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Optimized postconditioning algorithm protects liver graft after liver transplantation in rats

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ABSTRACT

Background: Ischemia reperfusion injury (IRI) causes postoperative complications and influences the outcome of the patients undergoing liver surgery and transplantation. Postconditioning (PostC) is a known manual conditioning to decrease the hepatic IRI. Here we aimed to optimize the applicable PostC protocols and investigate the potential protective mechanism.

Methods: Thirty Sprague–Dawley rats were randomly divided into 3 groups: the sham group ($n=5$), standard orthotopic liver transplantation group (OLT, $n=5$), PostC group (OLT followed by clamping and re-opening the portal vein for different time intervals, $n=20$). PostC group was then subdivided into 4 groups according to the different time intervals: (10 s × 3, 10 s × 6, 30 s × 3, 60 s × 3, $n=5$ in each subgroup). Liver function, histopathology, malondialdehyde (MDA), myeloperoxidase (MPO), expressions of p-Akt and endoplasmic reticulum stress (ERS) related genes were evaluated.

Results: Compared to the OLT group, the grafts subjected to PostC algorithm (without significant prolonging the total ischemic time) especially with short stimulus and more cycles (10 s × 6) showed significant alleviation of morphological damage and graft function. Besides, the production of reactive oxidative agents (MDA) and neutrophil infiltration (MPO) were significantly depressed by PostC algorithm. Most of ERS related genes were down-regulated by PostC (10 s × 6), especially ATF4, Casp12, hspa4, ATF6 and ELF2, while p-Akt was up-regulated.

Conclusions: PostC algorithm, especially 10 s × 6 algorithm, showed to be effective against rat liver graft IRI. These protective effects may be associated with its antioxidant, inhibition of ERS and activation of p-Akt expression of reperfusion injury salvage kinase pathway.

Introduction

Liver transplantation is a life-saving procedure for patients with end-stage liver diseases. On the other side, liver resection, transplantation, and trauma can result in prolonged deprivation of tissue oxygen, converting cellular metabolism to anaerobic pathways [1]. Reperfusion and consequently the restoration of oxygen delivery can lead to local and systemic injury which is still the major factor of influencing the outcome of liver transplantation [1,2]. Overcoming the shortage of donor organ in modern era is one of the biggest challenges in the transplantation field. A number of therapeutic strategies including pharmaceutical agents, manual conditioning, controlled reperfusion etc. have been proposed to alleviate this series of events with the hope to improve the tolerance of liver induced by ischemia reperfusion injury (IRI) and expand the donor pool by increasing the number of marginal organs [3].

Experimentally, we proved that the ischemic preconditioning (IPC, 10 minutes pre-ischemia conditioning) initiated hepatocyte proliferation and alleviated IRI through TNF-alpha/IL-6/JNK pathway [4,5]. However, the widely clinical application of IPC in the or-

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thotopic liver transplantation (OLT) has been concerned, partly due to the unpredictability of the ischemic episode of the donor liver. The application of pretreatments of a donor-to-be before the legal determination of death is associated with serious ethical concerns. Compared to the IPC, ischemic postconditioning (PostC) procedure is clinically more valuable because of its therapeutic application at the onset of reperfusion. PostC was proposed by Na et al., [6] then was developed by Zhao et al. [7] who proved that it was as effective as IPC in preventing myocardium I/R injury in mammalian models, and later it was proved to be potentially effective to reduce reperfusion injury in brain, [8] intestine, [9] liver, [10] and testis [11] in other animal models. However, further studies are needed for the clinical application of PostC protocols as the underlying mechanisms are not clarified yet. Recent evidences from our laboratory and others have shown that the mechanisms responsible for PostC-induced organ protections are associated with a myriad of cellular and subcellular adaptive responses to reperfusion injury [12,13]. Until to date, the studies on the PostC of the liver graft are limited, which are partially due to the technical challenge of the rodent liver transplantation model [10,14]. The present study aimed to investigate the optimal algorithm of perfusion-occlusion sequences of the PostC on liver graft and try to clarify the underlying mechanisms, based on our previous well- established platform of rat liver transplantation model [15].

Methods

Adult male Sprague–Dawley rats (SD, 250–300 g) were kept in a temperature-controlled environment (25 °C to 30 °C), with water and food ad libitum. This study was approved by the Ethics Committee of Experimental Animals in Zhejiang University.

Experimental design and groups

SD rats were divided into three groups: the sham-operation group (sham), OLT (OLT followed by a persistent reperfusion for 180 min); PostC (OLT followed occlusion and open the portal vein for different time intervals, followed by a persistent reperfusion for 180 min). With different algorithm, the PostC group was then divided into 4 subgroups (10 s × 3, 10 s × 6, 30 s × 3, 60 s × 3, $n = 5$ in each subgroup) respectively (Fig. 1A). "S" stands for the unit of second. Ten-day liver function was observed in another two groups (OLT, $n = 3$; PostC 10 s × 6, $n = 3$).

Rat OLT and PostC models

The performance of OLT mainly keeps in line with the method of Kamada and Calne [16] details refer to our previous article. [17] The PostC protocols were followed by designed cycles of reperfusion and reocclusion of the portal vein applied immediately at the onset of portal vein reperfusion by a nontraumatic miniartery clamp (Fig. 1B). Upon awakening from anesthesia, rats had free access to sterilized water and standard rodent chow.

Histopathological examination and liver function test

Samples including blood and liver tissues were collected at 180 min after the OLT. Liver tissue was fixed, embedded, sectioned and stained according to the routine procedure. A grading scale of 0–4 was used for the histopathological assessment of I/R injury based on the degree of sinusoidal congestion, and necrosis of parenchyma cells according to the Suzuki classification [18] and our previous study. [19]

Serum was used for liver function test by Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan).

Levels of lipid peroxidation and myeloperoxidase activity

Lipid peroxidation of liver tissue was determined by the assay of malondialdehyde (MDA) and myeloperoxidase (MPO), the kits were from Nanjing Jiancheng Bioengineering Institute, Nanjing, China, as previous described [20]. MDA is an indirect measurement of oxidative damage induced by reactive oxygen species (ROS) [21] while MPO is a marker of polymorphonuclear neutrophil (PMN) infiltration [22].

In situ cell death detection

In situ cell death detection kit-POD (Roche, Basel, Switzerland) was used for apoptosis detection according to the instruction. Briefly, tissue section as described in histopathology examination was incubated for 30 min at 25 °C with Pro-teinase K working solution. TUNEL reaction mixture was prepared according to the instruction. 50 μL TUNEL reaction mixture was added to each sample, incubated at 37 °C for 60 min in a humidified atmosphere in the dark, 50 μL converter-POD was added and incubated in a humidified chamber for 30 min at 37 °C after section was dried. 100 μL POD substrates were added and incubated for 10 min at 25 °C. Samples were analyzed under a fluorescence microscope.

Measurement of phosphorylated Akt (P-Akt) expression in liver graft

The procedure of western blotting was in line with our previous method [17]. Briefly, equivalent proteins were loaded and separated on 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Guangzhou, China). The membranes were blocked with 5% fetal bovine serum and incubated with the rabbit polyclonal anti-P-Akt antibody (1:1000; Signalway antibody, Maryland, USA) or mouse monoclonal anti-β-actin antibody (1:1000; Dawen bioscience, Hangzhou, China) for 16 h at 4 °C. Finally, washed and incubated with HRP-conjugated anti-rabbit or antimouse secondary antibodies (1:2000; Dawen bioscience) for 1 h. Densitometric analysis was performed for quantification of the protein expression (Image J NIH).

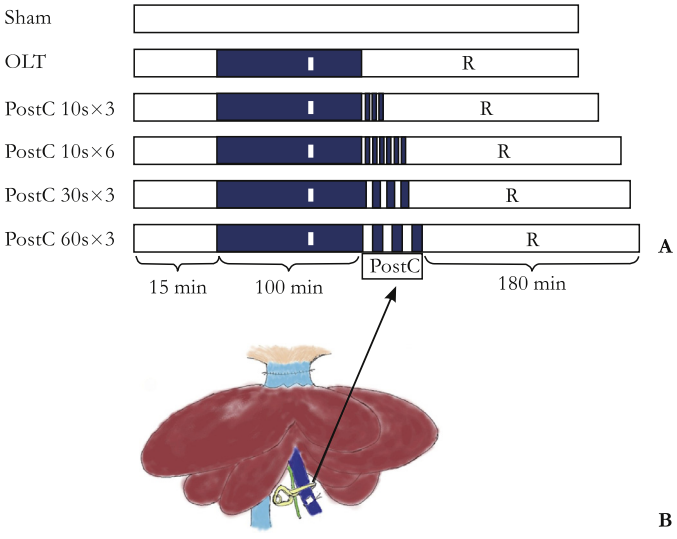

Fig. 1. Schematic diagram ischemic postconditioning (PostC) protocols. Sham: sham operated; OLT: orthotopic liver transplantation; PostC 10 s × 3: postconditioning 10 s × 3; PostC 10 s × 6: postconditioning 10 s × 6; PostC 30 s × 3: postconditioning 30 s × 3; PostC 60 s × 3: postconditioning 60 s × 3. A: The details of PostC; B: The location of PostC.