

Reperfusion, Conditioning and the On-Going Search for the Holy Grail

Robert L Yellon, Rob M Bell

Dr Robert L Yellon, Dr Rob M Bell

The Hatter Cardiovascular Institute

University College London

67 Chenies Mews, London

WC1E 6HX, UK

Email: r.yellon@ucl.ac.uk; rob.bell@ucl.ac.uk

Tel: 020 3447 9888

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Abstract

Ischaemic conditioning is the phenomenon of protection against reperfusion injury via the application of brief, repeated episodes of non-lethal ischaemia. This review has three aims: 1) to briefly explain the various categories of ischaemic conditioning; 2) to explore past clinical trials and their failures; 3) to explore the future of clinical trials in the realm of ischaemic conditioning.

Introduction

Protecting the myocardium against lethal ischaemia necessitates rapid reperfusion. Paradoxically however, reperfusion injures the myocardium, a phenomenon known as “ischaemia-reperfusion injury” (IRI). There is currently no effective clinical intervention for IRI in spite of the fact that it may contribute up to 50% of final myocardial infarct (MI) size¹. This review intends to describe past, present, and future clinical trials into ischaemic conditioning, which present one of the few avenues currently being explored clinically to curb IRI.

The Principles of Ischaemic Conditioning and the Supporting Proof-of-Concept Studies

Ischaemic Conditioning is a form of protection against IRI produced via brief cycles of non-lethal ischaemia. These cycles may be sub-classified thusly (see also figure 1):

- **Pre-conditioning**
 - **Classical Ischaemic Pre-conditioning (IPC):** brief cycles of non-lethal ischaemia induced locally and directly to the myocardium *prior* to an episode of lethal ischaemia, e.g. aortic clamping. IPC has been shown to reduce post-surgical markers of cardiovascular damage (e.g. ATP², Troponin T³) when performed during coronary artery bypass surgery (CABG). However, due to the inherent embolic risk of such intervention, it has not been carried over to large-scale clinical trials.
 - **Remote Ischaemic Pre-conditioning (RIPC):** brief cycles of non-lethal ischaemia induced at a “remote” limb via blood pressure cuff *prior* to an episode of lethal

myocardial ischaemia. The need to apply the conditioning prior to an ischaemic stimulus largely limits its application to the surgical arena where the time of onset of the injurious ischaemia can be readily predicted. Proof-of-concept studies have shown that RIPC has the capacity to reduce post-CABG troponin release in humans.^{4,5} Such an intervention is an attractive prospect due to its non-invasive nature. However, the recent outcome trials, RIPHEART and ERICCA, have been neutral and will be explored later in this review.

- **Per-Conditioning**

- **Pharmacological Per-conditioning (PPerC):** the use of pharmaceutical agents to mimic a conditioning stimulus *during* an ischaemic insult to protect against subsequent reperfusion injury. Proof-of-concept studies have shown cyclosporine-A (CsA)⁶ and metoprolol⁷ to be associated with a significant reduction in infarct size (measured by late-gadolinium enhancement on cardiac MRI). However, the large-scale CsA-based CIRCUS and CYCLE studies have also been neutral and are discussed in the next section (“The Failure of Large-Scale Ischaemic Conditioning Trials”).
- **Remote Ischaemic Per-conditioning (RIPerC):** brief cycles of non-lethal ischaemia induced at a limb via blood pressure cuff *during* an episode of lethal myocardial ischaemia. The proof-of-concept ERIC-LYSIS⁸ study randomised ST-elevation myocardial infarction (STEMI) patients to receive thrombolysis with or without RIPerC. The RIPerC cohort had a significant reduction in MI size 24 hours post-intervention. This was shown by a median 32% reduction in troponin-T (P = 0.020), and 19% reduction in creatinine kinase (P = 0.026) levels. The proof-of-concept CONDI⁹ trial randomised STEMI patients to receive PPCI with or without in-ambulance RIPerC. The RIPerC cohort had a significant reduction in all-cause mortality (P = 0.027) on follow-up (median = 3.8 years). The large-scale CONDI2/ERICPPCI studies are

underway and will be discussed in the final section (“The Future of Clinical Trials in Ischaemic Conditioning”).

- **Post-conditioning**

- **Pharmacological Post-conditioning (PPost):** the use of pharmaceutical agents to mimic a conditioning stimulus *following* onset of reperfusion to reduce subsequent reperfusion injury. The J-WIND study of 569 MI patients¹⁰ showed that those given an atrial natriuretic peptide (ANP) infusion post-reperfusion had a significantly reduced infarct size of 14.7% (P = 0.019) and a reduced incidence of readmission for heart failure relative to the control group (median follow-up period = 2.7 years) (HR = 0.267; P = 0.011). This same study showed that nicorandil had no effect on infarct size. In a study of 172 STEMI patients,¹¹ exenatide (glucagon-like-peptide-1 analogue) infusion during reperfusion attenuated myocardial infarction, resulting, on average, in a 15% greater salvage index (i.e. the difference between actual and potential infarct size). In spite of this however, no significant change in clinical outcome between the two cohorts was found.
- **Classical Ischaemic Post-conditioning (IPost):** brief cycles of non-lethal ischaemia applied *following* angioplasty of the culprit lesion, typically achieved via transient balloon inflation within the culprit vessel. Proof-of-concept studies have produced mixed results^{12,13} and the large-scale DANAMI 3-iPost (data presented at the American College of Cardiology 2016) clinical trial showed no significant difference in clinical outcomes. This trial will be explored in the next section (“The Failure of Large-Scale Ischaemic Conditioning Trials”).
- **Remote Ischaemic Post-conditioning (RIPost):** brief cycles of non-lethal ischaemia induced at a limb via blood pressure cuff following reperfusion therapy. Whilst proof-of-concept studies have shown some promise¹⁴, no large-scale clinical trials have yet been undertaken to explore RIPost.

The Failure of Large-Scale Ischaemic Conditioning Trials

Whilst proof-of-concept studies have provided a tantalising view of potential clinical efficacy with various ischaemic conditioning interventions, large-scale clinical trials have so far proved fruitless. This section will explore these failed trials and examine why ischaemic conditioning techniques have yet to fulfil their clinical potential.

- **RIPC** – The 2015 RIPHeart (“A Multicentre Trial of Remote Ischaemic Preconditioning for Hearth Surgery) study¹⁵ by Meybohm et al was a 1403 patient, multicentre, double-blinded, randomised controlled trial (RCT) exploring RIPC in elective surgical patients requiring cardiac bypass. The primary end-point was a composite of acute renal failure, stroke, non-fatal MI or all-cause death up to 14 days after surgery. Four cycles of true or sham RIPC were given post-anaesthetic induction and pre-surgical incision. No significant difference was seen between the primary end-points of either cohort. At approximately the same time, the ERRICA (“The Effect of Remote Ischaemic Conditioning on Clinical Outcomes in Coronary Artery Bypass Graft Surgery”) study¹⁶ by Yellon et al was published. This study was a 1612 patient, multicentre, double-blinded RCT of RIPC in patients undergoing CABG surgery. The primary end-point was the rate of major adverse cardiac and cerebral events 12 months after the intervention. Four cycles of true or sham RIPC were given post-anaesthetic induction and pre-surgical incision. Once again, no significant difference was seen between the primary end-points of either cohort.

Why was it that both of these trials failed to provide positive results? It may be that the injury suffered during CABG surgery is too small for RIPC to provoke a significant protective effect: it is well recognised from the pre-clinical literature that the smaller the primary injury, the smaller the benefit from a conditioning intervention is likely to be. It is also important to consider the other therapies patients undergoing surgical procedures will be exposed to: all patients in RIPHeart and >90% of patients in ERICCA were anaesthetised with propofol, an

agent which might in-fact abolish RIPC-based cardio-protection and an important potential confounder¹⁷.

- **PPerC** – The 2015 CIRCUS (“Cyclosporine to ImpRove Clinical oUtcome in ST-elevation Myocardial Infarction Patients”) study¹⁸ by Cung et al was a 970 patient, multicentre, double-blinded RCT exploring CsA as a preconditioning agent in patients undergoing PPCI to treat STEMI. The primary end point was a 1- and 3-year composite of heart failure progression, rehospitalisation for heart failure, adverse left ventricular modelling, and all-cause death. Prior to PPCI, patients were randomised to a CsA or placebo infusion. No significant difference in the primary end point was seen at 1 year: a neutral outcome. This could be due to the absence of data for LV end-diastolic volume in 17% of patients. This absent data, combined with the high incidence of adverse modelling in both cohorts, could have made it difficult to detect a significant difference in the other components of the primary end point composite. The 2016 CYCLE (“CYCLosporinE A in Reperfused Acute Myocardial Infarction”) study¹⁹ by Ottani et al was a 410 patient, multicentre, Prospective/Randomised/Open-label/Blinded-Endpoint (PROBE) trial which also explored CsA as a perconditioning agent in patients undergoing PPCI to treat STEMI. The primary end point was the incidence of $\geq 70\%$ ST-segment resolution 60 min after Thrombolysis in Myocardial Infarction (TIMI) flow grade 3. Prior to PPCI, patients were randomised to a CsA or placebo injection. Once again, no significant difference between the cohorts was detected. As such, the future use of CsA as a perconditioning agent appears doubtful.
- **IPost** – DANAMI 3-iPost (“DANish Study of Optimal Acute Treatment of Patients with ST-segment Elevation Mycocardial Infarction) is a treatment subgroup of a 2016 trial programme comparing multiple treatment strategies for STEMI. The iPost arm of the trial was a 1234 patient, multicentre, PROBE trial comparing standard PCI to PCI plus IPost. The primary end point was a composite of hospitalisation for heart failure and all-cause death within 2 years. Patients were randomised to PCI, or PCI plus four 30-second balloon inflations within the

reperfused coronary artery following initial reperfusion. No significant difference was detected between the two cohorts (data presented at the American College of Cardiology, 2016). The duration of ischaemia is critical: in the pre-clinical literature, there is a clear “therapeutic window”: ischaemic post-conditioning is ineffective where the index ischaemia duration is either very short or very long²⁰ and it would seem that in clinical trials, the therapeutic window is typically in the order of 1-4 hours from symptom onset to revascularisation. Whether or not ischaemic time played a role in the neutral DANAMI 3-iPost study will require further analysis upon official publication.

The Future of Clinical Trials in Ischaemic Conditioning

A number of large outcome trials exploring ischaemic conditioning are currently underway and may yet still provide evidence for the introduction of conditioning techniques into clinical practice.

RIPerC in Primary PCI

The CONDI 2 (Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing pPCI) and ERIC-PPCI (Effect of Remote Ischaemic Conditioning on Clinical Outcomes in STEMI Patients Undergoing pPCI) are a pair of collaborative studies investigating the use of RIPerC in STEMI patients.²¹ The studies are both multicentre, multinational, double-blinded RCTs with 2300 patients and 2000 patients respectively. Both trials share identical primary endpoints of hospitalisation for heart failure or cardiovascular mortality at 1 year. In these trials, STEMI patients will be randomised to pPCI with or without RIPerC. The RIPerC will be delivered either in-ambulance or on arrival to the pPCI centre depending on average national transit time. The results of these trials are hoped to be released in approximately 18 months.

RIPerC in Thrombolysis

Though the ERIC-lysis⁸ study provided evidence for clinically applied RIPerC, no large-scale trials have explored RIPerC in STEMI patients treated with thrombolysis. Whilst thrombolysis as an intervention

has largely been superseded by pPCI in the developed world, in the developing world thrombolysis is remains an important first-line therapy for STEMI. Large-scale human trials are still needed to provide evidence for a cost-neutral, life-saving therapeutic adjunct to thrombolysis.

PPerC in pPCI

Though the aforementioned CIRCUS¹⁸ study of PPerC was neutral at 1 year, the trial has not yet completed follow-up. It may yet show an effect of CsA at the 3-year end-point and as such, full conclusions may not currently be drawn. It is also worth noting that CsA is not the sole pharmacological agent: there are multiple potential pharmacological targets within the increasingly well characterised cell-death pathway that may yet yield an efficacious PPerC option. Indeed, repurposing existing cardiovascular drugs may yield significant benefit to improve cardiovascular outcomes following an acute myocardial infarction.

Conclusion

Reperfusion injury plays a significant role in the evolution of the final myocardial infarct size yet physicians have no effective tools with which to combat the phenomenon. Recent small-scale human trials exploring the variety of ischaemic-conditioning modalities have shown some promise. If large-scale trials can provide the evidence, reperfusion injury may yet become amenable to treatment. In remote conditioning exists a potentially cheap and non-invasive method of therapy, but as with pharmacological conditioning, the holy grail of an effective clinical intervention against IRI has yet to be realised. However, the search for an effective adjunct to reperfusion, if successful, will be well-rewarded by further reductions in cardiovascular mortality and morbidity and the consequent reduction upon the burgeoning socioeconomic healthcare burden worldwide.

Figure legends

Figure 1: Cartoon summarising the various forms of conditioning – ischaemic, remote and pharmacological – and the terminology relative to the onset of injurious ischaemia and subsequent reperfusion. Modalities applied prior to injurious ischaemia are termed “preconditioning”. A modality applied during the ischaemic injury is “perconditioning”. Any modality applied following the restoration of blood flow and reperfusion is regarded as “postconditioning”.

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Figure 1

