

Contents lists available at [ScienceDirect](http://ScienceDirect.com)

Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm

Document heading doi:

The effect of ischemic precondition to IL-6 on rat liver ischemia–reperfusion injury in transplantation

Lin–Zhong Cui, Biao Wang, Li–Yan Chen, Jie Zhou*

Department of Hepatobiliary Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, 510000, Guangdong Province, China

ARTICLE INFO

Article history:

Received 21 February 2013

Received in revised form 25 February 2013

Accepted 10 March 2013

Available online 20 May 2013

Keywords:

Hypoxic preconditioning

Liver transplantation

Rat

IL-6

ABSTRACT

Objective: To investigate the effect of ischemic precondition to protect ischemia–reperfusion injury and reduce IL-6 expression in the rats liver transplantation. **Methods:** The rat portal vein infusion of autologous liver transplantation model were used. The rats were divided into ischemic preconditioning rats liver transplantation group (A group), the rats liver transplantation group (B group) and the normal rat control group (C group). Then we analyzed the changes of liver function, liver microstructure and the expression of IL-6, SOD and MDA within 48 h. **Results:** The pathology of liver in group A showed lobular architecture essentially normal, the liver cells was slightly swell and no significant changes in postoperative 12 h. In transmission electron microscope (46 000 \times), the mitochondria of liver cells in group A became swelling, elliptical can cristae partially broken. But there still has a small amount of arrangement. While that in group, the mitochondria were swollen, became round, serious visible crest reduce or ruptured. The result of over function test showed that the serum ALT and AST levels in group A and B were both higher than that in group C at each time period, but the serum ALT and AST levels in group A were lower than that in group B. The expression changes of IL-6 in group B were higher than that in group A and B ($P < 0.05$). The expression of MDA in group A is more obvious than that in group B ($P < 0.05$). **Conclusions:** Ischemic precondition could alleviate part of ischemia–reperfusion injury in the rat liver transplantation, and also could reduce IL-6 expression to protect the liver cells against liver damage and inflammatory cytokine production.

1. Introduction

Ischemia/reperfusion injury (IRI) and related inflammatory response are the main contributors to decreased liver function after liver transplantation. Avoiding the ischemia–reperfusion injury is very important for the treatment and prognosis of the disease. Ischemic preconditioning (IPC) is a method which brief interruption, then reinstatement of an organ's blood supply protects that organ from subsequent injury^[1]. IL-6 is a pleiotropic cytokine which produced during immune responses elicits a wide spectrum of physiological and pathogenic events and immune response^[2,3]. It can enhance the effect of other cytokines at physiological concentration, while induce immune injury at pathological condition^[4]. IL-6 is an important

indicator which reflects the severity of inflammation and tissue damage^[3,4]. Our study aims to investigate the effect of ischemic precondition to protect ischemia–reperfusion injury and related inflammatory response after liver transplantation.

2. Materials and methods

2.1. Animals

SD rats of either gender aged 8–11 weeks, weighted 240–300 g were purchased from Animal Center of Southern Medical University. The rats were randomly divided into 3 groups: ischemic preconditioning group (A group, $n = 24$), occlude liver transplantation group (B group, $n = 24$) and the control group (C group, $n = 24$). In A group, the animals underwent liver autotransplantation after occluding the hepatic artery portal vein 15 min and reperfusion 20 min. In B group, the animals underwent liver autotransplantation

*Corresponding author: Prof. Jie Zhou, Department of Hepatobiliary Surgery, Nanfang Hospital, Southern Medical University.

Tel: +8613632101856

E-mail: gdwkczl@163.com

directly. In C group, the animals do not accept any treatment.

Table 1

The characters of the SD rats *n*(%).

| Characters | | Group A | Group B | Group C |
|-------------|---------|-----------|-----------|-----------|
| Gender | Male | 10(41.70) | 8(33.30) | 15(62.50) |
| | Female | 14(58.30) | 16(66.70) | 9(37.50) |
| Age (weeks) | 8-9 | 11(45.80) | 7(29.20) | 8(33.30) |
| | 9-10 | 13(54.20) | 17(70.80) | 16(66.70) |
| Weight (g) | 250-275 | 15(62.50) | 6(25.00) | 13(54.20) |
| | 275-300 | 9(37.50) | 18(75.00) | 11(45.80) |

2.2. Instruments and agents

Instrument for microsurgery, 0/8 Vascular suture; vascular clamp; ringers solution; AV800 automatic biochemical analyzer were purchased from Hitachi Ltd, optical microscopy were purchased from OLYMPUS; high speed freezing centrifuge were purchased from the United States department general instrument company; X-ray film were purchased from SGSBG Shanghai Photosensitive material factory; finnpipette were purchased from Eppendorf; TRIzol Reagent (invitrogen), KGA1203 kit and RT-PCR kit; KGA1303 were purchased from BOSTER Wuhan, KGT003 Malondialdehyde testing kit were purchased from keyGEN bioTECH Nanjing, A001-3 superoxide dismutase (SOD) testing kit were purchased from Jiancheng Bioengineering Institute, Nanjing.

2.3. Experimental method

The model was a modification of orthotopic liver autotransplantation model with the portal vein (PV) used for reperfusion[6]. The rats were anesthetized by ketamine 100 mg/kg i.p., atropine 0.03 mg i.m. 10 min before surgery, and the anesthesia was maintained with ether continuous inhalation. Open the abdomen, free the ligaments around liver, porta hepatic artery, hepatic vein, and portal vein. Occlude the hepatic artery, hepatic vein, and porta hepatic artery by using vascular clips individually, and then release the vascular clips after 6 min for reperfusion for 6 times[5]. The rats were given Penicillin 1.6 million IU i.p. before suture. After surgery, the animals were housed in an air-conditioned room at 38 °C. The surgery is succeed if rats wake up and stands up instantly, maintain effective breathing and heartbeat. Perfusion via the portal vein, hepatic warm ischemia time is negligible (<5 s). In A group, the animals underwent liver autotransplantation after occluding the hepatic artery portal vein 15 min and reperfusion 20 min. In B group, the animals underwent liver autotransplantation directly; imitate ischemia-reperfusion injury in liver transplant. In C group, the animals do not accept any treatment. There were 24 rats in each group; six rats were killed at 2, 6, 12, and 24 h after surgery. The central lobe of the liver was removed for IL-6, MDA, SOD and liver function detection. The liver tissues underwent

pathologic examination.

2.4. Detection

2.4.1. Postoperative conditions

Three milliliters of blood was drawn from the IVC to test liver function. The central lobe of the liver was removed for pathologic examination. Electron microscopy specimens: fixed in 2.5% glutaraldehyde fixative, then dehydrated, soaked, embedded, polymerization, sliced, stained, and observed by electron microscopy.

2.4.2. RNA isolating and RT-PCR analysis

Total RNA from liver was isolated with Trizol, and 2 μL RNA was used for cDNA synthesis. Using β-actin as internal standard control, 2 μL cDNA was used as a template for PCR amplification to detect the target gene mRNA expression levels. According the kites instruction, 20 μL PCR system was established: 2 μL cDNA, 10 μL SYBR Green Mix (Applied Bio systems, Foster City, CA), 0.5 μL upstream primer and downstream primer(10 μmol/L). PCR amplification was performed with 32 cycles of 30 s at 95 °C, 30 s at 55 °C, 35 s at 72 °C: IL6 primer (594 bp) Sense primer: 5'GTC AAC TCC ATC TGC CCT TC 3' Anti-sense primer: 5'CTT GGT CCT TAG CCA CTC CT 3'

2.4.3. MDA detect

Liver tissue was weighed after surgery, and the tissue homogenates were centrifuged at 3 000 r/min for 15 min, Take supernatant for MDA detecting.

2.4.4 SOD detect

Liver tissue was weighed after surgery, and the tissue homogenates were centrifuged at 3 000 r/min for 15 min, Take supernatant for SOD detecting.

2.5. Statistical analysis

All measurement data are presented as $\bar{x} \pm s$ (standard error of the mean). The difference between means was analyzed using *t*-test. All statistical analyses were performed using SPSS 13.0 software. *P* values <0.05 were considered statistically significant.

3. Result

3.1. Pathological change of hepatocytes

The rats in group A shows pathological damage in hepatocytes: 12 h postoperative, lobular architecture is nearly normal, liver cells become swelling, no significant changes in liver tissue (Figure 1); The rats in group B show significantly damage in liver cells 12 h postoperative: liver cells is swollen around the central veins, hepatic sinusoid is narrowing, lobular architecture is not obvious, accompanied

by red blood cells and thrombus, surrounded by the infiltration of inflammatory cells (Figure 2).

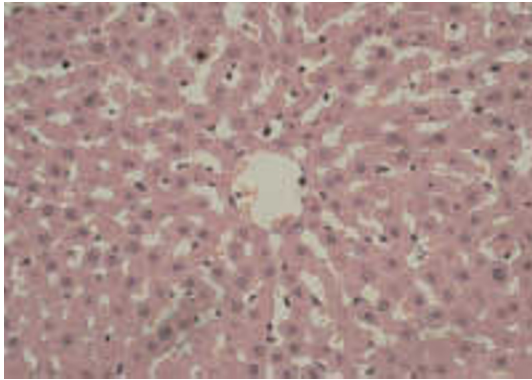


Figure 1. Group A shows pathological damage in hepatocytes (HE × 400).

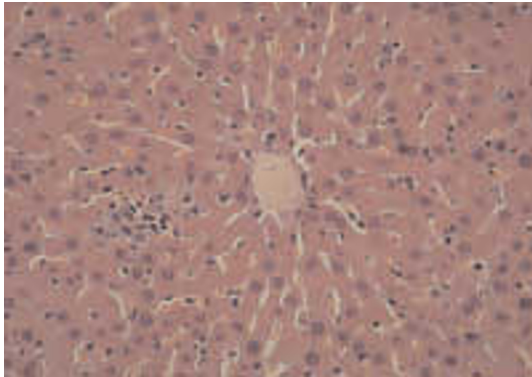


Figure 2. Group B show significantly damage in liver cells 12 h postoperative (HE × 400).

3.2. Ultrastructural damage of the liver cells under electron microscope

Under a transmission electron microscope (46 000×) mitochondrial visible in every group: after 12 h, group A rat liver mitochondria slight swelling, oval, cristae partial tear, and the majority are the normal arrangement. Endoplasmic reticulum exist (Figure 3); Group B rat liver mitochondria was markedly swollen state, round, with vacuolar degeneration, damaged, or disappear. The endoplasmic reticulum is unclear (Figure 4).

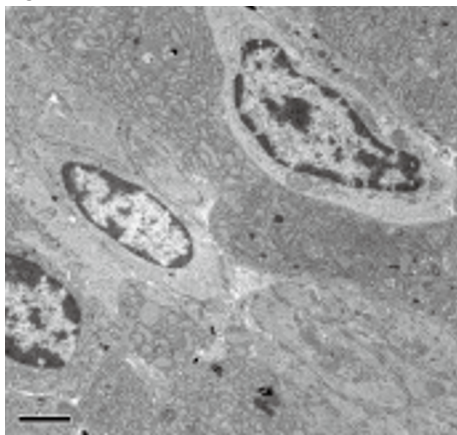


Figure 3. Group A rat liver mitochondria slight swelling, oval, cristae partial tear, and the majority are the normal arrangement. Endoplasmic reticulum exist (46 000×).

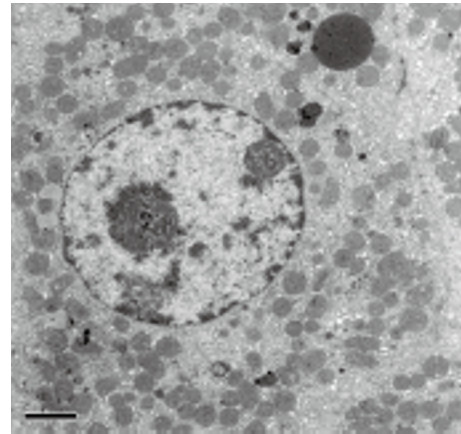


Figure 4. Group B rat liver mitochondria were markedly swollen state, round, with vacuolar degeneration, damaged, or disappear. The endoplasmic reticulum is unclear (46 000×).

3.3. Expression of ALT and AST

Postoperative liver function results show that, the ALT and AST were at the highest level after 2 h, and slowly returned to normal after 24 h. In each period, the serum ALT and AST levels in group A and group B were significantly higher than group C, but group A serum ALT and AST levels were significantly lower than group B ($P < 0.05$) (Figure 5&6). The indicators show that the degree of postoperative liver damage in group A was slight than group B.

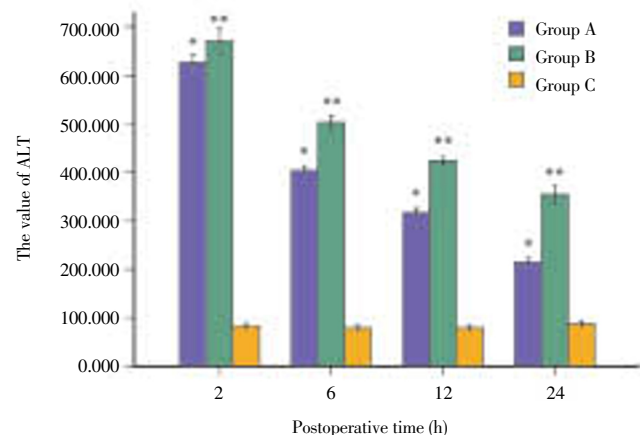


Figure 5. The expression of ALT in different time. The expression of ALT in group A and group B tend to decrease, it was at the highest level after 2 h, then decreased. *: compared with group C, $P < 0.05$, **: compared with group A and C, $P < 0.05$.

3.4. The expression of IL-6

An RT-PCR result shows that: The expression of IL-6 in group A and group B of were significantly higher than the group C, ($P < 0.05$) (Figure 7). While the expression of IL-6 in the group B was highest at 12 h postoperative, then decreased. The expression of IL-6 in group A was significantly less than the group B at 2 h, 6 h and 12 h postoperative ($P < 0.05$). Less IL-6 expressed in group C, no significant fluctuations in each time period.

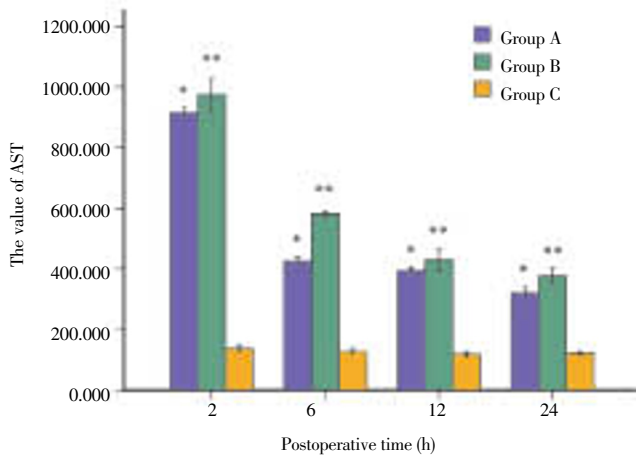


Figure 6. The expression of AST in different time. The expression of AST in group A and group B tend to decrease, it was at the highest level after 2 h, then decreased. *: Compared with group C, $P < 0.05$, **: Compared with group A and C, $P < 0.05$.

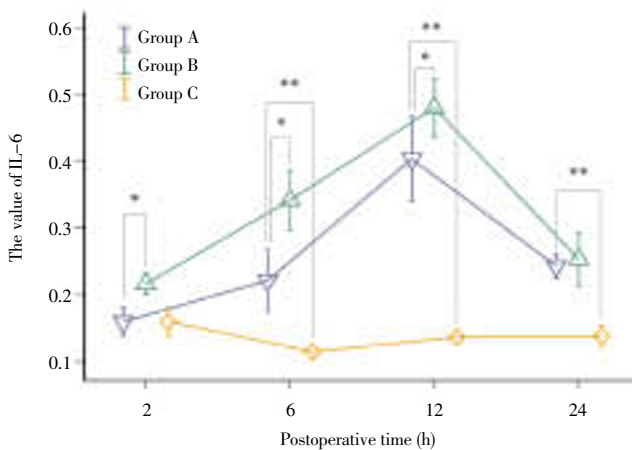


Figure 7. The expression of IL-6 in different time. The expression of IL-6 in group A and group B was highest at 12 postoperative, then tend to decrease, *: Compared with group C, $P < 0.05$, **: Compared with group A and C, $P < 0.05$.

3.5. The expression of SOD and MDA

The expression of SOD in group A and group B of were significantly higher than the group C, ($P < 0.05$); While group A was significantly lower than group B at 6 h and 12 h postoperative ($P < 0.05$) (Table 2). The MDA in group A and group B of were significantly lower than the group C, ($P < 0.05$); While group A was significantly higher than group B ($P < 0.05$) (Table 3).

Table 2
Activity of SOD (U/mg prot)($\bar{x} \pm s, n=6$).

| Group | 2 h | 6 h | 12 h | 24 h |
|-------|-------------|--------------|--------------|-------------|
| A | 82.43±3.22 | 94.77±3.01* | 91.78±1.33* | 81.81±4.69 |
| B | 84.18±2.44 | 103.16±5.43▲ | 95.99±1.24▲ | 84.16±3.70 |
| C | 80.11±0.82▲ | 83.75±4.10*▲ | 78.42±2.41*▲ | 87.28±4.10* |
| F | 4.436 | 30.798 | 166.317 | 2.590 |
| P | 0.031 | <0.001 | <0.001 | 0.108 |

▲: Compared with group A, $P < 0.05$; *: Compared with group B, $P < 0.05$.

Table 3
MDA concentration (nmol/mg prot)($\bar{x} \pm s, n=6$).

| Group | 2 h | 6 h | 12 h | 24 h |
|-------|-------------|-------------|-------------|-------------|
| A | 4.11±0.07 | 4.04±0.07 | 4.59±0.18 | 4.67±0.49 |
| B | 4.08±0.06 | 3.96±0.11 | 4.35±0.23 | 4.65±0.24 |
| C | 4.75±0.46*▲ | 4.81±0.17*▲ | 5.00±0.19*▲ | 5.16±0.15*▲ |
| F | 11.624 | 85.184 | 16.025 | 4.617 |
| P | 0.001 | <0.001 | <0.001 | 0.027 |

▲: Compared with group A, $P < 0.05$; *: Compared with group B, $P < 0.05$.

4. Discussion

Ischemia-reperfusion injury in the process of liver transplantation is inevitable, increase liver damage opportunity, especially for through an injury process such as hot ischemia, cold ischemia-reperfusion injury[6]. Ischemia-reperfusion injury is a continuous process and eventually leads to liver cell damage[7]. Liver transplantation postoperative complications affect liver transplantation patients postoperative survival rate seriously, and getting more and more attention. Murry[8] proved the protective effect of ischemic precondition for the first time, which can produce protection for all kinds of animals and organization after precondition 1-3 h. Ischemic precondition could alleviate cold ischemia-reperfusion, inhibit excessive production of oxygen radical, improve liver antioxidant ability and play a protective effect[5].

Interleukin-6 (IL-6) is a kind of cell factor with multiple immune adjustment function. Because of inflammation and anti-inflammatory two-way function, IL-6 plays an important role in biological damage and the pathological process of occurrence, development of the SIRS. Its role and the content of the organization related, the normal level on the body can be beneficial, but overproduction can cause a series of inflammatory damage[9]. IL-6 is an acute phase protein, that can enhance T, B lymphocytes and NK cell activity, and can be produced by liver cell in the acute phase of disease. There are some injure to the structure and function of liver cell after liver transplantation, the severity liver ischemia-reperfusion injury affect the success rate of liver transplantation directly, how to reduce liver cell injure and necrosis is the key of disease prognosis. Liver ischemia-reperfusion injury is a complex pathophysiological process, that variety of pathway play a role and multiple factors involved. Liver transplantation not only experiences the same thermal ischemia-reperfusion injury as other conditions, but also experience cold ischemia-reperfusion injury[10]. That laboratory testing of IL-6 can reveal the severity of ischemia or oxygen deficit of the body early, help to judge and dispose of liver early after liver transplantation, and then reduce inflammatory factor injure[11,12].

Our study experienced ischemia-reperfusion injury to the rats, test expression of IL-6 in different period after liver transplantation, and found that control group C have a small amount of IL-6 expression, have no obvious fluctuation anytime; the quantity of IL-6 expression in group A and group B postoperative is higher than those in control group. But IL-6 expression of group A is slightly lower than group

B, the difference having statistical significance. IL-6 level of human is low in physiological condition, but in pathological condition, IL-6 secretion increased and various kinds of inflammation caused by the factors of the waterfall release can cause inflammation reaction and tissue cell injure^[13]. IL-6 can reflect the degree of inflammation and injure. IL-6 is key ingredients to inflammatory medium network, plays an important role in inflammation. The serum IL-6 level can show tissue injures level and a variety of diseases and the pathophysiological process was related closely^[14]. The test result of ALT and AST for liver function show that suggest that group B is higher than group A significantly, autologous transplantation group of liver injure is heavier, and IL-6 expression has positive correlation to Liver function injure. Some research result show that precondition reduce reactive oxygen species (ROS) exploded by breathing in the process of ischemia-reperfusion, MDA is an important indicators to show ROS increase in the organization, precondition can reduce super oxygen anion of ischemia-reperfusion injury, and also reduce serum MDA at the same time^[15]. ROS play an important role in ischemia-reperfusion injury; it is a triggered factor to activated mitochondrial permeability transport hole (MPTP) and shut down the signaling pathways^[16]. Compare the mitochondrial injure among the groups by transmission electron microscope, it is obviously that different mitochondrial sizes of autologous transplantation, obvious swelling, approximate circular, degeneration, or cristae reduce, fracture or disappear. Observe liver cell injure in autologous transplantation group 12 h postoperation from pathological perspective, we can see the around the markedly swollen of liver cells around central vein, the gap widened, hepatic sinusoids narrow, hepatic lobule structure fuzzy, fill of red blood cells and blood clots, inflammatory cells infiltration. And hepatic lobule structure of group A appears normal, swelling slightly, no significant changes of liver tissue^[17,18]. It is suffice to show that ischemic precondition could alleviate ischemia-reperfusion injury in the rat liver transplantation.

In conclusion, liver function recovery in rats liver transplantation and degree of ischemia-reperfusion injury and protection measures exhibiting a close relationship. Ischemic precondition could enhance resistant ability to anaerobic and ischemia in rat liver transplantation postoperative, reduce IL-6 expression, reduce liver cell mitochondria injury, and alleviate ischemia-reperfusion injury in the rat liver transplantation, the function and significance is more important in clinical.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Ye Meng, Zhang Jiwei. Progress in the study of ischemic preconditioning in surgery. *J Surg Concepts Pract* 2004; **9**(1): 78-80.
- [2] Ravaglia G, Forti P, Maioli F. Associations of the 174g/c interleukin-6 gene promoter polymorphism with blood interleukin-6 and mortality in the elderly. *Biogerontology* 2005; **6**: 415-423.
- [3] Boniface K, Lecron JC, Bernard FX, Dagregorio G, Guillet G, Nau F, et al. Keratinocytes as targets for interleukin-10-related cytokines: a putative role in the pathogenesis of psoriasis. *Eur Cytokine Netw* 2005; **16**: 309-319.
- [4] Murano M, Maemura K, Hirata. Therapeutic effect of intronically administered nuclear factor kappa B(p65) antisense oligonucleotide on mouse dextran sulphate sodium(DSS) induced colitis. *Clin EXP Immunol* 2000; **120**(1): 51-58.
- [5] Huang HF, Song F, Zhang YJ. Protective effects of ischemic postconditioning against ischemia/reperfusion injury in rats undergoing liver transplantation. *World Chin J Digestol* 2010; **18**(34): 3627-3631.
- [6] Moon KH, Hood BL, Mukhopadhyay P, Rajesh M, Abdelmegeed MA, Kwon YI, et al. Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion. *Gastroenterology* 2008; **135**(4): 1344-1357.
- [7] Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, et al. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *Clin Invest* 1999; **104**(11): 1631-1639.
- [8] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124-1136.
- [9] Tian ZQ, Deng L. The expression of TNF- α , IL-6 Systemic inflammatory response syndrome. *J SNAKE* 2008; **20**(4): 275-278.
- [10] Zhao HF, Zhang GW, Zhou J, Lin JH, Cui ZL, Li XH. Biliary tract injury caused by different relative warm ischemia time in liver transplantation in rats. *Hepatobiliary Pancreat Dis Int* 2009; **8**(3): 247-254.
- [11] Chen C, Li WM, Jiang HW, et al. Study on the protective effect of liver and limbs ischemic preconditioning against liver ischemia/reperfusion injury in the rat. *Chin J General Surg* 2010; **19**(1): 25-27.
- [12] Wang Bing, Wang GS, Chen GH. The mechanism of liver ischemia reperfusion injury. *Organ Transplantation* 2010; **9**(1): 317-320.
- [13] Wilson MR, Choudhury S, Takata M. Pulmonary inflammation induced by high-stretch ventilation is mediated by tumor necrosis factor signaling in mice. *Am J Physiol Lung Cell Mol Physiol* 2005; **288**(4): L599-L607.
- [14] Luppi P, Licata A, Haluszczak C, Rudert WA, Trucco G, McGowan FX, et al. Analysis of TCR Vbeta repertoire and cytokine gene expression in patients with idiopathic dilated cardiomyopathy. *J Autoimmun* 2001; **16**(1): 3-13.
- [15] Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, et al. Inhibition of myocardial injury by ischemic post conditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003; **285**(2): 579-588.
- [16] Zhao ZQ, Vinten JJ. Postconditioning: reduction of reperfusion induced injury. *Cardiovasc Res* 2006; **70**(2): 200-211.
- [17] Varyani N, Tripathi S, Thukral A, Mishra M, Garg S, Tripathi K, et al. Correlation of serum endothelial dysfunction markers with CT angiographic findings in ischemic stroke. *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S11-S15.
- [18] Behera S, Mangaraj M, Mohapatra PC. Diagnostic efficacy of ischemia modified albumin and its correlation with lipid profile, oxidative stress in acute myocardial infarct patients on admission. *Asian Pac J Trop Dis* 2012; **2**(1): 62-65.