

# Role of Ischemic Preconditioning in Liver Transplant: A Review of Literature

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## Abstract

**Interruption of blood flow and subsequent organ reperfusion lead to significant tissue damage. This well-studied phenomenon is recognized as ischemia/reperfusion injury. Ischemic preconditioning refers to a mechanism in which a prior, short, ischemic period induces some protection against a subsequent prolonged ischemic and reperfusion damage. The mechanisms involved in ischemic preconditioning and its applications for clinical and basic research are discussed in this paper.**

**Key words:** Liver transplantation, Ischemic-reperfusion injury, Postoperative outcomes

Liver transplant is widely accepted as an effective therapeutic modality for irreversible acute and chronic liver diseases. Prolonged liver ischemia followed by reperfusion, which occurs during liver transplant, results in severe injury that contributes to increased morbidity and mortality in liver transplant. This phenomenon is defined as *ischemia-reperfusion injury*. Ischemia-reperfusion injury involves multifactorial mechanisms and distinct metabolic pathways leading to severe microischemia-reperfusion injury, circulatory dysfunction, organ failure, and death (1). This is likely increased by use of extended criteria donors, a suitable approach to deal with the dramatic shortage of organ donors. The consequences of ischemia-reperfusion injury over

grafts has led to the development of new strategies to protect the graft; thereby reducing the magnitude of this injury, and improving postoperative outcomes.

Among those strategies, ischemic preconditioning represents a feasible and reliable tool. The technique is based on the fact that resistance of organs or tissue may be obtained by means of brief and repetitive episodes of ischemia, followed by reperfusion, before a more-sustained episode of ischemia and reperfusion (2). First described in the canine ischemia heart model, it is currently accepted as a universal phenomenon among organs and tissues. Here, we emphasize the pathophysiological mechanisms and clinical relevance of liver ischemic preconditioning.

## Materials and Methods

### Mechanisms of ischemic preconditioning

Ischemic preconditioning can be achieved by gene or pharmacological therapy, heat-shock treatment, or surgery. In this paper, we will consider only the surgical ischemic preconditioning.

The mechanism of ischemic preconditioning is uncertain, but it is considered to be a biphasic event. The early stage of protection lasts about 2 hours after the trigger, while a second phase of protection occurs during the following 24 hours. Once the effects can be observed shortly after the stimulus, the mechanisms involved might be related to pre-existing molecules as effectors of natural defense events against subsequent ischemic injury.

### Oxidative stress

According to some previous studies, oxygen-free radicals are produced by cytosolic xanthine oxidase, or are released by Kupffer cells, adherent leukocytes, or mitochondrial origin. These are the most-relevant pathways of tissue damage after reperfusion of ischemic organs or tissues (3). Ischemic

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preconditioning modulates oxidative stress for limiting the accumulation of xanthine, and for reducing the conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase requires molecular oxygen introduced on tissue reperfusion to convert hypoxanthine to xanthine with superoxide release. The action of ischemic preconditioning over xanthine oxidase-derived oxidant stress inhibits this pathway of oxidative injury (4).

Recently, it has been shown that ischemic preconditioning reduces oxidative stress by suppression of free radicals released by Kupffer cells, because ischemic preconditioning has no benefit on Kupffer cell-depleted rat livers. Ischemic preconditioning decreases endothelial adhesion molecule expression (P-selectin) in warm ischemia-reperfusion injury of the rodent liver, thereby diminishing the neutrophil adhesion and resulting in decrease of oxidative neutrophil-mediated damage (5, 6).

#### **Adenosine, nitric oxide, and intracellular kinases**

Adenosine is an endogenous compound produced by action of enzymes related to adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate. In case of sustained oxygen deficit, adenosine triphosphate is rapidly consumed to generate energy for cellular metabolism, thereby resulting in adenosine production. At some stage in reperfusion, adenosine is washed out of the tissues, depriving them of its known beneficial effects. Moreover, adenosine is transformed to hypoxanthine and xanthine leading to oxidative radical production. Adenosine-related mechanisms during ischemic preconditioning seem to be mediated by A<sub>2</sub> receptors, because their blockade results in inhibition of nitric oxide production and subsequent cessation of protective effects of ischemic preconditioning (6, 7).

Adenosine activates endothelial (constitutive) nitric oxide synthase, resulting in nitric oxide synthesis. It is widely accepted that nitric oxide is an important mediator of ischemia-reperfusion injury associated with improvement of liver microcirculation and tissue oxygenation by attenuating neutrophil adherence and inhibiting platelet aggregation. Nitric oxide inhibits the release of endothelins—a member of a family of potent and long-acting vasoconstrictive mediators (8). Endothelins counteract the beneficial effects of nitric oxide. Inhibition of nitric oxide synthesis blocks the

protective effect of ischemic preconditioning (9, 10). Ischemic preconditioning stimulates the activity of various intracellular kinases via activation of cell membrane receptors by adenosine and other effectors. Protein kinase C (PKC) and p38 mitogen-activated protein kinase (p38MAPK) have been shown to reduce hepatocyte injury. This is mainly due to activation of another intracellular kinase (protein kinase B (Akt/PKB), which has antiapoptotic effects and stimulates nitric oxide synthesis via endothelial (constitutive) nitric oxide synthase (11, 12).

#### **Remote ischemic preconditioning**

Ischemic preconditioning of an organ may protect others organs from ischemia-reperfusion injury. This mechanism is recognized as remote ischemic preconditioning. It was first described in a model of canine of heart ischemia, and involves the release of effectors into systemic circulation. Liver preconditioning-induced resistance to ischemia-reperfusion injury damage in remote organs is thought to be elicited by decreased hepatic TNF- $\alpha$  release.

Recently, it was shown that remote ischemic preconditioning of the hind limb reduces warm ischemia-reperfusion injury in the livers of rabbits and rats. Although the mechanism of remote ischemic preconditioning remains poorly understood—adenosine, bradykinin, and opioids are released in systemic circulation after sublethal ischemic injury seems to be involved in the process (13, 14).

#### **Delayed ischemic preconditioning**

Delayed ischemic preconditioning onsets 24 hours after reperfusion and protection may persist for several days. Gene expression and synthesis of proteins have been investigated in delayed ischemic preconditioning. The activation of nuclear factor kappa B and other transcription factors can induce the synthesis of inducible nitric oxide synthase, heat-shock proteins, and antioxidants. Heat-shock proteins contribute to the diminishment of TNF- $\alpha$  synthesis and reduce inflammatory injury in livers subjected to ischemic preconditioning (15, 16).

#### **Medical relevance of ischemic preconditioning**

Because of the advantages shown in experimental models of ischemic preconditioning, clinical trials of

ischemic preconditioning have been done over the past decade. Several clinical studies of ischemic preconditioning have demonstrated that ischemic preconditioning has beneficial effects in liver surgery, thereby improving both clinical and laboratory parameters in patients with normal or cirrhotic livers. However, the majority of studies failed to demonstrate improvement of mortality and morbidity in preconditioned versus nonpreconditioned patients (17).

The liver donor shortage has led transplant surgeons to accept livers that were previously considered unsuitable. Use of these extended criteria grafts is related to higher incidence of primary liver dysfunction, or in its most-severe form, primary liver failure and death. Ischemic preconditioning may represent an attractive option to deal with ischemia-reperfusion injury in extended criteria grafts, mainly in steatotic grafts. Steatosis is considered a major parameter to classify a graft as extended criteria. Moreover, steatotic livers have decreased tolerance to ischemia-reperfusion injury and ischemic preconditioning may have beneficial effect in human steatotic liver.

## Conclusions

The advantageous effects of liver ischemic preconditioning have been shown in experimental models and more recently, in clinical trials. However, the role of ischemic preconditioning remains unclear, and investigation to elucidate these effects seems to represent a promising research field in the future. New cellular mediators, molecules, and metabolic pathways related to ischemic preconditioning remain to be clarified. These new data should lead to the synthesis of drugs that will eventually reduce effects reperfusion injury to the liver. Clinical trials and experimental models should provide important insights into the role of remote ischemic preconditioning to reduce ischemia-reperfusion injury, thereby improving clinical outcomes. Transplant surgeons must clearly define the clinical relevance of ischemic preconditioning when using extended criteria grafts and hepatic surgery in the near future.

## References

1. Kupiec-Weglinski JW, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. *Transplant Proc.* 2005;37(4):1653-1656.
2. Grande L, Roselló-Catafau, J, Peralta C. Liver ischemic preconditioning: molecular basis and clinical application. *Cir Esp.* 2006;80(5):275-282.
3. Glantzounis GK, Salacinski HJ, Yang W, Davidson BR, Seifalian AM. The contemporary role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: a review. *Liver Transpl.* 2005;11(9):1031-1047.
4. Cavalieri B, Perrelli MG, Aragno M, et al. Ischemic preconditioning attenuates the oxidant-dependent mechanisms of reperfusion cell damage and death in rat liver. *Liver Transpl.* 2002;8(11):990-999.
5. Yadav SS, Howell DN, Steeber DA, Harland RC, Tedder TF, Clavien PA. P-Selectin mediates reperfusion injury through neutrophil and platelet sequestration in the warm ischemic mouse liver. *Hepatology.* 1999;29(5):1494-1502.
6. Sawaya DE Jr, Brown M, Minardi A, et al. The role of ischemic preconditioning in the recruitment of rolling and adherent leukocytes in hepatic venules after ischemia/reperfusion. *J Surg Res.* 1999;85(1):163-170.
7. Peralta C, Hotter G, Closa D, et al. The protective role of adenosine in inducing nitric oxide synthesis in rat liver ischemia preconditioning is mediated by activation of adenosine A2 receptors. *Hepatology.* 1999;29(1):126-132.
8. Arai M, Thurman RG, Lemasters JJ. Contribution of adenosine A(2) receptors and cyclic adenosine monophosphate to protective ischemic preconditioning of sinusoidal endothelial cells against Storage/Reperfusion injury in rat livers. *Hepatology.* 2000;32(2):297-302.
9. Shah V, Kamath PS. Nitric oxide in liver transplantation: pathobiology and clinical implications. *Liver Transpl.* 2003;9(1):1-11.
10. Peralta C, Rull R, Rimola A, et al. Endogenous nitric oxide and exogenous nitric oxide supplementation in hepatic ischemia-reperfusion injury in the rat. *Transplantation.* 2001;71(4):529-536.
11. Carini R, De Cesaris MG, Splendore R, et al. Signal pathway involved in the development of hypoxic preconditioning in rat hepatocytes. *Hepatology.* 2001;33(1):131-139.
12. Carini R, Albano E. Recent insights on the mechanisms of liver preconditioning. *Gastroenterology.* 2003;125(5):1480-1491.
13. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation.* 1993;87(3):893-899.
14. Kanoria S, Jalan R, Davies NA, Seifalian AM, Williams R, Davidson BR. Remote ischaemic preconditioning of the hind limb reduces experimental liver warm ischaemia-reperfusion injury. *Br J Surg.* 2006;93(6):762-768.
15. Kume M, Yamamoto Y, Saad S, et al. Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. *J Lab Clin Med.* 1996;128(3):251-258.
16. Clavien PA, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg.* 2000;232(2):155-162.
17. Jassem W, Fuggle SV, Cerundolo L, Heaton ND, Rela M. Ischemic preconditioning of cadaver donor livers protects allografts following transplantation. *Transplantation.* 2006;81(2):169-174.