

From bench to bedside: Seeing the future of ischaemic post-conditioning as a novel cardioprotective therapy

Alex von Klemperer and Sandrine Lecour

Hatter Cardiovascular Research Institute, Cardioprotection Group,
Department of Medicine, Faculty of Health Sciences,
University of Cape Town, South Africa

Address for correspondence:

Sandrine Lecour
Hatter Cardiovascular Research Institute
Department of Medicine
Faculty of Health Sciences
University of Cape Town
Observatory
7925
South Africa

Email:

sandrine.lecour@uct.ac.za

INTRODUCTION

Cardiovascular disease is the second leading cause of death in South Africa, accounting for more than 10% of total deaths per year.⁽¹⁾ These data counteract the perception that cardiovascular disease only affects the population in developed countries. Following acute coronary occlusion, current cardiological practice dictates that rapid reperfusion, with the use of coronary angioplasty or thrombolysis, is vital to salvage the myocardium and reduce the infarct within the limited window available.

The benefits of reperfusion, however, come at a price. Lethal reperfusion injury manifests itself clinically as stunned myocardium, arrhythmias, endothelial damage and most importantly, a significant increase of up to 50% of the final size of the infarct.⁽²⁾ Although still unclear, the exact mechanisms behind reperfusion injury involve multiple processes such as increased oxidative stress,⁽³⁾ inflammatory damage,⁽⁴⁾ changes in myocyte osmolarity,⁽⁵⁾ calcium overloading,^(6,7) and changes in pH.⁽⁸⁾ The opening of the mitochondrial permeability transition pore (mPTP) is proposed as the endpoint

ABSTRACT

In order to improve the survival of patients suffering from an acute myocardial infarction, it is crucial to limit the size of the infarct. In the laboratory, several promising treatment strategies have been developed, but very few of these have been successfully translated from bench to bedside. This review aims to evaluate the translation of a novel therapy, ischaemic post-conditioning that can reduce infarct size and salvage myocardial function after acute myocardial ischaemia, from bench to bedside. The phenomenon of post-conditioning refers to staccato bouts of ischaemia at the onset of reperfusion. By elucidating the signalling mechanisms involved in the post-conditioning process, it has been possible to determine several pharmacological agents that deliver an equivalent level of protection. Following a large number of successful initial laboratory tests, small clinical trials suggest a promising future for this therapy. However, the breakthroughs required for this effective laboratory phenomenon to translate into meaningful clinical therapies can only be achieved by careful application of data obtained in basic research and controlled trials. SAHeart 2010; 7:166-171

for reperfusion injury,⁽⁹⁾ causing swelling and rupture of the mitochondria, release of calcium and uncoupling of energy production leading to further damage and ultimately cell death.⁽⁹⁻¹¹⁾

There is a clear need for effective and novel therapeutic strategies to protect the heart against reperfusion injury. In this regard, the recent experimental discovery of ischaemic post-conditioning⁽¹²⁾ represents a promising therapy for cardioprotection in patients with acute myocardial infarction (AMI).

THE DISCOVERY OF POST-CONDITIONING THERAPY IN ANIMAL MODELS

In 1986, Murray et al introduced the concept of ischaemic preconditioning, whereby brief episodes of a sub-lethal ischaemic insult offer significant protection from a subsequent lethal ischaemic insult.⁽¹³⁾ This powerful protective mechanism reduces infarct size,⁽¹³⁾

preserves vascular endothelial function⁽¹⁴⁾ and limits apoptosis.⁽¹⁵⁾ Unfortunately, the clinical application of this intervention is limited as it needs to be applied prior to the ischaemic insult. However, ischaemic post-conditioning, achieved by repetitive brief bouts of ischaemia at the onset of reperfusion, protects against reperfusion injury and offers a more practical clinical approach.⁽¹²⁾ Three episodes of 30 sec of reperfusion and 30 sec of ischaemia performed at the onset of reperfusion, following a 60 min ischaemic insult in dog hearts, protected against reperfusion injury.⁽¹²⁾ Its infarct limiting effect is comparable to ischaemic preconditioning and it can reduce the infarct size by up to 80%.⁽¹²⁾ Post-conditioning has been successful in multiple animal species such as canines, rats, mice and rabbits.⁽¹⁴⁾ Interestingly, ischaemic pre- and post-conditioning, used in combination, did not produce any significant benefit over the strategies used separately, which may suggest the activation of similar protective mechanisms by both phenomena.⁽¹⁷⁾

MECHANISM OF ISCHAEMIC POST-CONDITIONING

The delineation of the signalling mechanisms involved in ischaemic pre- and post-conditioning is the focus of intense experimental research as it may lead to the development of novel therapeutic drugs which would mimic the cardioprotective effect of ischaemic post-conditioning (Figure 1).

Multiple signalling pro-survival cascades are activated, leading ultimately to the activation of the mitochondrial potassium adenosine 5'-triphosphate (ATP)-dependent channel,⁽¹⁸⁾ and/or the inhibition of the mPTP opening.⁽¹⁹⁾ The activation of the reperfusion injury salvage kinase (RISK) pathway, which involves the phosphorylation of kinases such as Akt and Erk at the time of reperfusion, is a powerful protective path to limit cell death in both

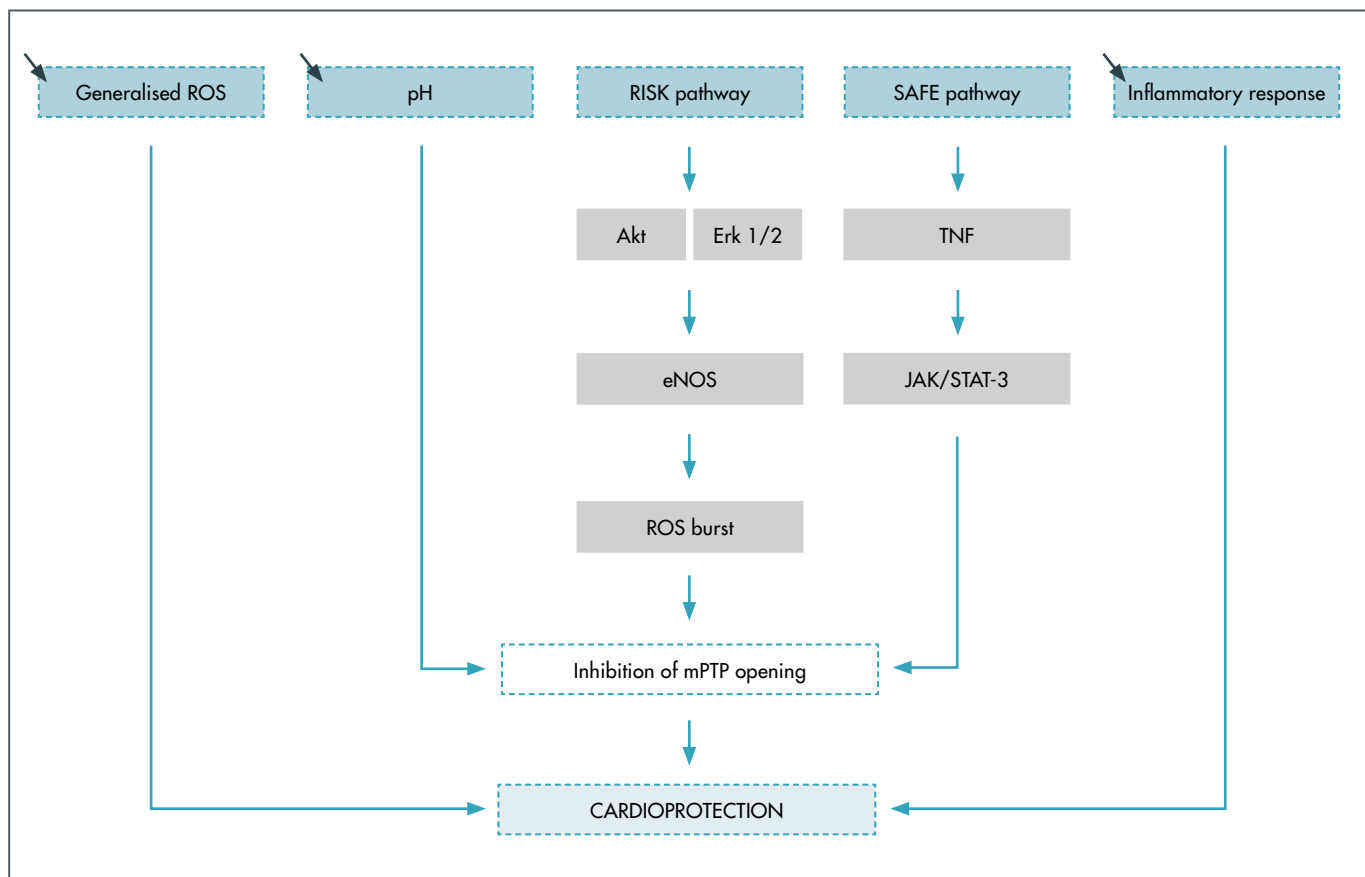


FIGURE 1: Proposed protective signalling events in ischaemic post-conditioning.

Akt: Akt/Protein kinase B, eNOS: Endothelial nitric oxide synthase, Erk 1/2: Extra-cellular signal-regulated kinase 1/2, JAK/STAT-3: Janus kinase/Signal transducer and activator of transcription-3, mPTP: Mitochondrial permeability transition pore, RISK: Reperfusion injury survival kinase (Pathway), ROS: Reactive oxygen species, SAFE: Survivor activating factor enhancement (Pathway), TNF: Tumour necrosis factor.

ischaemic pre- and post-conditioning.⁽²⁰⁾ Activated by G-protein coupled receptors, the RISK pathway will limit cell death by activation of endothelial nitric oxide synthase^(21,22) and anti-apoptotic molecules.⁽²³⁾ Similarly, the survivor activating factor enhancement (SAFE) pathway confers protection by activation of the cytokine tumour necrosis factor alpha (TNF α), the signal transducer and activator of transcription 3 (STAT-3) and inactivation of several pro-apoptotic molecules such as Bad.⁽²⁴⁻²⁷⁾ Whilst post-conditioning may reduce the overall levels of reactive oxygen species (ROS), small bursts of ROS at the onset of reperfusion act as an important signalling mediator in the post-conditioning pathway.⁽¹⁶⁾ In addition, the protective effect of post-conditioning may be related to a direct anti-inflammatory and/or antioxidant effect. Also, the maintenance of a low pH during reperfusion is vital for the success of post-conditioning as it may directly inhibit mPTP opening.^(28,29)

ISCHAEMIC POST-CONDITIONING: A SUCCESSFUL TRANSLATION FROM BENCH TO BEDSIDE

Considering that post-conditioning was only discovered in 2003,⁽¹²⁾ it is remarkable how quickly it made the leap to proof-of-concept clinical trials (Table 1). In 2005, Staat et al. published a landmark study whereby post-conditioning, applied during the first minutes of reperfusion to patients with AMI undergoing emergency percutaneous coronary intervention (PCI), reduced myocardial damage measured through creatine kinase release over 72 hours.⁽³⁰⁾ After reperfusion by direct stenting, post-conditioning simply performed within the first minute of reperfusion by 4 cycles of 1 min inflation/deflation of the angioplasty balloon, reduced the infarct by 36%. Using more specific endpoints, the same group later confirmed that their post-conditioning protocol was associated with a reduction of the infarct size (measured by 201 thallium single photon emission computed tomography technique) and improved myocardial contractile function (measured by echocardiography) for several months.⁽³¹⁾ At 1 year, their pilot study, performed on 38 patients only, showed a 7% increase in the left ventricular ejection fraction in patients subjected to the post-conditioning protocol.⁽³¹⁾ Similarly, post-conditioning the human heart with three cycles of 30 sec inflation and 30 sec deflation of the angioplasty balloon,

within the first 3 min of reperfusion, reduced the infarct size by 27%.⁽³²⁾ Animal studies have shown that optimising the post-conditioning protocol is an important process for the success of the therapy (see review).⁽³³⁾ In a retrospective analysis of patients undergoing primary angioplasty, the release of creatine kinase in patients who received 4 or more balloon inflations was lower than in patients who received between 1 and 3 balloon inflations.⁽³⁴⁾

The benefit of post-conditioning can also be extended to cardiac surgery. In patients undergoing a valve replacement under cold blood cardioplegic arrest, post-conditioning (performed by 3 cycles of 30 sec ischaemia and 30 sec of reperfusion using aortic clamping) reduced the creatine kinase release, transcardiac neutrophil count and the use of inotropic agents during reperfusion.⁽³⁵⁾

Remote post-conditioning, whereby the post-conditioning protocol applied in one part of the body results in protection of a remote region undergoing ischaemia-reperfusion, is successful in animal models⁽³⁶⁾ and may have its application in humans. Similar to remote ischaemic preconditioning,⁽³⁷⁾ remote post-conditioning, performed by transient limb ischaemia on the contra-lateral arm or leg before ischaemia, prevents the reduction in flow-mediated dilatation induced by the ischaemic insult.⁽³⁸⁾ Following coronary artery bypass graft surgery, remote ischaemic preconditioning reduces myocardial damage (measured with troponin T).⁽³⁹⁾ Similarly, remote ischaemic preconditioning performed on the upper arm decreases troponin I levels and chest pain in patients subjected to PCI.⁽⁴⁰⁾ It also reduces the risk of myocardial injury, myocardial infarction and renal impairment following repair of an abdominal aortic aneurysm.⁽⁴¹⁾ However, further clinical studies are needed to test whether ischaemic remote post-conditioning can be as protective as ischaemic remote preconditioning in patients subjected to PCI or cardiac surgery.

PHARMACOLOGICAL POST-CONDITIONING AS A PROMISING THERAPEUTIC TOOL

The delineation of signalling mechanisms involved in the cardioprotective effect of ischaemic post-conditioning have recently been translated into very promising clinical trials.

TABLE 1: Major studies investigating the effect of post-conditioning in patients following AMI.

Study	N	Endpoints	Result compared to control
Ischaemic post-conditioning in reperfusion following PCI ⁽³⁰⁾	30	Infarct size	CK released in 1st 72 hours reduced by 36%
		ST – segment shift	Trend towards lower shift
		Reperfusion	Increased
Ischaemic post-conditioning in reperfusion following PCI ⁽³¹⁾	37	Short term infarct size	CK release in 1st 72 hours reduced by 40% Troponin I released in 1st 72 hours reduced by 47%
		Long term infarct size	On SPECT imaging reduced by 39%
		Functional recovery 1 year post AMI	LVEF improvement WMSI reduced by 0.2 or 14.3%
Ischaemic post-conditioning in reperfusion following emergency PCI ⁽⁵²⁾	94	Infarct size	CK-MB reduced by 37%
		Arterial endothelial function 1 day post AMI	Vasodilation of Brachial Artery improved function
		Ventricular function at 8 weeks	ΔWMSI increased by 0.16 or 13.3%
		Lipid oxidation	Plasma MDA levels lower than control
Retrospective trial comparing patients with balloon inflations in emergency PCI ⁽³⁴⁾	115	Infarct size	Peak CK lower in ≥4 inflations than 3 inflations
Ischaemic post-conditioning in reperfusion following PCI ⁽³³⁾	41	Infarct size	CK release 1st 72 hours reduced by 27% SPECT imaging at 7 days reduced by 27%
		Ventricular function	Trend towards LVEF improvement
Ischaemic post-conditioning during cold cardioplegic arrest in valve surgery ⁽³⁵⁾	50	Post-operative inotropic requirements	Reduced by 40%
		Post-operative infarct size increase	CK-MD release reduced Trend of Troponin I release reduced
		Transcardiac WBC count	Improved by 7.5%

AMI: Acute myocardial infarction, PCI: Percutaneous coronary intervention, CK: Creatine kinase, CK-MB: Creatine kinase-MB isoform, LVEF: Left ventricular ejection fraction, MDA: Malonaldehyde, N: Number of patients, SPECT: Single photon emission computer tomography, WBC: white blood cell, WMSI: Wall motion score index.

As mentioned earlier, an endpoint target of ischaemic post-conditioning is the inhibition of the opening of the mPTP and experimental studies in animals have shown that cyclosporine, given at the onset of reperfusion, could reduce the infarct size⁽⁴²⁾ and improve left ventricular ejection fraction⁽⁴³⁾ by inhibiting the opening of the mPTP.

In 2008, these findings were confirmed in a landmark small proof-of-concept clinical study. 58 patients with acute ST-elevation myocardial infarction who received an intravenous bolus of 2.5mg/kg of cyclosporine immediately before undergoing PCI, significantly reduced the release of creatine kinase by 40% within

the first 72 hours.⁽⁴⁴⁾ Infarct size, assessed on day 5 (by measuring the area of hyper enhancement on magnetic resonance imaging) was significantly reduced. Cyclosporine, routinely used as an immunosuppressive agent, is well known for its toxic side-effects, such as renal and hepatic toxicity and increased susceptibility to infections and cancers. A single bolus injection of cyclosporine did not show any of these side-effects, but larger and longer clinical trials are required to prove the safety and efficacy of cyclosporine as a therapeutic agent following AMI.

Two clinical trials have explored the effect of adenosine in patients with acute myocardial infarction (AMISTAD and AMISTAD II) but

the results were mitigated by the haemodynamic effect of the drug.^(45,46) Although adenosine can successfully reduce the infarct size, it has a vasodilatory and negative chronotropic effect, causing hypotension and bradycardia, thus limiting its clinical application. However, recent experimental studies using polyethylene glycol liposomal adenosine in rats protected against ischaemia-reperfusion and reduced the haemodynamic effect of adenosine.⁽⁴⁷⁾ If this protocol can be applied in a clinical setting, it may limit the side effects of adenosine.

Erythropoietin successfully reduced the infarct size in animal models but its clinical application still needs to be confirmed.⁽⁴⁸⁾

Also of interest, lipid lowering agents such as atorvastatin, are beneficial to patient prognosis following acute myocardial infarction,^(49,50) and animal studies suggest that its cardioprotective effect occurs via the RISK pathway.⁽⁵¹⁾

CONCLUSION

The ultimate aim of basic medical research is to lead to an effective and safe clinical application. The impressive speed at which ischaemic post-conditioning leapt from animal studies to successful clinical trials, combined with its cost-effectiveness and the relative ease with which existing protocols can be modified to include it, seem to indicate that we can expect to see a transition to a meaningful clinical treatment evolving out of this phenomenon in a shorter time frame than alternative procedures such as cell therapy.

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