Protective ischaemia in patients: preconditioning and postconditioning

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Infarct size can be limited by reducing the determinants of infarct size or increasing collateral blood flow by treatment initiated before the ischaemic event. Reperfusion is the definitive treatment for permanently reducing infarct size and restoring some degree of contractile function to the affected myocardium. Innate survival mechanisms in the heart can be stimulated by short, non-lethal periods of ischaemia and reperfusion, applied either before or after the ischaemic event. Preconditioning, a series of transient intervals of ischaemia and reperfusion applied before the lethal ‘index’ ischaemic event, sets in motion molecular and cellular mechanisms that increase cardiomyocyte survival to a degree that had not hitherto been seen before. The cardioprotective ischaemic-reperfusion protocol applied at onset of reperfusion, termed ‘postconditioning’ (Postcon), is also associated with significant cardioprotection that can be applied at the point of reperfusion treatment in the catheterization laboratory or operating room. Both preconditioning and Postcon have been successfully applied to the clinical setting and have been found to reduce infarct size and other attributes of post-ischaemic injury. This review will summarize the physiological preclinical data on preconditioning and Postcon that are relevant to their translation to clinical therapeutics and treatment.

KEYWORDS
Preconditioning; Postconditioning; Infarct size; Myocardial protection; Coronary intervention; Cardiac surgery

1. Introduction

The relationship between infarct size and incidence of acute mortality, morbidity, and heart failure suggests that reducing infarct size may be an important therapeutic goal. Hence, limitation of infarct size has major ramifications on outcomes as well as the financial burden of healthcare. Infarct size is determined by a number of interacting factors, which include (i) the duration of ischaemia (coronary artery occlusion), (ii) the abundance of collateral blood vessels to the area at risk, (iii) nutritional status, (iv) temperature, and (v) the presence of comorbidities such as age, hypercholesterolaemia, diabetes, and hypertension. There has been vigorous debate for decades whether the process of reperfusion itself contributes to the pathophysiology of infarction (reperfusion injury) and to infarct size. The determinants or mechanisms of reperfusion injury have been reviewed elsewhere and will not be discussed in this review. However, the observation that reperfusion injury has its pathogenesis in the early moments of reperfusion is very important, especially in postconditioning (Postcon) in which the first minutes of reperfusion are intervened. There are few studies that have attempted to demonstrate reperfusion injury as an entity separate from ischaemic injury. Matsumura et al. using uptake of C-deoxylglucose administered 5 min after reperfusion and F-2-deoxyglucose give 3 h after reperfusion to distinguish viable from non-viable tissue showed no-reflow myocardium was largely non-viable by 5 min of reperfusion, thereby potentially reflecting either rapid cell death during reperfusion or ischaemic cell death. At 5 min, 45% of myocardial samples taken from infarcted myocardium were non-viable, increasing to 98% after 3 h of reperfusion. In addition, Freude et al. found that necrosis was the predominant cell death pathway after ischaemia. On the other hand, apoptosis was largely absent during ischaemia, but progressively increased during reperfusion. Zhao et al. showed a similar rapid increase in infarct size during the first 24 h of reperfusion which was virtually unchanged thereafter, whereas apoptosis in peri-infarcted myocardium increased progressively over 72 h post-reperfusion. A temporal progression of reperfusion injury is the substrate on which Postcon, and perhaps preconditioning, exerts its cardioprotective mechanisms.

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1.1 Myocardial protection by endogenous mechanisms

Infarct size can be limited by endogenous mechanisms applied at three major time points: first, cardioprotective treatments can be applied before the ischaemic event occurs. The most successful pre-emptive treatment modality has been to stimulate adaptive mechanisms by 'preconditioning' the heart before the major (index) ischaemic event. In preconditioning, a series of short ischaemic periods separated by brief reperfusion is applied immediately before (early preconditioning)14 or 24 h before (delayed preconditioning or second window of preconditioning) the ischaemic event. Secondly, endogenous 'conditioning' mechanisms can be applied during the ischaemic event ('perconditioning'). Thirdly, an alternative treatment opportunity is to intervene at reperfusion, e.g. by thrombolysis, percutaneous coronary intervention (PCI), or surgery. Myocardial protection strategies address reperfusion injury in cardiac surgery. However, the strategies used in thrombolysis and PCI do not address reperfusion injury specifically.16

This review will provide an overview of the two endogenous cardioprotective strategies, preconditioning and Postcon, and focus on their clinical translation. However, the review will refer to the pathophysiological mechanisms of reperfusion injury which are altered by either preconditioning or Postcon. In addition, the molecular mechanisms of preconditioning and Postcon will not be discussed in detail, as space will not allow a comprehensive discussion. The review will focus on the physiological mechanisms that are translatable to the human condition.

2. Preconditioning

2.1 Background

Over 20 years ago, Murry et al.14 first described the phenomenon of ischaemic preconditioning (IPC) by which brief, intermittent periods of coronary artery ischaemia separated by reperfusion precede the more sustained myocardial ischaemia, resulting in highly significant cardioprotection. The landmark study by Murry et al.14 exposed anesthetized, open-chest dogs to four cycles of 5 min coronary artery occlusions followed by 5 min of reperfusion before the onset of 40 min of coronary occlusion and 4 days of reperfusion. The animals receiving the 'IPC' displayed significantly smaller infarct sizes when compared with the control animals. The original paper by Murry et al.14 has been cited over 3200 times, demonstrating the importance of this paradoxical discovery that ischaemia protects from itself. Since this remarkable discovery in 1986, there has been a plethora of experimental investigations to define the cellular and molecular signals and pathways that elicit the reduction in infarct size. Numerous studies have provided tremendous insights into the mechanisms of IPC in a variety of animal species including both in vitro and in vivo model systems. Please see the elegant reviews by Downey et al.,17 Das and Das,18 Hausenloy et al.,19 and Bolli et al.20 for a detailed discussion of the signalling mechanisms involved in the various forms of myocardial preconditioning. Major signalling pathways and mediators involved in IPC are: phosphatidylinositol-3-kinase (PI3-K)-Akt, nitric oxide (NO*)-PKG, mitochondrial KATP channels, adenosine, oxygen-derived free radicals,21,22 and the mitochondrial permeability transition pore (mPTP). At present, there is a clear consensus that IPC is cardioprotective across all animal species investigated and is considered to be the most protective intervention against myocardial ischaemia-reperfusion injury to date.20,23 This section of the review will highlight the most recent attempts at clinical translation of this highly efficacious intervention in the setting of vascular and cardiovascular diseases.

It is important to delineate the two distinct phases or ‘windows’ of IPC that have been described as early as 1993,24,25 The protective effects of IPC are transduced within minutes and triggered by the release of several mediators, and depend upon the activation of complex second messenger signalling cascades including a number of survival kinases.19,20,25 The effects of the ‘first window’ or early preconditioning, last 1–2 h, after which the protection wanes. The ‘second window’ or late preconditioning occurs 24 h following the initial preconditioning ischaemia and lasts for 48–72 h.20 A major distinction between the two phases of IPC is that early preconditioning has been shown to result in the modification of existing myocardial proteins, whereas late preconditioning is exerted by newly synthesized cytoprotective proteins in the heart.20 Experimental evidence also suggests that there are differences in the cardioprotective actions of early and late preconditioning. Early IPC clearly protects against myocardial infarction, but fails to limit the degree of myocardial contractile dysfunction or stunning. In contrast, late IPC protects against both myocardial cell death and preserves post-ischaemic left ventricular function.20 Therefore, the second window of IPC may be of more clinical benefits as a result of the more significant protective actions in the ischaemic-reperfused myocardium as well as the longer duration of the protective effects (48–72 h vs. 1–2 h).

2.2 Pharmacological preconditioning

Following the initial discovery of IPC, it became clear that pharmacological agents could also impart cardioprotection when administered prior to the onset of sustained myocardial ischaemia.23 This is significant since pharmacological agents could be more readily applied to clinical practice as a means of protecting the heart and other organs against ischaemia-reperfusion injury rather than imposing ischaemia directly. The number of agents that have been shown to induce myocardial preconditioning is rather large and includes nitric oxide related agents such as nitric oxide donors26 and sodium nitrite,27 phosphodiesterase inhibitors,28 adenosine monophosphate-activated protein kinase activators,29 and the G-protein-coupled receptor agonists adenosine,30,31 bradykinin,32 opioid agonists33,34 and other G-protein agonists,17,18 muscarinic agents,35,36 angiotensin AT1 agonists,37 and endothelin.38,39 In general, these agents activate the cytoprotective signalling pathways in the myocardium. In addition, a number of noxious stimuli (heat stress, rapid pacing, reactive oxygen species, cytokines, and endotoxins) when applied spuriously can trigger myocardial preconditioning. The use of pharmacological agents to induce myocardial preconditioning is very clinically relevant, since the delivery of a single dose of a well-characterized and safe pharmacological agent is far more feasible and often safer than using ischaemia to elicit
myocardial preconditioning. Surgeons would be very hesitant to cross-clamp the aorta repeatedly, especially in patients with atherosclerotic disease in this great vessel.

On the basis of the remarkable results of both IPC and pharmacological preconditioning, it is feasible to consider the use of gene therapy to induce a more permanent and long-lasting preconditioning phenotype in the heart. Animal studies have demonstrated significant cardioprotection following gene transfer of mediators or mimetics of IPC such as eNOS,40 iNOS,23 COX-2,20 and ecSOD20,23 to the heart. The most extensively studied gene therapies to induce preconditioning have been eNOS and iNOS gene therapy in murine model systems.

2.3 Remote IPC

A more recent development in the field of IPC is the discovery that remote ischaemia at a distant site can elicit the same protective effects of IPC of the local coronary artery of interest. Experiments performed in 1993 by Przyklenk et al.41 first demonstrated that brief ischaemic episodes of the left circumflex coronary artery significantly reduced myocardial infarct size following sustained occlusion of the left anterior descending coronary artery. Following the initial discovery of remote IPC (RIPC) in the heart, it was reported that brief, intermittent ischaemia of distant organs such as the skeletal muscle,42 kidney,43 and intestine44 could also induce preconditioning of the heart against prolonged myocardial ischaemia and reperfusion. Additionally, Elrod et al.45 recently reported that chronic genetic overexpression of eNOS protein in the murine myocardium resulted in significant protection of the liver following ischaemia–reperfusion injury. The cardioprotection was linked to an increase in nitric oxide in the liver following transport of this salubrious factor from the heart to the liver in the form of nitrite and nitrosothiols. This is yet another example of remote preconditioning (pharmacological in nature) against ischaemia–reperfusion mediated injury.

From a mechanistic standpoint, RIPC very closely resembles traditional IPC and depends on the very same trigger mechanisms and second messenger signalling pathways to promote cardioprotection. At present, there are three main hypotheses regarding the central mechanism responsible for RIPC.46,47 The first theory is the neural hypothesis, which proposes that the ischaemic remote organ releases endogenous substances (i.e. adenosine, bradykinin) that activate local afferent neural pathways that, in turn, activate efferent neural pathways to trigger end-organ protection. A second theory is the humoral hypothesis, which suggests that the remote organ releases humoral mediators such as adenosine and bradykinin into the blood stream, which are then transported to the remote organ where the humoral factor directly triggers the intracellular survival pathways. A third theory is termed as the ‘inflammatory suppression theory’, which proposes that the transient, remote organ ischaemia produces a systemic anti-inflammatory phenotype that protects the distant organ against subsequent ischaemia–reperfusion injury. It is not clear which of the three proposed hypotheses accounts for the majority of cardioprotective effects mediated by remote IPC. However, it is possible that all three of these hypotheses are correct, and there are interactions between endogenous substances such as adenosine, and local afferent neural pathways that synergize to promote cardioprotection and subsequently produce systemic suppression of inflammation. Future experiments utilizing highly specific pharmacological agents and/or gene-targeted animals can be employed to define the role(s) of humoral, neural, and inflammatory mediators in RIPC.

3. Clinical translation of preconditioning

Unlike many interventions that have proven to elicit cardioprotection in animal models and failed to do so in humans, IPC has been shown to protect the human myocardium. The major limitation to the clinical use of IPC is that the intervention must be performed prior to the onset of the clinical symptoms of acute myocardial infarction. Therefore, the majority of clinical investigations that have been performed have been restricted to various cardiovascular surgical procedures, including both vascular and cardiac operations in which the ischaemic period is predictable. Seminal work by Yellon et al.48 was the first to demonstrate the feasibility of IPC in the human heart in cardiac surgery. In a small number of patients (n = 7 per group) undergoing coronary artery bypass surgery, the heart was subjected to two periods of aortic cross-clamping, each lasting 3 min separated by 2 min of reperfusion. The hearts were then subjected to 10 min of cross-clamping with electrical fibrillation. Myocardial biopsies were obtained prior to IPC, at the end of IPC and following the 10 min ischaemia, and were assessed for ATP levels as an index of myocardial protection. Interestingly, the two periods of IPC significantly preserved myocardial ATP levels following a more prolonged period of ischaemia, suggesting that the hearts would exhibit greater tolerance to ischaemia–reperfusion injury. Since this study48 was reported in 1993, the cardioprotective effects of IPC have been demonstrated in various vascular and cardiac surgical procedures, including coronary artery bypass surgery and transplantation (reviewed in49). The imposition of ischaemia directly in the heart and other organs has, to some extent, made the clinician hesitant to use IPC in the clinical setting.

As an alternative to preconditioning, RIPC offers a simple and highly cost-effective procedure to provide systemic protection against organ injury in patients undergoing complex surgical procedures that could reduce perioperative and post-operative complications. An early proof of concept study on RIPC by Kharbanda et al.50 employed upper arm ischaemia (three times for 5 min.) induced by inflation of a blood pressure cuff on one arm and then subjected the other arm to 20 min of upper arm ischaemia. The 20 min of upper arm ischaemia and reperfusion resulted in significant endothelial dysfunction as assessed by response to acetylcholine in the control group, which was largely abrogated in the group receiving RIPC of the contralateral arm. This early study provides very strong evidence that RIPC affords significant vascular protection at a distant site in humans. The authors also demonstrated in the same study that RIPC triggered by hind limb ischaemia in a porcine model significantly reduced myocardial infarct size following ischaemia and reperfusion. This same research team also investigated the effects of early and late IPC on vascular function following ischaemia in a small group (i.e. 16
In this clinical study, the non-dominant arm was rendered ischaemic by inflation of a blood pressure cuff for 5 min of ischaemia followed by 5 min of reperfusion for three cycles. Similar to the earlier study, the contralateral arm was then subjected to 20 min of ischaemia via cuff inflation followed by critical assessment of conduit vessel vasoreactivity. The study clearly demonstrated that both ‘early’ and ‘late’ phases of IPC were present in the RIPC variant and that the effects were in part mediated via neuronal activity since treatment with the autonomic blocker, trimetaphan, abolished the preservation of flow-mediated vasodilatation in subjects that received the RIPC protocol.

In 2007, Hausenloy et al.52 investigated the effects of RIPC as a potential therapeutic strategy to reduce myocardial injury in the setting of cardiopulmonary bypass surgery. Fifty-seven patients undergoing elective coronary artery bypass graft surgery (CABG) were randomized to either RIPC or no intervention. The RIPC group was subjected to three 5 min cycles of upper arm ischaemia that was induced by an automated cuff-inflator that was inflated to 200 mmHg three times followed by reperfusion just prior to the start of CABG surgery. The total troponin-T released from the heart during the 72 h study period was significantly attenuated by 43% in the RIPC group when compared with control. This small study provides the rationale for a larger randomized clinical trial for patients undergoing CABG as well as isolated valve and combined CABG-valve procedures.

Another study in 2007 by Ali et al.53 investigated the effects of RIPC on both myocardial and renal injury following abdominal aortic aneurysm repair in 82 patients (41 per study group). The patients were randomized to abdominal aortic repair with or without RIPC at the time of surgery. The RIPC protocol consisted of two cycles of intermittent cross-clamping of the common iliac artery with 10 min of ischaemia and 10 min of reperfusion. The study clearly demonstrated significant reductions in both plasma troponin-I and creatine in the first week following the surgery. The area under the curve for troponin-I was significantly lower in the RIPC group, and the absolute risk for myocardial injury decreased by 27% with a 22% reduction in the risk of myocardial infarction. Similar results were observed for renal impairment with a 23% reduction in the risk of renal impairment and a significant reduction in the mean creatinine area under the curve in the patients subjected to RIPC compared with no intervention. This study provides significant support for the use of RIPC in a variety of high-risk surgical procedures and suggests that RIPC provides global organ protection that is not limited only to the heart. The use of RIPC can be extended to paediatric cardiac surgical patients with congenital defect repairs.54

A recent article by Walsh et al.55 published in 2008 performed a systematic review and meta-analysis of all randomized clinical trials investigating IPC in the human heart. In this very thorough and extensive review of the literature, the authors examined a total of 6506 citations and concluded that only 22 reports were eligible for meta-analyses. The authors concluded that IPC reduces arrhythmias, inotropic requirements as well as critical care stay following cardiac surgery. The authors did point out that there was evidence of heterogeneity and bias with respect to a number of outcomes in the meta-analysis, and this was to be expected since the primary trials were not designed with the intention to assess the effect of IPC on clinical end-points but were designed to confirm the existence of IPC protection in the setting of cardiac surgery in humans. The authors believe that a large scale trial is required to determine the effect of IPC or RIPC on clinical outcomes following cardiac or other forms of major surgery, and estimated that a study cohort of 3800 patients in each arm would be necessary to detect a reduction in mortality from 1.2 to 0.6%. The authors conclude that preconditioning represents a very inexpensive and readily available intervention that requires no significant modifications to existing surgical protocols. Clearly, larger prospective clinical trials that are adequately powered are required to determine whether preconditioning (either ischaemic or pharmacological) can significantly reduce complications and improves outcomes in the setting of cardiac surgery.

4. Postconditioning

4.1 Historical perspective on Postcon

Unpublished studies on Postcon were first conducted in 1992 by Zhao and colleagues in the Vinten-Johansen Laboratory, in an anesthetized rabbit model of coronary artery occlusion–reperfusion, in which the Postcon algorithm was 5 min of ischaemia followed by 5 min of reperfusion (parallel the durations used in preconditioning) repeated for several such cycles at the onset of reperfusion. These studies were subsequently discontinued on the basis of equivocal effects on infarct size compared with a control group. However, the Vinten-Johansen Laboratory resumed these studies in 2001, with cycles of reperfusion and re-occlusion that were compressed from 5 min to 30 s each based on an emerging understanding of the rapid time course of reperfusion injury (Figure 1). Vinten-Johansen (in reference 56) and Zhao et al.57 first formerly introduced Postcon for the reduction of infarct size in 2003. Earlier, stimulated by a clinical case report by Grech and Ramsdale,58 Na et al.59 independently reported that transient ischaemia applied at the onset of arrhythmias during reperfusion suppressed the incidence of ventricular fibrillation in anesthetized cats with 20 min of coronary occlusion. The term ‘Postcon’ was actually used in this report.59 Indeed, Halkos et al.60 recapitulated the
arrestion suppressing effects of Postcon in a canine model of necrosis caused by 1 h left anterior descending artery (LAD) occlusion–reperfusion.

4.2 Physiological effects of Postcon

In the experimental laboratory setting, Postcon was performed by sequentially releasing and reapplying an external ligature around the coronary artery, thereby imposing transient periods of ischaemia followed by reperfusion. More recent studies have used fluoroscopically guided angioplasty balloon catheters to sequentially occlude and reperfuse the target vessel in closed-chest preparations, a model which more closely simulates the clinical situation of PCI for acute myocardial infarction. Since its inception, other methods have been used to (i) simulate the alternating intervals of reperfusion and ischaemia (hypoxia-reoxygenation, acidic-neutral pH perfusion), i.e. ‘hypoxic’ Postcon, (ii) stimulate the release of endogenous ligands to G-protein-coupled neutral pH perfusion), i.e. ‘hypoxic’ Postcon, (ii) stimulate the release of endogenous ligands to G-protein-coupled receptors (adenosine, bradykinin, and PAR2 agonists) that trigger Postcon, or (iii) pharmacologically induce a ‘postconditioned state’ (i.e. G-protein-coupled receptor agonists and inhalational anaesthetics). In fact, applying ‘postconditioned state’ (i.e. G-protein-coupled receptor agonists and inhalational anaesthetics) has been shown by some to be triggered by reperfusion after transient ischaemia, and extends the total infarct size. In addition, Postcon reduced reperfusion arrhythmias and neutrophil adherence to the post-ischaemic coronary artery vascular endothelium, neutrophil accumulation in the area at risk myocardium, and endo-thelial injury. The preservation of the post-ischaemic vascular endothelium may be a critical event that attenuates the inflammatory response that is initiated at the onset of reperfusion. The role of the vascular endothelium and neutrophils will be discussed below.

One physiological mechanism by which Postcon may protect the heart is by delaying the normalization of tissue pH. Although hydrogen ions (H⁺) accumulate intracellularly and interstitially during ischaemia, reperfusion rapidly washes out the accumulated interstitial H⁺, and quickly restores the intracellular pH. The washout of hydrogen ions in the interstitium restores the transmembrane H⁺ gradient and hence activates the sodium–hydrogen exchanger, which expels H⁺ while translocating interstitial Na intra-cellularly. The intracellular accumulation of Na⁺ activates the Na⁺/Ca²⁺ antiporter, thereby resulting in Ca²⁺ accumulation, which in turn activates enzyme systems and triggers contracture of the cardiomyocyte during the early moments of reperfusion. Postcon delays the realkalization of the

4.2.1 Physiological effects of Postcon

Postcon has broad-spectrum physiological effects on post-ischaemic pathology. A major effect is a reduction of infarct size. Infarct size is reported to be reduced by up to 50% in canines, and 25% in rodent models (Table 1). Postcon was also shown to reduce apoptosis; apoptosis has been shown by some to be triggered by reperfusion after transient ischaemia, and extends the total infarct size. In addition, Postcon reduced reperfusion arrhythmias and neutrophil adherence to the post-ischaemic coronary artery vascular endothelium, neutrophil accumulation in the area at risk myocardium, and endothelial injury. The preservation of the post-ischaemic vascular endothelium may be a critical event that attenuates the inflammatory response that is initiated at the onset of reperfusion. The role of the vascular endothelium and neutrophils will be discussed below.

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### Table 1 The efficacy of preconditioning and postconditioning in various species related to the experimental protocol used

<table>
<thead>
<tr>
<th>First author</th>
<th>Model</th>
<th>Ischaemia/reperfusion protocol</th>
<th>Postcon protocol</th>
<th>Postcon infarct size reduction % or % dead cells</th>
<th>IPC infarct size reduction %</th>
<th>IPC + Postcon infarct size reduction %</th>
</tr>
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<tbody>
<tr>
<td>Zhao et al. 2003</td>
<td>In vivo dog</td>
<td>60/180 min</td>
<td>3 × 30/30 s cycles</td>
<td>44</td>
<td>40</td>
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<td>Argaud et al. 2005</td>
<td>In vivo rabbit</td>
<td>30/240 min</td>
<td>4 × 60/60 s cycles</td>
<td>52</td>
<td>70</td>
<td>–</td>
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<td>Yang et al. 2004</td>
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<td>30/180 min</td>
<td>4 × 30/30 s cycles</td>
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<td>62</td>
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<tr>
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<td>In vivo rat</td>
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<td>3 × 10/10 s cycles</td>
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<td>67</td>
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<td>3 × 30/30 s cycles</td>
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<td>46</td>
<td>50</td>
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<td>Tsang et al. 2004</td>
<td>In vitro rat</td>
<td>35/120 min global</td>
<td>6 × 10/10 s cycles</td>
<td>38</td>
<td>46</td>
<td>41</td>
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<td>Dosenko et al. 2006</td>
<td>Rat cell culture</td>
<td>30/60 min</td>
<td>3 × 60/60 s cycles</td>
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<td>13</td>
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<td>6 × 10/10 s cycles</td>
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<td>69</td>
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<td>6 × 10/10 s cycles</td>
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<td>63</td>
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<tr>
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<td>3 × 30/30 s cycles</td>
<td>49</td>
<td>51</td>
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<tr>
<td>Tang et al. 2006</td>
<td>In vivo rat</td>
<td>30 min/24 h</td>
<td>20 × 10/10 s cycles</td>
<td>47</td>
<td>72</td>
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<td>Donato et al. 2007</td>
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<td>45 min/24 h</td>
<td>11</td>
<td>47</td>
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<tr>
<td>Lim et al. 2007</td>
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<td>2</td>
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<td>Sivaraman et al. 2007</td>
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<td>4 × 60/60 s cycles</td>
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<td>2</td>
<td>16</td>
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</table>

\[a\]Non significant.
\[b\]Contractile recovery.
heart during early reperfusion; tissue pH remains acidic longer after Postcon compared with an abruptly reperfused heart,74 and coronary effluent is more acidic during the early moments of reperfusion in postconditioned hearts.75 Cohen et al.76 demonstrated in an elegant set of experiments in the isolated rabbit heart that perfusion with an acidic buffer reduced infarct size to the same extent as conventional Postcon; the infarct sparing effect of Postcon was reversed when the heart was simultaneously perfused with alkalotic (vs. neutral pH) perfusate during Postcon. However, it is not clear how maintaining tissue acidosis influences the sodium–hydrogen exchanger, the antioxidant effect, and the numerous molecular cascades (PI 3-kinase, ERK1/2) that are stimulated by Postcon.

### 4.2.2 Antioxidant mechanisms and inhibition of the mPTP

Postcon attenuates superoxide anion generation in area at risk myocardium after 3 h of reperfusion.62,66 However, reactive oxygen species are also required as a signalling mechanism for Postcon, since blocking them during the stimulus phase abrogates cardioprotection.77 It is thought that low levels of oxidants acts as a signal for endogenous protection, whereas higher levels induce damage to myocardial cells and coronary vascular endothelium. Studies have also revealed that intracellular and intramitochondrial calcium accumulation is reduced after ‘hypoxic’ Postcon in vitro62 which, in addition to the reduced reactive oxygen species generation, suggested that triggers of mitochondrial transition pore opening were reduced. The mPTP opens during the early moments of reperfusion in response to Ca2+ and oxidant accumulation,78,79 which initiates a cascade that culminates in either necrosis when ATP is depleted or apoptosis when ATP is present. Indeed, studies have shown that Postcon attenuates apoptosis57,80 in addition to necrosis, both of which ostensibly contribute to overall infarct size. A summary of potential mechanisms involved in IPC and Postcon is shown in Table 2.

### 4.2.3 Inhibition of neutrophil functions

Early events of reperfusion can be compared with an inflammatory-like response involving activation of neutrophils and a subsequent interaction with the vascular endothelium.72 Evidence strongly suggests a role of neutrophils in the pathogenesis of both necrosis and apoptosis in vivo72,81 through the release of reactive oxygen species (via NADPH oxidase activity), cytokines, and proteolytic enzymes. However, the involvement of neutrophils in ischaemia-reperfusion injury is currently debated in the literature.82–84 Indeed, some of the physiological attributes of protection reported with Postcon after coronary artery occlusion are consistent with an anti-inflammatory affect. These observations include (i) a reduction in neutrophil accumulation;57,60 (ii) less endothelial cell activation and dysfunction (attenuated expression of endothelial cell adhesion molecules, attenuated adhesion of neutrophils; and (iii) reduced levels of plasma pro-inflammatory mediators such as TNF-α, IL-6, and IL-8 that are involved in activation and recruitment of neutrophils.67 However, it is not clear whether less of an inflammatory response after Postcon reduces infarct size or whether a smaller infarct size recruits a smaller inflammatory response. Confirmation of a direct inhibitory effect of Postcon on neutrophils has heretofore been lacking.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Displaying the physiological, cellular, subcellular, and molecular differences and similarities of preconditioning and postconditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPC</td>
<td>Postcon</td>
</tr>
<tr>
<td>Physiological effect</td>
<td></td>
</tr>
<tr>
<td>Reduces infarct size</td>
<td>+</td>
</tr>
<tr>
<td>Reduces stunning</td>
<td>+</td>
</tr>
<tr>
<td>Reduces apoptosis</td>
<td>+</td>
</tr>
<tr>
<td>Reduces tissue oedema</td>
<td>+</td>
</tr>
<tr>
<td>Reduces vascular injury</td>
<td>+</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>+</td>
</tr>
<tr>
<td>Blood flow defects</td>
<td>+</td>
</tr>
<tr>
<td>Delays washout of adenosine</td>
<td>–</td>
</tr>
<tr>
<td>Cellular and subcellular effects inhibits</td>
<td></td>
</tr>
<tr>
<td>PMN accumulation</td>
<td>+</td>
</tr>
<tr>
<td>PMN adherence to EC</td>
<td>+</td>
</tr>
<tr>
<td>EC activation</td>
<td>+</td>
</tr>
<tr>
<td>EC &quot;O2&quot;-generation</td>
<td>+</td>
</tr>
<tr>
<td>Myocyte &quot;O2&quot;-generation</td>
<td>+</td>
</tr>
<tr>
<td>Cytokine generation</td>
<td>+</td>
</tr>
<tr>
<td>&quot;O2&quot;-signalling</td>
<td>+</td>
</tr>
<tr>
<td>Activates KATP channels</td>
<td>+</td>
</tr>
<tr>
<td>Attenuates mPTP threshold</td>
<td>+</td>
</tr>
<tr>
<td>Tissue realalkalinization</td>
<td>?</td>
</tr>
<tr>
<td>Activates G-protein-coupled receptors</td>
<td></td>
</tr>
<tr>
<td>Adenosine mediated</td>
<td>+</td>
</tr>
<tr>
<td>Opioid mediated</td>
<td>+</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>+</td>
</tr>
<tr>
<td>Protease activated receptor 2 (PAR2)</td>
<td>?</td>
</tr>
<tr>
<td>Molecular effects pathways</td>
<td></td>
</tr>
<tr>
<td>PKC</td>
<td>+</td>
</tr>
<tr>
<td>P3 kinase–Akt</td>
<td>+</td>
</tr>
<tr>
<td>GSK3</td>
<td>+</td>
</tr>
<tr>
<td>ERK1/2-MEK</td>
<td>–</td>
</tr>
<tr>
<td>P70S6K</td>
<td>+</td>
</tr>
<tr>
<td>eNOS</td>
<td>+</td>
</tr>
<tr>
<td>STAT</td>
<td>+</td>
</tr>
</tbody>
</table>

± indicates controversial data; ? indicates no available data.

In a rat model of 30 min left coronary artery occlusion and 3 h of reperfusion, Granfeldt et al.35 showed that systemic neutrophil depletion alone, achieved by rabbit anti-rat anti-serum injected 7.5 h before the onset of ischaemia, was associated with the same reduction in infarct size as that observed in non-neutropenic postconditioned hearts. However, when Postcon was performed in neutrophil depleted rats, only a slight and (insignificant) further reduction in infarct size was observed. A direct inhibitory effect on neutrophil activation by Postcon was observed in an anesthetized canine model of LAD occlusion–reperfusion. Blood taken from the anterior interventricular vein draining the area at risk at 3 and 24 h of reperfusion was immediately analysed for superoxide anion production using luminol-enhanced chemiluminescence. In abruptly reperfused canine hearts, the chemiluminescence signal increased five-fold after 3 h and 16-fold after 24 h of reperfusion relative to baseline before ischaemia. However, in postconditioned hearts, the signal was not significantly different from baseline at 3 and 24 h of reperfusion. Indeed, this direct inhibition of neutrophil activation is intriguing in that the neutrophils must either be affected systematically by circulating inhibitory factors released by...
postconditioned myocardium (such as adenosine), or locally as they pass through the area at risk myocardium. The mechanisms underlying both short- and long-term inhibitions of neutrophil function by Postcon have yet to be elucidated.

4.2.4 Pharmacological Postcon
The potential benefit of Postcon applicable to patients undergoing either primary angioplasty or cardiac surgery, while its applicability is less clear to patients receiving thrombolytic therapy in which the onset of reperfusion (and hence the Postcon ‘window’) is not known. Studies have identified certain drugs, when given at reperfusion or re-oxygenation, that reduce reperfusion injury, an approach termed ‘pharmacological Postcon’. Indeed, pharmacological intervention at the onset of reperfusion in experimental models has been tested for years, but none has been adopted for routine clinical practice clinically.

Agents such as inhalational anaesthetics, bradykinin, the chemotherapeutic agent cyclosporine A, erythropoietin (EPO), authentic nitric oxide (NO) and NO donors, hydrogen sulfide, and adenosine have demonstrated cardioprotective effects when given at reperfusion in experimental studies. Adenosine infusion at the onset of reperfusion by either coronary route, intravenous infusion, or intra-atrial infusion has demonstrated cardioprotective effects in part by attenuating neutrophil–endothelium interactions.

EPO, inhalational anaesthetics, bradykinin, and a host of other agents activate the so-called reperfusion injury survival kinases (RISK) pathway. These kinases, including PI3-K and the MAP kinase ERK1/2 ultimately inhibit the myocardial transition permeability pore (mPTP). Another strategy used by Jiang et al. to enhance Postcon with concomitantly delivered pharmacological agents. The combination of Postcon and a protease activated receptor-2 agonist, both applied at the onset of reperfusion, reduced infarct size to a greater extent than either intervention alone. Hence, pharmacological agents may not only mimic Postcon, but also they can enhance its effects by triggering different pathways, lowering the threshold of Postcon’s effects, or extending its effects beyond the early reperfusion phase. The potential enhancement of or mimicking of Postcon with pharmacological agents will be fertile ground for future research.

4.2.5 Remote Postcon
‘Remote’ Postcon refers to inducing the Postcon stimulus by applying the re-occlusion and reperfusion algorithm to an organ distant from the ischaemic site, for example, in the renal artery or peripheral limbs. Remote Postcon was first reported in 2005 by Kerendi et al. who demonstrated that a 5 min occlusion of the renal artery followed by reperfusion applied 1 min before reperfusion of the left coronary artery in a rat model of ischaemia–reperfusion reduced infarct size by nearly 50% compared with a control group with abrupt reperfusion. This remote protection was abrogated by an adenosine receptor antagonist. Andreka et al. reported similar myocardial infarct sparing after applying iterative limb ischaemia–reperfusion as the remote Postcon stimulus in a porcine model of coronary artery occlusion. These observations suggest that Postcon releases soluble and circulating mediators such as adenosine, that can sustain the transit time between a remote organ and an ischaemic-reperfused organ. Indeed, remote Postcon has been applied in clinical settings (see below).

4.3 Postcon in models of comorbidities
Unfortunately, the majority of the studies investigating the effects of Postcon have been performed in models of healthy young animals without comorbidities. This difference between experimental models and clinical patient population draws into question whether Postcon (and most other cardioprotective strategies) is effective in human populations with comorbidities that affect the tolerance to ischaemia–reperfusion.

4.3.1 Ageing
Age is an important prognostic indicator of cardiovascular death, and the incidence of sudden cardiac death increases with age in both men and women. With increasing age, endothelial dysfunction is more prevalent, and this dysfunction is thought to be related to an increase in oxidative stress, an increased production of oxidants, and a concomitant attenuation of endogenous antioxidant defenses or NO production.

Studies by Lauzier et al. and Dow et al. using an accelerated senescence-prone mouse model reveal that Postcon was still protective in aged models, but may require a higher threshold. Nevertheless, the ideal Postcon protocol in young and aged models remains to be determined.

4.3.2 Hypercholesterolaemia
Hypercholesterolaemia is regarded as a key risk factor for myocardial infarction secondary to endothelial dysfunction and vascular injury. Conflicting results have been presented regarding the efficacy of preconditioning in models with hypercholesterolaemia. Similarly, the efficacy of Postcon in hypercholesterolaemic animals is also inconsistent. In a report by Donato et al. using an in vitro rabbit model fed a cholesterol supplemented diet for 4 weeks; the cardioprotective effect of Postcon was preserved relative to a control cohort. However, in three other studies, a cardioprotective effect of Postcon was not observed in aged rats or C57Bl6/J mice. However, Postcon was still protective when a protocol of three cycles of 5’s ischaemia–reperfusion was applied at the onset of reperfusion. This suggests that Postcon may still be protective in aged models, but may require a higher threshold. Nevertheless, the ideal Postcon protocol in young and aged models remains to be determined.
99% of the area at risk and may not have been salvageable by any intervention. More experimental work needs to be done to understand whether Postcon is effective in hypercholesterolaemic models that mimic the human condition.

### 4.3.3 Diabetes and obesity

The number of patients hospitalized due to diabetes and obesity is increasing, and globally both diabetes and obesity are major risk factors for acute myocardial infarction. Animal studies exploring whether the heart is more susceptible to ischaemia–reperfusion due to diabetes are conflicting, but clinical studies demonstrate a worse outcome after myocardial infarction in diabetic patients.\(^1\)\(^2\) In a recent paper by Bouhidel et al.,\(^1\)\(^2\) the effect of Postcon in the genetically obese ob/ob murine model was investigated. This strain of mice is leptin-deficient, leading to hyperphagia, obesity, hyperglycaemia, and hypercholesterolaemia. In the ob/ob cohort, the infarct size was greater when compared with a wild-type cohort, and a protective effect of Postcon was not observed. However, leptin is known to have cardioprotective effects by activating the salvage kinase pathway, and whether the results obtained by Bouhidel et al.,\(^1\)\(^2\) are due to the deficiency in an endogenous cardioprotective agent is unknown.\(^1\)\(^2\) Similar results were reported by Wagner et al.,\(^1\)\(^4\) using Wistar-Ottawa-KarlsruheW rats characterized by the triple threat of obesity, dyslipidaemia, and hyperinsulinaemia. In an abstract by Przyklenk et al.,\(^1\)\(^5\) the effect of Postcon was investigated in streptozotocin-induced diabetes in mice. The protective effect of Postcon was lost in the diabetic mice at both 2 and 4 weeks. However, when normal blood glucose levels were restored by an islet cell transplantation, the effect of Postcon was re-established.\(^1\)\(^2\) Whether the restoration of normal blood glucose or insulin levels is responsible for re-establishing cardioprotection is currently unknown, and the clinical relevance of this model is not clear since blood glucose levels in patients seldom reach this high level, and high glucose levels often co-exist with insulin treatment.

### 5. Clinical studies of Postcon

#### 5.1 Postcon in PCI

Despite the few experimental studies showing no benefit from Postcon, the clinical data obtained to date are remarkably consistent regarding the protective effect of Postcon. Seven clinical studies have evaluated the effect of Postcon in PCI; six of these were prospective randomized trials with a total of 244 patients (summarized in Table 3).\(^1\)\(^6\)–\(^1\)\(^3\)

The subjects reflect a cross-section of acute myocardial infarction patients with similar age, gender, and comorbidity demographic profiles. The first reports were published from Laskey et al.,\(^1\)\(^6\) and Staat et al.,\(^1\)\(^7\) Staat et al.,\(^1\)\(^7\) used four episodes of alternating reperfusion and re-occlusion by inflating the angioplasty balloon after reperfusion in 30 patients with documented acute myocardial infarction presenting within 6 h of symptoms onset; each Postcon phase lasted for 1 min. The Postcon protocol was associated with a 36% reduction in creatine kinase levels assessed as the area under the curve over the first 72 h after intervention. Blush grade, a marker of myocardial reperfusion, was significantly higher than in control PCI patients. Since the studies

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**Table 3** A summary of the results from the seven clinical trials using postconditioning in PCI

<table>
<thead>
<tr>
<th>Investigator/contact</th>
<th>Ischaemia time (h)</th>
<th>Patients</th>
<th>Protocol</th>
<th>Results Postcon vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laskey et al.,(^1)(^6)</td>
<td>Control: 5.7 Postcon: 6.0</td>
<td>Control (n=10) Postcon (n=14)</td>
<td>Two cycles of 90 s inflation/deflation each separated by 5 min deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Staat et al.,(^1)(^7)</td>
<td>Control: 5.4 Postcon: 5.0</td>
<td>Control (n=12) Postcon (n=17)</td>
<td>Three cycles of 30 s inflation/deflation each separated by 60 s deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Ma et al.,(^1)(^8)</td>
<td>Control: 5.4 Postcon: 5.0</td>
<td>Control (n=13) Postcon (n=17)</td>
<td>Four cycles of 60 s inflation/deflation each separated by 60 s deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Darling et al.,(^1)(^9)</td>
<td>Control: 5.4 Postcon: 5.0</td>
<td>Control (n=13) Postcon (n=17)</td>
<td>Four cycles of 30 s inflation/deflation each separated by 60 s deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Yang et al.,(^1)(^2)</td>
<td>Control: 5.4 Postcon: 5.0</td>
<td>Control (n=13) Postcon (n=17)</td>
<td>Four cycles of 30 s inflation/deflation each separated by 60 s deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Thibault et al.,(^1)(^3)</td>
<td>Control: 5.4 Postcon: 5.0</td>
<td>Control (n=12) Postcon (n=14)</td>
<td>Three cycles of 30 s inflation/deflation each separated by 60 s deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
<tr>
<td>Laskey et al.,(^1)(^6)</td>
<td>Control: 5.4 Postcon: 5.0</td>
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<td>Two cycles of 90 s inflation/deflation each separated by 5 min deflation/deflation</td>
<td>Coronary flow reserve; 1ST-segment resolution</td>
</tr>
</tbody>
</table>

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\(^1\) Coronary flow reserve; \(^2\) 1ST-segment resolution; \(^3\) Flow velocity; \(^4\) PEAK-CK; \(^5\) CK-MB; \(^6\) Wall motion (8 weeks); \(^7\) Average 7; \(^8\) PEAK-CK; \(^9\) Ejection fraction (echo) at 1 year; \(^10\) Resolution of ST-segment changes; \(^11\) Doppler velocimetry in infarct-related artery; \(^12\) Not prospective/randomized

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by Laskey et al.\textsuperscript{126} and Staat et al.\textsuperscript{127} several studies have confirmed the clinical efficacy of Postcon to reduce infarct size estimated by enzyme release\textsuperscript{129,132} or thallium\textsuperscript{201} single photon emission computed tomography and to improve post-ischaemic left ventricular function\textsuperscript{129} six months to a year after intervention.\textsuperscript{133} Although some investigators and clinicians have suggested that the inflation and deflation of the angioplasty balloon could induce damage to the vascular endothelium, no clinical study has yet reported such complications. However, all studies to date are relatively small proof-of-concept studies. Large clinical trials are warranted; a meta-analysis of the currently completed trials would provide more insight into the clinical efficacy in the interim. Any advantages in clinical outcomes (mortality, morbidity, incidence of heart failure, recovery of productive work days, costs of hospitalization, and treatment) of Postcon have not yet been determined.

Cyclosporine A is a potent inhibitor of the mPTP and has been used in experimental studies to attenuate lethal opening of the mPTP during reperfusion.\textsuperscript{93,134} Recently, a clinical study including 58 acute myocardial infarction patients explored the effect of cyclosporine A on reperfusion injury.\textsuperscript{135} Cyclosporine (25 mg/mL) was administered \textless{} 10 min before stenting, thereby mimicking pharmacological Postcon. Cyclosporine A treatment resulted in a 40% reduction in creatine kinase and a 20% reduction in the area of hyperenhancement on magnetic resonance imaging compared with a control group with only PCI. Even though this is a relatively small study, it once again confirms the existence of reperfusion injury in the setting of acute myocardial infarction and opens up new pharmacological targets (mPTP) for inhibition of reperfusion injury by ‘pharmacological Postcon’ as predicted by experimental studies.\textsuperscript{136}

### 5.2 Postcon in cardiac surgery

The question naturally arises whether the innate cardioprotection of Postcon can protect the myocardium during cardiac surgery in which other forms of cardioprotection are used concomitantly, i.e., hypothermia and cardioplegia. There is significant mortality and morbidity in cardiac surgery despite the use of cardioplegia and adjuncts.\textsuperscript{137,138} Postcon can potentially marshal the cardioprotection of endogenous autacoids and other mechanisms to reduce the multiple manifestations of reperfusion injury after removal of the cross-clamp and reanimation. In a sense, the ‘hot shot’ reperfusate\textsuperscript{139} that precedes cross-clamp removal\textsuperscript{140} can be altered to include the Postcon algorithm, and to which drugs can be used adjunctively to attenuate reperfusion injury. As such, Postcon can be applied by (i) briefly removing and reapplying the cross-clamp in a cyclical manner to induce reperfusion and ischaemia; and/or (ii) delivering a reperfusate via the cardioplegia line in a cyclical manner, i.e. in 30–60 s pulses that mimic the cyclical perfusion pattern of Postcon. Many surgeons may be hesitant to practice repeated aortic clamping, particularly in elderly patients who have some degree of atheromatous material in the aorta, which carries the fear of showering embolic debris distally and precipitating stroke and focal ischaemia. Nonetheless, cyclical removal and reapplication of the cross-clamp has been used in paediatric\textsuperscript{141} and adult patients.\textsuperscript{142} In surgical correction of tetrology of Fallot in children, Luo et al.\textsuperscript{141} used two 30 s cycles of aortic declamping (reperfusion) and reclamping (ischaemia) before complete reperfusion was restored. Plasma troponin I and CK levels were significantly lower than a control cohort at 4 h post-reperfusion. In the adult patients undergoing valve replacement using cardiopulmonary bypass and a crystalloid cardioplegia solution, plasma CK-MB was significantly lower in the postconditioned hearts compared with a control group with standard cardioplegia and resuscitation protocols at 4 and 8 h after aortic declamping. Both surgical studies demonstrate that cardioplegic arrest, and de-clamping increases biomarkers for myocardial morphological injury despite the use of cold blood cardioplegia and that Postcon attenuated the release of these biomarkers. The Postcon protocol is reported to be without complications; however, the number of patients was small and needs to be verified in larger multi-centre trials. Hence, the reperfusion phase can be altered to apply Postcon and combination therapy to protect the myocardium in cardiac surgery. Postcon may be useful in other surgical procedures such as correction of ascending aortic aneurysm that are associated with significant cardiac dysfunction and mortality.\textsuperscript{143}

### 6. Concluding remarks and future directions

Preconditioning and Postcon both harness innate cardioprotective mechanisms. Both seem to engage similar molecular mechanisms such as G-protein-coupled receptors and kinases that converge on the mPTP, although with subtle differences\textsuperscript{144} and at a different times (before vs. after ischaemia).

A primary limitation of preconditioning is the difficulty in applying it to clinical situations in which the ischaemic episode cannot be predicted; surgery is one example in which IPC and RIPC can be applied since the ischaemic event during cardioplegia is predictable. Postcon, on the other hand, can be applied even when the ischaemic event is not predictable. The brief Postcon algorithm will not unduly delay catheterization laboratory procedures and will not impact the door-to-balloon time which has now become a measure of a hospital’s efficiency at handling the acute myocardial infarction patient. Indeed, if Postcon reduces infarct size and essentially reduces a 6 h infarct size to a 3 h infarct, the cardiologist may well be less preoccupied with door-to-balloon time and more concerned with how reperfusion is initiated. Since clinicians are reticent to induce ischaemia, heat stress, or endotoxaemia as pre- or postconditioning stimuli, pharmacological approaches to harness the protective effects of both IPC and Postcon may be more clinically applicable. Future studies are required to identify pharmacological agents which, when given either before or after ischaemia, stimulate not only the molecular cascades that are part of the endogenous salvage kinase pathways, but also to inhibit the more physiological events of reperfusion such as inflammation, the sodium/hydrogen exchanger and the mPTP. Indeed, the combination of pre- and/or Postcon and pharmacological agents should be explored to overcome the potential limitations of either cardioprotective manoeuvre. The basic question of what is the optimal duration of ischaemia–reperfusion algorithm (and why) in Postcon has not been answered. Furthermore, the ultimate importance of the RISK pathways as a target of conventional or pharmacological pre- and...
postconditioning in the in vivo model (animal and human) should be confirmed in the light of data from Skyschally et al.\textsuperscript{51} showing a discordance between expression of these kinases and infarct size reduction. In addition, studies should resolve the differences in efficacy of Postcon between experimental models and human subjects with comorbidities. Finally, larger clinical trials are necessary to confirm the comparative efficacy of preconditioning and Postcon in surgical and non-surgical revascularization settings and transplantation.

Conflict of interest: A.G. and D.J.L. have no conflict of interest. J.V.-J. has patents pending on the concept of postconditioning and pharmacological postconditioning. He is also founder of Reperfusion Therapeutics, Inc. (RTx) which is a research and development company exploring clinical applications of postconditioning and other cardioprotective strategies, and has equity in RTx.

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References


Translation of pre- and postconditioning to humans


