# Ischaemic conditioning – are we there yet?

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## Learning objectives:

1. To recognise that acute myocardial ischaemia/reperfusion injury is a neglected therapeutic target for cardioprotection that is responsible for the ongoing morbidity and mortality of patients with ischaemic heart disease.

2. To be aware that cardiac bypass surgery and STEMI are the major clinical settings in which the heart is subjected to acute ischaemia/reperfusion injury.

3. To be familiar with the concept of ‘ischaemic conditioning’, in which the heart is protected against acute ischaemia/reperfusion injury by subjecting it to cycles of brief ischaemia and reperfusion, a therapeutic strategy which has been demonstrated in proof-of-concept studies to be beneficial in patients with ischaemic heart disease.

## 1. INTRODUCTION - THE NEED FOR CARDIOPROTECTION

Ischaemic heart disease (IHD) is the leading cause of death and disability worldwide. Despite current therapies, patients still experience significant morbidity and mortality when undergoing cardiac bypass surgery or when presenting with an ST-segment elevation myocardial infarction (STEMI). This is partly attributed to the detrimental effects of acute ischaemia/reperfusion injury (IRI) on the heart, which in combination mediate cardiomyocyte death, resulting in impaired left ventricular (LV) systolic function and increased risk of heart failure. Although a number of strategies exist for reducing the ischaemic component of acute IRI injury in cardiac bypass surgery (such as cardioplegia and hypothermia) and STEMI (such as prompt reperfusion with primary percutaneous intervention - PPCI), paradoxically, reperfusing previously ischaemic myocardium leads to further cardiomyocyte death – termed 'myocardial reperfusion injury' and for which there is currently no effective therapy. Therefore, novel therapeutic interventions are required to protect the heart from acute IRI in these clinical settings in order to improve clinical outcomes. In this regard, 'ischaemic conditioning', in which the heart is rendered tolerant to acute IRI by subjecting it to cycles of brief ischaemia and reperfusion, provides an endogenous form of cardioprotection. In this article, we review the role for ischaemic conditioning as a therapeutic strategy for attenuating cardiomyocyte death, preserving myocardial function, and improving clinical outcomes in patients subjected to acute myocardial IRI.

## 2. MYOCARDIAL REPERFUSION INJURY – A NEGLECTED THERAPEUTIC TARGET

Although myocardial reperfusion is essential to salvage viable myocardium following the onset of acute myocardial ischaemia, the restoration of coronary blood flow comes at a price, paradoxically inducing myocardial injury and cardiomyocyte death – termed

‘myocardial reperfusion injury’. Four types of myocardial reperfusion injury have been described:

1. *Reperfusion-induced arrhythmias:* These occur on reperfusing previously ischaemic myocardium and comprise idioventricular rhythm and ventricular arrhythmias, the majority of which are self-terminating or are easily treated.
2. *Myocardial stunning*: This refers to the reversible contractile dysfunction that occurs on reperfusing acute ischaemic myocardium and is believed due to be due to myocardial oxidative stress and intracellular calcium overload.
3. *Coronary no-reflow and microvascular obstruction:* Coronary no-reflow is indicative of underlying microvascular obstruction (MVO) – defined as the “inability to reperfuse a previously ischaemic region”[1]. MVO is present on cardiovascular magnetic resonance imaging (CMR) in 40 to 60% of PPCI patients, despite the presence of normal coronary flow (TIMI 3) within the infarct-related artery post-PPCI[2 3]. The presence of MVO is associated with adverse LV remodelling and worse clinical outcomes post-PPCI.
4. *Lethal myocardial reperfusion injury:* Ischaemia/Reperfusion induces cytosolic and mitochondrial calcium overload, oxidative stress, rapid restoration in intracellular pH, which on a background of relative adenosine triphosphate (ATP) depletion, culminates in the opening of the mitochondrial permeability transition pore (MPTP) and irreversible cardiomyocyte hypercontracture – the hallmark of lethal myocardial reperfusion injury. Cardiomyocytes that were still viable at the end of the index ischaemic insult undergoes necrosis and this accounts for up to 50% of the final MI size, thereby mitigating the benefits of reperfusion, and making lethal myocardial reperfusion injury a therapeutic target for cardioprotection (Figure 1).

Crucially, there is currently no effective therapy for reducing either MVO or lethal myocardial reperfusion injury. In the following sections we review the therapeutic potential

of ischaemic conditioning, a collective term given to the different forms of endogenous cardioprotection, which have been described, and these include ischaemic preconditioning, ischaemic postconditioning and remote ischaemic conditioning.

## 3. ISCHAEMIC PRECONDITIONING – LIMITED CLINICAL APPLICATION

In 1986, Murry et al[4] made the intriguing observation that following an acute coronary artery occlusion the resultant myocardial infarct (MI) size could be significantly reduced by 'preconditioning' the heart with brief episodes of ischaemia and reperfusion. In that landmark experimental study, four cycles of 5-minutes alternating left anterior descending (LAD) coronary artery occlusion and reflow applied immediately prior to a 90-minutes LAD occlusion and a 3-days reperfusion period, led to a 25% reduction in MI size in the canine heart[4]. This endogenous form of cardioprotection, which was termed ischaemic preconditioning (IPC), has been shown to be ubiquitous in several other organs and all species tested (reviewed in [5] [6]). The IPC stimulus is known to elicit two windows of cardioprotection, the first one occurring immediately and lasting 2-3 hours, and the second one appearing 12-24 hours later, and lasting up to 72 hours. In several clinical scenarios the heart is able to protect itself by IPC – e.g. (1) 'Warm-up angina' refers to the phenomenon in which a patient with stable IHD is able to exercise more following an episode of angina followed by a period of rest[7]; (2) 'Pre-infarct angina' refers to the cardioprotective effects of an episodes of angina immediately prior to an MI resulting in smaller MI size and better clinical outcomes[8]. As a cardioprotective strategy for protecting the heart against acute IRI, its clinical application has been limited by the need to apply the IPC stimulus directly to the heart, and prior to the index ischaemia, which cannot be predicted in acute MI patients. In this regard, the discovery of ischaemic postconditioning has provided a cardioprotective strategy which can be applied at the time of reperfusion to protect the heart against acute IRI.

## 4. ISCHAEMIC POSTCONDITIONING – PROTECTION AT TIME OF REPERFUSION

In 2003, Zhao et al [9] discovered that by interrupting myocardial reperfusion with several short-lived episodes of myocardial ischaemia could reduce MI size to a similar extent as IPC – a phenomenon which has been termed 'ischaemic postconditioning' (IPost)[6] [10]. They[9] found that by applying three cycles of 30-seconds LAD coronary artery occlusion and reflow at the onset of myocardial reperfusion could reduce MI size by 44% in the canine heart[9]. The idea of modifying reperfusion as a strategy to limit MI size was first proposed in the 1980s and 1990s using gentle[11] and gradual[12] reperfusion instead of sudden reperfusion. The discovery of IPost as a therapeutic strategy which could be applied at the onset of myocardial reperfusion has rekindled interest in lethal myocardial reperfusion injury as a target for cardioprotection, and was rapidly translated into the clinical setting within two years of discovery[13]. It has also provided confirmatory evidence for the existence of lethal myocardial reperfusion injury in man. However, both IPC and IPost require an intervention applied to the heart directly, which may not always be feasible depending on the clinical situation, and therefore, the strategy of remote ischaemic conditioning, in which the cardioprotective stimulus is applied to an organ or tissue away from the heart is vastly more attractive as a clinical application.

## 5. REMOTE ISCHAEMIC CONDITIONING – CARDIOPROTECTION MADE EASY

In 1993, Przyklenk et al[14] made the interesting observation that the cardioprotective effect of IPC was not restricted to one particular coronary artery territory as it could be transferred to another coronary artery territory. This gave rise to the concept of 'remote ischaemic conditioning' (RIC), the term given to the cardioprotection induced by applying cycles of brief ischaemia and reperfusion to an organ or tissue away from the heart. Using the canine heart, these authors found that brief occlusions and reflow in the circumflex

coronary artery were able to reduce MI size by 63% following LAD coronary artery occlusion[14]. This idea was soon extended beyond the heart with the demonstration that the RIC stimulus (cycles of brief ischaemia and reperfusion) could be applied to the kidney to limit MI size, giving rise to the concept of inter-organ protection against acute IRI[15]. Two key properties of RIC have facilitated its translation into the clinical setting:

(1) **Feasibility**: The discovery in an experimental animal MI model that the RIC stimulus could be applied to the hind limb to protect the heart against acute IRI[16 17], and the finding that the RIC stimulus could be delivered non-invasively in human volunteers by simply inflating a blood pressure cuff on the upper arm to induce cycles of brief ischaemia and reperfusion[18], has greatly increased its feasibility in the clinical setting.

(2) **Flexibility:** The application of IPC and IPost are restricted in terms of their 'timings', as the protective stimulus has to be applied either prior to ischaemia or at the onset of reperfusion, respectively) and 'target' (the protective stimulus has to be applied to the heart directly), whereas RIC can be applied at any time (either prior to, after the onset of, or at the end of ischaemia) and to an organ or tissue away from the heart, thereby making it much more flexible as a cardioprotective strategy (Figure 2).

Therefore, most clinical studies applying RIC as a cardioprotective strategy have used cycles of brief ischaemia and reperfusion in the upper or lower limb (henceforth referred to as limb RIC).

## 6. OVERVIEW OF MECHANISMS UNDERLYING ISCHAEMIC CONDITIONING

The mechanisms underlying ischaemic conditioning are rather complex and have been the subject of intensive investigation over the last 20 to 30 years (for comprehensive reviews please see [5 6 19 20]). With respect to IPC and IPost, the current paradigm proposes that the cycles of brief ischaemia and reperfusion, which make up the protective stimulus generate autacoids in the interstitium which then activate a number of signal transduction pathways

by binding to their respective receptors on the cardiomyocyte plasma membrane. This in turn results in the recruitment of a number of pro-survival protein kinase pathways (such as the Reperfusion Injury Salvage Kinase [RISK][21] [22], Survivor Activator Factor Enhancement [SAFE][23], and cGMP-PKG pathways[24]), which converge on and protect mitochondria from dysfunction induced by acute IRI. However, it is important to appreciate that there are some differences between IPC and IPost in terms of it signalling components. With IPC there is an additional signalling pathway responsible for the 'memory' effect of classical IPC (the activation of protein kinases such as protein kinase C) and of the second window of protection (the transcription of new proteins such as cyclooxygenase-2, inducible nitric oxide synthase and so on)[6] [20]. The mechanisms underlying RIC are even more complex given the added dimension of having to convey the cardioprotective signal from the remote organ or tissue to the heart. Once at the heart, the signalling pathways alluded to above for IPC and IPost are also recruited by RIC. The details of the pathway linking the remote organ or tissue to the heart remain unresolved, but are believed to involve the release of local autacoids stimulating the sensory afferent neural pathway in the remote organ or tissue leading to the production of a circulating transferrable blood-borne factor(s), which is able to confer cross-species cardioprotection. The identity of the cardioprotective factor(s) remains unknown, but is probably a peptide <30 kilodaltons in size (reviewed in [25] [26]).

## 7. CLINICAL APPLICATION OF ISCHAEMIC CONDITIONING

Ischaemic conditioning has been investigated in several clinical settings in which the heart is subjected to acute myocardial IRI including cardiac bypass surgery, elective percutaneous coronary intervention (PCI) and PPCI (Figure 2).

### *7.1. Cardiac bypass surgery as a clinical setting for cardioprotection*

In patients undergoing cardiac bypass surgery the heart is subjected to global ischaemic injury (when the aorta is clamped and the heart is put onto cardiopulmonary bypass) followed by global reperfusion injury (when the aorta is unclamped and the heart is taken off cardiopulmonary bypass)[27]. However, acute IRI is not the only cause of myocardial injury during cardiac bypass surgery as direct handling of the heart, coronary embolisation, and the inflammatory response to cardiopulmonary bypass can all contribute. The perioperative myocardial injury (PMI) which occurs during cardiac bypass surgery can be quantified by measuring serum cardiac enzymes (Creatine Kinase MB isoenzyme, Troponin T and I)[28 29], and can be detected as late gadolinium enhancement on cardiovascular magnetic resonance imaging (CMR)[30]. Given that the presence of PMI has been associated with worse clinical outcomes post-cardiac surgery[28 29], the measurement of serum cardiac enzymes has been used as a surrogate endpoint to assess the cardioprotective efficacy of novel therapies in patients undergoing cardiac bypass surgery.

*IPC and IPost in cardiac bypass surgery*

IPC was the first cardioprotective strategy to be investigated in the setting of cardiac bypass surgery. In 1993, Yellon's group[31] first demonstrated that brief episodes of global ischaemia induced by clamping and declamping the aorta prior to cardiopulmonary bypass preserved myocardial ATP levels in patients with IHD. Since then a number of studies have investigated the role of IPC as a cardioprotective strategy in cardiopulmonary bypass surgery. In a recent meta-analysis it was shown that IPC significantly reduced ventricular arrhythmias, decreased inotrope requirements and shortened the intensive care unit stay[32]. However, despite these potential beneficial effects, the need to intervene on the heart directly and the risk of embolisation arising from clamping an atherosclerotic aorta have prevented IPC from being adopted in this clinical setting.

Using a similar approach to cardioprotection at the time of aorta unclamping it has been reported that IPost induced by re-clamping and declamping the aorta to stutter global myocardial reperfusion attenuated perioperative myocardial injury in terms of serum cardiac enzyme release[33]. There is the potential to apply this cardioprotective strategy to children (in whom the aorta is not yet atherosclerotic) undergoing cardiopulmonary bypass surgery to correct congenital cardiac defects[34].

*Limb RIC in cardiac bypass surgery*

Limb RIC was first demonstrated to show benefit in the clinical setting of cardiac bypass surgery in 2006 by Cheung et al[35] in children undergoing corrective cardiac surgery for congenital heart defects. Subsequent studies have reported beneficial effects of RIC in adult patients undergoing coronary artery bypass graft surgery (CABG) and/or valve surgery in terms of attenuated perioperative myocardial injury as evidenced by decreased serum cardiac enzyme release (Table 1). However, there have been several neutral studies[36-38] including at least one very large study[39]. The reasons for this discrepancy are not clear but may relate to the following factors: patient selection (CABG vs valve surgery, stable vs unstable IHD patients); timing of the limb RIC protocol (prior to vs after surgical incision); blinding of the RIC protocol (proper vs limited blinding); the intensity of the RIC protocol (3 vs 4 cycles of limb RIC and inflation of cuff to 200mmHg vs 15mmHg above systolic blood pressure); and the presence of confounding factors (Table 5). The results of several recent meta-analyses have confirmed the cardioprotective effects of RIC in this clinical setting in terms of attenuating perioperative myocardial injury[40 41]. The ongoing RIPHeart[42] and ERICCA[43] multicentre clinical trials, which are currently investigating the effect of RIC on clinical outcomes post cardiac bypass surgery (Table 1), should hopefully provide definitive evidence of the cardioprotective effects of RIC in this clinical setting.

### *7.2. Elective PCI as a clinical setting for cardioprotection*

Ischaemic conditioning has been investigated as a cardioprotective strategy for protecting the heart against periprocedural myocardial injury in patients undergoing elective PCI. It is important to appreciate that PCI-related injury, which occurs in about 20 to 30% of patients undergoing elective PCI and measured by the release of serum cardiac enzymes, is not due to acute IRI, but mainly by acute ischaemic injury (distal branch occlusions, and coronary embolisation) - complications which are more frequent following multi-vessel and complex PCI[44]. Limb RIC administered prior to elective PCI has been reported to be beneficial in IHD patients, in terms of reducing serum levels of cardiac enzymes (Table 2), although there have been a number of neutral studies[45 46] and one negative study[47]. The reasons for this discrepancy are not clear but may relate to the following factors: the setting itself (acute IRI not a major component of myocardial injury during elective PCI); patient selection (stable vs unstable IHD patients); the timing of RIC in relation to PCI procedure (prior to vs after) and the PCI procedure itself (simple vs multi-vessel or complex PCI). A recent meta-analysis has suggested that limb RIC may be beneficial if lower limb RIC is used and in the setting of multi-vessel or complex PCI[48].

### *7.3. PPCI as a clinical setting for cardioprotection*

For patients presenting with STEMI, the most effective therapy for limiting MI size and preserve LV systolic function is myocardial reperfusion by PPCI. However, the restoration of coronary blood flow in the infarct-related artery induces myocardial reperfusion injury, thereby providing a target for cardioprotection for ischaemic conditioning strategies such as IPost and RIC.

#### *IPost in STEMI patients*

IPost was rapidly translated into the clinical setting by Staat et al in 2005 only 2 years after its discovery in the original animal experimental study[13]. They demonstrated in a small proof-of-concept study of 30 patients that IPost could reduce enzymatic MI size by 36% (Table 3). The IPost protocol was applied following direct stenting of the infarct-related artery by inflation of the angioplasty balloon to low pressure upstream of the stent to interrupt coronary flow for one minute followed by deflation of the angioplasty balloon for one minute to allow coronary reflow and repeated 4 times in total. A number of clinical studies have gone on to confirm the cardioprotective effect of IPost using echocardiographic, myocardial SPECT and CMR endpoints (Table 3). However, not all the studies have been positive[49 50], and the reasons for this are not clear but may be due to a number of factors including: patient selection (only patients with complete occlusion of the infarct-related artery and no coronary collateralisation should be included), the stenting technique (most benefit seen with direct stenting), the IPost protocol itself (which should not be delivered within the stent); the endpoints used to assess cardioprotection (ST-segment resolution vs MI size). The results of recent meta-analyses of IPost in PPCI patients have also produced mixed results[51-54]. Whether IPost can actually improve clinical outcomes following PPCI is currently being investigated in the DANAMI-3 trial (ClinicalTrials.gov Identifier: NCT01435408)(Table 3).

*Limb RIC in STEMI patients*

Several proof-of-concept studies have reported cardioprotective effects with limb RIC in STEMI patients treated by PPCI (Table 3). It appears to be effective when given in the ambulance by paramedics[55], on arrival at the hospital prior to PPCI[56 57], and even at the onset of reperfusion at the time of PPCI[58]. Whether limb RIC can improve clinical outcomes in PPCI patients is currently being investigated in the ERIC-PPCI and CONDI2 trials (ClinicalTrials.gov Identifier: NCT01857414), which are investigation, in collaboration,

whether RIC can reduce the rates of cardiac death and hospitalisation for heart failure at 12 months.

*Pharmacological conditioning – mimicking ischaemic conditioning*

The elucidation of the signal transduction pathways underlying ischaemic conditioning have resulted in the identification of new targets for cardioprotection, some of which can be modulated by pharmacological agents (reviewed in [59] [60]). In this regard, the most promising of these pharmacological conditioning strategies include atrial natriuretic peptide[61], cyclosporin-A (CsA)[62], exenatide[63], and metoprolol[64], all of which have been reported in proof-of-concept clinical studies to reduce MI size and preserve LV systolic function (Table 4). Whether CsA therapy can improve clinical outcomes post-PPCI is currently being tested in two clinical outcome studies (the CYCLosporinE A in Reperfused Acute Myocardial Infarction (CYCLE) (ClinicalTrials.gov NCT01650662) and Cyclosporine and Prognosis in Acute Myocardial Infarction Patients (CIRCUS) (ClinicalTrials.gov NCT01502774) multi-centre randomised clinical trials.

## 8. NEW AVENUES FOR ISCHAEMIC CONDITIONING

### *8.1. Other clinical settings of acute myocardial IRI*

There are other clinical settings in which acute myocardial IRI is a critical determinant of outcome. In patients having a cardiac arrest the whole body including the heart is subjected to acute global ischaemia, and in those patients which are successfully resuscitated, the whole body is then subjected to acute global reperfusion injury. Whether limb RIC is a therapeutic option in patients who are successfully resuscitated following a cardiac arrest remains to be tested. The added benefit of limb RIC in this setting is multi-organ protection against acute IRI.

For patients undergoing cardiac transplantation acute IRI is a major determinant of graft dysfunction post-transplantation. In this setting, the heart is subjected to global ischaemic injury as it is removed from the donor, followed by global reperfusion injury as it is transplanted into the recipient. In this setting there is the opportunity to perform limb RIC to the donor prior to organ harvesting and to the recipient prior to transplantation, but whether this approach is beneficial remains to be tested.

### *8.2. Limb RIC and cardiac function*

A number of clinical studies are investigating the cardioprotective effects of limb RIC on cardiac function. Limb RIC has been reported to attenuate ST-segment depression and prevent myocardial stunning in chronic renal failure patients undergoing haemodialysis during which the heart is subjected to repeated bouts of acute myocardial ischemia resulting in myocardial stunning and chronic LV systolic impairment [65].

Whether repeated episodes of limb RIC, applied as a daily therapy, is beneficial in the clinical setting is not known. One experimental study demonstrated that repeating limb RIC daily for 28 days prevented adverse post-MI LV remodelling in the rat heart[66]. There are currently two clinical studies investigating the effect of daily RIC continued for 4 weeks on post-MI LV remodelling (Daily REmote Ischaemic Conditioning following Acute Myocardial Infarction (DREAM, ClinicalTrials.gov Identifier: NCT01664611) and the Chronic Remote Ischemic Conditioning to Modify Post-MI Remodeling (CRIC-RCT; ClinicalTrials.gov Identifier:NCT01817114) trials. The CONDI-HF study (ClinicalTrials.gov Identifier:NCT02248441) is currently investigating the effect of daily RIC on LV ejection fraction in chronic heart failure patients.

### *8.3. Increasing exercise performance by limb RIC*

Interestingly, limb RIC has been reported to improve exercise performance in elite swimmers[67], presumably by rendering skeletal muscle more tolerant to acute ischaemia, although the actual mechanism is not clear. In the setting of heart failure however, limb RIC failed to improve exercise capacity and oxygen consumption, with the suggestion that heart failure patients were already chronically preconditioned, as plasma dialysate obtained from both sham and RIC patients reduced murine MI size compared to plasma dialysate from historical healthy controls [68].

### *8.4. Limb RIC protection of other organs*

The key advantage of limb RIC as a therapeutic strategy is that it offers multi-organ protection against acute IRI. As such limb RIC has been shown to be beneficial in a number of non-cardiac organs including the brain (against acute ischaemic stroke[69]), the kidney (protection against acute kidney injury induced by cardiac bypass surgery[38 70 71], and induced by contrast following coronary angiography[72]) and the liver (during acute liver resection [ClinicalTrials.gov Identifier: NCT007965880] and liver transplantation [Remote Ischaemic PreCOnditioning in Liver Transplant or RIPCOLT. The recently completed REnal Protection Against Ischaemia-Reperfusion in transplantation (REPAIR) trial (ISRCTN30083294) has found that limb RIC of both the donor and recipient preserved estimated glomerular filtration rate of the transplanted kidney at 6 months in recipient patients undergoing live-donor related renal transplantation, suggesting limb RIC to be a potential therapeutic strategy for preserving renal graft function post-transplantation.

## 9. OPTIMISING THE TRANSLATION OF CARDIOPROTECTION

The field of cardioprotection has a chequered history with a disappointing large number of neutral clinical studies in which a novel cardioprotective therapy has failed to improve clinical outcomes in IHD patients subjected to acute IRI. The failure to translate the large

number of cardioprotective therapies discovered in laboratory studies into patient benefit has been the subject of several recent articles [73-76]- the major issues are highlighted in Table 5.

## 10. SUMMARY AND CONCLUSIONS

Ischaemic conditioning is an endogenous form of cardioprotection which can be elicited by cycles of brief ischaemia and reperfusion to the heart directly or to an organ or tissue away from the heart. A number of proof-of-concept clinical studies have shown beneficial effects with ischaemic conditioning in the settings of cardiac bypass surgery, elective PCI and PPCI, with reduced myocardial injury and preservation of cardiac function. Whether ischaemic conditioning can actually improve clinical outcomes in CABG and PPCI patients is currently being investigated in several large multi-centre randomised clinical trials. As a result, in the next couple of years we should know whether ischaemic conditioning could benefit IHD patients in terms of reducing morbidity and mortality and potentially change clinical practice.

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## *Figure legends*

### Figure 1

**The clinical impact of myocardial reperfusion injury in reperfused STEMI patients**

This hypothetical scheme depicts the magnitude and clinical impact of myocardial reperfusion injury on patients with ischaemic heart disease who are subjected to acute ischaemia/reperfusion injury. The thick blue curve shows the extent of myocardial salvage (which equates to the area-at-risk subtract the myocardial infarct size and is expressed as the % of the left ventricular volume) in a theoretical patient presenting with an acute ST-segment elevation myocardial infarction (STEMI) reperfused by primary percutaneous coronary intervention (PPCI) or thrombolysis – as expected in the absence of reperfusion the extent of myocardial salvage declines with time. Although myocardial reperfusion is essential for myocardial salvage following a STEMI, the process of restoring coronary blood flow within the infarct-related artery, can paradoxically induce cardiomyocyte death – a phenomenon which has been termed ‘myocardial reperfusion injury’. As a result, following reperfusion, the extent of myocardial salvage is actually smaller than expected given the duration of acute myocardial ischaemia – this attenuation in myocardial salvage is due to the presence of myocardial reperfusion injury, which can contribute up to 50% of the final myocardial infarct size.

**Figure 2**

**Ischaemic conditioning in the clinical setting**

This figure illustrates the variety of clinical settings in which ischaemic conditioning has been tested (in orange) including: cardiac bypass surgery, elective percutaneous coronary intervention (PCI) and non-ST-segment elevation myocardial infarction (NSTEMI) patients undergoing PCI, and STEMI patients. There is the potential to investigate the role of ischaemic conditioning in other clinical settings (in yellow) such as heart transplantation, heart failure and cardiac arrest. The term ischaemic conditioning encompasses a number of endogenous forms of cardioprotection including ischaemic preconditioning (IPC, which has to be delivered within 2-3 hours of the index ischaemia), ischaemic postconditioning (IPost, which has to be delivered in the first minute of reperfusion) and remote ischaemic conditioning (RIC, using transient limb ischaemia and reperfusion). RIC can be divided according to the timing of the intervention into remote ischaemic preconditioning (RIC stimulus applied prior to index ischaemia), RIPerC (RIC stimulus applied after the onset of index ischaemia but prior to reperfusion) and RIPost (RIC stimulus applied at the onset of reperfusion).

**Table 1:** Major clinical studies investigating limb RIC in cardiac bypass surgery

| Study | RIC protocol | Number | Main outcomes | Comments |
|---|---|---|---|---|
| Cheung et al 2006[35] | Four x 5 minutes thigh cuff inflations/deflations | 37 | Smaller peak Trop T, less inotrope support and lower airway pressures | First study to report beneficial effect of limb RIC in CABG |
| Hausenloy et al 2007[77] | Three x 5 minutes upper arm cuff inflations/deflations | 53 | 43% reduction in 72 hr AUC Trop-T | First study to report beneficial effect of limb RIC patients undergoing CABG |
| Candilio et al 2014[71] | Two x 5 minutes simultaneous upper arm and thigh cuff inflations/deflations | 180 | 27% less 72 hr AUC Trop T. 54% Less AF 48% Less AKI and 1 day less ICU stay | First study to report beneficial effect of limb RIC on short-term clinical outcomes |
| Heusch et al 2013[78] | Three x 5 minutes upper arm cuff inflations/deflations | 329 | 21% less 72 hr AUC Trop I. 73% reduction in all-cause mortality | First study to report beneficial effect of limb RIC on long-term clinical outcomes. |
| ***Ongoing studies*** | | | | |
| Meybohm et al RIPHEART[42] | Four x 5 minutes cycles of upper arm cuff inflation/deflation | 2070 | Primary endpoint of death, non-fatal MI, stroke, AKI at 30 days | First study which will test effect of RIC on 30 days primary endpoint following CABG<br>Results available March 2015 |
| Hausenloy et al ERICCA[43] | Four x 5 minutes cycles of upper arm cuff inflation/deflation | 1610 | Primary endpoint of death, non-fatal MI, revascularisation, stroke at 12 months | First study which will test effect of RIC on 12 months primary endpoint following CABG<br>Results available March 2015 |

*RIC: remote ischaemic conditioning; Trop T: troponin T; CABG: cardiac bypass surgery; AUC: area under curve; AF: atrial fibrillation; AKI: acute kidney injury; ICU: intensive care unit; MI: myocardial infarct*

**Table 2:** Major clinical studies investigating limb RIC in elective PCI

| Clinical study | RIC protocol | Number of patients | Outcome | Comments |
|---|---|---|---|---|
| Hoole et al 2009[79] | Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI | 242 | 63% reduction in median Trop I | First study to test effect of limb RIC in PCI |
| Davies et al 2013[80] | Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI | 192 | 42% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 6 years | First study to test effect of limb RIC on long-term clinical outcomes following PCI |
| Zografos et al 2014[81] | One x 5 minutes upper arm cuff inflations/deflations Immediately prior to PCI | 94 | 80% reduction in 24 hr serum levels of Trop I. Also reduced incidence of PCI-related MI by 56%. | First study to test effect of one cycle of limb RIC |
| Liu et al 2014[82] | Three x 5 minutes upper arm cuff inflations/deflations 18 hours prior to PCI | 200 | 40-60% reduction in 24 hr serum levels of Trop I and CK-MB. Also less chest pain and ST-segment deviation with PCI. | First study to test effect of second window of protection of limb RIC |
| EURO-CRIPS [83] | Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI | Planned | | Also investigating the effect of limb RIC on contrast-induced AKI |

*RIC: remote ischaemic conditioning; PCI: percutaneous coronary intervention, Trop I: troponin I; MI: myocardial infarction; TIA: transient ischaemic attack; HHF: hospitalisation for heart failure; CK-MB: creatine kinase MB isoenzyme; AKI: acute kidney injury*

**Table 3:** Major clinical studies investigating IPost and limb RIC in STEMI patients

| Clinical study | Treatment protocol | Number of patients | Outcome | Comments |
|---|---|---|---|---|
| ***Ischaemic postconditioning in STEMI patients*** | | | | |
| Staat et al 2005[13] | Four x 60 seconds angioplasty balloon inflations/deflations | 30 | 36% reduction in 72 hours AUC CK | First study to report beneficial effect of IPost in PPCI patients |
| Thibault et al 2008[84] | Four x 60 seconds angioplasty balloon inflations/deflations | 38 | 41% reduction in 72 hours AUC CK-MB<br>39% reduction in MI size at 6 months by SPECT<br>LVEF improved by 7% echocardiogram at one year | First study to report long-term cardioprotective effects of IPost in PPCI patients |
| Lonborg et al 2010[85] | Four x 30 seconds angioplasty balloon inflations/deflations | 118 | 9% reduction in MI size at 3 months by CMR<br>31% increase in myocardial salvage index | First study to report cardioprotective effects of IPost in PPCI patients using CMR |
| Thuny et al [86] | Four x 60 seconds angioplasty balloon inflations/deflations | 50 | 40% reduction in MI size and 21% reduction in myocardial oedema on CMR | First study to report effect of IPost on myocardial oedema on CMR |
| Engstrom et al DANAMI-3 ClinicalTrials.gov Identifier: NCT01435408 | Four x 30 seconds angioplasty balloon inflations/deflations | 2000 Completed recruitment | All-cause mortality, heart failure at 4 years Results awaited | First study which will report effects of IPost on long-term clinical outcomes |
| ***Remote ischaemic conditioning in STEMI patients*** | | | | |
| Botker et al 2010[55] | Four x 5 minutes upper arm cuff inflations/deflations in the ambulance prior to PPCI | 142 | Increase in myocardial salvage index at 30 days<br>No difference in MI size (SPECT or peak Troponin) | First study to test effect of RIC in PPCI-treated STEMI patients. Reduced MI size in LAD STEMI. |
| Rentoukas et al 2010[56] | Three x 4 minutes cuff inflations/deflations at the hospital prior to PPCI | 93 | Better ST resolution and lower peak Troponin I. Synergistic effects with morphine. | |
| Crimi et al 2013[58] | Three x 5 minutes thigh cuff inflations/ deflations at onset of reperfusion | 100 anterior STEMI only | 20% reduction in 72 hours AUC CK-MB.<br>% reduction in myocardial oedema by CMR | First study to show effect of RIC given at onset of reperfusion via PPCI. Also, first study to report effect of RIC on enzymatic MI size and myocardial oedema. |
| White et al 2014[57] | Four x 5 minutes upper arm cuff inflations/deflations at the hospital prior to PPCI | 197 | 27% reduction in MI size by CMR<br>19% reduction in myocardial oedema by CMR | First study to show effect of RIC given prior to PPCI on MI size and myocardial oedema by CMR |
| Hausenloy et al ERIC-LYSIS | Four x 5 minutes upper arm cuff | 519 | 17% reduction in enzymatic MI size (CK- | Only study to test effect of RIC in |

| | | | | |
|---|---|---|---|---|
| (ClinicalTrials.gov Identifier: NCT02197117) | inflations/deflations at hospital prior to thrombolysis | | MB and Trop-T) | thrombolysed STEMI patients |
| Sloth et al 2014[87] | Four x 5 minutes upper arm cuff inflations/deflations in the ambulance prior to PPCI | 251 | 51% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 3.8 years | First study to test effect of RIC on long-term outcomes following PPCI |
| Botker CONDI-2 Hausenloy ERIC-PPCI<br><br>ClinicalTrials.gov Identifier: NCT01857414 | Four x 5 minutes upper arm cuff inflations/deflations prior to PPCI | 4300 ongoing | Primary endpoint of cardiac death and HHF at 12 months | Collaboration between UK, Denmark. First study to test effect of RIC on long-term clinical outcomes at primary endpoint following PPCI |

*IPost: Ischaemic postconditioning; RIC: remote ischaemic conditioning; STEMI: ST-segment elevation myocardial infarction; AUC: area under curve; CK: creatine kinase; PPCI: primary percutaneous coronary intervention; CK-MB: creatine kinase MB isoenzyme; SPECT: single-photon emission computed tomography; LVEF: left ventricular ejection fraction; PPCI: primary percutaneous coronary intervention; MI: myocardial infarct; LAD: left anterior descending artery; CMR: cardiovascular magnetic resonance imaging; Trop I: Troponin I; TIA: transient ischaemic attack; HHF: hospitalisation for heart failure;*

**Table 4:** Major clinical studies investigating promising pharmacological conditioning agents in STEMI patients

| Clinical study | Therapeutic intervention | Number | Outcome | Potential mechanisms underlying cardioprotection |
|---|---|---|---|---|
| Kitakaze et al 2007[61] J-WIND | 72 hours IV carperitide (atrial natriuretic peptide analogue) infusion started prior to PPCI | 569 | 15% reduction in 72 hours AUC total CK<br>2.0% absolute increase in LVEF | Atrial natriuretic peptide targets pro-survival kinase pathways such as the cGMP and RISK pathways |
| Piot et al 2008[62] | IV cyclosporin A (2.5mg/kg) bolus 10 minutes prior to PPCI | 58 | 44% reduction in MI size (72 hours AUC total CK)<br>20% reduction in MI size (CMR subset)<br><br>28% reduction in MI size and smaller LVESV on CMR at 6 months[88] | Cyclosporin-A inhibits the opening of the mitochondrial permeability transition pore, a critical determinant of lethal myocardial reperfusion injury |
| Lonborg et al 2012[63] | IV infusion of exenatide started 15 minutes prior to PPCI and continued for 6 hr | 107 | Increase in myocardial salvage index (0.62 to 0.71)<br>23% reduction in MI size at 3 months on CMR<br><br>Patients presenting with short ischaemic times (≤132 minutes) had greater myocardial salvage[89] | Exenatide, a GLP-1 analogue, targets pro-survival kinase pathways such as the RISK pathway |
| Ibanez et al 2013[64] | IV metoprolol (3x5mg) in ambulance prior to PPCI | 270 | Reduction in MI size by CMR at one week. Increased LVEF at 6 months<br>Improvement in clinical outcome at 2 years<br><br>Reduced: incidence of severely depressed LVEF (<35%) at 6 months by 60%; less need for ICD by 65% at 6 months and reduced HF at 2 years [90] | The mechanism of cardioprotection is not currently clear |

*STEMI: ST-segment elevation myocardial infarction; IV: intravenous; AUC: area under curve; CK: creatine kinase; LVEF: left ventricular ejection fraction; cGMP: cyclic guanosine monophosphate; RISK: reperfusion injury salvage kinase; MI: myocardial infarct; CMR: cardiovascular magnetic resonance imaging; LVESV: left ventricular end-systolic volume; GLP-1: glucagon-like peptide-1; AAR: area at risk; HF: heart failure*

## **Table 5:** Improving the translation of cardioprotection for patient benefit

| | STEMI-PPCI trials | Cardiac bypass surgery trials |
|---|---|---|
| **Patient selection** | Select patients which are most likely to benefit from the cardioprotective therapy:<br><br>1. Large AAR (>30% of the left ventricle)<br>2. No coronary collateralisation (Rentrop<1)<br>3. Fully occluded artery prior to PPCI (TIMI<1)<br>4. Onset of symptoms 3-6 hours | Select patients which are most likely to benefit from the cardioprotective therapy:<br><br>1. Patients undergoing on-pump cardiac surgery who are subjected to global acute IRI.<br>2. Patients with longer cardiopulmonary bypass times are at greater risk of perioperative myocardial injury.<br>3. Higher risk patients who are at greater risk of perioperative myocardial injury. |
| **Confounding factors** | Be aware of confounding factors such as prior chest pain, age, diabetes mellitus, hypertension, hyperlipidaemia and concomitant medication (nitrates, morphine), which can interfere with cardioprotection. | Be aware of confounding factors such as prior chest pain, age, diabetes mellitus, hypertension, hyperlipidaemia and concomitant medication (sulphonylureas, nitrates, morphine, nicorandil, volatile anaesthetics), which can interfere with cardioprotection. |
| **The intervention** | 1. Only test therapies having shown conclusive cardioprotection in pre-clinical studies.<br>2. Administer the therapy prior to myocardial reperfusion via PPCI. | 1. Only test therapies having shown conclusive cardioprotection in pre-clinical studies<br>2. Option to administer therapy prior to surgical incision, in cardioplegic solution, or at time of aortic declamping. |
| **Clinical endpoints** | Select relevant clinical endpoints for assessing cardioprotective efficacy:<br><br>1. MI size (enzymatic or CMR)<br>2. Myocardial salvage index (more sensitive than MI size reduction)<br>3. Microvascular obstruction<br>4. LV remodelling (LVH, LVEF and indexed LVEDV or LVESV)<br>5. Cardiac death<br>6. Hospitalisation for heart failure. | Select relevant clinical endpoints for assessing cardioprotective efficacy:<br><br>1. Perioperative myocardial injury (enzymatic or CMR)<br>2. LVEF<br>3. Amount of inotrope support required<br>4. Cardiac death<br>5. Hospitalisation for heart failure |

*STEMI: ST-elevation myocardial infarction; PPCI: percutaneous coronary intervention; AAR: area at risk; CMR: cardiovascular magnetic resonance imaging; AUC: area under the curve; MI: myocardial infarction, LVH: left ventricular hypertrophy; LVEF: left ventricular ejection fraction; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume*

**Figure 1**

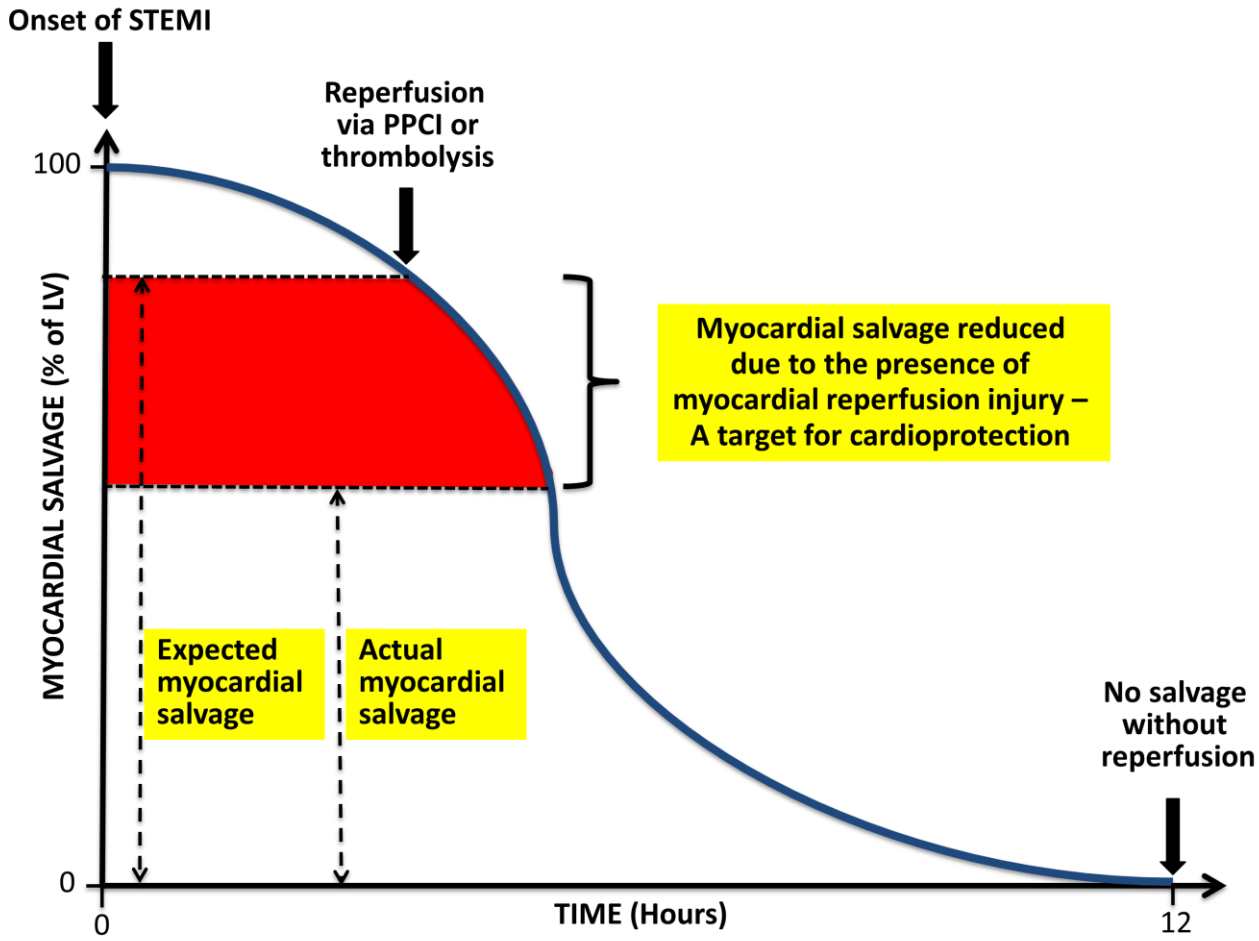
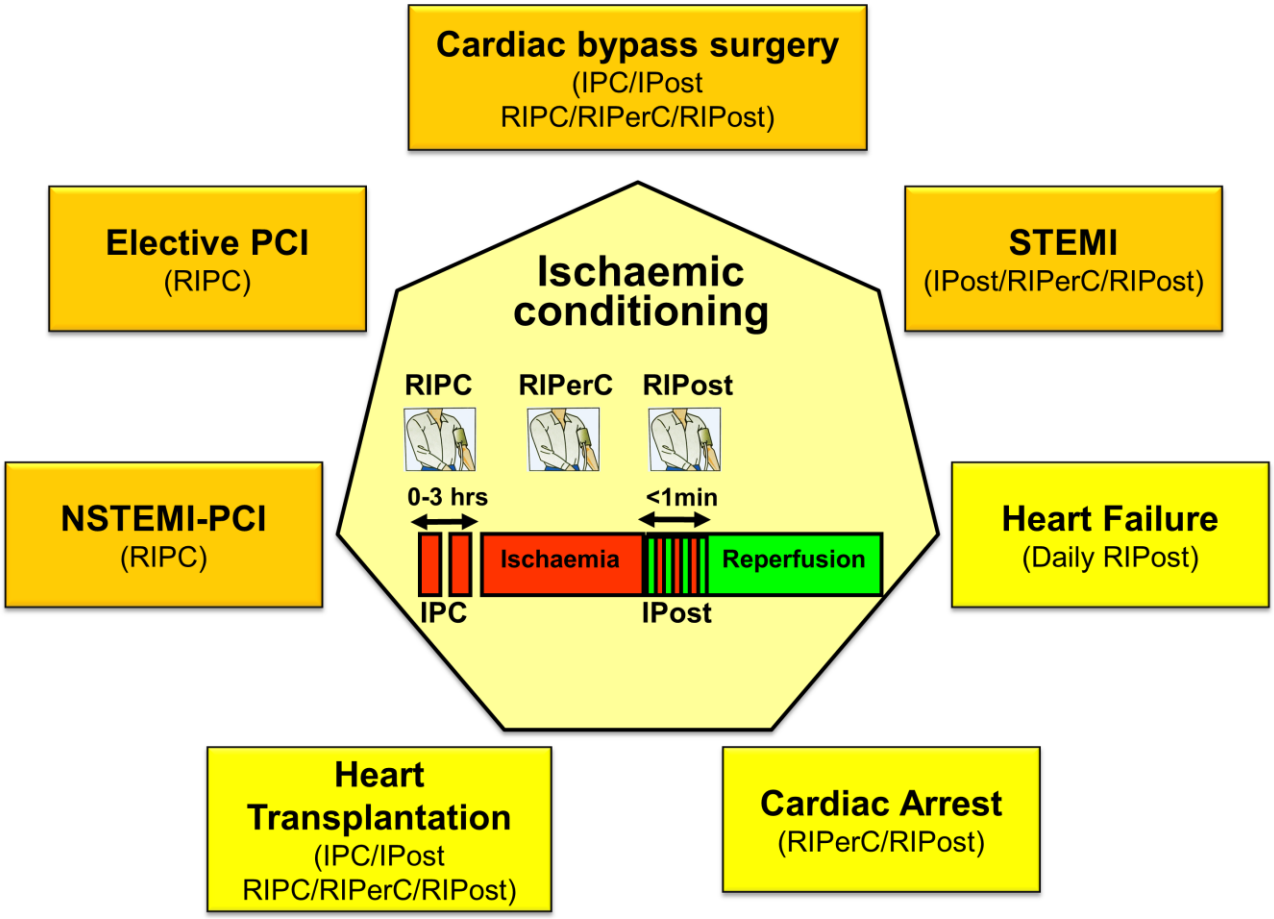

Onset of STEMI
Reperfusion via PPCI or thrombolysis
100
MYOCARDIAL SALVAGE (% of LV)
Myocardial salvage reduced due to the presence of myocardial reperfusion injury – A target for cardioprotection
Expected myocardial salvage
Actual myocardial salvage
No salvage without reperfusion
0
0
TIME (Hours)
12

**Figure 2**


Cardiac bypass surgery
(IPC/IPost
RIPC/RIPerC/RIPost)
Elective PCI
(RIPC)
STEMI
(IPost/RIPerC/RIPost)
Ischaemic conditioning
RIPC
RIPerC
RIPost
0-3 hrs
<1min
Ischaemia
Reperfusion
IPC
IPost
NSTEMI-PCI
(RIPC)
Heart Failure
(Daily RIPost)
Heart Transplantation
(IPC/IPost
RIPC/RIPerC/RIPost)
Cardiac Arrest
(RIPerC/RIPost)

## Ischaemic conditioning in the clinical setting: key points

- 'Ischaemic conditioning' has the therapeutic potential to protect the heart against acute ischaemia/reperfusion (I/R) injury and improve clinical outcomes in patients with ischaemic heart disease (IHD), the leading cause of death and disability worldwide.
- Ischaemic conditioning is mediated by applying cycles of brief ischaemia and reperfusion to either the heart itself or to an organ/tissue remote from the heart - it can be reproduced by certain pharmacological agents (termed 'pharmacological conditioning').
- Ischaemic and pharmacological conditioning have been reported in proof-of-concept studies to be beneficial in three major clinical settings in which the heart is subjected to acute I/R injury: cardiac bypass surgery, elective percutaneous coronary intervention (PCI), and ST-segment elevation myocardial infarction (STEMI) patients treated by primary PCI.
- Whether this therapeutic strategy can improve patient outcomes in these clinical settings should be known in the next few years with the availability of results from several large multi-centre clinical trials.
- The translation of promising cardioprotective therapies discovered in the research laboratory into the clinic has been hampered by the use of inadequate animal models and poorly designed clinical studies – this can be overcome by increased interaction between basic scientists and clinicians, thereby facilitating the translation of novel cardioprotective therapies into the clinical setting for patient benefit.