathological changes of the tissue were observed under optical microscope after dehydration, transparency and sealing.

Blood Index Detection

6 h after reperfusion, for each group, blood of 2 mL in the aorta abdominals was collected; then, standing for 1 h and centrifuged at 2500 rpm for 10 min. Blood serum was collected and detected by enzyme-linked immune sorbent assay (ELISA) for serum alanine transaminase (ALT), aspartic transaminase (AST), alkaline phosphatase (AKP), interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α . ELISA kits were purchased from Wuhan Boster Co. Ltd., (Wuhan, China). The wavelength was set as 450 nm during the detection.

Detection of the Expression of JAK2mRNA and STAT3mRNA

6 h after reperfusion, 5 rats were euthanized. Their ischemia-reperfusion hepatic tissues were collected and stored in liquid nitrogen. RT-PCR technology was used to detect the expression level of JAK2mRNA and STAT3mRNA. The total RNA of hepatic tissues was firstly extracted for the next mRNA expression detection by RT-PCR. SYBR Premix Ex Taq II was purchased from Beijing Think-Far Technology Co., Ltd. (Beijing, China). The primers were designed and provided by Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China): JAK2 (upstream: 5'-TGACAGAA-ATGGAGGGAACC-3', downstream: 5'-GCCCA-AGAGAATGGTACAGG-3'), STAT3 (upstream: CAGCCAGCAAAGAGTCACAC', downstream: TTCGCAGGTTGTGCTGATAG). Thermal Cycler

Dice Real Time System amplification was produced by Roche Diagnostics Co., Ltd., (Basel, Switzerland). The expression contents of mRNA were tested by $\Delta\Delta CT$ method.

Detection of JAK2 and STAT3 and Their Phosphorylated Proteins

The expression levels of JAK2, STAT3, p-JAK2, and p-STAT3 proteins were detected through Western-blot assay. Firstly, the total proteins of the tissue were extracted, and solved with tissue lysate. Samples of 30 μg were taken for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) transfer, and blocking at room temperature for 2 h. Incubation was carried out overnight at 4°C after the addition of 500 x-diluted antibodies JAK2, STAT3, p-JAK2 and p-STAT3. The membrane was washed with phosphate-buffered saline (PBS) and combined with second antibodies for the next 1 h-incubation followed by development. DXI800 luminescence machine was obtained from Porton (Cranbury, NJ, USA). The ratio of the gray level of the target protein band to the internal reference (GAPDH) represents the protein expression level.

mPTP Opening Detection

The openness was tested with Cobalt Quenching Technology. The mPTP detection kit (Shanghai Genmed Technology Co., Ltd., Art. No. GMS10095.1, Shanghai, China) was used to reflect the openness through the extent of loss of mitochondrial calcein signals. The test procedures were strictly in accordance with the specifications. Observation was conducted under optical microscope for 30 min in the manner of once every 5 minutes to detect intensity value of the mitochondrial calcein fluorescence (set emission wavelength as 505 nm and excitation wavelength as 488 nm) reflecting the openness of mPTP.

Statistical Analysis

SPSS 19.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data was expressed in mean value $\pm$ standard deviation ($\bar{x} \pm s$). One-way analysis of variance was used for comparison among groups and LSD test for pairwise comparison. $p < 0.05$ was considered as statistically significant differences.

Results

Liver Morphological Observation

From the observation under optical microscop-

pe, liver tissues of rats in the sham group showed clear structure and normal hepatic lobule. No hepatic sinusoid hyperemia or inflammatory cell infiltration was found. In the HIRI group, it was showed the hepatic lobule had its structure damaged and much inflammatory cell infiltration, mainly lymphocyte and mononuclear macrophage, were found in portal area. Inflammation occurred in blood vessel endothelium, and there was visible cells edema. In the SF group, the structure of the liver was basically normal. Inflammation found in the hepatic lobule and portal area was more moderate than that in HIRI group. In the AG490 group, it showed abnormal liver structure, hepatic sinusoid expansion, severe hepatic congestion, inflammatory cell infiltration in the portal area and between the hepatic cells, and obvious hepatic cell edema (Figure 1).

Comparisons of Expression Levels of JAK2mRNA and STAT3mRNA Among the Four Groups

The expression levels of JAK2mRNA and STAT3mRNA in HIRI group were lower than that in the sham group ($p < 0.05$). The expression content of each mRNA in SF group was higher than those in the HIRI group ($p < 0.05$). Expression level of each mRNA in AG490 group was lower than those in the SF group ($p < 0.05$) (Figure 2).

Comparisons of Levels of JAK2 and STAT3 and Their Phosphorylated Proteins Among the Four Groups

Protein levels in HIRI group in respect of the JAK2, pJAK2, STAT3 and pSTAT3 were lower than those of the sham group ($p < 0.05$). Expression contents of proteins above in SF group were higher than that in the HIRI group ($p < 0.05$). Expression levels of such proteins in AG490 group were lower than those in the SF group ($p < 0.05$) (Figure 3).

Comparisons of Liver Function and Levels of Inflammatory Cytokines Among The Four Groups

The levels of ALT, AST, AKP, IL-1 β , IL-6 and TNF- α in HIRI group were higher compared with the sham group ($p < 0.05$) and the SF group ($p < 0.05$), which was also lower than that in the AG490 group ($p < 0.05$) (Table I).

Comparisons of mPTP Openness Among The Four Groups

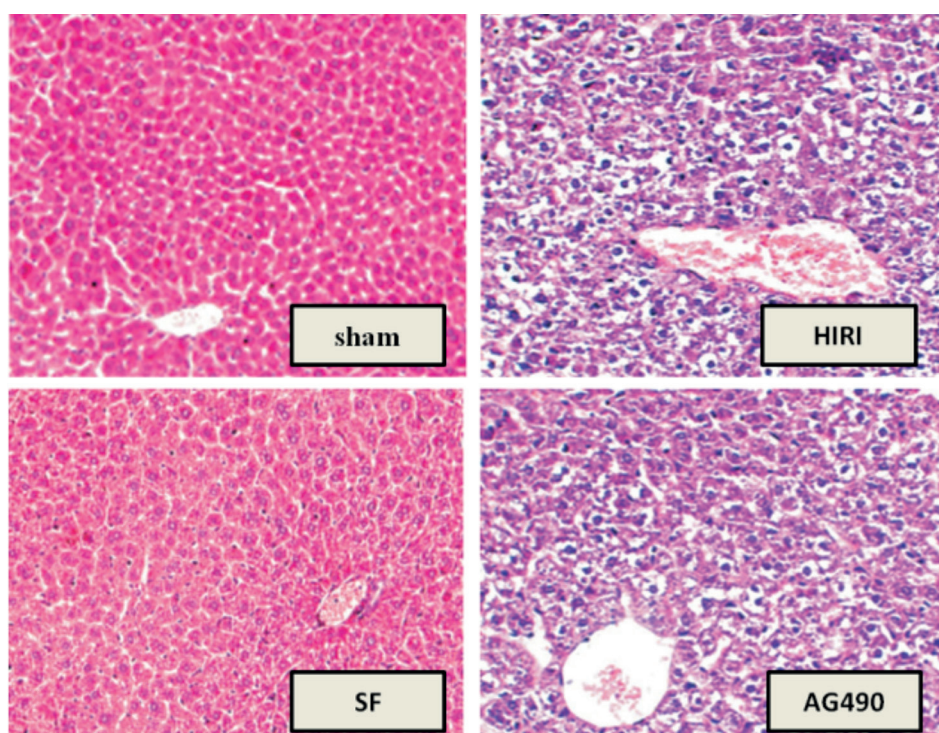


Figure 1. Morphological changes of liver in four groups (HE staining, $\times 400$).

The degree of openness in the HIRI group was higher compared with the sham group ($p < 0.05$) and the SF group, which was also lower than that in the AG490 group ($p < 0.05$) (Figure 4).

Discussion

As showed by previous studies, immune inflammation is one of the critical pathogenesis of HIRI⁹. Kupffer cells are macrophages in the liver that play important roles in the occurrence and development of HIRI. During the ischemia and reperfusion, the Kupffer cells may be activated to

secrete inflammatory cytokines (e.g. IL-1 β , IL-6, TNF- α) and oxygen free radicals¹⁰. In this paper, it was found that compared with the sham group, serum IL-1 β , IL-6 and TNF- α levels in the rats of HIRI group were significantly increased, indicating that the liver had an immuno-inflammatory response. That is consistent with the results of Wang et al¹¹. Under light microscope, it was found that in the HIRI group, the hepatic lobule had its structure damaged and much inflammatory cell infiltration existed, mainly lymphocyte and mononuclear macrophage, in portal area. Inflammation occurred in blood vessel endothelium, and there was visible cells edema. In addi-

Table I. Comparison of liver function and inflammatory cytokines in four groups ($n=10 \bar{x} \pm s$).

Group	ALT (U/L)	AST (U/L)	AKP (U/L)	IL-1 β (pg/mL)	IL-6 (pg/mL)	TNF- α (pg/mL)
Sham group	63.25 $\pm$ 12.81	125.24 $\pm$ 22.11	62.01 $\pm$ 8.64	62.35 $\pm$ 9.87	42.65 $\pm$ 6.25	66.35 $\pm$ 10.11
HIRI group	305.21 $\pm$ 32.01*	320.25 $\pm$ 34.58*	112.35 $\pm$ 26.21*	185.23 $\pm$ 33.24*	125.35 $\pm$ 18.64*	156.35 $\pm$ 20.11*
SF group	200.56 $\pm$ 14.77 [#]	246.38 $\pm$ 29.00 [#]	75.36 $\pm$ 10.00 [#]	85.36 $\pm$ 14.35 [#]	70.26 $\pm$ 22.01 [#]	75.39 $\pm$ 24.01 [#]
AG490 group	304.23 $\pm$ 24.67 [@]	318.24 $\pm$ 20.04 [@]	124.36 $\pm$ 30.84 [@]	188.36 $\pm$ 26.25 [@]	130.22 $\pm$ 24.68 [@]	152.39 $\pm$ 39.45 [@]

Note: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AKP: alkaline phosphatase; IL: interleukin; TNF: tumor necrosis factor. *compared with sham group, $p < 0.05$; compared with HIRI group, $p < 0.05$; @ compared with SF group, $p < 0.05$.

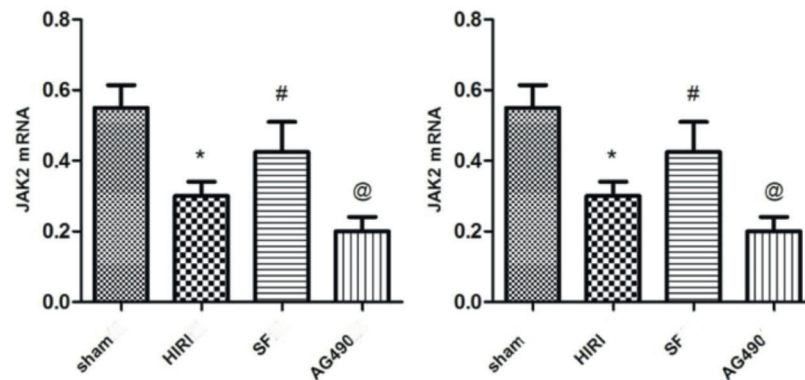


Figure 2. Comparison of JAK2mRNA and STAT3mRNA levels in each group. *compared with sham group, $p < 0.05$; compared with HIRI group, $p < 0.05$; @ compared with SF group, $p < 0.05$.

tion, the serum ALT, AST and AKP levels also increased with the results suggesting that immune inflammatory response may lead to hepatic cell and liver damage. Sevoflurane is clinically a common inhalational anesthetic and may prevent the occurrence of HIRI, but the mechanism is unknown yet¹². Researches related to the mechanism of sevoflurane preventing HIRI inflammation are few. Duan et al¹³ found that pulmonary ischemia

reperfusion injury led to HIRI in rats, increased the expression of TNF- α , IL-1 β , monocyte chemoattractant protein-1 and NF- κ B protein, and decreased that of IL-10 protein. In our research we discovered that compared with the HIRI group, the levels of IL-1 β , IL-6 and TNF- α in SF group were lower, showing that the sevoflurane may prevent the occurrence of HIRI inflammation. The liver showed basically normal structure

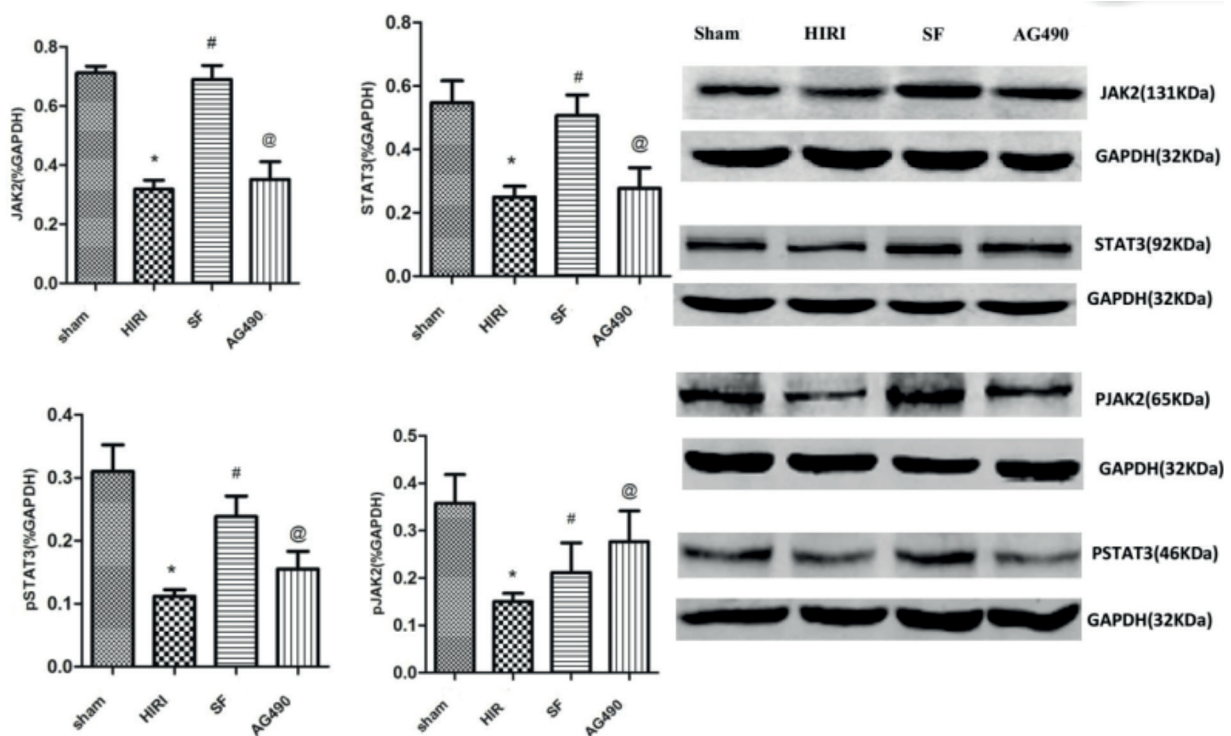


Figure 3. Comparison of JAK2 and STAT3 and their phosphorylated proteins in each group. *compared with sham group, $p < 0.05$; compared with HIRI group, $p < 0.05$; @ compared with SF group, $p < 0.05$.

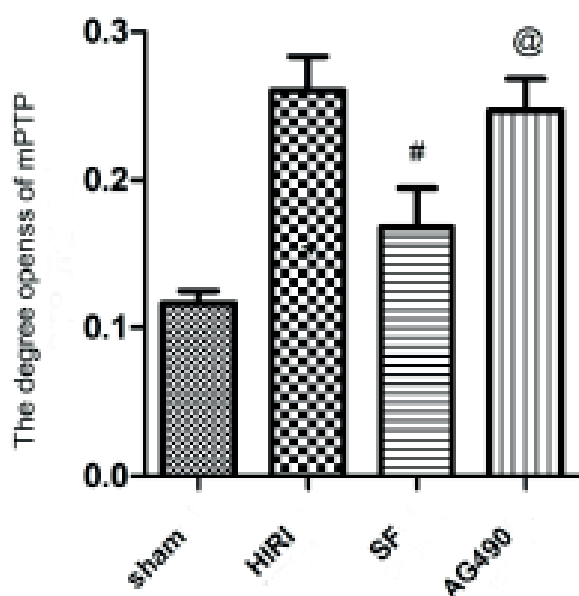


Figure 4. Comparison of the openness of the four groups of mPTP. *compared with sham group, $p < 0.05$; compared with HIRI group, $p < 0.05$; @ compared with SF group, $p < 0.05$.

and inflammation in the lobule and portal area was more moderate compared to the HIRI group after sevoflurane intervention. The JAK2-STAT3 pathway mediates the development of inflammation and plays an important role in the pathogenesis of ischemia-reperfusion injury^{12,14}. The JAK family consists of JAK1, JAK2, JAK3, and TYK; the STAT family includes STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. However, JAK2-STAT3 pathway received much concern. Its decreased expression level gives a major impact on the occurrence of cardiac, renal and cerebral ischemia-reperfusion injury^{4,15,16}. Moreover, some drugs such as isoliquiritigenin prevent ischemia-reperfusion injury occurrence in connection with the activation of JAK2-STAT3 pathway¹⁷. AG490 is a JAK2-STAT3 pathway-specific antagonist. It was observed, after intervention and under the optical microscope, that there were abnormal liver structures, hepatic sinusoid expansion, severe hepatic congestion, inflammatory cell infiltration in the portal area and between the hepatic cells, and obvious hepatic cell edema. In addition, the liver function and inflammation markers in the AG490 group were also higher than those in the SF group. In this paper, following the sevoflurane intervention to rats with HIRI, it was found that the protein levels of JAK2, pJAK2, STAT3 and p STAT3 increased and the liver function and inflammation mar-

kers improved, suggesting the sevoflurane may have remitted the HIRI by activating the JAK2-STAT3 pathway. Previous studies¹⁸ confirmed the myocardial ischemia reperfusion injury is possibly related to the over-opening of mPTP. Smith et al¹⁹ found that AG490, JAK2-STAT3 pathway inhibitor, can improve the myocardial Ischemia reperfusion injury in rats through inhibiting the over-opening of mPTP. The openness of mPTP is also connected with HIRI. Huang et al²⁰ found hydrogen sulfide pretreatment can improve the HIRI condition in rats by activating Akt-GSK-3 β signal pathway and inhibiting the over-opening of mPTP. In this study, the openness of HIRI after HIRI in rats significantly increased, while after sevoflurane pretreatment significantly decreased; then, combined together with AG490 pretreatment and playing its part again, it was significantly higher. The results showed that the mechanism of sevoflurane improving the openness of HIRI in rats with HIRI is probably related to the activation of JAK2-STAT3 pathway.

Conclusions

We found that sevoflurane could significantly improve the HIRI in rats and decrease hepatic immune inflammatory responses, through activating the JAK2-STAT3 pathway and inhibiting the over-opening of mPTP.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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