

Protection of organs other than the heart by remote ischemic conditioning

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Abstract

Organ or tissue dysfunction due to acute ischemia-reperfusion injury (IRI) is the leading cause of death and disability worldwide. Acute IRI induces cell injury and death in a wide variety of organs and tissues in a large number of different clinical settings. One novel therapeutic non-invasive intervention, capable of conferring multi-organ protection against acute IRI, is 'remote ischemic conditioning' (RIC). This describes an endogenous protective response to acute IRI, which is triggered by the application of one or more brief cycles of non-lethal ischemia and reperfusion to one particular organ or tissue. Originally discovered as a therapeutic strategy for protecting the myocardium against acute IRI, it has been subsequently demonstrated that RIC may confer protection against acute IRI in a number of different non-cardiac organs and tissues including the kidneys, lungs, liver, skin flaps, ovaries, intestine, stomach and pancreas. The discovery that RIC can be induced non-invasively by applying the RIC stimulus to the skeletal tissue of the upper or lower limb, has facilitated its application to a number of clinical settings in which organs and tissues are at high-risk of acute IRI. In this article, we review the experimental studies which have investigated RIC in organs and tissues other than the heart, and we explore the therapeutic potential of RIC in preventing organ and tissue dysfunction induced by acute IRI.

Keywords:

Ischemia, reperfusion, remote ischemic preconditioning, remote ischemic perconditioning, remote ischemic postconditioning.

1. Introduction

Organ or tissue dysfunction arising from acute IRI is the leading cause of death and disability worldwide. Acute IRI induces cell injury and death in a large number of different clinical settings and in a wide variety of organs and tissues including the heart, kidneys, liver, intestine, lungs, brain, skeletal muscle, pancreas, skin, and ovaries. The pathophysiology of IRI and its effects on cellular function and viability are largely similar in these different organs and tissues. It follows then that novel therapeutic strategies for protecting against acute IRI appear to be similarly efficacious in these different organs and tissue. This appears to be the case with the endogenous protective response of remote ischemic conditioning (RIC), in which the application of one or more brief cycles of non-lethal ischemia and reperfusion in one organ or tissue confers protection in remote organs or tissues from a sustained lethal acute episode of IRI ^{1, 2}. The majority of the experimental studies investigating RIC as a protective therapeutic strategy against acute IRI have revolved around the heart, and this topic is reviewed elsewhere in this special issue. In this article, we focus on the experimental studies which have investigated RIC in organs and tissues other than the heart, and we discuss the therapeutic potential application of RIC for preventing organ and tissue dysfunction in the clinical setting.

2. Remote ischemic conditioning as an evolving protective strategy

The discovery that the myocardium could be protected against a sustained lethal episode of acute IRI, by subjecting it to one or more brief cycles of non-lethal ischemia and reperfusion, was originally made in 1986 by Murry et al ³. This endogenous protective response against acute IRI, which was termed 'ischemic preconditioning' (IPC), has been reported to confer cross-species protection in all

organs and tissue it has been investigated in ⁴. However, the major disadvantage of IPC is the need to intervene before the index ischemic event, thereby limiting its clinical application ⁵. This was overcome by the introduction of the concept of ischemic postconditioning in 2003 by Zhao et al. ⁶ who demonstrated that interrupting myocardial reperfusion with several short-lived episodes of intermittent myocardial ischemia, so as to 'stutter' the reperfusion phase, could reduce myocardial infarct size in the canine heart, providing a therapeutic cardioprotective strategy which could be applied at reperfusion. However, the disadvantage of both IPC and IPost as treatment strategies is that both approaches require the intervention to be applied to the organ or tissue directly, which in some cases may not be feasible or practical ⁷. This obstacle was overcome by the discovery of 'remote ischemic preconditioning' (RIPC) in 1993 ¹, a therapeutic strategy for protecting organs and tissues against acute IRI by applying the protective stimulus away from the target organ or tissue.

The concept of protecting an organ or tissue against acute IRI at a distance was first conceived in a seminal experimental study in 1993 by Przyklenk et al ¹. These authors demonstrated in the canine heart, that applying four 5-minute episodes of left circumflex branch occlusion and 5-minute reflow, reduced myocardial infarct (MI) size induced by 1 hour of sustained left anterior descending (LAD) coronary artery occlusion and 4.5 hours of reflow ¹. The concept that protection against acute IRI could be transferred from one coronary artery territory to another was then extended to protecting the heart at a distance by applying the preconditioning stimulus to an organ 'remote' from the heart by McClanahan et al in 1993 ⁸. These authors demonstrated that subjecting the kidney to brief cycles of non-lethal ischemia and reperfusion (IR) could limit subsequent MI size in the rat heart ⁸.

Later in 1997, Birnbaum et al ⁹ made the important discovery that RIPC could be induced by subjecting the skeletal muscle of the hind-limb to brief cycles of non-lethal ischemia and reperfusion. These authors observed, that briefly restricting blood flow to the skeletal muscle of the lower limb and pacing the gastrocnemius leg muscle prior to an acute coronary artery occlusion, reduced MI size in the rabbit heart ⁹. A non-invasive method for applying the RIPC stimulus to the hind-limb using external compression with a tourniquet was then introduced by Oxman et al ¹⁰, a finding which has greatly facilitated the translation of RIPC as an experimental phenomenon into a potential non-invasive therapeutic strategy in the clinical setting.

In this regard, the first clinical study to investigate RIPC in patients was by Gunaydin et al in 2000 ¹¹, who inflated and deflated a standard blood pressure cuff placed on the upper arm to non-invasively induce the RIC stimulus in patients prior to coronary artery bypass graft (CABG) surgery. However, the study was underpowered to detect any meaningful differences in the effect of RIC on peri-operative myocardial injury. Later in 2002, Kharbanda et al ¹² characterized in human volunteers a simple non-invasive RIPC protocol (three-5 min inflations/deflations of an upper arm blood pressure cuff) using flow-mediated dilatation as the measured endpoint of endothelial function.

Crucially, experimental studies have demonstrated that the RIC stimulus does not have to be applied prior to the index ischemic event to be effective (as in 'remote ischemic preconditioning' or RIPC), but can be applied after the onset of index ischemia (termed 'remote ischemic perconditioning' or RIPerC) ¹³, and even at the time of reperfusion (termed 'remote ischemic postconditioning' or RIPost) ^{14, 15}. The finding that the RIC stimulus can be effective when delivered at different time-points during the index episode of acute IRI has also increased its applicability to a number

of different clinical settings. The term, remote ischemic conditioning (RIC), has been introduced to refer to these different protective phenomena and will be used from this point forward.

The recognition that a single RIC stimulus could confer multi-organ and tissue protection against acute IRI has led to it being extensively investigated not only in the field of myocardial protection but also in a wide variety of other non-cardiac organs or tissues such as the kidneys, lungs, liver, brain, stomach, intestine, pancreas and ovaries, in which acute IRI also causes significant morbidity and mortality. The mechanisms underlying RIC protection against acute IRI remain uncertain and are discussed elsewhere in this special issue. Throughout this review article, the reader will note the widely varying RIC protocols that have been used in the experimental studies. Unfortunately, insufficient work has been undertaken to ascertain the optimal RIC protocol in terms of its efficacy. If RIC is to succeed as a clinical applicable intervention both these issues will need to be addressed by further experimental study.

3. Remote ischemic conditioning and the kidney

Acute kidney injury (AKI) due to acute IRI is a frequent cause of morbidity and mortality following a number of medical conditions and operative procedures. AKI is a condition frequently encountered in the period that follows major surgery and which is associated with significant morbidity and mortality. In the setting of cardiac surgery it can affect up to 30% of patients resulting in renal dialysis, in 1-2% of cases ¹⁶, the repercussions of which include an eight-fold increase in death rate ¹⁷. AKI is also a common complication of major vascular surgery involving the abdominal aorta, and can occur in up to 10% of these patients ¹⁸, where it is an independent predictor of

death ¹⁹. Aortic cross-clamping and de-clamping during the surgical procedure subjects the kidneys to acute IRI ²⁰.

Different mechanisms have been hypothesized to explain the etiology underlying renal injury following cardiac surgery and these include hemodynamic effects related to cardiopulmonary bypass (CBP), metabolic factors, neuro-hormonal stimulation, the systemic inflammatory response to CBP, exogenous and endogenous toxins, and the production of micro-emboli and oxidative stress ¹⁶. Importantly, despite a number of different preventative strategies being investigated to preserve renal function following cardiac surgery, the results have been disappointing ¹⁶. Therefore, novel renoprotective strategies are required to protect the kidneys against acute IRI in order to reduce the incidence of AKI and improve clinical outcomes in these patients.

3.1. The experimental data for RIC as a renoprotective strategy

A number of experimental studies have investigated the effect of IPC as a therapeutic strategy for limiting acute renal IRI (reviewed in ²¹). However, only a few experimental studies have explored the effect of RIC in preventing acute IRI in the kidney (see Table 1a). Beneficial effects have been reported using a diverse variety of RIC protocols applied to different organs or tissues including the liver, small intestine, subphrenic aorta and the hindlimb. The study by Wever et al ²², which used limb RIC, attempted to characterize the RIC stimulus, and as expected they were able to show that the protective effect was augmented when the RIC stimulus was applied to both hindlimbs instead of one. Importantly, Kadkhodae et al ²³ demonstrated that the RIC limb stimulus was effective even when applied after the onset of index renal ischemia (RIPerC) and even at the onset of renal reperfusion

(RIPost), thereby broadening the clinical application of RIC as a therapeutic renoprotective strategy. Finally, the concept of combining renoprotective stimuli has been recently investigated by Wever et al ²⁴, who demonstrated the synergistic effects on preserving renal function using RIPost and a local IPost protocol in combination.

3.2. The clinical data for RIC as a renoprotective strategy

Several clinical studies have investigated the effect of RIC using repeated cycles of upper arm or leg ischemia and reperfusion on preventing renal dysfunction and the development of acute kidney injury (AKI) following a number of medical and surgical procedures including percutaneous coronary intervention (PCI), CABG surgery, renal transplantation, abdominal aortic aneurysm surgery, and other major vascular surgery (see Table 1b). However, not all the clinical studies have reported a beneficial effect of RIC as a renoprotective strategy with some studies being neutral. The reasons for the mixed results are multi-factorial and have been discussed in relation to myocardial protection in several recently published articles ²⁵⁻²⁸. The majority of the clinical studies have investigated the protective effect of RIC against acute renal IRI, although a recently published trial reported beneficial effects against contrast-induced AKI during elective PCI ²⁹, a condition which affects up to 12% of patients with coronary heart disease (CHD) undergoing elective PCI.

Whether RIC can actually improve clinical outcomes following acute renal IRI is currently being investigated in two large multi-centre randomized controlled clinical trials. The ERICCA trial (ClinicalTrials.gov Identifier: NCT01247545), is currently investigating the effect of RIC (four-5 min upper arm cuff inflations/deflations) on post-operative renal function, renal biomarkers, the development of AKI, and major

adverse cardiovascular events in adult patients undergoing CABG plus or minus valve surgery ³⁰. The REPAIR trial (ISRCTN30083294) is currently investigating the effect of RIC (three-5 min upper arm cuff inflations/deflations) applied to both the donor and recipient on post-operative renal function and renal biomarkers in patients undergoing live donor-related renal transplantation.

4. Remote ischemic conditioning and the lungs

Acute lung injury is a major cause of morbidity and mortality in a number of clinical settings including cardiac surgery, orthopedic surgery, lung resection surgery and lung transplantation. In the setting of CABG surgery, the main contributory mechanisms underlying pulmonary dysfunction include cardiopulmonary bypass (CBP)-induced systemic cytokine-mediated inflammatory response and acute lung IRI ³¹, resulting in poor alveolar oxygenation and increased pulmonary vascular resistance leading to prolonged requirement for artificial ventilation ^{32, 33}.

4.1. The experimental data for RIC as a lung protective strategy

One of the first experimental studies to investigate the effect of RIC on acute lung injury was by Peralta et al in 2002 ³⁴ who demonstrated in the rat that applying a RIC stimulus to the liver reduced systemic inflammation and attenuated neutrophil accumulation in the lung and other organs following a sustained liver IRI. Since this study a number of studies have examined the effect of RIC in preventing acute lung IRI in a wide variety of experimental models (see Table 2a).

4.2. The clinical data for RIC as a lung protective strategy

A number of clinical studies have investigated the effect of RIC on pulmonary function following mainly cardiac surgery, although there has been one study in orthopaedic surgery (see Table 2b). The first clinical study to demonstrate a beneficial effect of RIC on pulmonary function was by Li et al in 2001³⁵ who demonstrated that RIC of the heart could preserve pulmonary function following adult cardiac valve surgery. However, in the majority of the clinical studies, the main objective has been to evaluate the effect of RIC on cardioprotection, and the endpoints of pulmonary function, were usually secondary objectives of the study. In this regard, RIC appears to have some beneficial effects on preserving pulmonary function in pediatric cardiac surgery and following orthopedic surgery. However, it appears that RIC has little effect on ventilation times following adult cardiac surgery, which may be expected given that there are a large number of factors other than acute IRI which contribute to the length of ventilation post-cardiac surgery. It will be interesting to examine the effect of RIC in clinical settings in which acute lung IRI is the predominant insult such as in cases of lung resection surgery and lung transplantation. In this regard, on-going clinical studies are currently evaluating the effects of RIC on pulmonary function in the setting of pulmonary lobectomy (ClinicalTrials.gov identifier: NCT01307085) and elective abdominal aortic aneurysm repair (ClinicalTrials.gov NCT01344239).

5. Remote ischemic conditioning and the liver

During major liver surgery, intra- or post-operative blood loss with subsequent requirement of blood transfusion is associated with worse clinical outcomes^{36, 37}. Vascular clamping of the hepatic territory can prevent or minimise this risk although it

is itself associated with acute liver IRI due to clamping and subsequent unclamping of the portal triad^{38, 39}. The mechanism underlying acute hepatic IRI is likely to be related to the significant amount of oxygen-derived free radicals accumulating in the liver during reperfusion, particularly in those cases of resected or cirrhotic liver when acute IRI is poorly tolerated due to impaired compensatory mechanisms, leading to post-operative hepatic and multi-organ failure³⁸.

5.1. The experimental data for RIC as a liver protective strategy

The first experimental study to demonstrate benefit with limb RIC was by Lai et al⁴⁰ who found that four-10 min cycles of hind-limb ischemia and reperfusion reduced acute liver IRI as evidenced by an attenuated increase in the serum liver enzyme ALT (alanine aminotransferase). In this initial study, RIC protection was associated with the activation of hemoxygenase-1 (HO-1), a known mediator of direct IPC⁴⁰. Since this publication, a number of experimental studies, using a wide variety of different RIC protocols, have confirmed the protective effects of limb RIC against acute liver IRI in the rat, rabbit and mouse (see Table 3 for summary). The mechanism underlying the protective effect is unclear, although potential mediators include components of the nitric oxide pathway (sGC, cGMP, eNOS)⁴¹⁻⁴³ and the Toll-4 receptor signalling pathway. An exciting recent study has suggested that limb RIC may not only be able to protect against acute liver IRI, but it may actually promote liver regeneration in small liver grafts following liver transplantation⁴⁴. Wang et al⁴⁴ demonstrated that a standard limb RIC protocol increased the proliferation index in the liver graft, findings which were associated with enhanced expression of interleukin-6 mRNA and suppressed expression of TNF- α .

5.2. The clinical data for RIC as a liver protective strategy

Two randomised controlled clinical trials are currently investigating the therapeutic potential of RIC in the clinical setting of acute hepatic IRI. In the first study, based in the UK, Davidson and colleagues are exploring whether RIC (three-10 min cycles thigh cuff inflation/deflation) can reduce acute liver IRI (serum hepatic and tissue biomarkers) in patients undergoing liver surgery and orthotopic liver transplantation (ClinicalTrials.gov identifier: NCT00796588). In the second study, based in the USA, Koneru and colleagues will evaluate short and long-term clinical outcomes in patients receiving liver, kidney or pancreas transplantation from deceased organ donors previously randomised to either RIC (two-10 min cycles of inflation/deflation of a blood pressure cuff applied sequentially on each leg) (Remote Ischemic Preconditioning In Abdominal Organ Transplantation [RIPCOT]; ClinicalTrials.gov identifier: NCT00975702). The results from these two studies should inform us of the therapeutic potential of RIC in preventing acute liver IRI in patients undergoing major abdominal surgery or organ transplantation.

6. Remote ischemic conditioning and the brain

There are a number of clinical settings in which the brain is subjected to acute IRI including the setting of stroke and during carotid endarterectomy (CEA). Stroke is currently the second most common cause of death worldwide, after coronary artery disease ⁴⁵, and is responsible for nearly 10% of all deaths around the world ⁴⁶. In 2002, stroke-related disability was estimated as the fifth most common cause of reduced disability-adjusted life-years (DALYs—the sum of life-years lost as a result of premature death and years lived with disability adjusted for severity) in low-income and middle-income countries ⁴⁶. CEA has been recognised as an effective strategy

for stroke prevention in both symptomatic and asymptomatic patients⁴⁷⁻⁵⁰. However CEA itself carries important peri-operative risks, with an estimated mortality rate of 1%, perioperative stroke rate of 3-5%, and a major cardiac complication rate (myocardial infarction, unstable angina, pulmonary edema, or ventricular tachycardia) of 4%^{51, 52}.

6.1. The experimental data for RIC as a neuroprotective strategy

As a potentially novel neuroprotective intervention, RIC has been extensively studied in the pre-clinical literature. Experimental studies have evaluated RIC from a number of different perspectives: (1) a number of different species have been investigated including mouse, rat, rabbit and pig; (2) the timing of the RIC stimulus in relation to the acute IRI; (3) site of the RIC stimulus (mainly the limb but also other organs); (4) A variety of conditions including acute brain IRI (cerebral infarction, cerebral asphyxia, hypothermic circulatory arrest, and neonatal cerebral hypoxia) and spinal injury (Table 4). The first experimental study to evaluate RIC as a neuroprotective strategy was by Dave et al⁵³ who found that RIC (either a single episode of 15 or 30 minutes of hindlimb ischemia and reperfusion) could protect the rat brain against acute IRI induced by asphyxial cardiac arrest. Crucially, the RIC stimulus has been shown to be effective at a number of different time-points in relation to the cerebral IRI broadening its scope for application in the clinical setting. The RIC stimulus can be applied one or two days prior to the index cerebral ischemia (delayed RIPC), immediately prior to the index cerebral ischemia (classical RIPC), after the onset of the index cerebral ischemia (RIPerC), at the onset of cerebral reperfusion (RIPost)⁵⁴ and even when delayed up to 3 hours into the reperfusion phase (delayed RIPost)⁵⁴ (Table 4). The ability to protect the brain against acute IRI with the RIC delivered 3

hours into reperfusion would have important clinical implications. Whether delaying the RIC stimulus into reperfusion is effective in protecting other organs or tissue against acute IRI is unknown, although this approach does appear to contradict the current paradigm that, for a protective intervention to be effective it needs to be applied at the onset of reperfusion. Interestingly, a recent experimental study has demonstrated that local IPost applied direct to the heart could limit myocardial infarct size in a murine model of acute IRI, even when delivered up to 30 min into reperfusion ⁵⁵.

6.2. The clinical data for RIC as a neuroprotective strategy

Several small proof-of-concept randomised controlled clinical trials have been published investigating the neuroprotective effect of limb RIC. In a phase 1b study, Koch et al ⁵⁶ demonstrated that RIC could be safely applied to 33 critically ill subjects presenting with aneurysmal subarachnoid haemorrhage (SAH). Hu et al ⁵⁷ demonstrated significant reductions of post-operative serum markers of cerebral ischemic injury (S-100B and neurone-specific enolase) and improvements in neurological recovery in 20 patients with cervical spondylotic myelopathy undergoing elective cervical decompression, when compared to similar control subjects. Walsh et al ⁵⁸ evaluated the effects of RIC (10 minutes of ischemia-reperfusion applied with a blood pressure cuff sequentially to each leg) prior to clamping of the carotid artery in 70 patients undergoing elective CEA. This study failed to demonstrate any significant effect on saccadic latency deteriorations (a marker of cerebral injury following CEA). A recent clinical trial has evaluated the effect of RIC (four-5 min arm cuff inflations/deflations) on cerebral infarct size in 151 acute ischemic stroke patients undergoing thrombolysis, but this ground-breaking clinical trial failed to find

any significant effect on cerebral infarct size or neurological recovery ⁵⁹. There are currently a number of on-going clinical trials (www.clinicaltrials.gov) investigating the neuroprotective potential of RIC in a wide variety of clinical settings including pediatric and adult cardiac surgery.

7. Remote ischemic conditioning and other organs and tissues

7.1. Remote ischemic conditioning and the gastrointestinal system

The intestine, stomach and pancreas are all vulnerable to the detrimental effects of acute IRI. Of these the intestine is most commonly affected by acute IRI in a number of clinical settings including cardiac arrest ⁶⁰, hemorrhagic shock ⁶¹, burn trauma ⁶², and major vascular and cardiac surgery ⁶³⁻⁶⁵, the effects of which are increased intestinal epithelial barrier permeability and translocation of pathogenic bacteria and endotoxins ⁶⁶ with subsequent inflammation, sepsis and multi-organ failure ⁶⁷.

In 2002, Dickson et al ⁶⁸ were the first to report that RIC of the rabbit heart could protect jejunal intestinal contractile function, beneficial effects which were blocked in the presence of either naloxone or glibenclamide, thereby implicating possible involvement of opioids and the K_{ATP} channel, known mediators of cardioprotection. Saeki et al ⁶⁹ went on to demonstrate that RIC (15 minute infrarenal aortic occlusion and reflow) could protect against intestinal IRI in rats receiving small bowel transplantation. Interestingly, Zitta et al ⁷⁰ demonstrated that serum from human volunteers treated with RIC (four-5 minute cycles of arm ischemia and reperfusion) protected intestinal cells against simulated IRI via matrixmetalloproteinase (MMP)-2 and MMP-9. Brzozowski et al ⁷¹ were the first to demonstrate that RIC (two-5 min cycles of myocardial or hepatic ischemia and

reperfusion) could protect the stomach against acute IRI. RIC gastric protection was found to be abrogated in capsaicin-denervated or vagotomised rats.

Combined pancreas-kidney transplantation has recently been successfully implemented for patients with type 1 diabetes and end-stage renal failure ⁷². This operation can be complicated by graft pancreatitis, a contributing factor of which is acute IRI, resulting in graft loss ⁷³. Oehmann et al ⁷⁴ were the first to demonstrate that RIC (15 minutes of infrarenal aortic ischemia and reperfusion) could protect the pancreas against acute IRI as evidenced by improved circulation, reduced inflammatory tissue response and histology damage. RIC-mediated protection against acute pancreatic IRI has been confirmed by a subsequent study ⁷⁵, although not all studies have been positive ⁷⁶.

In terms of clinical application, a large randomised controlled clinical trial is underway investigating the effect of RIC on clinical outcomes following combined liver, kidney or pancreas transplantation (ClinicalTrials.gov identifier: NCT00975702).

7.2. Remote ischemic conditioning and skin flaps

In the setting of trauma, plastic and reconstructive surgery, soft tissue coverage is usually performed with the use of local or free skin flaps, a surgical procedure which is associated with significant morbidity due to the risk of necrosis of the implanted flap in 1-5% of cases ^{77, 78}. This is likely to be caused by prolonged post-operative flap ischemia when the flap is exposed to during the surgery. Küntscher et al ⁷⁹⁻⁸¹ were the first to demonstrate that hindlimb RIC (10 min clamping of femoral artery) could protect muscle flaps against IRI. Whether, this RIC is beneficial in the clinical setting of plastic surgery is currently being tested in patients undergoing skin flap transplantation to the anterolateral thigh (ClinicalTrials.gov identifier: NCT01235286).

It is interesting to note that upper arm RIC has been recently demonstrated to improve cutaneous tissue oxygen saturation, arterial capillary blood flow and post-capillary venous filling pressure in the skin of the contralateral thigh in human volunteers ⁸².

7.3. Remote ischemic conditioning and the ovaries

Ovarian auto-transplantation is an increasingly used procedure for maintaining fertility in women receiving cytotoxic treatment for malignant disease or immunosuppressive disorders, which ultimately cause early ovary failure ^{83, 84}. This has been observed in up to 34% of women receiving chemotherapy and 92% of patients having radio- or chemo-therapy for bone marrow transplantation ⁸⁵. Acute IRI of the graft ovary is one of the major factors responsible for preventing graft revascularization and success of ovarian transplantation ⁸⁶. It has been demonstrated that hindlimb RIC in the rat improves ovarian viability, vascularization and vasodilatation, findings which were associated with increased serum oestradiol levels, improved graft morphology and follicular maturity ^{87, 88}. This novel application of RIC in improving the success of ovarian transplantation remains to be explored in the clinical setting.

8. Conclusions

Although remote ischemic conditioning (RIC) was first discovered as a non-invasive therapeutic strategy for protecting the heart from a distance, its potential for conferring widespread protection against acute IRI in a number of different non-cardiac organs and tissues has now been realized. Experimental studies have demonstrated beneficial effects of RIC against acute IRI in a wide variety of organs

and tissues including kidney, liver, brain, skin flaps, ovaries, intestine, stomach and the pancreas. Further study is now needed to characterize the optimal RIC protocol in terms of efficacy and to elucidate the mechanisms underlying its protective effect. The ability to induce RIC by simply applying the protective stimulus to the arm or leg has facilitated its translation into a number of clinical settings in which vital organs such as the kidney, brain and liver are vulnerable to acute IRI. Large multi-centre randomized controlled clinical trials are needed to investigate whether RIC can improve clinical outcomes in patients at risk of IRI-induced organ and tissue dysfunction.

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Conflicts of interest

None to disclose.

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Figure legend

This figure demonstrates the non-cardiac organs and tissue in which remote ischemic conditioning (RIC, inflations and deflations of a blood pressure cuff placed on the upper arm or thigh to induce cycles of non-lethal ischemia and reperfusion) may offer protection against acute ischemia-reperfusion injury (IRI). In each organ and tissue, there are number of clinical settings in which RIC has been or is currently being investigated as a therapeutic strategy or in which the potential for clinical application exists.



SKIN FLAP

Trauma surgery
Plastic surgery
Reconstructive surgery



BRAIN

Stroke
Subarachnoid hemorrhage
Carotid endarterectomy
Cervical decompression surgery
Cerebral asphyxia



LUNGS

Resection surgery
Transplantation
Cardiac surgery
Vascular surgery



LIVER

Resection surgery
Transplantation



KIDNEY

Cardiac surgery
Vascular surgery
Transplantation
Contrast-induced AKI



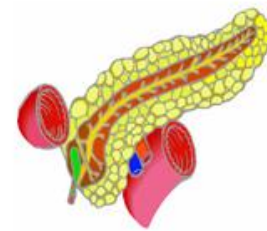
SMALL INTESTINE

Ischemic bowel



STOMACH

Gastric ischemia



PANCREAS

IRI Pancreatitis
Transplantation

Table 1a: Major experimental studies investigating the effect of RIC on preventing acute renal IRI

Experimental study	Experimental setting	RIC protocol	Result of RIC
Ates et al 2002 ⁸⁹	Rat model of renal acute IRI (45 min I /24 hr R)	One-10 min cycle of hepatic IR	Reduced BUN levels at 24 hours and improved histological changes.
Song et al 2007 ⁹⁰	Rat model of renal acute IRI (45 min I /24 hr R)	Three-cycles of 8 min I and 5 min R of small intestine	Reduced levels of Cr, BUN and less renal morphologic change.
Lazaris et al 2009 ⁹¹	Rat model of renal acute IRI (45 min aortic cross-clamp/45 min R)	One-15 min cycle of aortic clamping and declamping	Reduced levels of renal blood lactate and MDA and less renal tissue MDA.
Kadkhodae et al 2011 ²³	Rat model of renal acute IRI (45 min I /24 hr R)	Four-5 min cycles of IR to one hindlimb during acute renal ischemia (RIPerC) and at the onset of reperfusion (RIPost).	Reduced Cr and BUN levels at 24 hours.
Wever et al 2011 ²²	Rat model of renal acute IRI (25 min I /48 hr R)	Four RIC protocols prior to acute renal IRI: One-12 min or three-4 min cycles of IR to one or both hind-limbs.	All RIC protocols except 12 min IR to one limb improved renal function. Protective effect dependant on tissue mass.
Wever et al 2012 ²⁴	Rat model of renal acute IRI (25 min I /48 hr R)	Three-5 min cycles of IR to both hindlimbs at the onset of reperfusion (RIPost).	Improved renal function. RIC effect synergistic with local IPost

I-ischemia, R-reperfusion, IR-ischemia and reperfusion, IRI-ischemia-reperfusion injury, RIC-remote ischemic conditioning, RIPerC- remote ischemic preconditioning, RIPost- remote ischemic postconditioning, IPost- ischemic postconditioning, BUN-blood urea nitrogen, Cr-creatinine, MDA- malondialdehyde

Table 1b: Major clinical studies investigating the effect of RIC on preventing acute renal IRI

Clinical study	Clinical setting	N	RIC protocol	Result of RIC
Ali et al 2007 ⁹²	Elective AAA repair	82	10 min clamping of the right common iliac artery followed by clamping of the left common iliac artery.	23% absolute risk reduction in the incidence of renal impairment (defined as peak serum Cr level of 177 µmol/L (2.0 mg/dL)).
Walsh et al 2009 ⁹³	Elective EVAR	40	10min cuff inflation of the right thigh followed by 10 min cuff inflation of the left thigh.	Attenuated increase in the renal injury biomarker, urinary retinol binding protein, and a non-significant reduction in the urinary albumin:Cr ratio.
Walsh et al 2010 ⁹⁴	Elective open infrarenal AAA repair	40	10 min clamping of the right common iliac artery followed by clamping of the left common iliac artery.	No effect on either urinary retinol binding or albumin:Cr ratio
Venugopal et al 2010 ⁹⁵	Adult CABG surgery	78	Three-5 min upper arm cuff inflations/deflations	Retrospective study showing a reduction in the incidence of AKI (according to AKIN criteria) from 25 to 11%.
Thielmann et al 2010 ⁹⁶	Adult CABG surgery	53	Three-5 min upper arm cuff inflations/deflations	No overall difference in Cr levels and eGFR over 72 hrs. Improved post-operative peak Cr levels in RIC group Lower post-operative minimum eGFR in control group.
Rahman et al 2010 ⁹⁷	Adult CABG surgery	162	Three-5 min upper arm cuff inflations/deflations	No effect on AKI or renal biomarkers
Choi et al 2011 ⁹⁸	Adult complex valve surgery	76	Three-10 min thigh cuff inflations/deflations	No effect on AKI or renal biomarkers (serum Cr, cystatin C and NGAL).
Zimmerman et al 2011 ⁹⁹	Adult CABG surgery	120	Three-5 min thigh cuff inflations/deflations	Reduction from 47% to 20% in the incidence of AKI (defined as an increase in serum Cr levels by at least 0.3 mg/dL or 50% more than the baseline value within 48 hr of surgery). No difference in plasma NGAL at 3 hours.
Pedersen et al 2011 ¹⁰⁰	Paediatric cardiac surgery for complex congenital heart disease	103	Four-5 min thigh cuff inflations/deflations	No effect on AKI or renal biomarkers.
Whittaker et al 2011 ¹⁰¹	Adult patients with mildly impaired renal function undergoing primary PCI	43	>4 inflations/deflations of angioplasty balloon	Retrospective study showing preserved renal function in those receiving angioplasty balloon inflations/deflations at time of primary PCI.
Hong et al 2012 ¹⁰²	Adult OPCABG surgery	70	Four-5 min cycles of thigh cuff ischemia and reperfusion prior to after anastomoses.	No difference in post-operative renal dysfunction
Er et al 2012 ²⁹	Adult patients with moderately impaired renal function undergoing elective PCI	100	Four-5 min upper arm cuff inflations/deflations	Reduction from 40% to 16% in the incidence of contrast-AKI (defined as an increase in serum Cr ≥25% or ≥0.5 mg/dL above baseline at 48 hr

AAA- abdominal aortic aneurysm, EVAR- endovascular aortic aneurysm repair, CABG- coronary artery bypass graft, PCI- percutaneous coronary intervention, OPCABG- off-pump coronary artery bypass graft, RIC-remote ischemic conditioning, Cr- creatinine, µmol- micromoles, L- litre, dL- decilitre, mg- milligram, AKI- acute kidney injury, AKIN- acute kidney injury network, NGAL- neutrophil gelatinase-associated lipocalin, eGFR- estimated glomerular filtration rate

Table 2a: Major experimental studies investigating the effect of RIC on preventing acute lung IRI

Experimental study	Experimental setting	RIC protocol	Result of RIC
Peralta et al 2001 ³⁴	Rat model of acute liver acute 90 (90 min I /R)	One-10 min cycle of hepatic IR	Attenuated neutrophil accumulation in lungs.
Harkin et al 2002 ¹⁰³	Porcine model of acute lung IRI (2 hr I /2.5 hr R)	Three-5 min cycles of bilateral hindlimb IR	Reduced markers of systemic inflammation (TNF and interleukin-6, and attenuated phagocytic cell priming and pulmonary oedema/respiratory failure.
Xia et al 2003 ¹⁰⁴	Sheep model of repeated myocardial ischemia induced by 10 min clamping of LAD, LCx then RCA.	Three-5 min cycles of iliac artery IR	Attenuated increase in pulmonary vascular resistance and pulmonary artery pressure and a smaller reduction in PaO ₂
Waldow et al 2005 ¹⁰⁵	Porcine model of acute lung IRI (1.5 hr I /5 hr R)	Three-5 min cycles of left common femoral artery IR	Prevention of acute lung IRI and pulmonary hypertension.
Olguner et al 2006 ¹⁰⁶	Rat model of acute hindlimb IRI (4 hr I /2 hr R)	Three-10 min cycles of hindlimb IR	Attenuated neutrophil accumulation in lungs. Reduced histological changes in lung.
Kharbanda et al 2006 ¹⁰⁷	Porcine model of cardiopulmonary bypass	Four-5 min cycles of hindlimb IR	Improved lung compliance, attenuated increase in pulmonary resistance and a lower peak inspiratory pressure.

I-ischemia, R-reperfusion, IR-ischemia and reperfusion, IRI-ischemia-reperfusion injury, LAD- left anterior descending, LCx- left circumflex, RCA- right coronary artery, RIC-remote ischemic conditioning ,TNF- tissue necrosis factor, PaO₂- partial pressure of oxygen

Table 2b: Major clinical studies investigating the effect of RIC the effect of RIC on preventing acute lung IRI

Clinical study	Clinical setting	N	RIC protocol	Result of RIC
Li et al 2001 ³⁵	Adult CABG surgery	40	Two cycles of 3 min aortic cross clamping and 2 min declamping	Reduced ventilation requirements, pulmonary oedema and haemorrhage, and decreased leukocyte count found on lung biopsies taken after 1 hour of reperfusion.
Cheung et al 2006 ¹⁰⁸	Pediatric cardiac surgery for congenital heart disease	37	Four-5 min cycles of thigh cuff ischemia and reperfusion	Lower airway resistance at 6 hr postoperatively.
Rahman et al 2010 ³⁷	Adult CABG surgery	162	Three-5 min cycles of arm cuff ischemia and reperfusion	No effect on ventilation time and preoperative and postoperative PaO ₂ /FiO ₂ ratios.
Zhou et al 2010 ¹⁰⁹	Pediatric cardiac surgery for congenital heart disease	60	Three-5 min cycles of arm cuff ischemia and reperfusion 24 hrs and 1 hr pre-operatively	Better lung compliance (Cs) and dynamic lung compliance.
Li et al 2010 ¹¹⁰	Adult valvular heart surgery	81	Three-4 min cycles of thigh cuff ischemia and reperfusion	No effect on ventilation time.
Thielmann et al 2010 ⁹⁶	Adult CABG surgery	53	Three-5 min cycles of arm cuff ischemia and reperfusion	No effect on ventilation time.
Lin et al 2010 ¹¹¹	Lower limb orthopaedic surgery with 75 min lower leg ischemia.	30	Three-5 min cycles of leg cuff ischemia and reperfusion	Reduced pulmonary injury.
Young et al 2012 ¹¹²	Adult high-risk CABG surgery	96	Three-5 min cycles of arm cuff ischemia and reperfusion	Increased ventilation time
Hong et al 2012 ¹⁰²	Adult OPCAB surgery	70	Four-5 min cycles of thigh cuff ischemia and reperfusion prior to after anastomoses.	No effect on PaO ₂ /FiO ₂ ratio
Kim et al 2012 ¹¹³	Adults complex valvular heart surgery	54	Three-10 min cycles of thigh cuff ischemia and reperfusion prior to after bypass.	No effect on PaO ₂ /FiO ₂ or incidence of acute lung injury (was defined as PaO ₂ /FiO ₂ <300 mmHg, the detection of bilateral pulmonary infiltrates on frontal chest radiography and no clinical evidence of further elevation in the left atrial pressure.
Lomivorotov et al 2012 ¹¹⁴	Adult CABG surgery	80	Three-5 min cycles of arm cuff ischemia and reperfusion	No effect on ventilation time

CABG- coronary artery bypass graft, OPCABG-off-pump coronary artery bypass graft, RIC-remote ischemic conditioning, PaO₂/FiO₂-oxygen tension/Fraction of inspired oxygen ratio, Cs- static compliance, mmHg- millimetres of mercury

Table 3: Major experimental studies investigating the effect of RIC on preventing acute liver

IRI

Experimental study	Experimental setting	RIC protocol	Effect of RIC
Lai et al 2006 ⁴⁰	Rat model of partial liver (left lobe only) IRI 45 min I/240 min R	Four-10 min cycles of IR to hindlimb	Attenuated increase in serum ALT. Increased hepatocyte expression of HO-1
Kanoria et al 2006 ¹¹⁵	Rabbit model of liver acute IRI 25 min I/ 120 min R	Three-10 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels Preserved PLBF Increased hepatic nitrite/nitrate levels.
Gustafsson et al 2006 ¹¹⁶	Rat model of mild liver IRI 60 min I/60 min R (occlusion of hepatic artery only) severe liver IRI 60 min I/60 min R (occlusion of hepatic vascular supply).	One 10 min episode of hindlimb ischemia	Attenuated increase in serum ALT levels but no difference in PLBF following mild ischemia. No difference in ALT or PLBF in severe hepatic ischemia.
Tapuria et al 2009 ¹¹⁷	Rat model of partial liver IRI 45 min I/180 min R	Four-4 min cycles of IR to hindlimb	Increased red blood cell velocity, sinusoidal flow and sinusoidal perfusion along with decreased neutrophil adhesion and cell death.
Wang et al 2010 ⁴¹	Murine model of partial liver ischemia 60 min I/R	One 10 min episode of hindlimb ischemia.	Attenuated increase in serum ALT. Reduced serum and hepatocyte expression of TNF- α , and less apoptotic cell death. Increased expression of serum HMG-B1, which acts on TLR4. No RIC in TLR4 deficient mice.
Abu-Amara et al 2011 ¹¹⁸	Murine model of partial liver acute IRI 40 min I/ 120 min R	Six-4 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels. Preserved electron microscopic/histological appearances RIC blocked by NO inhibitor
Abu-Amara et al 2011 ⁴²	Murine model of partial liver acute IRI 40 min I/ 120 min R	Six-4 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels. Preserved electron microscopic/histological appearances No RIC in eNOS deficient mice.
Kanoria et al 2012 ¹¹⁹	Rabbit model of liver acute IRI 25 min I/ 120 min R	Three-10 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels Increased mitochondrial oxygenation, and elevated serum bicarbonate and hepatic nitrite/nitrate levels.
Abu-Amara et al 2012 ⁴³	Murine model of partial liver acute IRI 40 min I/ 120 min R	Six-4 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels Preserved electron microscopic/histological appearances RIC dependant on sGC-cGMP
Wang et al 2012 ⁴⁴	Rat model of liver graft transplantation	Four-4 min cycles of IR to hindlimb	Attenuated rise in ALT/AST levels. Preserved electron microscopic/histological appearances. Increased graft proliferation and enhanced expression of interleukin-6 mRNA and suppressed expression of TNF- α .

I-ischemia, R-reperfusion, IR-ischemia and reperfusion, IRI-ischemia-reperfusion injury, RIC-remote ischemic conditioning, ALT- alanine aminotransferase, AST- aspartate aminotransferase, HO-1- hemoxygenase-1, PLBF- peripheral liver blood flow, TNF- α - tissue necrosis factor alpha, HMG-B1- High Mobility Group-Box 1, TLR4- toll-like receptor-4, NO- nitric oxide, eNOS- endothelial nitric oxide synthase, sGC- soluble guanylate cyclase, cGMP- cyclic guanosine monophosphate, mRNA- messenger ribonucleic acid

Table 4: Major experimental studies investigating the effect of RIC on preventing acute brain

IRI

Experimental study	Experimental setting	RIC protocol	Effect of RIC
Dave et al 2006 ⁵³	Rat model of ASA for 8 min and assessment at 7 days.	One 15 or 30 min episode of ischemia applied to both hindlimbs 48 hrs prior to ASA.	Preservation of CA1 hippocampal neurones
Gurcun et al 2006 ¹²⁰	Rabbit model of abdominal aortic occlusion (40 min) and reflow (24 hrs) to induce acute spinal cord IRI	Two cycles of 5 min renal I and 15 min renal R.	Improved neurological recovery.
Zhao et al 2007 ¹²¹	Rat model of middle cerebral artery occlusion/reflow (2 hr I, 24 hr R).	Three-10 min cycles of hindlimb IR	Reduced neurological dysfunction score. Reduced cerebral infarct size when RIC given 1,2 or 24 hrs prior to middle cerebral artery occlusion but not 12 or 48 hrs prior, confirming the two windows of neuroprotection with RIC.
Rehni et al 2007 ¹²²	Murine model of bilateral carotid artery occlusion (10 min I/24 hr R)	One 15 min episode of intestinal IR	Reduced cerebral infarct size Less deterioration in motor function and short-term memory. RIC protection abrogated by antagonists of K _{ATP} channel and adenosine.
Ren et al 2008 ¹²³	Rat model of cerebral IRI (permanent left distal middle cerebral artery occlusion + bilateral common carotid artery occlusion for 30 minutes).	Three-15 min cycles of hindlimb IR	Reduced cerebral infarct size when RIC administered 24 hr, 12 hr and immediately prior to acute cerebral IRI.
Ren et al 2009 ¹²³	Rat model of permanent cerebral IRI (permanent left distal middle cerebral artery occlusion + bilateral common carotid artery occlusion for 30 minutes).	Three-15 min cycles of hindlimb IR applied at the onset of cerebral reperfusion (RIPost).	Reduced cerebral infarct size when RIC immediately and 3 hours after cerebral reperfusion but not at 6 hours after reperfusion. First demonstration that RIPost and delayed RIPost effect. RIC blocked by capsaicin and cycloheximide implicating afferent nerve stimulation and protein synthesis in the mechanism.
Saxena et al 2009 ¹²⁴	Rat model of transient GCI, 8 min bilateral common carotid artery occlusion and hypotension.	Five-5 min cycles of hindlimb IR	No effect on CA1 hippocampal neurones. This is probably because of the index ischemic event not being insufficient.
Yannopoulos et al 2010 ¹²⁵	Piglet model of cardiopulmonary bypass and hypothermic circulatory arrest	Four-5 min cycles of hindlimb IR	Reduced cerebral lactate, glucose and glycerol levels. More rapid recovery of EEG activity.
Xu et al 2011 ¹²⁶	Rat model bilateral carotid artery occlusion (60 min I/5-8 days R)	Three-10 min cycles of hindlimb IR	Improved neurocognitive function. Increased expression for anti-apoptotic protein Bcl2.
Malhotra et al 2011 ¹²⁷	Rat model of middle cerebral artery occlusion (2 hrs) and reflow (24 hrs)	Three-10 min cycles of infrarenal aortic occlusion and reflow.	Reduced cerebral infarct size when RIC stimulus applied 24 hrs but not 48 or 72 hrs prior to acute cerebral IRI. Improved neurological outcomes RIC protection abrogated by hexamethonium implicating a neural pathway in protection.
Jensen et al 2011 ¹²⁸	Piglet model of cardiopulmonary bypass and hypothermic circulatory arrest	Four-5 min cycles to hindlimb IR	Reduced cerebral lactate levels. Less cerebral injury on histological analysis. More rapid recovery of EEG activity. Improved neurological recovery.
Zhou et al 2011 ¹²⁹	Neonatal rat model cerebral hypoxia (unilateral carotid ligation and 2 hrs of hypoxia)	Four-10 min cycles of IR to both hindlimbs at the onset of cerebral reoxygenation/ reperfusion (RIPost).	Reduced cerebral infarct size Improved neurological recovery. RIC blocked by naloxone and wortmannin implicating opioid receptor and PI3K signalling in protective response. RIC associated with PKB activation and less BAX.
Hahn et al 2011 ¹³⁰	Rat model of middle cerebral artery occlusion (2 hrs) and reflow (24 hrs)	Four-5 min cycles to hindlimb IR	Reduced cerebral infarct size when RIC stimulus applied prior to and after the onset of cerebral ischemia. First demonstration that RIPerC in brain.
Sun et al 2012 ¹³¹	Rat model of middle cerebral artery occlusion (90 min) and reflow (72 hrs)	Three-5 min cycles of IR to both hindlimbs (RIPost).	Reduced cerebral infarct size when RIC stimulus applied at 3 or 6 hours into reperfusion. RIC protection via mitochondrial K _{ATP} channel.
Geng et al 2012 ¹³²	Rat model of intracerebral haemorrhage using collagenase.	Repeated episodes of IR to both hindlimbs.	No beneficial effects on cerebral blood volumes, brain water content, Evans blue extravasations, and expressions of AQP-4 and MMP-9 or

			neurological function at either 24 or 72 hrs.
Wei et al 2012 ¹³³	Rat model of permanent cerebral IRI (permanent left distal middle cerebral artery occlusion + bilateral common carotid artery occlusion for 30 minutes).	Three-15 min cycles of hindlimb IR.	Reduced cerebral infarct size at 2 and 60 days with improved neurological recovery at 2 months. Less cerebral edema and reduced blood brain-barrier permeability. RIC protection via afferent neural pathway. Attenuated increase in inflammatory protein galectin-9 and T-cell immunoglobulin domain and Tim-3 and less iNOS and nitrosylation.
Hu et al 2012 ¹³⁴	Rat model of middle cerebral artery occlusion (90 min) and reflow (72 hrs)	Three-5 min cycles of hindlimb IR	Reduced cerebral infarct size when RIC stimulus applied at 3 or 6 hours into reperfusion. RIC protection via mitochondrial K_{ATP} channel.

I-ischemia, R-reperfusion, IR-ischemia and reperfusion, IRI-ischemia-reperfusion injury, RIC-remote ischemic conditioning, RIPost- remote ischemic postconditioning, RIPerC- remote ischemic preconditioning, ASA- asphyxial cardiac arrest, GCI- global cerebral ischemia, CA1- cornu ammonis-1, KATP- adenosine triphosphate-sensitive potassium, EEG- electroencephalography, bcl-2- B-cell lymphoma-2, PI3K- phosphatidylinositol 3-kinase, PKB- protein kinase B, BAX- b-cell lymphoma-2-associated X protein, AQP4- aquaporin-4, MMP-9- matrix metalloproteinase-9, Tim-3- mucin-domain-containing molecule-3, iNOS- inducible nitric oxide synthase,



SKIN FLAP
Trauma surgery
Plastic surgery
Reconstructive surgery



BRAIN
Stroke
Subarachnoid hemorrhage
Carotid endarterectomy
Cervical decompression surgery
Cerebral asphyxia



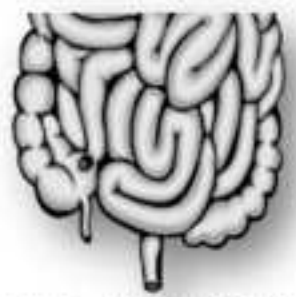
LUNGS
Resection surgery
Transplantation
Cardiac surgery
Vascular surgery



LIVER
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