Remote ischemic preconditioning protects liver ischemia-reperfusion injury by regulating eNOS-NO pathway and liver microRNA expressions in fatty liver rats

Yun-Fei Duan, Yong An, Feng Zhu and Yong Jiang

Changzhou, China

BACKGROUND: Ischemic preconditioning (IPC) is a strategy to reduce ischemia-reperfusion (I/R) injury. The protective effect of remote ischemic preconditioning (RIPC) on liver I/R injury is not clear. This study aimed to investigate the roles of RIPC in liver I/R in fatty liver rats and the involvement of endothelial nitric oxide synthase-nitric oxide (eNOS-NO) pathway and microRNA expressions in this process.

METHODS: A total of 32 fatty rats were randomly divided into the sham group, I/R group, RIPC group and RIPC+I/R group. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and nitric oxide (NO) were measured. Hematoxylin-eosin staining was used to observe histological changes of liver tissues, TUNEL to detect hepatocyte apoptosis, and immunohistochemistry assay to detect heat shock protein 70 (HSP70) expression. Western blotting was used to detect liver inducible NOS (iNOS) and eNOS protein levels and real-time quantitative polymerase chain reaction to detect miR-34a, miR-122 and miR-27b expressions.

RESULTS: Compared with the sham and RIPC groups, serum ALT, AST and iNOS in liver tissue were significantly higher in other two groups, while serum NO and eNOS in liver tissue were lower, and varying degrees of edema, degeneration and inflammatory cell infiltration were found. Cell apoptosis number was slightly lower in the RIPC+I/R group than that in I/R group. Compared with the sham group, HSP70 expressions were significantly increased in other three groups (all $P<0.05$). Compared with the sham and RIPC groups, elevated miR-34a expressions were found in I/R and RIPC+I/R groups ($P<0.05$). MiR-122 and miR-27b were found significantly decreased in I/R and RIPC+I/R groups compared with the sham and RIPC groups (all $P<0.05$).

CONCLUSION: RIPC can reduce fatty liver I/R injury by affecting the eNOS-NO pathway and liver microRNA expressions.

(*Hepatobiliary Pancreat Dis Int 2017;16:387-394*)

**KEY WORDS: fatty liver;
ischemia-reperfusion;
remote ischemic preconditioning;
nitric oxide;
heat shock protein 70;
endothelial nitric oxide synthase;
inducible nitric oxide synthase;
liver microRNA**

Introduction

Fatty liver disease was primarily caused by obesity and alcohol consumption and might increase the risk of progressive liver injury, such as nonalcoholic steatohepatitis, fibrosis and cirrhosis.[1] Ischemia-reperfusion (I/R) injury is an essential cause of liver damage occurring during hepatic resections or liver transplantation, and is an important reason for post-operative liver dysfunction.[2] Hepatic steatosis contributes to liver damage because fatty livers vulnerable to I/R injury.[3] Fatty infiltration can exacerbate mitochondrial oxidative injury and reduce tolerance to I/R injury.[4]

Ischemic preconditioning (IPC) is a strategy to reduce I/R injury, which submits tissues to controlled periods of ischemia and reperfusion prior to the longer periods of I/R injury and is initially proven to be beneficial when applied to a different tissue (remote).[5, 6] Remote ischemic preconditioning (RIPC) has recently emerged and been investigated mainly on myocardium, and has also

Author Affiliations: Department of Hepatobiliary Surgery, The Third Affiliated Hospital of Soochow University, Changzhou 213003, China (Duan YF, An Y, Zhu F and Jiang Y)

Corresponding Author: Yong Jiang, PhD, Department of Hepatobiliary Surgery, The Third Affiliated Hospital of Soochow University, Changzhou 213003, China (Tel/Fax: +86-519-68870000; Email: jy_sz3h@126.com)

doi: 10.1016/S1499-3872(17)60006-7
Published online April 24, 2017.

been shown to have some protection against liver I/R injury.[7] Circulating mediators and/or signals mediate the effect of RIPC on liver I/R injury by modulating oxidative stress, inflammatory cells, and cytokines.[6] Heat shock protein 70 (HSP70) is highly expressed in cells under a large variety of stress and plays a pivotal role in maintaining and repairing cellular homeostasis under I/R stress.[8] Overexpression of HSP70 could protect the brain from ischemic damage and thus HSP70 expression could be a potential marker for the treatment of I/R.[9]

IPC can increase the content of nitric oxide (NO) and attenuate the I/R induced inflammation, and can protect liver I/R injury via Akt-eNOS-NO-HIF pathway.[10] Endothelial nitric oxide synthase-nitric oxide (eNOS-NO) pathway is important in the pathogenesis of myocardial I/R injury, and IPC, via activating eNOS, increases NO which protects cells from the insults.[11]

MicroRNA (miR)-34a regulates the expression of sirtuin 1 which protects heart and brain from I/R injury, and ameliorates inflammation and apoptosis through NF-κB/p65 and p53 deacetylation in primary hepatocytes.[12] MiR-122 is a potent marker of hepatocyte apoptosis and liver injury.[13] MiR-27b overexpression regulated by transforming growth factor-β involves in the cardiac hypertrophy and dysfunction and thus miR-27b can be a therapeutic target for heart diseases.[14]

The present study aimed to investigate the roles of RIPC in liver I/R of fatty liver rats and the effects of RIPC on eNOS-NO pathway and microRNA expressions.

Methods

Fatty liver rat model

Specific-pathogen free male Sprague-Dawley rats (n=32; weighted 190-210 g) were purchased from Changzhou CAVENS Laboratory Animals, Co., Ltd. All the animal experiments were approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University. A stable fatty liver rat model was established according to Kim et al's method.[15] Briefly, animals were fed under a temperature-controlled environment with a 12-hour diurnal cycle and had free access to a high fat feed (basic feed 82.3%, lard 10%, sucrose 5%, cholesterol 2%, sodium cholate 0.5%, and propylthiouracil 0.2%; provided by the Soochow University Experimental Animal Center) for 5 weeks. The fatty liver model was successfully established at the end of 5 weeks.

Animal grouping and I/R treatment

The 32 fatty liver rats were divided into four groups. Animals fasted for 12-hour preoperatively and had free access to water, were anaesthetized with 2% sodium pentobarbital (0.3 mL/100 g, Merck, Germany) by intraperitoneally injection. Sham group: rats were subjected to only laparotomy and liver flapping; RIPC group: the hind legs were tied tightly by a tourniquet until the color of the hind legs turned purple and cannot touch the femoral pulse. Blood flow was blocked for 5 minutes, and then was restored after the tourniquet was released for 5 minutes, which repeated six times, followed by reperfusion of the hind legs for 160 minutes; I/R group: an electric blanket was used to keep the body temperature at 37.0±0.5 ℃. After laparotomy incision, the periphery liver ligament was isolated, and a microvascular clip was used to block the blood supply of left and central hepatic lobes for 40 minutes to cause ischemia, resulting in approximately 70% hepatic ischemia, and liver reperfusion was conducted for 2 hours; RIPC+I/R group: rats were subjected to I/R treatment immediately after RIPC, and the reperfusion was the same as I/R rats.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measurement

The rats were cultured for one week after successful modeling, and blood (2 mL) was taken by puncture from hepatic inferior cava vena to nearly hepatic vein, stewed at 4 ℃ for 1 hour, followed by centrifugation at 3000 r/min for 10 minutes. After that, the serum was collected, preserved in a -20 ℃ refrigerator, and measured within one month. HITACHI 7600-100 automatic biochemical analyzer was used to measure serum ALT and AST contents.

Serum NO content measured by nitrate reductase

Serum was collected, the standards and samples were prepared at 85 μL/well according to the manual operating procedures of NO detection kit (Biovision, California, Paroo, USA). Nitrate reductase mixture (5 μL) and enzyme cofactors (5 μL) were added, and the whole system was incubated at room temperature for 1 hour to enable nitrate in each well to be converted to nitrite; enhancers (5 μL) were added into each well, and the whole system was incubated for 10 minutes; Griess Reagent R1 (50 μL) and Griess Reagent R2 (50 μL) were added into each well, and the whole system was colored at room temperature for 10 minutes; and the absorbance at 540 nm was detected within 1 hour using a microplate. The formula was as follows: NO content (μmol/L)=(OD_{sample}-OD_{blank})× standard concentration (100 μmol/L)×dilution factor of samples before test/ ($OD_{standard}$-OD_{blank}).

Rat liver histology

The rats were sacrificed after the establishment of model and observation for one week. Liver tissues were rapidly removed, fixed with 10% formalin, dehydrated