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Ischemic postconditioning lightening ischemia/reperfusion apoptosis of rats *via* mitochondria pathway

W.-W. CHU, X.-Y. HE, A.-L. YAN, S.-W. WANG, S. LI, S. NIAN, Y.-L. WANG, F.-L. LIANG

Department of Pathology, Xi'an Medical University & Shaanxi Key Laboratory of Brain Disorders, Xi'an, P.R. China

Abstract. – OBJECTIVE: To explore whether ischemic postconditioning will lighten hepatic apoptosis caused by hepatic ischemia/reperfusion injury by inhibiting the mitochondria pathway.

MATERIALS AND METHODS: Pathomorphology of hepatic tissues in rats was observed under an optical microscope after hematoxylin-eosin (HE) staining. Hepatic apoptosis was detected using agarose gel electrophoresis (AGE) with DNA fragments and flow cytometry. Changes in morphology structure of mitochondria in hepatocytes of rats were observed under an electron microscope. Changes in mitochondria transmembrane potential of hepatocytes of rats were detected using a laser scanning confocal microscope (LSCM). Western blotting was adopted to detect changes in the expression of caspase-3 and cytochrome C protein in hepatocytes of rats.

RESULTS: Compared with that in I/R group, swelling degree of mitochondria in most hepatocytes of rats in ischemic postconditioning (IPOST) group and IPC group was lighter. Changes in expression of caspase-3 and cytochrome C protein in hepatic cells of rats: caspase-3 was lowly expressed and cytochrome C was highly expressed in S group. The expression of caspase-3 was evidently higher in I/R group than that in S group and expression of cytochrome C protein was evidently lower than that in S group ($p<0.05$). The expression of caspase-3 protein was evidently decreased in IPOST group and IPC group and the expression of cytochrome C protein was evidently increased ($p<0.05$).

CONCLUSIONS: IPOST can reduce hepatic apoptosis caused by hepatic ischemia/reperfusion injury in rats, which may be achieved by inhibiting the mitochondria pathway.

Key Words:
Hepatic ischemia/reperfusion, Ischemic postconditioning, Ischemic preconditioning, Mitochondria, Apoptosis.

Introduction

Ischemia/reperfusion (I/R) injury is a pathophysiology phenomenon in which the original hypoxic-ischemic injury is worsening instead of lighting after the ischemic livers regain blood or oxygen supply. In clinics, generally in hepatic surgery, such as hepatectomy, liver transplantation, chemoembolization of hepatic vessels, thrombolytic therapy for portal veins, infectious, and hemorrhagic shock, I/R hepatic injury is often an important cause of hepatic function damage, failure or primary graft dysfunction[1,2]. Therefore, further exploration into the pathophysiology of I/R hepatic injury and various measures for preventing I/R hepatic injury has always an important research direction in hepatic surgery.

Ischemic preconditioning (IPC) in one of the widely used mechanical intervention strategies in past research and it can play the protective role for I/R livers by initiating the endogenous protection mechanism in the body[3-5]. However, IPC must be implanted before hepatic ischemia, which has restricted its clinical application and promotion in clinics. In recent years, ischemic postconditioning (IPOST) has drawn the attention of many researchers due to its role in lighting myocardial damage caused by reperfusion[6-8]. The implementation methods of IPOST are similar to IPC in some points, but its implementation time is more reasonable. Therefore, it is more tempting in clinical application and promotion.

Some research has found that apoptosis plays an important role in the process of hepatic ischemia-reperfusion injury. Apoptosis is a form of active cell death regulated by genes. Apoptosis is mainly achieved via two pathways, death receptor pathway (extrinsic route) and mitochondrial

Corresponding Author: Weiwei Chu, MD; e-mail: chuweiwei61@163.com

pathway (intrinsic route)[9-12]. During the process of ischemia-reperfusion, the above pathways are both involved in the occurrence of apoptosis. However, ischemia-reperfusion injury, as a kind of pathophysiology process, is primarily the damaged caused by ischemia and anoxia, while mitochondria are the center of energy and metabolism of eukaryotic cells. Ischemia and anoxia of tissue cells will first change the structure and functions of mitochondria which is also an organelle that plays a key role in apoptosis signaling transduction pathway when apoptosis occurs to cells.

When the organs go through long term ischemia, calcium overload and a large generation of reactive oxygen can break the electron transference of respiratory chain in mitochondria, generate free radical, hinder energy supply, decrease the transmembrane potential of mitochondria, and even lose the release of protein via the adventitia. On the one hand, cytochrome C and other pro-apoptotic proteins in the interval of the mitochondrial membrane that are released into the cytoplasm trigger caspase cascade reaction and result in apoptosis[13-15]. On the other hand, the abnormally expressed Bcl-2 protein family on the mitochondrial outer membrane can regulate the permeability of mitochondria membrane, thus leading to apoptosis. The latest research has found that preventing mitochondria being damaged has become an action target of IPOST for preventing myocardial ischemia-reperfusion injury and hepatic ischemia-reperfusion injury[16-18]. However, there are few reports about IPOST in this field.

In the present study, IPOST was compared with IPC to explore whether it can lighten apoptosis of hepatic cells in rats with I/R by inhibiting the mitochondria pathway, thus providing a theoretical basis for further exploration into the protective mechanism of IPOST and targets of anti-hepatic ischemia/reperfusion injury.

Materials and Methods

Copy of Hepatic I/R Model in Rats

Healthy male SD rats of SPF level weighing 220-260 g were selected and were fasted and supplied with water at 12 h before surgery. The rats were narcotized with an intraperitoneal injection of 0.3% pentobarbital sodium (30 mg/kg). The left jugular vein was cannulated for liquid infusion or blood collection. The copy of 70% hepatic I/R models in rats[13]: an incision was made in the middle of the upper abdomen to expose hepatoduodenal ligament. The exposed parts were carefully separated to enter the hepatic pedicle of the left hepatic lobe and middle lobe. The vessels were interdicted with a non-invasive small vessel clamp for 60 min, causing ischemia in nearly 70% of livers. Then, the clamp was loosened for 3 h of reperfusion. This research was approved by the Ethics Committee of Xi'an Medical University.

Experimental Grouping

1) Sham surgery group (S group; n=10): open surgery was performed to expose the hepatoduodenal ligament without I/R treatment. It was observed for 60 min. 2) Ischemia-reperfusion group (I/R group; n=10): the methods were shown in the copy of hepatic I/R model. 3) Ischemic postconditioning group (IPOST group; n=10): 30 s of perfusion and 30 s of blockage were immediately performed in 3 cycles after 60 min of ischemia, followed by 3 h of reperfusion. 4. Ischemic preconditioning group (IPC group; n=10): 5 min of ischemia and 5 min of reperfusion were performed in 2 cycles. Next, 60 min of ischemia, followed by 3 h of reperfusion.

Changes in the Pathomorphology of Hepatic Tissues in Rats was Observed Under an Optical Microscope after Hematoxylin-Eosin (HE) Staining

Rats were executed via bloodletting at the scheduled time point. The hepatic tissue of the middle lobe was cut off, fixed with 10% neutral buffered formalin for 24 h, embedded in paraffin, cut into pieces, and stained using HE. Morphological changes of the hepatic tissues were observed under a light microscope.

Changes in the Activity of Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in Rats were Determined Via Biochemical Methods

A total of 0.5 mL venous blood was collected on the left neck of rats, with which the activity of ALT and AST was determined on Beckman's LN Synchron automatic biochemical analyzer using ALT and AST kits.

The Hepatic Apoptosis was Detected Using AGE with DNA Fragments

Hepatocellular DNA was extracted and determined for concentration. The DNA samples were added into the loading wells of sepharose gel (20 μg/well) and placed in 0.5×TBE buffer solution at 30 V for 4 h of electrophoresis, which made DNA

migrate to the positive pole. After 4 h, the gel imaging system was used for detection, with 100 bp DNA marker as reference. The results were recorded and photographed for preservation.

Hepatocyte Apoptosis in Rats Detected Via Flow Cytometry

The livers were taken out in aseptic condition and prepared into a single-cell suspension using the steel mesh grinding method. Annexin V/PI double staining method was adopted to determine the rates of survival, apoptotic, and necrotic hepatocytes in hepatic tissues of SD rats after reperfusion.

Morphology Structure of Mitochondria in the Hepatocytes of Rats was Observed Under the Electron Microscope

The fresh hepatic tissues were rapidly collected, washed off the blood with cold normal saline, soaked in 3% glutaraldehyde for 1 h, fixed in 1% osmium tetroxide for 3 h after repeated washings. Then, dehydrated with graded ethanol, embedded in epoxy resin, cut into ultra-thin slices, which were observed with an electron microscope after dual staining with uranyl acetate and lead citrate.

Mitochondria Transmembrane Potential of Hepatocytes of Rats was Detected Using a LSCM

A total of 100000-60000 hepatic cell suspension was resuspended in 0.5 mL of cell culture medium, added with 0.5 mL of JC-1 staining solution, incubated in the cell incubator at 37°C for 20 min, centrifuged at 100 rpm and 4°C for 3-4 min. The precipitated cells were resuspended with a proper amount of JC-1 staining buffer solution (1×) and observed via the laser scanning confocal microscope (LSCM). The red and green fluorescence values were recorded and their ratio was used to measure the changing degree of the transmembrane potential of mitochondria.

Detection of Changes in Expression of Caspase-3 and Cytochrome C Protein in Hepatocytes of Rats via Western Blotting

Once the samples were prepared, the protein content was determined using the bicinchoninic acid (BCA) method. A total of 50 μg total protein was added in each lane to carry out sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis, were transferred onto the nitrocellulose membrane, sealed with 5% skim milk powder at room temperature for 2 h, added with mice-anti-rat caspase-3 and cytochrome C (1:1000) primary antibody and incubated overnight at 4°C. The membrane was washed using Tris-Buffered Saline and Tween-20 (TBST) for 4 times, 15 min for each time, added with secondary antibody (1:6000), marked by horseradish peroxidase (HRP), developed by enhanced chemiluminescence (ECL) system for 20 min and exposed in an x-ray film. With β-actin as the internal reference, the semi-quantitative analysis was performed using GDS-8000 gel imaging analysis system.

Statistical Analysis

The SPSS 12.0 statistical software pack was employed for analysis and detection. The variance analysis was adopted for intragroup comparison. When $p<0.05$, further pairwise comparison of means was tested with q. The results were presented a mean ± standard deviation ($\bar{x}\pm SD$) and $p<0.05$ suggested that the difference was statistically significant.

Results

Pathomorphology Changes in Hepatic Tissues of Rats

The hepatocyte and hepatic sinusoid structure of rats in S group was normal and the hepatic lobule structure in I/R group was incomplete, with scattered patchy necrosis and edema of hepatocytes, as well as inflammatory cell infiltration. The hepatic lobule structure of rats in IPC group and IPOST group was basically normal, with some hepatocyte edema and casual single hepatocyte necrosis (Figure 1).

Changes in the Activity of Serum ALT and AST of Rats

Changes in serum ALT and AST in rats: the activity of serum ALT and AST in rats from I/R group was evidently higher than that in S group ($p<0.05$). It was significantly lower in IPOST group than that in I/R group ($p<0.05$) and the difference was not statistically significant compared to that in IPC group (Table I).

DNA Fragment Gel Electrophoresis of Hepatocytes of Rats and Analysis Results of Flow Cytometer

There was a genome DNA band near the loading well of the electrophoresis lane in S group, without a broken DNA ladder. Typically broken DNA ladder could be seen in hepatocytes of rats in I/R

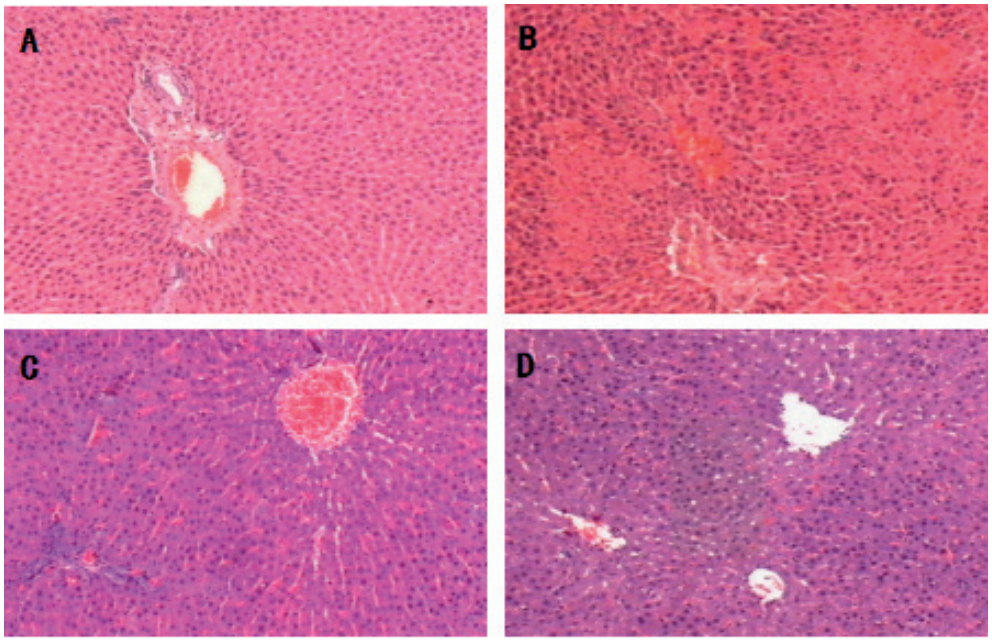

Figure 1. Representative figures of histology in livers of rats HE×100. **A,** S group; **B,** I/R group; **C,** IPOST group; **D,** IPC group.

group, which was manifested as 180-200 bp and its integral multiples. The strength of the broken DNA ladder in IPOST group and IPC group was weakened (Figure 2A).

From Figure 2B, the survival rate of hepatocytes (Q3) of rats was evidently decreased and the rate of early apoptotic cells (Q4) was increased after reperfusion in I/R group than those in S group. IPOST and IPC can significantly increase the survival rate of hepatocytes after reperfusion and decrease the rate of early apoptotic cells (Table II).

Changes in the Morphology Structure of Mitochondria and Changes in Mitochondria Transmembrane Potential of Hepatocytes in Rats

The morphology of hepatocytes in rats of S group was normal, with a complete structure of organelle. The mitochondria were oval in shape, with clear bilayers and cristaes. The volume of mitochondria in hepatocytes of rats in I/R group was increased of uneven size, with evident swelling and presenting globular or irregular outlines. In severe cases, cristae may decline or fade away. Compared with that in I/R group, the swelling degree of mitochondria in most hepatocytes of rats in IPOST group and IPC group was lighter (Figure 3A).

The mitochondrion potential of hepatocytes in rats was significantly decreased in I/R group compared with that in S group ($p<0.05$) and it was significantly higher in IPOST group than that in I/R group ($p<0.05$). There was no statistical significance comparing with IPC group ($p>0.05$) (Table III, Figure 3B).

Changes in The Expression of Caspase-3 and Cytochrome C Protein in Hepatocytes of Rats

Caspase-3 was lowly expressed and cytochrome C was highly expressed in S group. The

Table I. Changes in the activity of serum ALT and AST in rats in different groups.

Groups	No.	ALT	AST
S group	10	74.92±14.72	144.71±42.27
I/R group	10	1786.96±266.98*	1805.09±412.21*
IPOST group	10	994.55±535.02*#	1067.80±254.99*#
IPC group	10	1012.19±467.27*#	1165.80±473.19*#

group *$p<0.05$ *vs.* S; #$p<0.05$ *vs.* I/R group. Data are presented as Mean ± SD.

Table II. Analysis of the percentage of survival, early apoptotic and necrotic hepatocytes after reperfusion via Annexin V/PI double staining and flow cytometry.

Groups	Q1 (%)	Q2 (%)	Q3 (%)	Q4 (%)
S	0.1	0	98.5	1.3
I/R	0.4	1.3	78.7	20.2
IpostC	0.3	1.1	92.2	7.6
IPC	0.2	0.9	91.9	6.2

group *p<0.05 *vs.* S; #p<0.05 *vs.* I/R group. Data are presented as Mean ± SD.

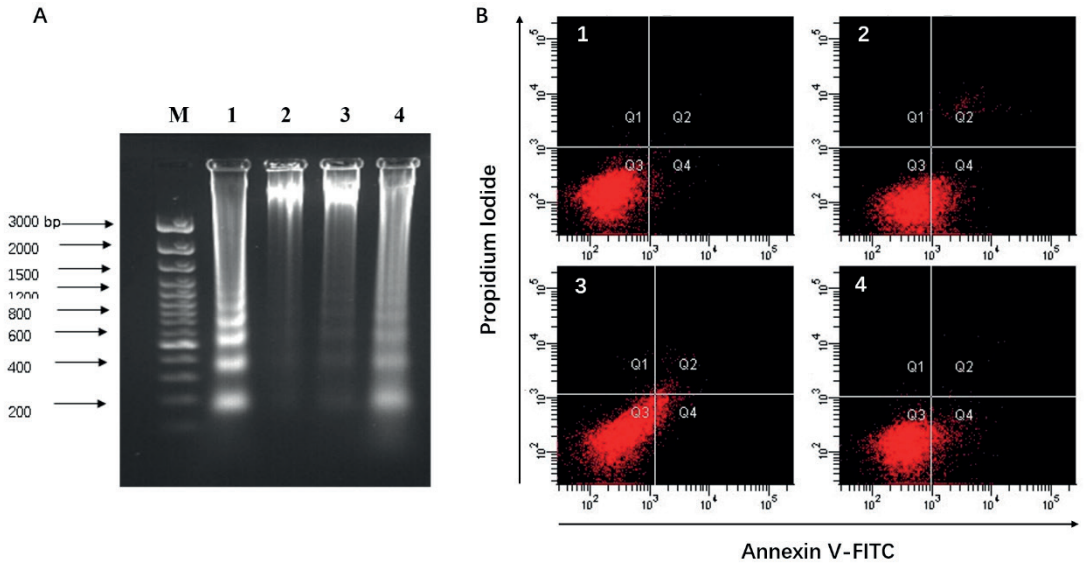

Figure 2. ***A,*** AGE of hepatocytes of rats in different groups. M: Standard molecular weight, 1: I/R group, 2: S group, 3: IPOST group, 4: IPC group. ***B,*** Analysis of the percentage of survival, apoptotic and necrotic hepatocytes via Annexin V/PI double staining and flow cytometry. Quadrant 1 (Q1) Annexin-V(-)/PI(+): necrosis part, Quadrant 2 (Q2) Annexin-V(+)/PI(+): advanced apoptotic and necrotic hepatocytes, Quadrant 3 (Q3) Annexin-V(-)/PI (-): normal survival hepatocytes, Quadrant 4 (Q4) Annexin-V(+)/PI(-): early apoptotic hepatocytes. 1: I/R group, 2: S group, 3: IPOST group, 4: IPC group.

expression of caspase-3 was evidently higher in I/R group than that in S group and the expression of cytochrome C protein was evidently lower than that in S group (p<0.05). Compared with that in I/R group, the expression of the caspase-3 protein was evidently decreased and the expression of cytochrome C protein was evidently increased in IPOST group and IPC group (p<0.05) (Figure 4).

Discussion

The hepatic injury caused by ischemia-reperfusion is often manifested as increased apoptosis of hepatocytes. Currently, various methods can be used to identify apoptosis, which include morphology observation, flow cytometer, DNA degradation analysis, etc. In the present study, apoptosis of hepatic tissues in the early stage of reperfusion was determined using Annexin V/ Propidium iodide (PI) double staining method and DNA AGE, which has high sensitivity and specificity. The results have shown that IPOST can decrease the apoptosis rate in the early stage of reperfusion and improve the survival rate of cells, indicating that IPOST intervened the occurrence of apoptosis, thus lightening the ischemia-reperfusion injury.

Apoptosis is mainly achieved via two pathways, death receptor pathway and mitochondrial pathway. During the process of ischemia-reperfusion, the above pathways are both involved in the occurrence of apoptosis. However, isch-

Table III. Changes in mitochondria membrane potential of hepatocytes in rats in different groups.

Groups	No.	Red fluorescence	Green Fluorescence	Red/Green Fluorescence
S group	10	25.44±1.57	6.37±0.82	3.79±0.28
I/R group	10	17.56±0.48*	25.16±3.56*	0.79±0.12*
IPC group	10	22.78±1.63*#	10.39±1.30*#	2.19±0.39*#
IPOST group	10	22.69±1.29*	11.67±3.08*#	2.05±0.58*#

*p<0.05 *vs.* S group; #p<0.05 *vs.* I/R group. Data are presented as Mean ± SD.

emia-reperfusion injury, as a kind of pathophysiology process, is primarily the damage caused by ischemia and anoxia, while mitochondria are the center of energy and metabolism of eukaryotic cells. Ischemia and anoxia of tissue cells will first change the structure and functions of mitochondria. When apoptosis occurs to cells, mitochondria are also the organelle that plays an important role in the transduction pathways of apoptosis signals, which are manifested as a breakage of the electron transference of respiratory chain in mitochondria, generation of free radical, blockage of energy supply, decrease in the transmembrane potential of mitochondria, and even losing the release of protein via the adventitia.

The decrease in the transmembrane potential of mitochondria is considered to be the earliest event during the process of apoptosis cascade reaction. Under the normal physiological conditions, the existence of the mitochondria transmembrane potential mainly depends on the off state of mitochondrial permeability transition pore (mPTP). MPTP, generally considered as a kind of protein complex, is a non-selective high conductivity pathway between the inner and outer membrane of mitochondria and plays an important role in apoptosis, which is thought to be a "kill switch" of cells. Especially after ischemia/reperfusion, the opening of mPTP is the common pathway of apoptosis and necrosis. Matrix Ca^{2+} overload, excessive production of reactive oxygen, and exhaustion of adenine nucleotide caused by stress conditions like ischemia/reperfusion injury can all lead to a decrease or even loss of mitochondria transmembrane potential, thus resulting in the opening of mPTP. The research has shown that Ca^{2+} overload and mass-produced reactive oxygen are inducers of mPTP opening, especially in the early stage of reperfusion. The opening of mPTP breaks the completeness of mitochondria membrane, makes the transmembrane potential decrease or even collapse and blocks mi-

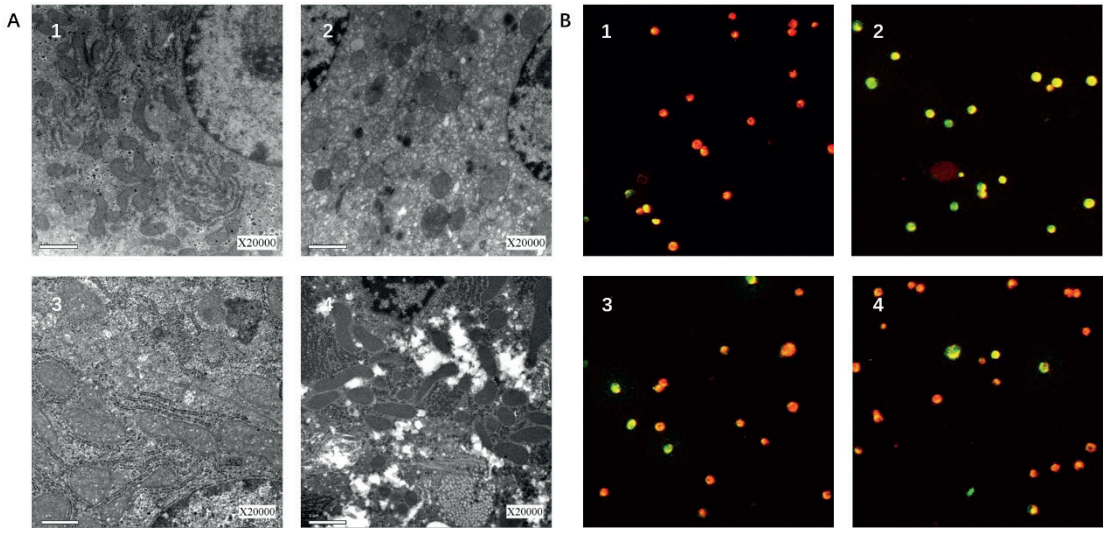

Figure 3. ***A,*** Changes in hepatocyte mitochondrion of rats in different groups (×20000). ***B,*** Fluorescence section of mitochondrion of hepatocytes in rats. Group 1: S group 2: I/R group 3: IPOST 4: IPC group.

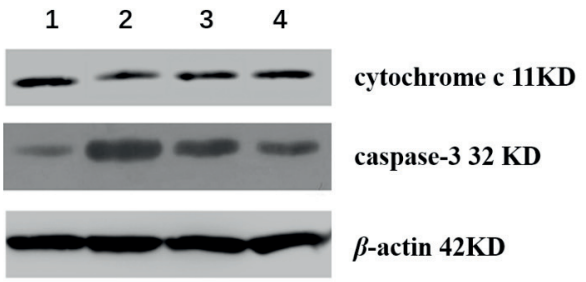

Figure 4. Expression of caspase-3 and cytochrome C protein in hepatocytes of rats in different groups. Group 1: S group 2: I/R group 3: IPOST 4: IPC group.

tochondria RNA and protein synthesis. Besides, the breakdown of mitochondria transmembrane potential may cause uncoupling of respiratory chains and release of cytochrome C, as well as other pro-apoptotic factors. The formation of cytochrome C, an apoptotic protein activator and caspase-9 activates caspase-3, which initiates the internal apoptosis cascade reaction, and it is an important event of ischemia/reperfusion injury. Currently, mPTP has become the treatment target of some diseases[19-21].

In the present study, changes in the mitochondria transmembrane potential of hepatocytes of rats were detected using a LSCM, with JC-1 as the fluorescent probe. The experimental results suggested that the mitochondria transmembrane potential of hepatocytes was notably decreased after hepatic ischemia/reperfusion injury, indicating that reperfusion injury in the early stage can lead to the opening of mPTP and a decrease in the stability of the membrane. In the experiments, the respiratory function and morphological changes of mitochondria in hepatocytes of rats in different groups were also observed, which indicated that the respiratory function of hepatocyte mitochondria was evidently decreased at 1 h after hepatic ischemia/reperfusion and is manifested as swelling in mitochondria and breakage or deficiency of the cristae. IPOST can effectively improve the morphology and respiratory function of hepatocyte mitochondria in rats after ischemia/reperfusion, as well as the mitochondria transmembrane potential, which is similar to the function of IPC. To further confirm the action mechanism of IPOST, it also detected the key factor cytochrome C released after the opening of mPTP and the key enzyme caspase-3 performed by apoptosis. Results suggested that the expression of cytochrome C in mitochondria of hepatocytes of rats was evidently decreased and the expression of caspase-3 was increased in I/R group compared with S group while it was on the contrary in IPOST group. It indicated that IPOST can reduce or slow the release of cytochrome C by inhibiting or delaying the decrease of mitochondria transmembrane potential, thus reducing the occurrence of haptic apoptotic.

Conclusions

We showed that IPOST is a kind of mechanical intervention measure used for the early stage of reperfusion, which is predictable and controllable. Its application is easy, convenient, and feasible. The results of the present work suggested that IPOST may lighten apoptosis of hepatic cells in rats with I-R injury by inhibiting mitochondria pathway of apoptosis. It can provide a scientific theoretical and experimental basis for further exploration into the protective mechanism of IPOST and targets of anti-hepatic ischemia/reperfusion injury.

Acknowledgments

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Saidi RF, Kenari SK. Liver ischemia/reperfusion injury: an overview. J Invest Surg 2014; 27: 366-379.
2) Karatzas T, Neri AA, Baibaki ME, Dontas IA. Rodent models of hepatic ischemia-reperfusion injury: time and percentage-related pathophysiological mechanisms. J Surg Res 2014; 191: 399-412.
3) Fawcett WJ, Quiney NF, Karanjia ND. Ischaemic preconditioning in transplantation and major resection of the liver (Br J Surg 2005; 92: 528-538). Br J Surg 2005; 92: 1299.
4) Farooqui W, Pommergaard HC, Rasmussen A. Remote ischemic preconditioning of transplant recipients to reduce graft ischemia and reperfusion injuries: a systematic review. Transplant Rev (Orlando) 2018; 32: 10-15.

5) Prieto I, Monsalve M. ROS homeostasis, a key determinant in liver ischemic-preconditioning. Redox Biol 2017; 12: 1020-1025.

6) Kin H, Zhao ZQ, Sun HY, Wang NP, Corvera JS, Halkos ME, Kerendi F, Guyton RA, Vinten-Johansen J. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. Cardiovasc Res 2004; 62: 74-85.

7) Stroethoff M, Behmenburg F, Spittler K, Raupach A, Heinen A, Hollmann MW, Huhn R, Mathes A. Activation of melatonin receptors by ramelteon induces cardioprotection by postconditioning in the rat heart. Anesth Analg 2018; 126: 2112-2115.

8) Frankenreiter S, Groneberg D, Kuret A, Krieg T, Ruth P, Friebe A, Lukowski R. Cardioprotection by ischemic postconditioning and cyclic guanosine monophosphate-elevating agents involves cardiomyocyte nitric oxide-sensitive guanylyl cyclase. Cardiovasc Res 2018; 114: 822-829.

9) Asweto CO, Wu J, Alzain MA, Hu H, Andrea S, Feng L, Yang X, Duan J, Sun Z. Cellular pathways involved in silica nanoparticles induced apoptosis: a systematic review of in vitro studies. Environ Toxicol Pharmacol 2017; 56: 191-197.

10) Teringova E, Tousek P. Apoptosis in ischemic heart disease. J Transl Med 2017; 15: 87.

11) Pedley R, Gilmore AP. Mitosis and mitochondrial priming for apoptosis. Biol Chem 2016; 397: 595-605.

12) Lopez-Neblina F1, Toledo AH, Toledo-Pereyra LH. Molecular biology of apoptosis in ischemia and reperfusion. J Invest Surg 2005; 18: 335-350.

13) Webster KA. Mitochondrial membrane permeabilization and cell death during myocardial infarction: roles of calcium and reactive oxygen species. Future Cardiol 2012; 8: 863-884.

14) Li Y, Liu X. Novel insights into the role of mitochondrial fusion and fission in cardiomyocyte apoptosis induced by ischemia/reperfusion. J Cell Physiol 2018; 233: 5589-5597.

15) Javadov S, Jang S, Parodi-Rullán R, Khuchua Z, Kuznetsov AV. Mitochondrial permeability transition in cardiac ischemia-reperfusion: whether cyclophilin D is a viable target for cardioprotection? Cell Mol Life Sci 2017; 74: 2795-2813.

16) Jašová M, Kancirová I, Waczulíková I, Ferko M. Mitochondria as a target of cardioprotection in models of preconditioning. J Bioenerg Biomembr 2017; 49: 357-368.

17) Hu C, Li L. Pre-conditions for eliminating mitochondrial dysfunction and maintaining liver function after hepatic ischaemia reperfusion. J Cell Mol Med 2017; 21: 1719-1731

18) Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annu Rev Pharmacol Toxicol 2017; 57; 535-565.

19) Mai EH, Lei T, Li SQ, Hu PG, Xu T, Jia FX, Zha ZM, Zhang SJ, Ding FH. MiR-34a affects hepatocyte proliferation during hepatocyte regeneration through regulating Notch/HIF-1α signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 3503-3511.

20) Yao R, Feng WT, Xu LJ, Zhong XM, Liu H, Sun Y, Zhou LL. DUXAP10 regulates proliferation and apoptosis of chronic myeloid leukemia via PTEN pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4934-4940.

21) Javadov S, Jang S, Parodi-Rullán R, Khuchua Z, Kuznetsov AV. Mitochondrial permeability transition in cardiac ischemia-reperfusion: whether cyclophilin D is a viable target for cardioprotection? Cell Mol Life Sci 2017; 74: 2795-2813.