Remote hindlimb preconditioning and hepatoprotection: NO-table strides against liver ischaemia/reperfusion injury

Narci C. TEOH
Gastroenterology and Hepatology Laboratory, Australian National University Medical School at The Canberra Hospital, Garran, Canberra, ACT 2605, Australia

ABSTRACT

Hepatic IR (ischaemia/reperfusion) injury is an important clinical problem complicating liver surgery and transplantation. IPC (ischaemic preconditioning) is a strategy whereby brief episodes of IR in an organ can induce an adaptive response to protect against subsequent prolonged IR injury. However, trauma to vessels supplying the target organ is unavoidable using the technique of direct IPC. One amenable strategy would be to apply the protective preconditioning stimulus to an organ distant or remote from the target organ of interest, a technique known as RIPC (remote IPC). In the present issue of Clinical Science, Abu-Amara and co-workers utilize hindlimb RIPC as a novel therapeutic strategy against liver IR injury and investigate the mechanistic contribution of NO to hepatoprotection by administering C-PTIO [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt], an NO scavenger. Their experiments set the stage for more definitive studies to demonstrate a discernible benefit for the utility of RIPC in liver surgery and transplantation.

Hepatic I/R (ischaemia/reperfusion) injury is an important clinical problem complicating liver surgery and transplantation. The pathogenesis underlying reperfusion injury after warm ischaemia is complex, encompassing a plethora of different cell types and signalling mechanisms innate and/or mobilized to the liver [1]. IPC (ischaemic preconditioning) is a strategy whereby brief episodes of IR in an organ can induce an adaptive response to protect against subsequent prolonged IR injury. IPC has now been described in the brain, intestine, skeletal muscle and liver. There are several underlying cytoprotective mechanisms modulating hepatic IPC; those implicated include adenosine, adenosine A2 receptors, NO (nitric oxide), abrogation in TNF-α (tumour necrosis factor-α) release, changes in energy metabolism, microcirculation and cytoprotection associated with accelerated cell-cycle entry [2]. Trauma to vessels supplying the target organ is unfortunately unavoidable and inherent on the technique of direct IPC. In contrast, a more appealing strategy would be to apply the protective preconditioning stimulus to an organ distant or remote from the target organ of interest.

RIPC (remote IPC) was first conceived in 1993 by Przyklenk et al. [3], who made the remarkable observation that inducing brief episodes of IR in the circumflex coronary artery territory had the capacity of reducing the size of the myocardial infarct arising from occlusion of the left anterior descending coronary artery. This form of RIPC was later extended to other non-cardiac organs and has now emerged as a feasible strategy of inter-organ protection against the detrimental effects of acute IR injury [4].

In the present issue of Clinical Science, Abu-Amara et al. [5] utilize hindlimb RIPC as a novel therapeutic

Key words: ischaemia/reperfusion, ischaemic preconditioning, liver, nitric oxide, reperfusion injury, warm ischaemia.

Abbreviations: C-PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt; EM, electron microscopy; I/R, ischaemia/reperfusion; IPC, ischaemic preconditioning; MAPK, mitogen-activated protein kinase; NOS, NO synthase; eNOS, endothelial NOS; RIPC, remote IPC; SEC, sinusoidal endothelial cell.

Correspondence: Associate Professor Narci C. Teoh (email narci.teoh@anu.edu.au).

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strategy against liver IR injury. Using a murine model, repeated cycles of mini-IR (4 × 4 min) of the hindlimb (induced by occlusion and release of the femoral vascular bundle) was protective against prolonged hepatic IR, confirmed by histology and EM (electron microscopy) studies, as well as by significant attenuation of plasma ALT (alanine aminotransferase) and AST (aspartate aminotransferase) release at 120 min of reperfusion. The protective effects of hindlimb RIPC were attributable to a marked increase in circulating NO levels in preconditioned animals and improved recovery of hepatic microcirculatory blood flow compared with naïve mice [5]. The beneficial microcirculatory impact of RIPC against liver IR injury was shown ultrastructurally by preservation of SECs (sinusoidal endothelial cells) and lack of red blood cell extravasation into the surrounding liver parenchyma, unlike liver from non-preconditioned animals. However, RIPC did not prevent hepatocyte damage inferred by endoplasmic reticulum dilatation, phagolysosomal and lipid droplet formation, as well as glycogen depletion as determined by transmission EM.

In order to investigate the mechanistic contribution of NO to hepatoprotection by hindlimb RIPC, the authors [5] administered C-PTIO [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt], an NO scavenger, immediately prior to the first cycle of RIPC. The protective effects conferred by RIPC were nullified by this compound [5]. In mice treated with C-PTIO, plasma transaminase release resumed to levels comparable with naïve animals subjected to hepatic IR, whereas histological indices of injury, such as hepatocyte cytoplasmic vacuolation, were significantly enhanced in the NO-scavenger-treated RIPC and IR cohorts. The observation that C-PTIO abrogates hepatoprotection by RIPC provides a logical explanation for the likely role of NO in mediating the effects of hindlimb RIPC.

Adenosine is an endogenous compound produced by the sequential action of various enzymes which dephosphorylate ATP, ADP and AMP. In the liver, an increase in extracellular adenosine, followed by the ligand-binding and activation of adenosine A₂a receptors, signals an increase in NO synthesis that is associated with the protective effects of preconditioning [6,7]. Both adenosine and NO are synthesized in vascular endothelium and are released into surrounding vascular and interstitial compartments during periods of IR. NO is a known major mediator of adenosine-induced vasodilatation. Administration of NO prior to hepatic IR has been shown to simulate the hepatoprotective effects of preconditioning [6]. Conversely, inhibition of the A₂a receptor or NO abolishes this protection. The signalling pathways responsible for the cytoprotective actions of NO have been investigated in rat hepatocytes treated with the NO donor NOC-9 and then exposed to hypoxia [7]. NOC-9-induced protection was underpinned by NO stimulation of Ras GTPase, activation of sGC (soluble guanylate cyclase) and cGK (cGMP-dependent kinase); these pathways ultimately converge on activation of PI3K (phosphoinositide 3-kinase) and p38 MAPK (mitogen-activated protein kinase).

NO may be synthesized by two possible NOS (NO synthase) isoforms in the liver. iNOS (inducible NOS) is not expressed under normal physiological conditions, but may be up-regulated during inflammation, such as post-I/R injury in hepatocytes, SECs, Kupffer cells, neutrophils and T-lymphocytes. In contrast, eNOS (endothelial NOS) is constitutively expressed in several cell types, including hepatocytes and SECs. In another recent publication, Abu-Amara et al. [8] have dissected further the role of eNOS by utilizing RIPC and IR in eNOS-deficient (eNOS⁻/⁻) mice. They report that hindlimb RIPC did not protect against liver IR injury in eNOS⁻/⁻ animals and that hepatic microcirculatory blood flow was not preserved by this technique when compared with RIPC-treated wild-type mice.

Several minor points pertaining to the design and analysis of the study by Abu-Amara et al. [5], however, warrant comment. The authors have not studied the sole effect of C-PTIO before induction of IR, nor was the efficacy of the compound evaluated if administered during reperfusion, and more precise measures of key signalling pathways of NO-mediated protection furnished by RIPC, such as p38 MAPK phosphorylation, adenosine A₂a, and PKC (protein kinase C) isoform activation, would have been desirable. Despite these minor shortcomings, the study [5] underscores the fundamental importance of NO and is a step in the right direction towards preventing liver IR injury. It confirms, in a murine partial liver IR injury model, the beneficial effects of NO in mediating hepatoprotection.

So, is hindlimb RIPC ready for prime time? Although we are encouraged by the promising results of this technique against hepatic IR injury and its relative ease in implementation in clinical practice [9], the study by Abu-Amara et al. [5] does not resolve several outstanding issues that remain, particularly in liver IR injury and transplantation. The RIPC experiments described by Abu-Amara et al. [5] were performed in lean healthy young mice. One of the major challenges today is the increasing use of ‘marginal’ grafts that are suboptimal for reasons such as steatosis, or grafts from elderly or non-heart-beating donors [10]. More definitive experiments are now necessary to demonstrate a discernible benefit of the utility of RIPC in liver surgery and transplantation.

REFERENCES


