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Remote ischemic preconditioning promotes early liver cell proliferation in a rat model of small-for-size liver transplantation

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ABSTRACT

Background: The size of the liver donor graft is a major concern in living donor liver transplantation. Rapid regeneration is essential for the survival of these grafts. The purpose of this study was to investigate the effect of remote ischemic preconditioning (RIPC) on liver regeneration in a rat small-for-size liver transplantation model.

Methods: We established rat models of small-for-size liver transplantation (30%) in the presence or absence (control) of remote ischemic preconditioning. We observed liver mass regeneration, serum alanine aminotransferase, hepatic pathologic alterations, flow cytometry, and Ki-67 antigen immunohistochemistry. In addition, using Western blotting and reverse-transcriptase–polymerase chain reaction, we assessed the activation of cell cycle progression as well as tumor necrosis factor-α and interleukin-6 expression.

Results: Compared with the control group, serum alanine aminotransferase activity was significantly lower and histopathology changes were significantly attenuated in the RIPC group. Remote ischemic preconditioning induced a high level of interleukin-6 mRNA in small grafts, but suppressed the expression of tumor necrosis factor-α. The proliferation index, indicated by the S-phase and G2/M-phase ratio [(S+G2/M)/(G0/G1+S+G2/M)], was significantly increased in the RIPC group at 24 h (58.25% $\pm$ 0.506% *versus* 53.405% $\pm$ 1.25%; $P = .007$). Meanwhile, cell cycle progression and regeneration (Ki-67) were initiated early in liver grafts treated with RIPC.

Conclusions: These results suggest that RIPC can protect liver cells against ischemia reperfusion injury in the small grafts and enhance liver regeneration. Interleukin-6 may be a critical mediator in the stimulatory effect on liver cell regeneration, which may make RIPC valuable as a hepatoprotective modality.

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1. Introduction

Living-related liver transplantation was developed to significantly improve clinical outcomes owing to the scarcity of cadaveric grafts [1–3]. The size of the graft is a major risk factor in adult-to-adult living donor liver transplantation. Graft failure may be characterized by coagulopathy, ascites, encephalopathy, cholestasis, and histological features of ischemia after implantation. Small graft size has been considered a dominant factor contributing to impaired postoperative graft function [4,5]. The mechanism leading to injury in a small-for-size graft has been studied in clinical and animal models [6–11]. Partial grafts must rapidly regenerate to enable hepatic mass recovery and normal function.

The ability of the liver to restore major tissue loss within a few weeks [12] involves numerous interacting cells and a complex network of mediators. Experiments using partial hepatectomy model have proved that early activation of tumor necrosis factor (TNF)-α and interleukin (IL)-6 responsive transcription factors is a critical process in the initiation of the regenerative response [8–10]. Recent studies have highlighted the IL-6/Stat3 pathway as a major signaling pathway involved in liver regeneration after hepatectomy. Studies have also demonstrated the importance of the IL-6/Stat3 activation pathway in the recovery of liver grafts from ischemia-reperfusion [13]. Ischemia-reperfusion injury is often seen in organ transplants, major organ resections, and shock. Ischemic-reperfusion injury significantly contributes to morbidity and mortality after liver surgery, especially in patients with liver disease.

Ischemic preconditioning (IPC), a brief period of ischemia followed by a short reperfusion before ischemia insult, serves to protect diverse organs against subsequent prolonged ischemic insults and reperfusion injury [14]. It has been considered to be beneficial not only for reducing hepatic ischemia-reperfusion injury in hepatectomy and liver transplantation [15–17], but also for enhancing liver regeneration [18,19]. However, its main disadvantage is trauma to major vessels and stress to the target organ. Remote preconditioning is a novel method in which brief ischemia of one organ has been shown to confer protection on distant organs without direct stress to the organ or blood vessels. Remote IPC (RIPC) of the limb reduces ischemia-reperfusion injury to the heart, lungs, and other organs in humans [20] and animals [21,22]. Studies have investigated the protective effect of RIPC of the hind limb in reducing the adverse effects of liver ischemia-reperfusion injury and underlying mechanism involved in increased hepatic blood flow, the role of heme oxygenase, modulation of the hepatic microcirculation, the role of High Mobility Group-Box1and, and others [23–26]. None of these studies addressed the hepatic proliferation *in vivo* seen in the effect of RIPC on the small-for-size liver graft. In the present study, we aimed to investigate the possible effect of RIPC on hepatocyte proliferation, and the underlying mechanism.

2. Materials and methods

2.1. Experimental design and surgical procedure

We used male Sprague–Dawley rats (250–300 g; Vital River Experimental Animal Co., Beijing, China) as donors and recipients. The animals were given free access to tap water and a standard rat diet. We housed the rats in accordance with institutional animal care policies. Research procedures complied with the Ethics Committee for animal experiments.

We randomly divided the animals into the RIPC group and the control group. We executed non-arterialized orthotopic liver transplantation as previously described [27]. In RIPC groups, the animal liver donators were subjected to RIPC treatment before the liver donor experienced laparotomy supplying the left and caudate lobes of the liver (approximately 30%). Control group animals underwent an identical experimental protocol without RIPC. Body temperature was maintained at 37°C and the graft was stored in Ringer's solution at 0°C to 4°C, with a mean time of 60 min (range, 51–67 min). The technique of RIPC involved a limb tourniquet, which we applied around the hind limb at the inguinal level without skin insult. We monitored the method of hind limb ischemia and perfusion using a pulse oximeter (Kangtai Medical-Technology Co.,Tianjin, China) modified for application in rats, and by the change in foot color. The procedure involved 5 min of ischemia followed by 5 min of reperfusion; this was repeated for four cycles. We based this on Tapuria and colleagues' study [28], which used a similar protocol of hind limb preconditioning before liver ischemia-reperfusion injury in a rat model. At each time point, we killed animals, collected liver tissue and serum samples, and froze them at −80°C. We also fixed liver tissues in 10% formalin for histology.

2.2. Serum alanine aminotransferase (ALT)

We obtained blood serum when we killed the animals after centrifugation. We measured the plasma level of ALT in a clinical laboratory to assess the extent of hepatocellular damage.

2.3. Histological investigation

We obtained liver tissue at each time point and fixed it in formalin. We cut a paraffin-embedded sample of the liver tissue into 4-μm sections and stained it with hematoxylin and eosin. The mitotic index is expressed as the percentage of mitotic hepatocytes per total number of hepatocytes in 20 high-power fields.

2.4. Detection of cell cycle

We performed hepatocyte isolation using an enzymatic technique previously described (Howard et al., 1967). We washed single cell suspensions of hepatocytes, fixed them