In this review, we examine the role of ischaemic conditioning in clinical practice has been less straightforward than expected.

ABSTRACT

Over the last quarter of a century, a huge effort has been made to develop interventions that can minimise ischaemia reperfusion injury. The most potent of these are the ischaemic conditioning strategies, which comprise ischaemic preconditioning, remote ischaemic preconditioning and ischaemic postconditioning. While much of the focus for these interventions has been on protecting the myocardium, other organs including the kidney can be similarly protected. However, translation of these beneficial effects from animal models into routine clinical practice has been less straightforward than expected. In this review, we examine the role of ischaemic conditioning strategies in reducing tissue injury from the 'bench to the bedside' and discuss the barriers to their greater translation.

KEYWORDS: preconditioning, postconditioning, remote ischaemic preconditioning

INTRODUCTION

It has been 101 years since Herrick [1] reported the first case of myocardial infarction due to coronary thrombosis, at which time he noted "The hope for the damaged myocardium lies in the direction of securing a supply of blood ... so as to restore so far as possible its functional integrity". This insight into the
The first pathway characterized is the reperfusion injury salvage kinase (RISK) pathway, a collection of pro-survival, anti-apoptotic protein kinases which are activated at the time of myocardial reperfusion to confer profound cardioprotection [16]. The main components of the RISK pathway are PI3K-Akt, JNK and Erk 1/2 [14]. Activation of this pathway leads to inhibition of caspases [17] cytochrome c release [18] and inhibition of the opening of the mitochondrial permeability transition pore (mPTP), which appears to be the final common effector pathway for cytoprotection. More recently, the survivor activating factor enhancement (SAFE) pathway involving TNFα and JAK-STAT was shown to lead to tissue protection independently of the RISK pathway [19, 20].

Following an IPC stimulus, cytoprotection is seen immediately and lasts for 1–2 h [21]; however, around 12–24 h after the initial IPC stimulus, the tissue-protective effect of IPC reappears and lasts for 24–48 h [22]. This led to the concept of early and late phases of cytoprotection. The delay in the late phase is thought to be due to the requirement for de novo protein synthesis such as COX-2 and iNOS and heme oxygenase-1 which are thought to mediate cardioprotection via the same common effectors as the early phase (see Hausenloy and Yellon [23] for a full review of the mechanism of the delayed IPC). Mechanistic insights into IPC have almost exclusively come from studies involving the myocardium. Given that IPC appears to be a ubiquitous cytoprotective mechanism and confers tissue protection in many different models of tissue ischaemia including the kidney, it is tempting to conclude that the mechanisms are the same across organs. However, there appears to be some disparity between the mechanisms of IPC in the heart and the kidney. Exogenous adenosine appears to be renoprotective, as in the myocardium, unlike [24] adenosine antagonists which do not appear to abolish the protective effects of IPC in the kidney [25, 26]. Additionally bradykinin and opiates failed to reduce renal IRI as they do in the myocardium [27]. However, there is evidence to suggest that members of the myocardial RISK pathway, such as PKC [27], ERK [28] and Akt [29] along with mediators of IPC in the heart such as iNOS [30] and heme oxygenase-1 [31] also appear to be involved in renoprotection from IPC.

MECHANISMS OF IPC

A detailed explanation of the mechanisms through which IPC leads to tissue protection is beyond the scope of this review. Hausenloy and Yellon [13–15] have written several excellent reviews on this subject.

In brief, IPC has been shown to be associated with the release of several autacoids (including adenosine, opioids and bradykinin) that trigger anti-apoptotic intracellular signal transduction cascades.
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<th>First author</th>
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<th>Primary outcome</th>
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<th>Conditioning protocol</th>
<th>Number of study groups</th>
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<th>Prespecified renal end point?</th>
<th>Renal outcome</th>
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</thead>
<tbody>
<tr>
<td>Clavien et al.</td>
<td>2003</td>
<td>IPC</td>
<td>Negative</td>
<td>Positive</td>
<td>100 (50/50)</td>
<td>Liver portal triad clamping</td>
<td>Major liver resection</td>
<td>1 cycle of 10 min I+R</td>
<td>2 groups</td>
<td>Cirrhosis</td>
<td>RIPC reduced peak post-op liver enzyme concentrations.</td>
<td>No</td>
<td>No difference in post-op renal creatinine concentration. 2 patients from each group developed post-op renal failure.</td>
</tr>
<tr>
<td>Azoulay et al.</td>
<td>2006</td>
<td>IPC</td>
<td>Negative</td>
<td>Negative</td>
<td>60 (30/30)</td>
<td>Liver portal triad clamping</td>
<td>Major liver resection</td>
<td>1 cycle of 10 min I+R</td>
<td>2 groups</td>
<td>Nil</td>
<td>RIPC had no effect on peak post-operative liver enzyme concentration, with no difference in post-op morbidity or mortality.</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Ali et al.</td>
<td>2007</td>
<td>RIPC</td>
<td>Positive</td>
<td>Positive</td>
<td>82 (41/41)</td>
<td>Common iliac occlusion</td>
<td>Open infra-renal AAA repair</td>
<td>2 × 10 min cross clamping</td>
<td>2 groups</td>
<td>Nicorandil or glibenclamide use, recent ACS or &gt;90 years old</td>
<td>RIPC reduced post-op incidence of myocardial injury (P = 0.005) and infarction (P = 0.006).</td>
<td>Part of composite secondary end point.</td>
<td>RIPC reduced incidence of post-op AKI (P = 0.009).</td>
</tr>
<tr>
<td>Hoole et al.</td>
<td>2009</td>
<td>RIPC</td>
<td>Negative</td>
<td>Positive</td>
<td>242 (125/17)</td>
<td>Upper limb</td>
<td>Elective PCI</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Emergency PCI, women of childbearing age, life expectancy &lt;6/12, on nicorandil or glibenclamide</td>
<td>RIPC led to a lower serum troponin 24 h post-op (P = 0.04) and at 6/12, fewer major adverse cardiac and cerebral events (P = 0.018).</td>
<td>No</td>
<td>No significant difference in renal function at 24 h post-op.</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2009</td>
<td>iPOST</td>
<td>Unclear</td>
<td>Positive</td>
<td>99 (48/23/25)</td>
<td>Intermittent aortic cross clamping</td>
<td>Tetralogy of Fallot</td>
<td>2 or 3 cycles of 30 s I+R</td>
<td>3 groups: control, 2 cycles, 3 cycles</td>
<td>Additional cardiac abnormalities</td>
<td>iPOST reduced troponin release (P = 0.026), reduced inotrope score (P = 0.001) and lactate release (P = 0.019) and reduced ICU stay (P = 0.048).</td>
<td>Part of composite primary end point.</td>
<td>Significant reduction in composite end point (P = 0.016), of which renal failure was one component, renal failure not classified. 1 episode of renal failure in control group, none in iPOST.</td>
</tr>
<tr>
<td>Walsh et al.</td>
<td>2009</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>40 (18/22)</td>
<td>Lower limb</td>
<td>Endovascular AAA repair</td>
<td>2 × 10 min cross clamping</td>
<td>2 groups</td>
<td>Pre-op Creatinine &gt;1.5 mg/dL or a past history of AKI</td>
<td>Nil</td>
<td>Primary end point: post-op urinary biomarkers of renal injury.</td>
<td></td>
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</tbody>
</table>

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Table 1. Continued

<table>
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<tr>
<th>First author</th>
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<th>Non-renal outcome</th>
<th>Prespecified renal end point?</th>
<th>Renal outcome</th>
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<tbody>
<tr>
<td>Rahman et al.</td>
<td>2010</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>160 (80/82)</td>
<td>Upper limb</td>
<td>Multivessel CABG</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Diabetes, dialysis or recent MI</td>
<td>RIPC did not alter troponin release haemodynamics.</td>
<td>No difference in dialysis requiring AKI or peak Cr between groups. Reduction in peak serum creatinine concentration in RIPC group (P &lt; 0.04).</td>
<td>Reduced incidence of AKI stages 1 or 2 or 3 (P = 0.005) in RIPC group.</td>
</tr>
<tr>
<td>Thielmann et al.</td>
<td>2010</td>
<td>RIPC</td>
<td>Positive</td>
<td>Positive</td>
<td>53 (26/27)</td>
<td>Upper limb</td>
<td>CABG</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Diabetes, Cr &gt;200 µmol/L, PVD, unstable angina or recent MI</td>
<td>Nil</td>
<td>Primary end point: reduction in AKIN score at 72 h post-op. Primary end point: post-op urinary biomarkers of renal injury.</td>
<td>No difference in markers of AKI (creatinine, cystatin c, serum NGAL) between groups.</td>
</tr>
<tr>
<td>Venugopal et al.</td>
<td>2010</td>
<td>RIPC</td>
<td>Positive</td>
<td>Positive</td>
<td>78 (38/40)</td>
<td>Upper limb</td>
<td>CABG</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Diabetes or CKD</td>
<td>Nil</td>
<td>Pre-op Creatinine &gt;1.5 mg/dL or a past history of AKI</td>
<td>No significant difference in urine output or creatinine concentration 24 h post-op.</td>
</tr>
<tr>
<td>Walsh et al.</td>
<td>2010</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>51 (22/18)</td>
<td>Common iliac occlusion</td>
<td>Open infra-renal AAA repair</td>
<td>2 × 10 min cross clamping</td>
<td>2 groups</td>
<td>Pre-op Creatinine &gt;1.5 mg/dL or a past history of AKI</td>
<td>RIPC reduced CK-MB release at 24 h (P = 0.017). Primary end point: incidence of AKI within 48 h post-op.</td>
<td>No significant difference in urine output or creatinine concentration 24 h post-op.</td>
<td></td>
</tr>
<tr>
<td>Choi et al.</td>
<td>2011</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>76(38/38)</td>
<td>Lower limb</td>
<td>Elective complex valvular cardiac surgery</td>
<td>3 × 10 min I+R</td>
<td>2 groups</td>
<td>Age &gt;80, creatinine &gt; 1.6 mg/dL (men), 1.4 mg/dL (women), heart, liver or lung disease, PVD, sulphonylurea or nicostranil use</td>
<td>RIPC reduced CK-MB release at 24 h (P = 0.017).</td>
<td>No significant difference in urine output or creatinine concentration 24 h post-op.</td>
<td></td>
</tr>
<tr>
<td>Ji et al.</td>
<td>2011</td>
<td>iPOST</td>
<td>Negative</td>
<td>Positive</td>
<td>80 (41/39)</td>
<td>Intermittent aortic cross clamping</td>
<td>Tetralogy of Fallot</td>
<td>3 × 30 s I+R</td>
<td>2 groups</td>
<td>Additional cardiac abnormalities</td>
<td>iPOST reduced peak troponin concentration (P &lt; .0001), lowered inotrope requirements (P &lt; 0.0001) and shortened ICU stay (P = 0.0003).</td>
<td>No significant difference in urine output or creatinine concentration 24 h post-op.</td>
<td>No significant difference in urine output or creatinine concentration 24 h post-op.</td>
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<tr>
<td>Study</td>
<td>Year</td>
<td>Technique</td>
<td>Endpoint</td>
<td>Adverse Events</td>
<td>Incidence</td>
<td>Limb Location</td>
<td>Surgery Details</td>
<td>Primary End Point</td>
<td>Secondary End Point</td>
<td>Additional Details</td>
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<tr>
<td>Zimmerman et al.</td>
<td>2011</td>
<td>RIPC</td>
<td>Positive</td>
<td>Positive</td>
<td>118 (59/59)</td>
<td>Lower limb</td>
<td>On pump cardiac surgery (CABG or valve or both)</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>PVD, ESRD</td>
<td>Nil</td>
<td>Primary end point: an elevation of serum creatinine of &gt; 0.3 mg/dL or &gt; 50% within 48 h after surgery. Reduced incidence of AKI within first 48 h (P = 0.005) in RIPC group.</td>
<td></td>
</tr>
<tr>
<td>Er et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Positive</td>
<td>Positive</td>
<td>100 (50/50)</td>
<td>Upper limb</td>
<td>Elective coronary angiogram</td>
<td>4 × 5 min I+R</td>
<td>2 groups</td>
<td>Nil</td>
<td>Nil</td>
<td>Primary end point: the incidence of contrast induced kidney injury</td>
<td></td>
</tr>
<tr>
<td>Hong et al.</td>
<td>2012</td>
<td>Combined RIPC+RPOST</td>
<td>Negative</td>
<td>Positive</td>
<td>75 (35/35)</td>
<td>Lower limb</td>
<td>Elective off pump CABG</td>
<td>2 × 5 min I+R</td>
<td>2 groups: control and RIPC+RPOST</td>
<td>Age &gt; 80, severe renal, liver, or heart failure</td>
<td>RIPC+RPOST reduced post op troponin release (P = 0.02). Secondary end point: creatinine level in first 5 days post-op.</td>
<td></td>
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<tr>
<td>Kim et al.</td>
<td>2012</td>
<td>RIPC+RPOST</td>
<td>Negative</td>
<td>Negative</td>
<td>54 (27/7)</td>
<td>Lower limb</td>
<td>Valvular heart surgery</td>
<td>3 × 10 min I+R</td>
<td>2 groups: control and RIPC+RPOST</td>
<td>PVD, liver or renal failure</td>
<td>No difference on post-op pulmonary function.</td>
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<td></td>
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<tr>
<td>Kottenberg et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Positive</td>
<td>72 (19/19/14/20)</td>
<td>Upper limb</td>
<td>On pump triple vessel CABG</td>
<td>3 × 5 min I+R</td>
<td>4 groups: RIPC or Control with isoflurane or propofol anaesthesia</td>
<td>Diabetes, serum Creatinine &gt; 2 mg PVD/ACS</td>
<td>Reduction in troponin release (P = 0.004) with RIPC in those patients who received isoflurane anaesthesia.</td>
<td></td>
<td></td>
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<tr>
<td>Lee et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>55 (28/27)</td>
<td>Lower limb</td>
<td>VSD repair</td>
<td>4 × 5 min I+R</td>
<td>2 groups</td>
<td>Renal, liver or lung disease or infection</td>
<td>RIPC did not alter cardiac enzyme release in first 24 h post-op.</td>
<td></td>
<td></td>
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<tr>
<td>Lomivorotov et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>80 (40/40)</td>
<td>Upper limb</td>
<td>On pump CABG</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Heart, liver, lung, or renal failure or diabetes.</td>
<td>RIPC did not alter myocardial enzyme release between groups.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucchinetti et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>55 (28/27)</td>
<td>Lower limb</td>
<td>On Pump CABG</td>
<td>4 × 5 min I+R</td>
<td>2 groups</td>
<td>Diabetes, PVD, BMI &gt; 35</td>
<td>RIPC did not alter peak post-op cardiac enzyme release.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedersen et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>105 (54/51)</td>
<td>Lower limb</td>
<td>Children undergoing heart surgery</td>
<td>4 × 5 min I+R</td>
<td>2 groups</td>
<td>Nil</td>
<td>Primary end point: Incidence of post-op AKI.</td>
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<tr>
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<th>Clinical setting</th>
<th>Conditioning protocol</th>
<th>Number of study groups</th>
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<th>Prespecified renal end point?</th>
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<tbody>
<tr>
<td>Young et al.</td>
<td>2012</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>96 (48/48)</td>
<td>Upper limb</td>
<td>On pump ‘High risk’ cardiac surgery</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>PVD</td>
<td>RIPC did not alter cardiac enzyme release or inotrope requirements.</td>
<td>No difference in peak post-op RIFLE score.</td>
<td></td>
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<tr>
<td>Chen et al.</td>
<td>2013</td>
<td>RIPC</td>
<td>Negative</td>
<td>Negative</td>
<td>60 (20/20/20)</td>
<td>Lower limb</td>
<td>Live donor renal transplantation</td>
<td>3 × 5 min I+R</td>
<td>3 groups: RIPC Donor, RIPC recipient, nil</td>
<td>Diabetes, PVD, previous transplant</td>
<td>Primary end point: renal function 72 h post-op.</td>
<td>No difference in renal function between the 3 groups at 72 h.</td>
<td></td>
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<tr>
<td>Deftereos et al.</td>
<td>2013</td>
<td>RPOST</td>
<td>Positive</td>
<td>Positive</td>
<td>225 (113/112)</td>
<td>Heart</td>
<td>NSTEMI patients having PCI within 72 h</td>
<td>4 × 30 s I+R</td>
<td>2 groups</td>
<td>Dialysis</td>
<td>Reduction in 30 day re-hospitalisation ($P = 0.047$).</td>
<td>58% reduction in AKI in RIPC group. NNT 6.</td>
<td></td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2013</td>
<td>RIPC</td>
<td>Positive</td>
<td>Negative</td>
<td>82 (41/41)</td>
<td>Lower limb</td>
<td>Laparoscopic nephrectomy for renal tumours</td>
<td>3 × 5 min I+R</td>
<td>2 groups</td>
<td>Heart, liver, lung or renal disease</td>
<td>Primary end point: absolute change in GFR of the affected kidney from baseline to 6 months.</td>
<td>No difference in primary end point. Secondary end points: better single kidney GFR at 1 month in RIPC group ($P = 0.04$) and reduction in 24 h post op RBP in RIPC group ($P &lt; 0.001$).</td>
<td></td>
</tr>
</tbody>
</table>

AAA, abdominal aortic aneurysm; CABG, coronary artery bypass graft; IPC, ischaemic preconditioning; iPOST, ischaemic postconditioning; MI, myocardial infarction; NGAL, neutrophil gelatinase-associated lipocalin; PCI, percutaneous coronary intervention; PVD, peripheral vascular disease; RBP, retinol-binding protein; RIPC, remote ischaemic preconditioning; RPOST, remote ischaemic postconditioning.
Candidate molecules involved in the neural pathway include nerves that terminate on the tissue at risk or when brief episodes of ischaemia release substances which stimulate efferent pathways [74, 75], thermolabile and is between 3.5 and 15 kDa [75]. The nature of this molecule (or molecules) is unknown, but several bloodstream to trigger distant cytoprotection. The precise humoral pathway is activated when a factor is released into the pathways have been proposed as possible mechanisms. The transduction from the remote organ to the organ at risk. Two remote organ to cytoprotection. First, the signal has to be transmitted from the remote organ to the kidney. In 1993, Przyklenk et al. [64] published a seminal paper which demonstrated that following IPC, protection was seen in vascular beds adjacent to the ischaemic vascular territory. The term ‘remote ischaemic preconditioning’ (RIPC) was used to describe this strategy.

As with IPC, RIPC appears to confer profound tissue protection in all organs studied. In animal studies, the kidney has been studied both as a trigger and a recipient of RIPC. As a trigger of RIPC, brief episodes of renal IRI have been shown to reduce myocardial infarct size in rabbits [65], rats [66] and pigs [67]. Similarly, the kidney can be protected by brief episodes of ischaemia and reperfusion in other organs: the liver [68], intestines [69], legs [70] and by infrarenal aortic clamping [71].

REMOTE ISCHAEMIC PRECONDITIONING

The one major limitation that has prevented a better translational capability of IPC and its more widespread use is the requirement for direct access to the blood supply of the organ at risk. This may explain the dearth of clinical trials involving IPC and the kidney. In 1993, Przyklenk et al. [64] published a seminal paper which demonstrated that following IPC, protection was seen in vascular beds adjacent to the ischaemic vascular territory. The term ‘remote ischaemic preconditioning’ (RIPC) was used to describe this strategy.

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MECHANISMS OF RIPC

As with IPC, the vast majority of studies of the mechanisms of tissue protection, as seen following RIPC, have focused on the myocardium. The precise mechanisms have yet to be fully elucidated and are beyond the scope of this review. Readers are directed to excellent reviews on the subject [72, 73]. Briefly, there are two steps in the transduction of the signal from the remote organ to cytoprotection. First, the signal has to be transduced from the remote organ to the organ at risk. Two pathways have been proposed as possible mechanisms. The humoral pathway is activated when a factor is released into the bloodstream to trigger distant cytoprotection. The precise nature of this molecule (or molecules) is unknown, but several elegant experiments have shown that the factor is hydrophobic [74, 75], thermolabile [75] and is between 3.5 [75] and 15 kDa [74] in size.

Secondly, the neural pathway is activated when brief episodes of ischaemia release substances which stimulate efferent nerves that terminate on the tissue at risk or when brief episodes of ischaemia directly stimulate nerves which then release local factors into the blood stream to induce cytoprotection. Candidate molecules involved in the neural pathway include adenosine, [76] bradykinin [77] and calcitonin gene-related peptide [78]. Thirdly RIPC may elicit a systemic anti-inflammatory tissue-protective response at the level of gene transcription [79, 80]. Once the signal has reached the target organ, the intracellular signal transduction is likely initiated via activation of cell surface G-protein-coupled receptors by the humoral/neural mediators. The cell surface receptors activate intracellular cascades which are thought to converge on the mitochondria [81, 82] possibly in a similar to fashion to IPC; however, the precise pathway from cell surface to cytoprotection has not yet been completely elucidated for RIPC and further investigation is necessary. Only two studies have examined the mechanisms of RIPC in the kidney; therefore, information is very limited. Weyer et al. reported that naloxone was able to abolish the effects of RIPC suggesting a role for opiates; however, unlike experiments involving the myocardium, blockade of cannabinoid, glucocorticoid, adenosine or noradrenalin pathways did not abolish the renoprotective effects of RIPC [70, 83].

CLINICAL TRANSLATION OF RIPC

The first study investigating the translation of RIPC from animal models to patients was published in 2000 [84]. Since then, there have been over 50 clinical trials investigating the potential benefit of RIPC in the context of tissue injury. Of these trials, the vast majority have examined the effect of RIPC on the heart and have on the whole demonstrated positive, if short-term, outcomes. However, some studies have additionally examined renal end points as part of their outcomes. A total of 18 clinical RIPC trials with renal parameters have taken place over the past 6 years (see Table 1). Of those, 13 have been in the context of heart surgery or PCI, 3 have dealt with AAA (abdominal aortic aneurysm) repair and 2 have focused on renal surgery. The lower limb, upper limb and common iliac occlusion were used to generate an RIPC signal in eight, eight and two of the trials, respectively. Only 6/18 demonstrated a significant reduction in renal injury. While this may appear disappointing, the trials have generally been small (median size 75) and often too underpowered to detect a difference in renal outcomes. Only 10/18 had renal indices as part of their primary outcome, with 3 studies investigating renal parameters as part of a secondary end point. Five of the 18 studies did not pre-specify renal outcomes in their end points.

Two large phase-3 trials are underway which will hopefully provide a definitive answer to the role of RIPC in AKI in the context of heart surgery: trial (ClinicalTrials.Gov NCT01247545) and the Remote Ischaemic Preconditioning for Heart Surgery (RIP-Heart) study (ClinicalTrials.Gov NCT01067703). Outside the arena of cardiac surgery, the role of RIPC in preventing contrast-induced AKI was examined by Er et al. [39] who demonstrated that RIPC reduced the incidence of contrast-induced AKI by 70% in patients with CKD (GFR < 60 mL/min) undergoing an elective PCI. This study opens the way for...
RIPC as a potential preventative therapy for contrast-induced AKI outside coronary angiography.

While many studies have demonstrated the beneficial effects of RIPC to prevent kidney injury, no experimental studies exist that specifically examine the role of RIPC in renal transplantation. A pilot study conducted in paediatric recipients of live donor kidneys indicated reduced urinary excretion of retinol-binding protein and a more rapid fall in serum creatinine. Additionally, improved graft function appeared to be maintained for up to 2 years following renal transplantation (unpublished data cited in the REPAIR study protocol). However, more recently, a small trial was published that failed to demonstrate a benefit of three 5-min cycles of leg ischaemia/reperfusion in the context of live donor renal transplantation [40].

There are several larger clinical trials underway examining the role of RIPC in renal transplantation. The Remote Ischaemic Preconditioning In Abdominal Organ Transplantation (RIPCOT) trial (Clinicaltrials.gov: NCT00975702), ongoing since 2009, aims to recruit 580 patients to examine whether two 10-min cycles of leg ischaemia before organ harvesting from a deceased donor lead to improved outcomes following renal, liver or pancreas transplantation. A related study the Remote Ischaemic Preconditioning In Neurological Death Organ Donors (RIPNOD) trial (Clinicaltrials.gov: NCT01515072) aims to recruit 320 neurological death donors to assess the efficacy of four 5-min cycles of RIPC applied immediately following confirmation of brain death and again at harvesting to investigate donor stability, organ quality, organ yield and early post-transplant clinical outcomes.

The Context trial (Clinicaltrials.gov: NCT01395719) is currently recruiting patients (estimated enrolment = 200) and is seeking to precondition the recipient rather than the donor to improve graft function. Finally, the Renal Protection Against Ischaemia Reperfusion in Transplantation (REPAIR) trial (Controlled-trials.com: 30083294), (ISRCTN30083294) will examine the effects of preconditioning both the live donor and the recipient either 24-h pre-operation, immediately pre-operation or combining both early and late preconditioning stimuli in the context of live kidney transplantation. Over the next few years the results of these three trials will give us a much better understanding of the role of RIPC in the context of transplantation.

**Ischaemic Postconditioning**

While RIPC removed the requirement for direct access to the blood supply of the organ at risk, most cases of IRI do not occur at a known time, which limits RIPC and IPC to elective procedures. However, in 2003 Zhao et al. [85] extended the conditioning paradigm by demonstrating in a dog model of myocardial ischaemia that interruption of reperfusion by three cycles of 30-s coronary artery occlusion/reperfusion could reduce the subsequent infarct size by 44%. Zhao coined the term ‘ischaemic postconditioning’ (iPOST) to describe this phenomenon. As with IPC and RIPC, iPOST appears to confer profound tissue protection across species [86–89], and while the vast majority of studies have examined the myocardium as the organ of interest [86–89], iPOST has been shown to be protective in many different organs including the kidney [90]. Following renal artery occlusion, iPOST has been shown to reduce apoptosis [90], tubular injury [91], serum urea and creatinine concentrations [90, 91] and led to a reduction in renal fibrosis 12 weeks post injury [92].

**Mechanisms of iPOST**

As with IPC and RIPC, the mechanisms by which alteration of reperfusion injury by iPOST leads to tissue protection have been studied almost exclusively in the heart, and the full details are beyond the scope of this review (the reader is directed to several reviews on this topic [13, 93–95]). In brief however, the mechanisms of myocardial protection from iPOST share many similarities to that of IPC: iPOST leads to activation of the SAFE [96] and RISK [97] pathways via autacoids, including adenosine [95, 98, 99], opioids [100], bradykinin [101, 102] and eNOS [103] leading to inhibition of mPTP opening, which, like IPC, is thought to be the final common effector pathway of iPOST [104, 105]. Additionally, interruption of reperfusion by iPOST delays the normalization of intracellular pH (rapid normalization of intracellular pH at the point of reperfusion is paradoxically harmful) [88], reduces calcium overload [106] and inhibits the oxidative burst seen at reperfusion [86, 106], all of which contribute to cytoprotection. There is very limited data examining the mechanisms of iPOST specifically in the kidney; however, as with the heart, iPOST in the kidney appears to involve opioid signalling [107], leads to phosphorylation of ERK and Akt [108] and involves the opening of the mitochondrial potassium ATP channel with inhibition of the opening of the MPTP [109].

**Clinical Translation of iPOST**

The almost universal protection seen in animal models of iPOST has been translated into over 30 clinical trials in the past 8 years. Almost all studies have been in the context of cardioprotection, and the majority have had positive outcomes. There have been no human studies of the role of iPOST on the kidney. However, there have been two small trials in children undergoing correction of tetralogy of fallot [41, 42] which used intermittent aortic cross clamping to postcondition the kidneys. These trials demonstrated cardioprotection but were unable to demonstrate any difference in renal outcomes (see Table 1).

There is some animal data to support the use of iPOST in renal transplantation. Jiang et al. [110] reported in a dog model of auto-transplantation (mimicking the warm and cold ischaemia of transplantation) that iPOST improved serum creatinine clearance and renal histology post transplant. A pilot study is underway to investigate this further (Dutch trial registry number NTR 3117), along with two studies examining the role of remote ischaemic postconditioning in transplantation (ISRCTN66437627 and clinicaltrials.gov NCT01363687).
**OTHER ISCHAEMIC CONDITIONING STRATEGIES**

More recently, researchers have combined the translational benefits of RIPC and iPOST by generating a remote ischaemic conditioning signal after the ischaemic insult has started, referred to as remote ischaemic postconditioning (RPOST). The renoprotective effects of RPOST have been reported in the context of PCI, with a 56% reduction in AKI (see Table 1) [43]. Finally, there have been studies that have combined two ischaemic conditioning protocols (RIPC+RPOST) to confer tissue protection (Figure 1). Recently, two small studies examined the effect of combining two ischaemic conditioning strategies (RIPC+RPOST) on cardiac and pulmonary outcomes following correction of tetralogy of Fallot (see Table 1). These studies contained renal end points; however, no differences were seen in the incidence of post-operation AKI when RIPC+RPOST were used (see Table 1). In addition, the term ‘remote ischaemic perconditioning’ has been used to describe tissue protection seen when a conditioning stimulus occurs during ischaemia but before reperfusion. There have been several promising clinical trials in the cardiology literature but none as yet has examined the role of perconditioning to reduce renal injury.

**FROM ‘BENCH’... BUT CURRENTLY MISSING THE ‘BEDSIDE’?**

For an intervention to have a good chance of clinical translation it should be effective in many different species, across different models and be easily replicated across different laboratories and backed up by human trial data. Conditioning strategies (IPC, RIPC, iPOST) represent ideal interventions that fulfil all of the above criteria, yet in the 25 years since IPC was first reported [4], these procedures have not become part of routine clinical practice.

To answer this question, we must first explain why animal models fail to represent patients and second why the clinical studies have failed to lead to a paradigm shift in the management of IRI in patients.

**NOT ALL ANIMALS ARE CREATED EQUAL... COMORBIDITIES AND MEDIATION USE**

The typical patient presenting to the emergency department with acute coronary syndrome is most likely to be older and a smoker with a history of ischaemic heart disease, obesity, hypertension, diabetes or chronic kidney disease and has been prescribed several different medications. This is in contrast to the vast majority of animal studies that use juvenile, healthy animals with no comorbidities and not on medications. These differences could explain why the most potent cytoprotective strategies known to science appear to translate into only moderate benefits in humans.

There has been much effort over the past decade to examine the effects of underlying comorbidities on the effectiveness of conditioning strategies.

Diabetes appears to abolish the tissue-protective effects of IPC in several animal species [111–114] including humans [115]; however, this abrogation is not universal and some authors have demonstrated preserved protection [116, 117]. An interesting study by Tsang et al. [118] demonstrated that the resistance to IPC caused by diabetes could be overcome with additional cycles of IPC. This concept may open the door to the use of conditioning strategies in the context of comorbidities by using an increased ‘dose’ of ischaemic conditioning by increasing the number or duration of the cycles.

In addition to diabetes, other comorbidities have been shown to confer resistance to IPC. Underlying hypertension [119], metabolic syndrome [120], dyslipidaemia [121–123] and senescence [124–126] abolish its effect in animal studies. However, as with diabetes, there have been other studies which have demonstrated preserved protection despite the presence of comorbidities [127–132].

The conflicting results for IPC in the context of comorbidities may be due to differing models of disease, different species or differing study protocols or indeed publication bias and have yet to be studied extensively in humans.

Broadly similar effects have been shown with iPOST in animals in terms of loss off efficacy of tissue protection in the presence of diabetes [133, 134], metabolic syndrome/obesity [135, 136], senescence [137], coronary artery stenosis [138] and hypertension [139, 140]. Interestingly, female gender also appears to abrogate the effects of IPC [141, 142] and iPOST [143–145] in animal models.

Very few studies have been published specifically examining the effect of underlying comorbidities in the efficacy of

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**FIGURE 1:** Schematic representations of ischaemic conditioning protocols. IPC, ischaemic preconditioning; several brief cycles of ischaemia and reperfusion; before the lengthy ischaemic insult. RIPC, remote ischaemic preconditioning; several brief cycles of ischaemia and reperfusion on a remote organ or tissue, before the lengthy ischaemic insult. iPOST, ischaemic postconditioning; several brief cycles of ischaemia and reperfusion occurring at reperfusion. RPOST, remote ischaemic postconditioning; several brief cycles of ischaemia and reperfusion occurring at a remote tissue or organ during reperfusion of the target organ.
RIPC; however, neither senescence [146] nor hypertension [146] appears to be a barrier to protection in humans.

We have previously examined the effect of underlying CKD on the tissue-protective effects of ischaemic conditioning strategies [147]. We demonstrated in a sub-total nephrectomy (SNx) model of moderate CKD that the tissue-protective effects of IPC were unaltered by chronic uraemia, despite a reduced ischaemia tolerance. Additionally, we demonstrated that, unlike diabetes [118], uraemia was not associated with an increased threshold for the protective effects of IPC. We also demonstrated that the effects of IPC were maintained in a model of advanced CKD (0.75% adenine diet). Finally, we went on to demonstrate that underlying uraemia (SNx model) did not affect the tissue-protective effects of either RIPC or iPOST. In addition, we have determined that iPOST remains effective in uraemia even when the duration of uraemia is extended to >6/12 [148].

Many common medications such as statins [149], nicorandil [150], opiates [151], sildenafil [152], erythropoietin [153] and ciclosporin [154] are thought to alter components of the conditioning signal transduction cascade, mimicking the beneficial effects ischaemic conditioning and leading to pharmacological preconditioning or blocking of the signal transduction cascade, as in the case of sulphonurreas [155], thereby inhibiting cytoprotection. However, it is currently unclear what the effects are of combining an IPC stimulus in the context of medication use in models of renal IRI.

The impact of polypharmacy combined with multiple comorbidities on the efficacy of ischaemic conditioning strategies is unknown but could possibly go some way towards explaining the gap in potency between animal models and clinical trials. In addition, other factors may be involved in the apparent reduction in efficacy between animals and human studies: in animal models of ischaemia reperfusion, the occlusion is rapid (commonly via external pressure on the artery supplying the organ or tissue), and the precise duration of ischaemia and reperfusion are known. In contrast, in humans the duration of ischaemia is often not known and the arterial occlusion may be staggered such as with crescendo angina, which itself may generate a preconditioning signal [156]. The duration of the ischaemic period is critical to the success of ischaemic conditioning strategies. If the ischaemic period is too long, there may be little tissue to salvage, whereas with very short ischaemic duration there is little injury. There is some evidence that these strategies may not improve outcomes and, in the case of iPOST, may even be harmful [157].

The second question is why, despite generally positive outcomes in human clinical trials, there has not been a paradigm shift in the use of IPC/RIPC/iPOST in routine clinical practice? The apparent simplicity and low cost of ischaemic conditioning strategies may be a 'double-edged sword' for clinical translation. Because these techniques do not involve a patentable pharmaceutical product, there has been a lack of industrial sponsorship of these techniques, which has led to small, single-centre trials with differing protocols and interventions. Much of the disease burden following a myocardial infarct, particularly for those patients with CKD, comes in the days and months following the event. However, many clinical trials, due to the prohibitive cost involved in undertaking studies of longer duration, use surrogate end points such as cardiac enzyme rise or short-term renal function, rather than hard end points such as survival, which would be a much more compelling indication for the widespread adoption of conditioning strategies.

CONCLUSIONS

Looking forward, there are still many questions that need to be resolved: what is the ideal number/duration of cycles in the arm or leg to maximize the benefit of RIPC? Are the mechanisms of ischaemic conditioning strategies the same across different organs?

More work needs to be done to establish the mechanisms of the intracellular signal transduction cascade in RIPC. We need to investigate the cellular basis of how comorbidities confer a resistance to ischaemic conditioning, examine the role of remote ischaemic per/post-conditioning in the context of renoprotection and examine the impact of medication use on renal ischaemic conditioning. Also, we need to investigate combining different ischaemic conditioning or pharmaceutical conditioning strategies to improve renal outcomes.

We need appropriately powered large-scale clinical trials run by clinical trial networks employing hard clinical end points to establish the role of conditioning strategies in those areas we have outlined. Thankfully, we appear to be moving into an era where the potential of ischaemic conditioning is being recognized by public funding organizations. Within the next few years, we will hopefully have data to address the role of RIPC in the prevention of AKI post-cardiac surgery and the role of iPOST/RIPC in the context of renal transplantation along with further studies expanding on the role of ischaemic conditioning with IV contrast. If backed up by the results of ongoing studies, we feel it is only a matter of time before these strategies will truly arrive from the bench to the bedside.

CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or in part, except in abstract form.

REFERENCES

23. Hausenloy DJ, Yellon DM. The second window of preconditioning (SWOP) where are we now? Cardiovasc Drugs Ther 2010; 24: 235–254

60. Young PJ, Dalley P, Garden A et al. A pilot study investigating the effects of remote ischemic preconditioning in high-risk cardiac surgery using a randomised controlled double-blind protocol. Basic Res Cardiol 2012; 107: 256
61. Huang J, Chen Y, Dong B et al. Effect of remote ischemic precondition-ing on renal protection in patients undergoing laparoscopic partial nephrectomy: a ‘blinded’ randomised controlled trial. BJU Int 2013; 112: 70–82
64. Przyklenk K, Bauer B, Ovize M et al. Regional ischemic ‘preconditioning’ protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993; 87: 893–899
70. Wever KE, Warle MC, Wagener FA et al. Remote ischemic precondition-ing by brief hind limb ischaemia protects against renal ischaemia-

140. Penna C, Tullio F, Perrelli MG et al. Ischemia/reperfusion injury is increased and cardioprotection by a postconditioning protocol is lost as cardiac hypertrophy develops in nandrolone treated rats. Basic Res Cardiol 2011; 106: 409–420


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