



# Ischemic Postconditioning Attenuates Ischemia/Reperfusion-induced Injury Through Activating Inflammatory Signaling Pathways

Tianyi Su<sup>1</sup>, Tian Su<sup>2</sup>

<sup>1</sup>Department of Surgery, Nanan District People's Hospital, Chongqing, P. R. China

<sup>2</sup>Department of Surgery, Chongqing Medical University, Chongqing, P. R. China

## Email address:

sabc1984@163.com (Tian Su), sutianycq@sohu.com (Tianyi Su)

## To cite this article:

Tianyi Su, Tian Su. Ischemic Postconditioning Attenuates Ischemia/Reperfusion-induced Injury Through Activating Inflammatory Signaling Pathways. *Cell Biology*. Vol. 9, No. 2, 2021, pp. 25-30. doi: 10.11648/j.cb.20210902.12

**Received:** July 14, 2021; **Accepted:** July 27, 2021; **Published:** August 5, 2021

---

**Abstract:** The role of post-treatment in protecting organ ischemia and re-perfusion damage is increasingly recognized, however, its mechanism of the action is not very clear, all in all, it still needs further research. The purpose of this experiment is to investigate whether IPO can reduce I/R-induced liver damage through inhibiting inflammatory signaling pathways in rats. Rats were randomly divided into sham, I/R, IPO and LY294002+IPO groups. The levels of AST and ALT were assessed. The expression levels of IL-1, Akt, NF- $\kappa$ B-P65 and TNF- $\alpha$  were analyzed using western blot analysis. The expression levels of ALT, AST, IL -1, TNF- $\alpha$  and NF- $\kappa$ B-P65 were significant reduction in the IPO group compared with those in the I/R group. Furthermore, the protein expression level of phosphorylated Akt was observed to be significant increase in the livers of the rats in the IPO group compared with those in the I/R group. Moreover, LY294002 was found to offset the advantages of IPO. To the best of our knowledge, this study provided the clear evidence to show that IPO significantly reduced the injury caused by I/R, and it might protect the liver from hepatic injury through activating the phosphoinositide 3-kinase pathway, which increased the expression of Akt, and inhibited the protein expression of IL-1, NF- $\kappa$ B-P65 and TNF- $\alpha$ .

**Keywords:** Ischemic Postconditioning, Inflammatory, Signaling Pathways

---

## 1. Introduction

It is well established that ischemia followed by reperfusion leads to liver injury. Ischemia-reperfusion (I/R) injury occurs during liver transplantation and trauma, as well as during liver resection. It has been proposed that inflammatory signaling cascades may be activated during the process of I/R. Inflammatory signaling cascades have been found to induce the activation of macrophages, as well as increase infiltration by neutrophils and lymphocytes [1]. These cells produce several mediators, including reactive oxygen species (ROS), proteases and cytokines, which cause cell damage. Kupffer cells are activated following reperfusion [2, 3] and have been reported to release inflammatory cytokines and free radicals [4-6]. It has previously been reported that tumor necrosis factor (TNF)- $\alpha$  [7], interleukin (IL) 1 [8], ROS [7, 9] and leukotrienes [10] recruit neutrophils to the liver [11], which may have an important role in acute liver injury and lead to

cell death, as well as activate internal signaling pathways, for example the cytochrome *c*-caspase signaling pathway. Therefore, attenuation of I/R injury is of critical importance in surgery. Several protective and treatment strategies have been reported, including ischemic pre- and postconditioning [12-14]. Such interventions may be performed at different time-points. Liver pre- and postconditioning may be used during liver transplantation and resection. Ischemic post conditioning (IPO) may be an important strategy to protect the liver from (I/R) injury. Zhao *et al* [15] were the first to discover that in the heart, postconditioning using the repetitive application of ischemia during early reperfusion had a cardio-protective effect through attenuating reperfusion injury. At the start of reperfusion, three cycles of 30 sec reperfusion and 30 sec left anterior descending artery reocclusion preceded the 3 h of reperfusion [15]. Infarct size was found to be significantly reduced in the pre- and postconditioning groups compared with the controls.

Postconditioning was observed to be as effective as preconditioning in reducing infarct size and preserving endothelial function. Therefore, postconditioning may be clinically applicable for coronary intervention, coronary artery bypass surgery, organ transplantation and peripheral revascularization where reperfusion injury is a problem [15]. However, there have been few reports on IPO in the liver and whether IPO in the liver is capable of activating inflammatory signaling pathways has yet to be elucidated. Therefore, the present study investigated the mechanisms underlying postconditioning in the liver.

## 2. Material and Methods

### 2.1. LY294002 and Experimental Animals

The phosphoinositol-3-kinase inhibitor 2-(4-morpholinyl)-8-phenyl-4 H-1-benzopyran-4-one (LY294002) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Male Sprague-Dawley rats (weight, 200-250 g;  $n=28$ ) were obtained from China. All procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with recommendations published in the Guide for Care and Use of Laboratory Animals by the National Research Council.

### 2.2. Experimental Groups and Protocols

Adult male Sprague-Dawley rats were used as the experimental animals and were maintained in a temperature-controlled environment (25-30°C) on a standard diet and water *ad libitum*. The rats were randomly divided into four groups ( $n=7$ /group): Sham, I/R, ischemia postconditioning (IPO) and LY294002+IPO groups. The rats in the I/R group underwent occlusion of the porta hepatis for 60 min, followed by persistent reperfusion for 2 h. In the rats in the IPO group, following the induction of ischemia, the livers were postconditioned using five cycles of reperfusion for 10 sec followed by ischemia for 10 sec, performed three times. The livers were then reperused for 2 h. In the rats in the LY294002+IPO group, ischemia was induced in LY294002 (15  $\mu$ mol/l)-treated livers, followed by IPO as described for the IPO group, subsequent to liver reperfusion for 2 h. Following a midline laparotomy incision, an atraumatic vascular clip was placed on the vessels blocking the portal venous and hepatic arterial blood supply to the median and left lateral lobes of the liver, which resulted in ~70% rat liver I/R injury. The rats in the sham group underwent the same surgical procedure as the rats in the other groups, except vessels clips were not applied. Blood samples and liver tissues were taken from the rats in each group for analysis following reperfusion for 2 h.

### 2.3. Serum Liver Function Assay

Blood samples were obtained from the rats following reperfusion for 2 h. Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) levels were measured using a standard clinical automated analyzer (ILab 600;

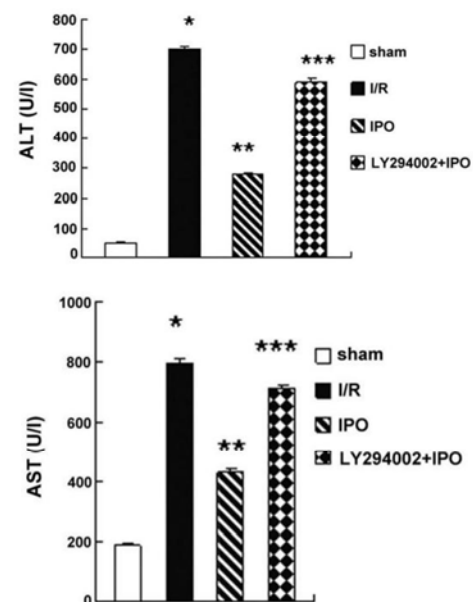
Shimadzu Corporation, Kyoto, Japan).

### 2.4. Phosphorylated (p)-Akt, IL-1, TNF- $\alpha$ and Nuclear Factor- $\kappa$ B-P65 Protein Expression

Total protein was extracted from the hepatic tissue samples and quantified using the Bradford assay. Equal quantities of protein (50  $\mu$ g) were separated using SDS-PAGE. Proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were incubated overnight at 4°C with anti-Akt, -IL-1, -TNF- $\alpha$  and NF- $\kappa$ B-P65 rabbit polyclonal antibodies, at a 1:500 dilution. Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (dilution, 1:2,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Membranes were re-incubated with anti- $\beta$ -actin antibodies at a 1:5,000 dilution (Santa Cruz Biotechnology, Inc.) to control for protein loading. Immunoreactive bands were visualized using the enhanced chemiluminescence Advance Western Blotting Detection Kit (GE Healthcare, Little Chalfont, UK) in a GeneGnome system (Synoptics Ltd, Cambridge, UK). Band intensity was measured using Quantity One® software (Bio-Rad, Hercules, CA, USA).

### 2.5. Statistical Analysis

Data are presented as the mean  $\pm$  standard deviation. Multiple comparisons were performed using analysis of variance. Comparisons between two groups were performed using Student's t-test. All analyses were conducted using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA).  $P<0.05$  was considered to indicate a statistically significant difference.

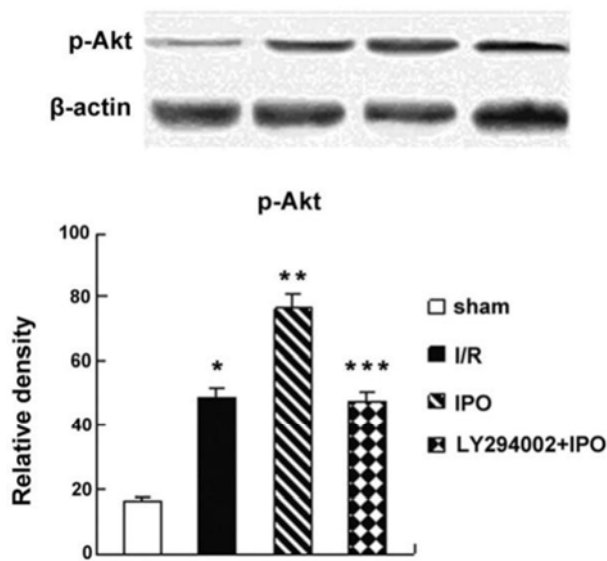


**Figure 1.** Serum levels of (A) ALT and (B) AST in rats. ALT and AST serum levels were assessed at the end of the treatment conditions. The levels of ALT and AST were significantly decreased in the IPO-treated rat liver tissues. LY294002 attenuated the IPO-induced decreases in ALT and AST. Data are presented as the mean  $\pm$  standard deviation. \* $P<0.05$  vs. sham; \*\* $P<0.05$  vs. I/R; \*\*\* $P<0.05$  vs. IPO ( $n=7$ /group). ALT, alanine aminotransferase; AST, aspartate transaminase; I/R, ischemia-reperfusion; IPO, ischemic postconditioning.

### 3. Results

#### 3.1. Physiological Function of IPO in Hepatic injury Caused by I/R (HIRI)

We observed that the obvious differences of Serum levels of ALT and AST measured after 2 h of reperfusion following 1h of ischemia occurred in these groups. The value of ALT and AST in the I/R group was observed to be significantly increased compared with those in the rats in the sham group. IPO treatment was found to significantly reduce ALT and AST serum levels compared with those in the I/R group (Figure 1A and B). Furthermore, LY294002 was not observed to inhibit the protective effect of IPO.

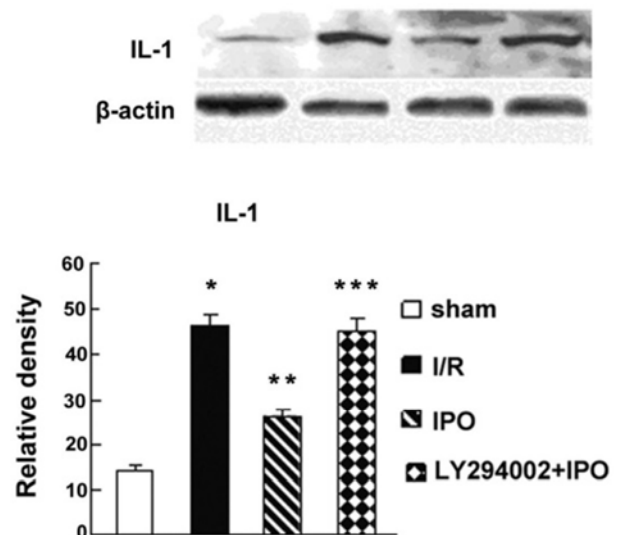


**Figure 2.** P-Akt expression detected using western blot analysis. Rats were randomly divided into sham, I/R, IPO and IPO+LY294002 groups. The rats in the I/R group underwent occlusion of the porta hepatis for 60 min, followed by persistent reperfusion for 2 h. In the rats in the IPO group, following the induction of ischemia, the livers were postconditioned using five cycles of reperfusion for 10 sec followed by ischemia for 10 sec, performed three times. The livers were then reperused for 2 h. In the rats in the LY294002+IPO group, ischemia was induced in LY294002 (15  $\mu$ mol/l)-treated livers, followed by IPO treatment, as described for the IPO group, subsequent to liver reperfusion for 2 h. At the end of the treatment, liver samples were obtained from the rats and subjected to western blot analysis using anti-p-Akt antibodies. Densitometric scanning was performed to assess p-Akt protein expression which was normalized to that of  $\beta$ -actin. Data are presented as the mean  $\pm$  standard deviation from four independent experiments ( $n=7$ /group). \* $P<0.05$  vs. sham; \*\* $P<0.05$  vs. I/R; \*\*\* $P<0.05$  vs. IPO. I/R, ischemia-reperfusion; IPO, ischemic postconditioning p-, phosphorylated; Akt, protein kinase B.

#### 3.2. p-Akt, IL-1, TNF- $\alpha$ and NF- $\kappa$ B-P65 Protein Expression

To assess whether IPO protected the liver against I/R injury through activating the prosurvival kinases PI3K and Akt, thereby inhibiting the expression of IL-1, TNF- $\alpha$  and NF- $\kappa$ B-P65, the protein expression of p-Akt, IL-1, TNF- $\alpha$  and NF- $\kappa$ B-P65 was analyzed using western blot analysis. p-Akt expression was found to be significantly increased in the liver tissue in the rats in the I/R group compared with that

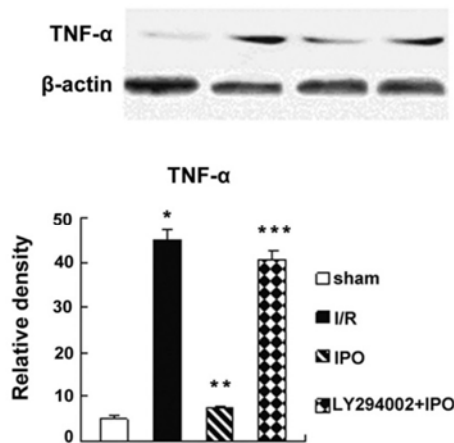
in the rats in the sham group. Furthermore, p-Akt expression was observed to be significantly higher in the liver tissues in the rats in the IPO group, compared with that in the I/R group. Moreover, LY294002 was found to significantly attenuate the IPO-induced increases in p-Akt expression (Figure 2). Inflammatory signaling pathways have been reported to be associated with the protective effects of preconditioning [16-18]. Therefore, the purpose of this experiment is to investigate whether IPO altered the activation of liver I/R-induced inflammatory signaling. The expression of IL-1, TNF- $\alpha$  and NF- $\kappa$ B-P65 was assessed. Compared with the rats in the sham group, IL-1, TNF- $\alpha$  and NF- $\kappa$ B-P65 protein expression were significantly increased in the rats in the I/R group. Furthermore, compared with the I/R group, IL-1, TNF- $\alpha$  and NF- $\kappa$ B-P65 protein expression was significantly decreased in the rats in the IPO group (Figures 3-5). LY294002 was observed to significantly attenuate the IPO-induced increases in p-Akt expression and decreases in IL-1, TNF- $\alpha$  NF- $\kappa$ B-P65 expression.



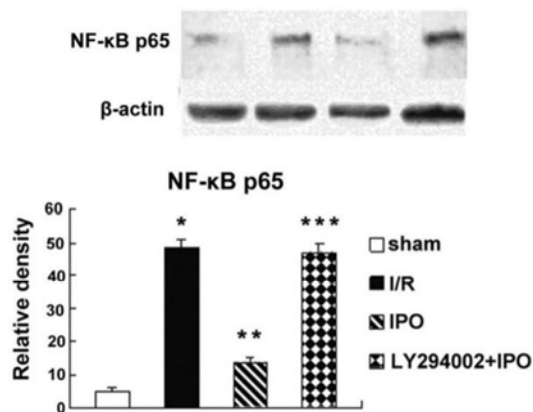
**Figure 3.** IL-1 expression detected using western blot analysis. After 60 min of ischemia and 2 h of reperfusion, IL-1 protein expression in IPO-treated hepatic tissues was determined using western blot analysis. I/R treatment activated inflammatory signaling in the rat liver. IPO treatment inhibited IL-1 expression, while LY294002 significantly attenuated the IPO-induced inhibition of IL-1 expression.  $\beta$ -actin was used as a loading control. (A) Western blot of IL-1 expression. (B) Ratio of IL-1 to  $\beta$ -actin expression. Data are presented as the mean  $\pm$  standard ( $n=7$ /group). \* $P<0.05$  vs. sham; \*\* $P<0.05$  vs. I/R; \*\*\* $P<0.05$  vs. IPO. I/R, ischemia-reperfusion; IPO, ischemic postconditioning; IL, interleukin.

### 4. Discussion

I/R injury is associated with hepatic resection and liver transplantation and is a serious complication in clinical practice, despite several attempts to solve the problem. It is well established that the redox balance, which is essential for normal tissue function and tissue integrity, is dysregulated during I/R and causes an accumulation of ROS [19, 20]. The formation of ROS and oxidative stress are the most commonly reported mechanisms involved in HIRI [21, 19].



**Figure 4.** TNF- $\alpha$  expression detected using western blot analysis. After 60 min of ischemia and 2 h of reperfusion, TNF- $\alpha$  protein expression was assessed in IPO-treated hepatic tissues using western blot analysis. I/R treatment activated inflammatory signaling in the rat liver which led to an increase in TNF- $\alpha$  protein expression. IPO treatment inhibited the I/R-induced increases in TNF- $\alpha$  expression and LY294002 significantly attenuated the effect of IPO.  $\beta$ -actin was used as a loading control. (A) Western blot of TNF- $\alpha$  protein expression. (B) Ratio of TNF- $\alpha$  to  $\beta$ -actin expression. Data are presented as the mean  $\pm$  standard deviation ( $n=7$ /group). \* $P<0.05$  vs. sham; \*\* $P<0.05$  vs. I/R; \*\*\* $P<0.05$  vs. IPO. I/R, ischemia-reperfusion; IPO, ischemic postconditioning; TNF, tumor necrosis factor.



**Figure 5.** NF- $\kappa$ B-P65 expression detected using western blot analysis. After 60 min of ischemia and 2 h of reperfusion, NF- $\kappa$ B-P65 protein expression was assessed in IPO-treated hepatic tissues using western blot analysis. I/R treatment activated inflammatory signaling in the rat liver which led to an increase in NF- $\kappa$ B-P65 protein expression. IPO inhibited the increase in NF- $\kappa$ B-P65 and LY294002 significantly attenuated the effect of IPO.  $\beta$ -actin was used as a loading control. (A) Western blot of NF- $\kappa$ B-P65 expression (B) Ratio of NF- $\kappa$ B-P65 to  $\beta$ -actin expression. Data are presented as the mean  $\pm$  standard deviation ( $n=7$ /group). \* $P<0.05$  vs. sham; \*\* $P<0.05$  vs. I/R; \*\*\* $P<0.05$  vs. IPO. IPO, I/R, ischemia-reperfusion; IPO, ischemic postconditioning; NF- $\kappa$ B, nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells.

The present study aimed to investigate postconditioning strategies to overcome HIRI. Zhao *et al* [15] investigated whether postconditioning using the repetitive application of ischemia during early reperfusion may have a cardio-protective effect through attenuating reperfusion injury. They found that postconditioning was as effective as preconditioning in reducing infarct size and preserving endothelial function [15]. Therefore, postconditioning may

be clinically applicable for coronary intervention, coronary artery bypass surgery, organ transplantation and peripheral revascularization where reperfusion injury is a problem. Studies have shown that IPO may have two predominant effects on I/R injury. The first of which may be a more passive effect, in which the gradual reperfusion associated with IPO reduces the generation of ROS, decreases neutrophil aggregation [22] and inhibits mitochondrial calcium overloading [23]. The second is proposed to be a more active effect, in which IPO activates PI3K-Akt [24], ERK1/2 and the downstream endothelial nitric oxide synthase [25], as well as p70S6K [26], thereby inhibiting mitochondrial permeability transition pore opening [27], cell death and protein transcription, and ultimately protecting the ischemic organ. In the present study, p-Akt was found to be increased in the livers of rats in the IPO group compared with those in the rats in the I/R group. This IPO-induced increase in p-Akt levels was observed to be significantly attenuated by LY294002, suggesting that the activation of the PI3K pathway may be involved in IPO-induced liver protection. Furthermore, compared with the rats in the I/R group, the levels of the serum liver enzymes ALT and AST were found to be significantly decreased in the IPO group, suggesting that IPO may reduce HIRI, but that LY294002 may significantly inhibit the protective effect of IPO. Therefore, IPO may reduce HIRI through activating the PI3K pathway.

The release of cytokines caused by HIRI is an important factor. A variety of cytokines have been reported to be involved in the pathophysiology of HIRI [28], which act in autocrine and paracrine manners to exert their biological effects and are capable of causing hepatic injury independently or synergistically. These cytokines include IL-1, -6 and -10, as well as platelet-activating factor and TNF- $\alpha$ . TNF- $\alpha$  has been reported to have a role in the initial development of HIRI and is highly correlated with HIRI [8]. TNF- $\alpha$  may not cause sinusoidal endothelial cell swelling directly, but may significantly increase the expression of adhesion molecules on the surface of sinusoidal endothelial cells, enhancing the interaction between polymorphonuclear neutrophils with sinusoidal endothelial cells, thus causing a microcirculation disorder [29]. Furthermore, TNF- $\alpha$  may activate the adhesion of polymorphonuclear neutrophils to the sinusoidal endothelial cells, resulting in the release of oxygen free radicals, causing liver damage [30]. IL-1 may not only induce Kupffer cells to produce TNF- $\alpha$  and increase the release of oxygen free radicals from polymorphonuclear neutrophils, but may cooperate with TNF- $\alpha$  on the endothelial cells, inducing the synthesis of thrombin and plasmin, thus destroying the endothelial cells [31] and increasing HIRI. The transcription factor NF- $\kappa$ B is a proinflammatory protein released by inflammatory mediators during HIRI. Activation of NF- $\kappa$ B has been reported to regulate the expression of a variety of molecules, including TNF- $\alpha$ , IL-6, IL-8, monocyte chemoattractant protein-1, inducible nitrogen oxide synthase, as well as adhesion molecules, including intercellular adhesion molecule 1, vascular cell adhesion molecule-1, E-selectin and P-selectin.

These molecules may directly or indirectly act on endothelial cells, leading to endothelial cell injury. Phosphorylation of inhibitor of NF- $\kappa$ B  $\alpha$  causes NF- $\kappa$ B activation. Activated NF- $\kappa$ B then passes through the nuclear membrane receptors into the nucleus where it binds its target genes, rapidly induces target genes transcription and increases the expression of a variety of molecules, which promote the release of inflammatory mediators, resulting in tissue damage. The above-mentioned factors may be activated though the toll-like receptor (TLR) 4-NF- $\kappa$ B pathway, which may activate the PI3K pathway and downstream Akt and inhibit glycogen synthase kinase 3 $\beta$  activity, thereby hindering the activation of NF- $\kappa$ B-P65 and the production of TNF- $\alpha$  and IL-1. The present study showed that the expression of NF- $\kappa$ B-P65, TNF- $\alpha$  and IL-1 were significantly increased in the I/R group and significantly reduced in the IPO group. However, LY294002 significantly inhibited the effect of IPO, with the expression of NF- $\kappa$ B-P65, TNF- $\alpha$  and IL-1 being significantly increased in the IPO+LY294002 group compared with those in the IPO group ( $P < 0.05$ ). It was reasoned that these factors may be primarily generated through this pathway, which reduced HIRI and protected livers.

## 5. Conclusions

All in all, we found that IPO had a protective effect on rat livers against I/R injury. IPO may activate the TLR4 receptor and in turn the PI3K pathway, promoting the phosphorylation and activation of Akt, thus inhibiting downstream NF- $\kappa$ B-P65, TNF- $\alpha$  and IL-1 expression. This process may ultimately reduce HIRI.

## Acknowledgements

The National Natural Science Foundation of China (nos. 30530360 and 30772098) supported our study.

## References

- [1] Brenner C, Galluzzi L, Kepp O and Kroemer G: Decoding cell death signals in liver inflammation. *J Hepatol* 59: 583-594, 2013.
- [2] Ngo BT, Beiras-Fernandez A, Hammer C and Thein E: Hyperacute rejection in the xenogenic transplanted rat liver is triggered by the complement system only in the presence of leukocytes and free radical species. *Xenotransplantation* 20: 177-187, 2013.
- [3] Ogawa K, Kondo T, Tamura T, Matsumura H, Fukunaga K, Oda T and Ohkohchi N: Influence of Kupffer cells and platelets on ischemia-reperfusion injury in mild steatotic liver. *World J Gastroenterol* 19: 1396-1404, 2013.
- [4] Llacuna L, Marí M, Lluís JM, García-Ruiz C, Fernández-Checa JC. and Morales A: Reactive oxygen species mediate liver injury through parenchymal nuclear factor-kappaB inactivation in prolonged ischemia/reperfusion. *Am J Pathol* 174: 1776-1785, 2009.
- [5] Yamaguchi Y, Matsumura F, Liang J, *et al*: Neutrophil elastase and oxygen radicals enhance monocyte chemoattractant protein- expression after ischemia/reperfusion in rat liver. *Transplantation* 68: 1459-1468, 1999.
- [6] Bautista AP: Acute ethanol binge followed by withdrawal regulates production of reactive oxygen species and cytokine-induced neutrophil chemoattractant and liver injury during reperfusion after hepatic ischemia. *Antioxid Redox Signal* 4: 721-731, 2002.
- [7] Spencer NY, Zhou W, Li Q, *et al*: Hepatocytes produce TNF- $\alpha$  following hypoxia-reoxygenation and liver ischemia-reperfusion in a NADPH oxidase- and c-Src-dependent manner. *Am J Physiol Gastrointest Liver Physiol* 305: G84-G94, 2013.
- [8] Gong WH, Zheng WX, Wang J, Chen SH, Pang B, Hu XM and Cao XL: Coexistence of hyperlipidemia and acute cerebral ischemia/reperfusion induces severe liver damage in a rat model. *World J Gastroenterol* 18: 4934-4943, 2012.
- [9] Elias- Miró M, Jiménez- Castro MB, Rodés J and Peralta C: Current knowledge on oxidative stress in hepatic ischemia/reperfusion. *Free Radic Res* 47: 555-568, 2013.
- [10] Yang SL, Chen LJ, Kong Y, Xu D and Lou YJ: Sodium nitroprusside regulates mRNA expressions of LTC<sub>4</sub> synthesis enzymes in hepatic ischemia/reperfusion injury rats via NF-kappaB signaling pathway. *Pharmacology* 80: 11-20, 2007.
- [11] Bajt ML, Farhood A and Jaeschke H: Effects of CXC chemokines on neutrophil activation and sequestration in hepatic vasculature. *Am J Physiol Gastrointest Liver Physiol* 281: G1188-G1195, 2001.
- [12] Zhai Y, Petrowsky H, Hong JC, Busuttil RW and Kupiec-Weglinski JW: Ischaemia- reperfusion injury in liver transplantation - from bench to bedside. *Nat Rev Gastroenterol Hepatol* 10: 79-89, 2013.
- [13] Zhang Q, Fu H, Zhang H, *et al*: Hydrogen sulfide preconditioning protects rat liver against ischemia/reperfusion injury by activating Akt-GSK-3 $\beta$  signaling and inhibiting mitochondrial permeability transition. *PLoS One* 8: e74422, 2013.
- [14] Yun N and Lee SM: Activation of protein kinase C delta reduces hepatocellular damage in ischemic preconditioned rat liver. *J Surg Res* 185: 869-876, 2013.
- [15] Song X, Zhang N, Xu H, Cao L and Zhang H: Combined preconditioning and postconditioning provides synergistic protection against liver ischemic reperfusion injury. *Int J Biol Sci* 8: 707-718, 2012.
- [16] Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA and Vinten-Johansen J: Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 285: H579-H588, 2003.
- [17] Huerta L, Rancan L, Simón C, *et al*: Ischaemic preconditioning prevents the liver inflammatory response to lung ischaemia /reperfusion in a swine lung autotransplant model. *Eur J Cardiothorac Surg* 43: 1194-1201, 2013.
- [18] Li J, Lai X, Chen Y, Niu B and Gong J: Endotoxin tolerance attenuates liver ischemia/reperfusion injury by down-regulation of interleukin-1 receptor-associated kinase 4 in kupffer cells. *Transplant Proc* 43: 2531-2535, 2011.

- [18] Sano T, Izuishi K, Hossain MA, *et al*: Hepatic preconditioning using lipopolysaccharide: association with specific negative regulators of the Toll-like receptor 4 signaling pathway. *Transplantation* 91: 1082-1089, 2011.
- [19] Elias-Miró M, Jiménez-Castro MB, Rodés J and Peralta C: Current knowledge on oxidative stress in hepatic ischemia/reperfusion. *Free Radic Res* 47: 555-568, 2013.
- [20] Schriewer JM, Peek CB, Bass J and Schumacker PT: ROS-mediated PARP activity undermines mitochondrial function after permeability transition pore opening during myocardial ischemia-reperfusion. *J Am Heart Assoc* 2: e000159, 2013.
- [21] Sasaki M and Joh T: Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant, protective agents. *J Clin Biochem Nutr* 40: 1-12, 2007.
- [22] Stewart RK, Dangi A, Huang C, *et al*: A novel mouse model of depletion of stellate cells clarifies their role in ischemia/reperfusion- and endotoxin-induced acute liver injury. *J Hepatol* 60: 298-305, 2014.
- [23] Watanabe T, Oowada S, Kobayashi HP, *et al*: The role of the new Ca<sup>2+</sup> antagonist, CV159, in hepatic I/R injury-the evaluation of hepatic organ reducing activity using in vivo and ex vivo EPR. *J Surg Res* 145: 49-56, 2008.
- [24] Wang Y, Zhang ZZ, Wu Y, Ke JJ, He XH and Wang YL: Quercetin postconditioning attenuates myocardial ischemia/reperfusion injury in rats through the PI3K/Akt pathway. *Braz J Med Biol Res* 46: 861-867, 2013.
- [25] Pignataro G, Esposito E, Sirabella R, Vinciguerra A, Cuomo O, Di Renzo G and Annunziato L: nNOS and p-ERK involvement in the neuroprotection exerted by remote postconditioning in rats subjected to transient middle cerebral artery occlusion. *Neurobiol Dis* 54: 105-114, 2013.
- [26] Vigneron F, Dos Santos P, Lemoine S, *et al*: GSK-3 $\beta$  at the crossroads in the signalling of heart preconditioning: implication of mTOR and Wnt pathways. *Cardiovasc Res* 90: 49-56, 2011.
- [27] Pagel PS, Krolikowski JG, Shim YH, *et al*: Noble gases without anesthetic properties protect myocardium against infarction by activating prosurvival signaling kinases and inhibiting mitochondrial permeability transition in vivo. *Anesth Analg* 105: 562-9, 2007.
- [28] Klune JR and Tsung A: Molecular biology of liver ischemia/reperfusion injury: established mechanisms and recent advancements. *Surg Clin North Am* 90: 665-677, 2010.
- [29] Menger MD, Richter S, Yamauchi J and Vollmar B: Role of microcirculation in hepatic ischemia/reperfusion injury. *Hepato gastroenterology* 46 (Suppl 2): 1452-1457, 1999.
- [30] Shibuya H, Ohkohchi N, Tsukamoto S and Satomi S: Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfused rat liver. *Hepatology* 26: 113-120, 1997.
- [31] Shito M, Wakabayashi G, Ueda M, *et al*: Interleukin 1 receptor blockade reduces tumor necrosis factor production, tissue injury, and mortality after hepatic ischemia-reperfusion in the rat. *Transplantation* 63: 143-148, 1997.