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SPOTLIGHT REVIEW

Endogenous cardioprotection by ischaemic postconditioning and remote conditioning

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Abstract Persistent myocardial ischaemia causes cell death if not rescued by early reperfusion. Millions of years in nature's laboratory have evolved protective responses that 'condition' the heart (and other tissues) to adapt to stressors, and these responses are applicable to the relatively new societal stress of myocardial ischaemia and reperfusion injury. Conditioning can be applied before (preconditioning), during (perconditioning), or after (postconditioning) the ischaemic stressor by imposing short periods of non-lethal ischaemia separated by brief periods of reperfusion. This conditioning protects multiple cell types and induces or rebalances a number of physiological and molecular pathways that ultimately attenuate necrosis and apoptosis. The seemingly disparate pathways may converge directly or indirectly on the mitochondria as a final effector, but other pathways not affecting mitochondria broaden the mechanisms of cardioprotection. The potential downsides of imposing even brief ischaemia directly on the heart somewhat tempered the enthusiasm for applying conditioning stimuli to the heart, but this hurdle was surmounted by applying ischaemia to remote organs and tissues in pre-, per-, and postconditioning. Although the clinical translation of remote per- and postconditioning has been rapid compared with classical preconditioning, there are numerous basic questions that require further investigation, and wider adoption awaits large-scale randomized clinical trials. Pharmacological mimetics may provide another important therapeutic approach by which to treat evolving myocardial infarction.

Keywords Reperfusion injury • Preconditioning • Postconditioning • Mitochondrial permeability transition pore • Myocardial infarction • Apoptosis • Endothelium

This article is part of the Spotlight Issue on: Reducing the Impact of Myocardial Ischaemia/Reperfusion Injury

1. Introduction

It is firmly established that the myocardium can be salvaged by early reperfusion.[1] Percutaneous coronary intervention (PCI) has become an important treatment modality to initiate that reperfusion. Smaller infarct size has been associated with lower mortality;[2] reduction of infarction size by pharmacological therapy in acute myocardial infarction patients undergoing percutaneous coronary intervention stenting has been associated with improved left ventricular performance and clinical outcomes (death, reinfarction).[3] Therefore, a reduction of infarct size is a clinically valuable strategy. However, reperfusion itself may contribute to the pathophysiology of infarction and post-ischaemic complications (reperfusion injury). Reperfusion injury may be responsible for up to 50% or more of the ultimate infarct size[4] and is an important contributor to post-surgical mortality and morbidity as well.[5] Clinically, the extent of myocardial salvage by early reperfusion may not be realized because of cell injury and death initiated by reperfusion itself.

The numerous mechanisms that contribute to myocardial reperfusion injury have been reviewed in depth elsewhere,[4,6] and select mechanisms are discussed in other articles in this focused issue. It is important to recognize that multiple mechanisms are initiated at the onset of reflow and that these mechanisms may act in concert to contribute to necrosis, apoptosis, and dysfunction of organelles, the coronary vascular endothelium, and contractile function. This review will discuss (i) the algorithm and mechanisms of ischaemic postconditioning from data derived from experimental studies, (ii) remote conditioning applied before, during, and after ischaemia, (iii) the clinical studies on direct and remote post- and perconditioning. The sections on clinical studies appear under the relevant conditioning stimulus, rather than discussing them under one section. However, clinical studies are summarized in a table for convenience. Where possible,

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commonalities between pre-, per-, and postconditioning will be discussed, thereby placing the three cardioprotective manoeuvres under the single umbrella of 'conditioning' responses to ischaemia–reperfusion.

2. General comments on 'conditioning' of the ischaemic myocardium

Conditioning of the myocardium describes the stimulation of innate cardioprotective mechanisms by short periods of non-lethal ischaemia that target lethal tissue injury caused by a longer period of ischaemia. The conditioning stimulus can be applied before (preconditioning), during (perconditioning), or immediately after (postconditioning) the longer 'index' ischaemia. Specifically to the heart, the original studies on preconditioning by Murry *et al.*[7] demonstrated that local intra-organ conditioning exerted by brief periods of coronary artery occlusion applied before the prolonged lethal ischaemia reduced infarct size. However, it has also been shown that ischaemia applied to a distant organ before index ischaemia (remote preconditioning) reduces infarct size. In this article, we will focus on ischaemic postconditioning (PostC) and remote preconditioning (rIPC), perconditioning (rPerC), and postconditioning (rPostC). Pharmacological postconditioning will be discussed elsewhere in this focused series.

3. Ischaemic postconditioning

3.1 Experimental studies

The PostC algorithm involves applying alternating cycles of brief reperfusion (or reoxygenation) interrupted by brief ischaemia (or hypoxia–anoxia) starting at the end of the index ischaemia. This manoeuvre exerts its effects during the immediate PostC period without affecting the pathophysiology of ischaemia. This clearly differs from the time course during which either ischaemic preconditioning (IPC) or perconditioning (PerC) exert cardioprotection, in which there may be a component of biochemical adaptation of the myocardium to ischaemia.

There are three aspects to the PostC cycle: the duration of the initial reperfusion phase, the duration of each subsequent ischaemia and reperfusion cycle, and the number of cycles applied. The PostC procedure should be initiated immediately upon the onset of reperfusion; the first phase of reperfusion is considered the first cycle. The duration of the index ischaemia itself may influence the effect of PostC. PostC may increase infarct size after brief periods of ischaemia while reducing infarct size after longer periods of index ischaemia.[8] In addition, there may be a duration of occlusion beyond which PostC does not reduce infarct size.[9] Finally, the benefits of PostC are lost if the onset of the first reperfusion cycle is delayed.[10]

A reduction in infarct size is the signature endpoint of PostC. In 2003, Zhao *et al.*[11] first reported a ~50% reduction of infarct size (relative to the area at risk) by PostC in an acute canine model of 60 min left anterior descending artery (LAD) occlusion followed by 3 h of reperfusion. Since this initial report, numerous studies have confirmed that PostC reduces infarct size in all species tested (reviewed in Vinten-Johansen *et al.*[12]), including humans (summarized in Hansen *et al.*[13] and Hausenloy and Yellon[14]). PostC has been confirmed in *in vitro*[15] and *ex vivo*[16] models. As with most cardioprotective manoeuvres, there are both positive and negative studies (reviewed in Vinten-Johansen *et al.*[12]). The usual suspects may be responsible for such variability, i.e. species variability, differences in the PostC protocol, and types of anaesthesia, but may also include gender, age, and presence of co-morbidities. There is some discrepancy on the magnitude of cardioprotection achieved by PostC relative to IPC. *In vivo* studies suggest that PostC exerts a similar reduction in either infarct size or enzyme release with IPC and PostC.[17,18] In addition, there may be a duration of ischaemia beyond which PostC is not effective, a so-called threshold phenomenon. Extending this threshold to exert cardioprotection after longer durations of ischaemia may require a greater number of cycles, longer durations of reperfusion or ischaemia cycles, or the addition of adjunctive drugs. However, the description and mechanisms of such a threshold remain unresolved.

PostC has also been reported to reduce apoptosis *in vivo*.[19] Taki *et al.*[20] reported that PostC attenuated the uptake of ^{99m}Tc-annexin V in the area-at-risk myocardium. *In vivo*, PostC attenuated known triggers of apoptosis such as oxidants [reactive oxygen species (ROS), peroxynitrite $OONO^-$] and Ca^{2+} overload, and by attenuating pro-apoptotic pathways[21,22] and regulatory factors.[23] PostC rebalances the apoptotic regulatory system by increasing anti-apoptotic regulators (Bcl-2)[23] and pathways [Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3)[24]]. However, few of the above studies have confirmed apoptosis by morphological criteria such as appearance of apoptotic bodies. Future studies should include this morphological endpoint.

3.1.1 Mechanisms of postconditioning

The concept of triggers, mediators, and effectors used in describing IPC is also applicable to PostC. The proximal triggers of PostC include autacoid substances released during PostC that activate cell surface G-protein-coupled receptors or other receptors (*Figure 1*). The transduction mechanisms then activate intermediary substances or pathways that converge on effectors which may include the mitochondrial permeability transition pore (mPTP) and K_{ATP} channel. There are also physiological effects, such as maintenance of tissue acidosis, that may be independent of these molecular pathways that exert protection. Therefore, multiple mechanisms are engaged by PostC, as summarized in *Figure 1*. Overall, the cardioprotective mechanisms can be divided into physiological mechanisms and prosurvival molecular pathways. The multiplicity and interactions between the physiological and molecular pathways triggered by PostC may explain why PostC successfully attenuates reperfusion injury compared with interventions that trigger a single mechanism or target.

3.1.2 Physiological mechanisms

Adenosine, bradykinin, and endogenous opioids may act as the initial trigger for PostC. These autacoids are present during and modulated by PostC.[25,26] Adenosine was the first such autacoid to be associated with PostC.[25] All four types of adenosine receptors have been implicated in PostC. However, data on receptor subtype involvement are inconsistent. While a specific A_1 receptor inhibitor did not abolish infarct size reduction by PostC,[25] specific knock-out of the A_1 receptor abolished PostC.[27] PostC can also be triggered by activation of other receptors, including the bradykinin B_2 receptor, opioid receptor, and the sphingosine-1-phosphate receptor.[28,29]

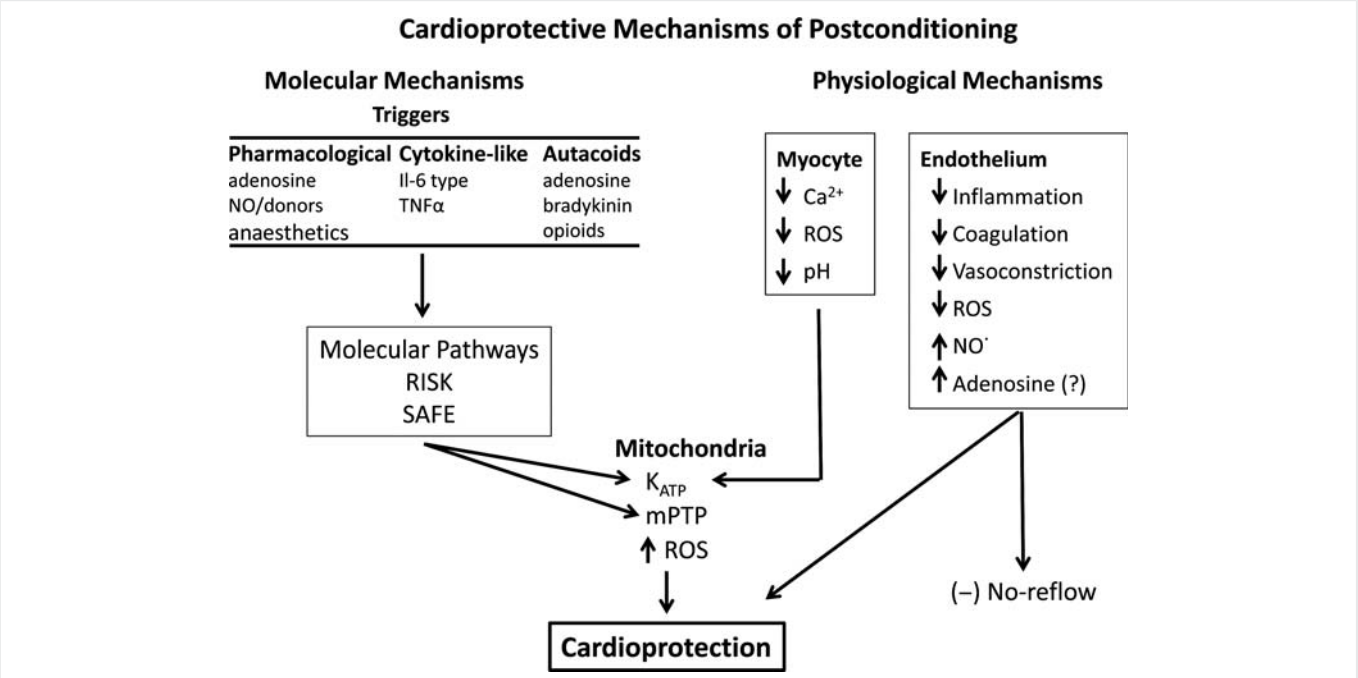

Figure 1 Overview of physiological and molecular mechanisms involved in postconditioning. The mechanisms are divided into two general categories: molecular and physiological. In the molecular category, the scheme of triggers, mediators, and effectors developed for preconditioning is also applicable to postconditioning, and likely to perconditioning and their respective remote counterparts. Note that elements of both categories converge on the mPTP, but this is not the sole factor leading to cardioprotection. RISK, reperfusion injury salvage kinase; SAFE, survivor activating factor enhancement; mPTP, mitochondrial permeability transition pore; K_{ATP}, ATP-sensitive potassium channels; ROS, reactive oxygen species; NO, nitric oxide.

3.1.3 Protection of the coronary vascular endothelium in postconditioning

The coronary vascular endothelium is important in regulating blood flow and maintaining a balance in local haemostasis (coagulation) and inflammation, in part through the release of endogenous factors such as adenosine, nitric oxide ($NO^{\cdot}$), endothelin-1, leucotrienes, and thromboxane A_2. Predictably, dysfunction of the coronary vascular endothelium results in (i) local vasoconstriction secondary to the impaired release of $NO^{\cdot}$ and increased release of vasoconstrictors; $NO^{\cdot}$ has potent anti-neutrophil effects which may be lost if $NO^{\cdot}$ generation is reduced or if $NO^{\cdot}$ is neutralized by $^{-}O_2^{\cdot}$ to form $ONOO^{-}$, (ii) increased coagulation and production of thrombotic material, (iii) activation of neutrophils and the inflammatory cascade, in part due to impaired $NO^{\cdot}$ bioavailability, and (iv) loss of barrier function with increased fluid and solute extravasation. The activation of neutrophils during ischaemia–reperfusion has been shown to contribute to the pathophysiological development of infarct size.[30,31] A study by Granfeldt *et al.*[32] reported that PostC directly inhibits superoxide anion production by neutrophils sampled from the area-at-risk coronary venous drainage.

PostC attenuates coronary vascular endothelial dysfunction observed after the index ischaemia.[11] Increased bioavailability of $NO^{\cdot}$ after PostC may be due to increased phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177,[9] reduced oxidant generation,[15,17] and preservation of endogenous antioxidants.[33] The ability of PostC to attenuate neutrophil adhesion and extravascular accumulation mirrors the cardioprotective effects of $NO^{\cdot}$ and adenosine.[25] PostC also attenuates the appearance of pro-inflammatory mediators in plasma after ischaemia–reperfusion.[19]

3.1.4 Maintaining tissue acidosis in postconditioning

Reperfusion quickly reverses tissue acidosis chiefly by activating the sarcolemmal Na^+/H^+ exchanger, and the Na^+ bicarbonate co-transporter, which favours the accumulation of Na^+ and secondarily Ca^{2+}, the latter by reverse activity of the Na^+/Ca^{2+} exchanger. The rapid normalization of pH, accumulation of intracellular Ca^{2+}, and a build-up of ROS increase opening of the mPTP. Heusch[34] first postulated that the cardioprotection of PostC was related to maintaining tissue acidosis. The studies of Cohen *et al.*[35] suggest that maintaining acidosis during early reperfusion inhibits opening of the mPTP. Reperfusing the ischaemic myocardium with acidic (re)perfusate mimicked PostC protection in isolated rabbit hearts[35] and pigs[36] while an alkalotic perfusate blocked PostC protection.[35] Indeed, PostC maintained tissue acidosis during early reperfusion and thereby delayed the onset of realkalinization.[12,37] Neutralizing tissue pH with $NaHCO_3$ not only abrogated the infarct-sparing effects of PostC, but also reduced the phosphorylation of the survival kinases Akt and ERK in the myocardium.[38] A recent study using[31] P-NMR demonstrates that PostC delayed intracellular pH recovery, reduced protease calpain-dependent α-fodrin proteolysis, improved left ventricular contractility, and decreased LDH release and infarct size. Maintaining acidosis during early reperfusion may also be related to inhibition of the Na^+/H^+ exchanger via a protein kinase G (PKG)-dependent pathway.[39]

3.1.5 Activation of survival pathways in postconditioning

There are two major molecular pathways that are recruited in PostC: the reperfusion injury salvage kinase (RISK) pathway and survivor

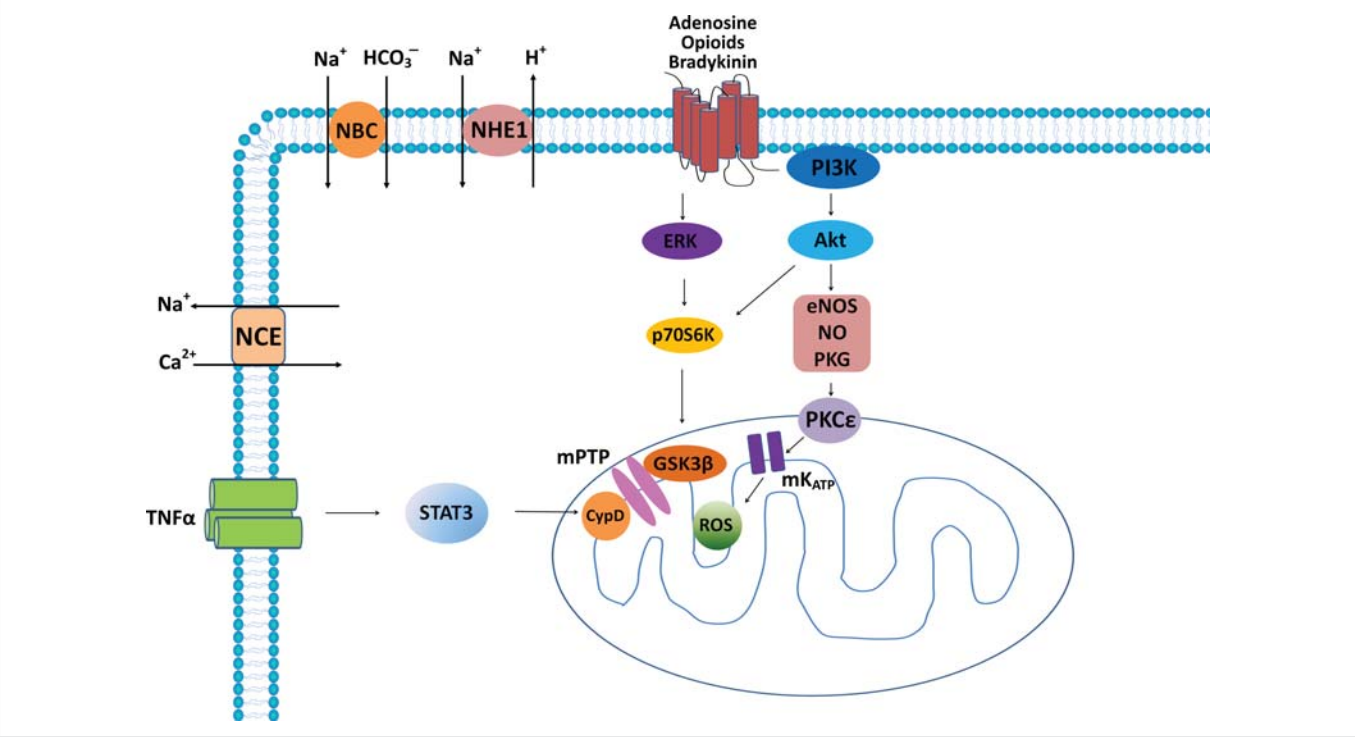

Figure 2 Major signalling pathways identified in ischaemic postconditioning (PostC). Several autacoid substances (adenosine, bradykinin, opioids, TNFα) are produced endogenously and act as triggers in PostC. These autacoids bind to G-protein-coupled receptors (GPCR) and stimulate RISK pathway which recruits PI3K/Akt and ERK, activates PKCε through eNOS/NO/PKG, and phosphorylates (inhibits) GSK3β, thereby delaying opening of mPTP. PKCε may stimulate ROS generation through activating mK_{ATP} channels, and consequentially inhibit mPTP opening. The RISK pathway also induces some other anti-apoptosis and anti-necrosis pathways. An alternative SAFE pathway recruits TNFα, which binds to TNF receptor resulting in STAT3 activation. STAT3 targets CYP-D leading to mPTP inhibition. PostC also inhibits the Na^+/H^+ exchanger in a PKG-dependent fashion and reduces Ca^{2+} accumulation, because the sarcolemmal Na^+/H^+ exchanger and the Na^+ bicarbonate co-transporter are activated at the onset of reperfusion, which causes intracellular Ca^{2+} accumulation through reverse activation of the Na^+/Ca^{2+} exchanger and induces cell apoptosis and necrosis. CYP-D, cyclophilin-D; eNOS, endothelial nitric oxide synthase; ERK, p42/p44 extracellular-regulated kinase; GPCR, G-protein-coupled receptor; GSK3β, glycogen synthase kinase-3β; mPTP, mitochondrial permeability transition pore; mK_{ATP}, mitochondrial K_{ATP} channel; NBC, Na^+ bicarbonate co-transporter; NCE, Na^+/Ca^{2+} exchanger; NHE1, Na^+/H^+ exchanger; NO, nitric oxide; PKCε, protein kinase C subtype ε; PKG, cGMP-dependent protein kinase; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription-3; TNFR, TNF receptor.

activating factor enhancement (SAFE) pathway.[40,41] The RISK pathway activated by PostC salvages the myocardium in most of mammal species except the porcine model.[42] PostC induces phosphorylation of phosphatidylinositol 3-kinases (PI3K) and extracellular signal-regulated kinase (ERK)1/2, and subsequently activates the downstream eNOS and p70S6 kinase, and inhibits (phosphorylates) glycogen synthase kinase-3β (GSK3β) (*Figure 2*). These molecules are involved in the RISK pathway.[40,41] A new study shows that the p85 subunit of PI3K is co-localized with caveolin 3 through MG53 which is also required for PostC.[43] The RISK pathway also includes p38MAPK and c-Jun N-terminal kinase (JNK), PKG, and protein kinase C (PKC).[40,41]

STAT3 is a member of the SAFE pathway and has previously been demonstrated to play a role in IPC.[44] A recent study shows that STAT3 is also involved in PostC because: (i) PostC increases STAT3 phosphorylation; (ii) JAK2 inhibition not only reduced STAT3 phosphorylation, but also abolished infarct reduction by PostC; and (iii) STAT3 deletion abolished cardioprotection of PostC.[45] Which molecules stimulate STAT3 phosphorylation during PostC is not well understood, but the pro-inflammatory cytokine tumour necrosis factor (TNFα) is a strong candidate.[46] Hence, the TNFα–TNF receptor type 2 (TNFR2)–STAT3 axis may underlie, in part, the cardioprotection of PostC by the SAFE pathway.[46] The final target of both pathways appears to be the mitochondria, where mPTP may be the final effector.[40,41]

3.1.6 The role of mitochondria in postconditioning

The above molecular signalling pathways may converge on the mitochondria. Mitochondria play a central role in lethal ischaemia–reperfusion injury largely by actions of the mPTP.[47] Many studies suggest that PostC delays mPTP opening potentially by reducing superoxide anion generation and intracellular Ca^{2+} overload, factors that stimulate opening of the mPTP.[48] Hearts deficient in CYP-D (CYP-$D^{-/-}$), an important regulatory subunit of the mPTP that is targeted by CsA, are resistant to infarct reduction conferred by PostC.[49] Therefore, PostC likely prevents opening of mPTP through regulating CYP-D, by decreasing ROS and Ca^{2+} accumulation, and by maintaining tissue acidosis during early reperfusion. Both RISK and SAFE pathways are linked to regulation of the mPTP in PostC. The direct regulators of the mPTP may be GSK3β (RISK)[50] and STAT3 (SAFE)[51] (*Figure 2*).

3.1.7 Mitochondrial K_{ATP} channels and ROS in postconditioning

The mitochondrial K_{ATP} (mitoK_{ATP}) channel also plays a role in PostC.[52] PostC induces active signalosome fractions that open mitoK_{ATP} channels in isolated-perfused rat hearts. The effect on the mitoK_{ATP} channel is PKG-dependent and may also be related to PKCε (*Figure 2*).[53] MitoK_{ATP} channel activation leads to generation of signalling ROS which may act as a cardioprotective signal.[54] PostC stimulates the generation of small concentrations of ROS at the onset of reperfusion which subsequently signal to inhibit the mPTP, probably through another type of PKCε.[55] On the other hand, PostC reduces the deleterious respiratory burst of ROS and salvages cardiomyocytes during early reoxygenation.[15] How the balance of these two actions is struck by PostC is not known.

3.1.8 Postconditioning in co-morbid models

Co-morbidities such as diabetes, hypercholesterolaemia, and hypertension have been reported to attenuate PostC protection. Myocardial protection by PostC was consistently less in different diabetic models.[12] The abnormality leading to failure of PostC to reduce infarct size is most likely related to impairment of the RISK pathway components ERK, PI3K, and eNOS. Hypercholesterolaemia affects the efficacy of PostC, but this depends on the disease severity. Animals (rat and swine) fed with 2% cholesterol or cholesterol combined with 6% corn oil were not protected by PostC.[56–58] However, animals (rat and rabbit) fed with 1% cholesterol were still protected by PostC.[59,60]

There is controversy over whether PostC is effective in salvaging the myocardium in aged hearts. While PostC is protective in aged rats,[61] it fails to reduce infarct size in isolated-perfused models testing aged murine hearts (>80 weeks of age) and in an *in vivo* model (>52 weeks of age).[45,62] The diminished myocardial salvage could be due to defects in ERK and STAT3 signalling.[45,62] The myocardial protection could be restored if the cycle number is increased, and the duration of the index occlusion/reperfusion was reduced, again consistent with a threshold concept.[45] However, PostC still improved other endpoints such as post-ischaemic contractile function and reduced ROS generation in the myocardium in senescent working mouse hearts.[63]

3.1.9 Clinical application of postconditioning

Initial clinical studies on PostC reported by Laskey[64] and Staat *et al.*[65] were conducted only 3 short years after its introduction by Zhao *et al.*[11] in experimental studies. To date, clinical studies have shown that PostC increases ST-segment resolution,[66,67] improves left ventricular function, and reduces infarct size.[65,67–73] A summary of the clinical trial outcomes using PostC is presented in *Table 1*. In a meta-analyses of the clinical PostC data, infarct reduction was surprisingly consistent among the studies, ranging from 18 to 39%.[13,74] Downey and Cohen[75] suggested that only 25% of the patients treated by PCI would benefit from pharmacological treatment to reduce infarct size; this 25% would include patients with large areas at risk. Accordingly, Sorensson *et al.*[76] showed that patients with large areas at risk benefited the most from PostC. However, not all clinical studies of PostC have been positive. Freixa *et al.*[77] recently reported a series of 79 first-time ST-segment elevation [ST-segment elevation myocardial infarction (STEMI)] patients presenting for PCI within 12 h of onset of symptoms. There were no group differences in infarct size (% of LV mass) or ejection fraction estimated by cardiac magnetic resonance at 7 days or 6 months. Since most studies on PostC in the setting of PCI have been conducted in patients with symptoms for less than 7 h,[13] the 12 h used in this study may represent a limitation in salvage with PostC in patients with prolonged occlusions using a specific algorithm, and perhaps suggests a threshold phenomenon. To date, there has been no report of adverse events reported that are directly attributed to PostC, and none have been referenced in the meta-analysis.[13] However, as with preconditioning,[78] there is concern that repeated inflations of the angioplasty balloon in the culprit vessel (PCI), or repeated cross-clamping of the aorta (cardiac surgery), could potentially contribute to both showering of atheromatous material and producing localized endothelial injury of the involved vessel. Certainly, PostC as a therapy should be tested for safety and efficacy in large-scale, multicentre randomized trials as concluded by the Working Group of Cellular Biology of the Heart of the European Society of Cardiology.[41]

4. Remote conditioning of the myocardium

The disadvantages of directly conditioning the heart are (i) limited access to conditioning protocols under all but in-hospital situations, (ii) the technical resources necessary to apply ischaemia directly to the heart, and (iii) the fear of creating further injury to the heart by repeated balloon inflations. The demonstration that the heart (and other organs) can be protected by brief ischaemia induced in a distant organ[79] was a key in overcoming a major conceptual hurdle in translating ischaemic conditioning to clinical applications. The conditioning stimulus can be applied to the distant organ or tissue before (rIPC), during (rPerC), or after (rPostC) the index ischaemic event. rPerC may be applied while the patient is being transported to the hospital by an ambulance or helicopter, in the cath lab before the infarct-related artery is opened, or in cardiac surgery *during* the period of aortic cross-clamping. rPostC may be applied in any of these cases where reperfusion is predictable.

4.1 Remote ischaemic preconditioning

rIPC was first reported by Przyklenk *et al.*[79] in 1993 using a canine model of LAD occlusion–reperfusion in which the remote site was the myocardium perfused by the left circumflex coronary artery. Infarct size was reduced by 60% compared with a control group with LAD occlusion–reperfusion only. The infarct reduction observed with rIPC is comparable to that reported for classic IPC.[80] The paradigm of intracardiac rIPC has subsequently been expanded to include the preconditioning stimulus originating from other organs or tissues, e.g. the kidney and skeletal muscle.

The traditional concept of triggers, mediators, and effectors may pertain to rIPC as it does to classical IPC and PostC. Adenosine, bradykinin, and opioids have been proposed as triggers involved in rIPC.[81] Some studies support a 'second window' of rIPC in which mesenteric preconditioning cycles applied 24 h before the index ischaemia reduced infarct size.[82] The protection is sensitive to inducible NOS inhibitors, suggesting that late rIPC is dependent on inducible NOS. Inhibition of inflammatory gene expression may also contribute to late rIPC.[83]

Table 1 Clinical studies of ischaemic postconditioning and remote conditioning

	Reference	Population (*n*)	Interventions	ST-segment resolution (STR)	Enzymes	Infarct size (SPECT, MRI)	LV function (V-gram, Echo)	Other endpoints
Ischaemic postconditioning	64	<12 h LAD, RCA, LCx (17)	90 s R/I × 2	Improved	→ Peak CK	N/A	N/A	↑ Coronary flow velocity
	65	<6 h LAD or RCA (30)	60 s R/I × 4	Improved	↓ (36%) 72 h CK	N/A	↑ Myocardial blush grade	—
	115	<12 h LAD, RCA, LCx (94)	30 s R/I × 3	N/A	↓ 72 h CK (NSD), ↓ MDA	N/A	↑ Wall motion (8 weeks)	Improved endothelial function
	73	<12 h LAD, RCA, LCx (41)	30 s R/I × 3	N/A	↓ (27%) 72 h CK	↓ 27% (1 week)	↑ EF (NSD)	—
	69	<6 h LAD or RCA (38)	60 s R/I × 4	N/A	↓ (40%) CK, ↓ (47%) TnI	↓ 39% (6 months)	↑ (7%) EF (12 months)	—
	67	<6 h LAD (24)	90 s R/I × 2	Improved	↓ Peak CK	N/A	→ Myocardial blush grade	↑ Coronary flow velocity
	68	<12 h LAD, RCA, LCx (75)	30 s or 60 s R/I × 3	N/A	N/A	N/A	N/A	↓ Apoptosis (7 days)
	74	<12 h LAD, RCA, LCx (118)	30 s R/I × 4	N/A	→ Peak TnT	↓ 13% (3 months)	→ EF	↑ (31%) myocardial salvage ratio ↓ (41%) heart failure
	116	<12 h LAD or RCA (43)	60 s R/I × 4	Improved	↓ CK-MB	↓ 46% (1 week)	↑ EF (1 week)	—
	76	<6 h LAD, RCA, LCx (76)	60 s R/I × 4	N/A	→ TnT, → CK-MB	↓ in patients with large AAR	N/A	—
	66	<12 h (118)	30 s R/I × 4	Improved	↓ Peak TnT in patients with complete STR	↓ in patients with complete STR (3 months)	↑ EF in patients with complete STR (3 months)	—
	117	<12 h LAD, RCA, LCx (75)	30 or 60 s R/I × 3	N/A	N/A	N/A	↑ EF (12 months), ↑ wall motion (12 months)	↓ TNF-α (1 week)
Remote ischaemic preconditioning	118	CABG, LAD, RCA, LCx (8)	Arm (3′–2′) × 2, 300 mmHg	N/A	↑ LDH at 5 min after declamping	N/A	N/A	—
	119	Children undergoing bypass surgery (37)	Leg (5′–5′) × 4, 15 mmHg > SBP	N/A	↓ TnI	N/A	N/A	↓ inotrope scores, ↓ airway resistance
	120	Elective PCI. LAD, RCA, LCx (41)	Both arms (5′–5′) × 3, 200 mmHg	N/A	↑ CK-MB, ↑ TnI	N/A	N/A	—
	101	Elective CABG (58)	Arm (5′–5′) × 3, 200 mmHg	N/A	↓ TnT	N/A	N/A	—
	121	Elective abdominal aortic aneurysm repair (82)	Leg (10′–10′) × 2, 200 mmHg	N/A	↓ TnI	N/A	N/A	↓ Incidence of MI, ↓ renal injury
	100	Elective PCI. LAD, RCA, LCx (242)	Arm (5′–5′) × 3, 200 mmHg	N/A	↓ TnI	N/A	N/A	↓ EKG change during stent implantation, ↓ MACCE rate at 6 months
rPerC	107,122	<12 h LAD, RCA, LCx (251)	Arm (5′–5′) × 4, 200 mmHg or 25 mmHg > SBP	→	N/A	↓	→ EF	May improve LV function in high-risk patients

Population, acute ST-elevation myocardial infarction with time from the onset of chest pain in LAD, RCA (right coronary artery), and LCx (left circumflex) artery, or other diseases listed; *n*, the number of patients; R/I, cycle of reperfusion/ischaemia; CK, creatine kinase; Echo, echocardiogram; EF, ejection fraction; LV, left ventricular; MACCE, major adverse cardiac and cerebral events; MDA, malondialdehyde; MI, myocardial infarction; MRI, magnetic resonance imaging; SBP, systolic blood pressure; SPECT, single-photon emission computed tomography; TnI, troponin I; TnT, troponin T; V-gram, ventriculogram; →, no significant change; ↑, increase; ↓, decrease; N/A, not studied; NSD, no significant difference.

4.1.1 Extracellular signal transduction in remote ischaemic preconditioning (rIPC)

There is an intervening communication or transfer step between these triggers from the remote site to the target organ[81] that is not required by either direct IPC or PostC. Dickson *et al.*[84] first showed that the myocardium could be preconditioned by transfer of coronary effluent from a preconditioned heart to a virgin isolated perfused acceptor heart, or by transfer of whole blood in a preconditioned rabbit heart to a naive rabbit. The transfer phenomenon for rIPC was confirmed by Shimizu *et al.*[85] in a rabbit model of transient limb ischaemia as the rIPC trigger and cardiac index ischaemia. Shimizu *et al.*[85] also extended the field by showing that plasma from preconditioned humans or rabbits could protect isolated perfused rabbit hearts and isolated rabbit cardiomyocytes, thereby demonstrating the cross-species effect. These communication factors may be humorally borne[85,86] or transmitted by neuro-humoral paths. Like direct IPC and PostC, rIPC stimulates receptors for adenosine, bradykinin B2, opioids (δ1 or κ), CB2 cannabinoid, and angiotensin AT1.[87] The mechanisms of protection of transferred factors may include suppression of blood-borne leucocyte activation[83,88] which would potentially attenuate the neutrophil-mediated component of ischaemia–reperfusion injury.[89] This, however, does not explain the transfer of protection in cell-free (isolated-perfused) systems.

There is evidence suggesting that a neural pathway exists in rIPC. For example, the cardioprotection conferred by local administration of bradykinin to the mesenteric artery is abolished by the ganglion blocker hexamethonium.[90] A neural pathway was further confirmed by Ding *et al.*[91] who showed that renal rIPC increased renal afferent nerve discharge and renal nerve ablation abolished renal rIPC. Further studies suggest that rIPC may produce adenosine locally which then stimulates afferent sensory nerves or activates capsaicin-sensitive sensory nerves to release calcitonin gene-related peptide.[87] However, some recent evidence argues against neural transmission of rIPC triggers. Kingma *et al.*[92] showed in a canine model of renal (remote) preconditioning on left ventricular infarct size that myocardial protection rendered by rIPC was not abrogated either by the autonomic blocking agent hexamethonium or by surgical decentralization of cardiac nerves.[92] In agreement, persistence of protection with rIPC by repetitive limb ischaemia–reperfusion in a porcine cardiac transplant model in which the donor heart is denervated also argues against the requirement for a neural pathway.[93] Therefore, the evidence supports that neural humour transmission seems adequate to achieve cardioprotection, and neural pathways, to the extent they exist in a given model, may be redundant.

4.1.2 Intracellular signal transduction in remote ischaemic preconditioning

rIPC shares some similar intracellular signal pathways as IPC and PostC. The involved primary kinases include p38, ERK1/2 or JNK1/2,[94] PI3K/Akt,[95] and PKCε.[96,97] The downstream target of PKC could be the mitoK_{ATP} channel; studies show that K_{ATP} channel inhibition abrogates rIPC protection.[87] Although inhibition of mPTP opening plays a central role in classical IPC and PostC, it is not understood whether mPTP inhibition contributes to rIPC.[87]

4.1.3 Clinical applications of remote ischaemic preconditioning

The clinical application of rIPC has recently been reviewed by Kharbanda *et al.*[98] and by Hausenloy and Yellon.[14] rIPC is induced clinically by repeatedly inflating and deflating a cuff occluder or tourniquet placed on the upper or lower limbs; therefore, skeletal muscle ischaemia–reperfusion provides the conditioning stimulus.[99] Patients with acute coronary occlusions are not candidates for rIPC because the ischaemic event is already underway. The Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study reported by Hoole *et al.*[100] showed that rIPC reduced infarct size estimated by cardiac troponin I (cTnI). Since these patients were elective and had undetectable cTnI levels before reperfusion, one could argue that this was actually rPerC rather than rIPC, but nonetheless remote conditioning reduced post-PCI injury.

In cardiac surgery, aortic cross-clamping with or without cardioplegia is a planned event, and therefore, conditioning before aortic cross-clamping can be strategically applied. Several cardiac surgery studies reported reductions in plasma biomarkers of injury when rIPC was achieved by transient limb occlusion before aortic cross-clamp and cardioplegia.[101,102] Applying a rIPC stimulus 18 h before surgery, i.e. within a 'second window', reduced cTnI release after cardiac surgery.[103] These data suggest that there is room for myocardial protection over and above that exerted by cardioplegia, hypothermia, and inhalational anaesthetics.

4.2 Remote perconditioning

4.2.1 Experimental studies

Practically, neither IPC nor rIPC can be applied to patients undergoing percutaneous or surgical interventions before the ischaemic event; PostC cannot be applied to those 30–40% of the STEMI patients who reperfuse spontaneously or after thrombolysis.[104] However, remote conditioning can be applied during ischaemia in the form of rPerC. The rPerC stimulus can be induced by occluding a limb using a tourniquet or inflatable cuff during transport to the emergency room or cath lab. The same communication mechanisms involved in rIPC may also be involved during rPerC.

Using a porcine model of angioplasty balloon catheter-induced LAD occlusion (40 min) and 120 min of reperfusion, Schmidt *et al.*[105] reported that four 5 min cycles of hind limb occlusion–reperfusion applied immediately after LAD occlusion (but before reperfusion) reduced infarct size compared with a control group. Infarct size reduction was reversed by the K_{ATP} channel antagonist glibenclamide given before rPerC, suggesting that the K_{ATP} channel is involved, which is consistent with both classical IPC and PostC. In the *in vivo* rat model of ischaemia–reperfusion, both rPerC and direct PostC alone reduced infarct size comparably, but less than that observed with IPC. However, combining rPerC and PostC achieved comparable infarct size reduction observed with IPC.[106] The reduction in infarct size has been associated with an increase in phospho-Akt and phospho-ERK1/2.[106]

4.2.2 Clinical application of perconditioning

rPerC has recently been translated to the clinical setting. Bøtker *et al.*[107] reported that rPerC during transport of patients with STEMI to the hospital reduced infarct size particularly in patients with large areas at risk. Rentoukas *et al.*[108] applied a rPerC stimulus in STEMI patients 10 min prior to estimated reperfusion with or without adjunctive morphine (5 mg) infusion at the onset of reperfusion. This study confirmed the infarct reduction (peak cTnI) observed by Bøtker *et al.*[107] but also showed that the incidence of full ST resolution was higher in the rPerC patients.

In adult patients undergoing cardiac surgery for valve replacement, Li *et al.*[109] applied remote conditioning after either induction of anaesthesia (rIPC) or during aortic cross-clamp (rPerC) by cuff occlusion of the lower limb. Peak plasma nTnI levels at 30 min to 4 h after cross-clamp removal and the incidence of ventricular fibrillation at reanimation were lower in the rPerC group compared with either controls or rIPC. However, whether salvage of myocardium by rPerC is associated with improved clinical outcomes (e.g. length of stay, mortality, or indices of morbidity) has yet to be determined by multicentre studies.

4.3 Remote postconditioning

Direct PostC of the heart may have the disadvantage of inducing additional myocardial ischaemia, although there have been no adverse events reported to date. rPostC was first reported by Kerendi *et al.*[110] in a rat model of prolonged coronary artery occlusion–reperfusion in which a single cycle of 5 min renal artery occlusion–reperfusion applied just prior to the onset of coronary artery reperfusion reduced infarct size by ~50% relative to controls. This observation was replicated in an elegant closed-chest porcine model using the hind limb as the rPostC stimulus organ.[111] rPostC induced by limb ischaemia–reperfusion was also reported to reduce apoptosis.[112] A remote conditioning stimulus has also been provided by occlusion of the opposite left coronary artery (intra-organ remote conditioning),[113] the carotid artery,[113] or femoral artery (limb stimulus).[114] Cardioprotection with rPostC has been reported to be equivalent to[114] or more potent[113] than direct PostC. At the time of writing, there have been no reported clinical trials of rPostC.

Like rIPC and rPerC, the transfer signal between the remote stimulus organ and the target organ may be humoral, neural, or systemically borne (cell signals). Kerendi *et al.* showed that rPostC could be blocked by the adenosine receptor antagonist 8-SPT, suggesting that adenosine was a humoral communication factor between the kidney and the heart. Adenosine released by the kidney may act directly on the heart or trigger neuronal stimuli that subsequently protect the heart. The communication factor(s) has not been identified as yet.

5. Concluding remarks: unanswered questions, future directions

Conditioning can be applied before, during, or after the ischaemic stressor, protects multiple cell types, and induces or rebalances a number of pathways that attenuate necrosis and apoptosis. This broadness is the conditioning response's strength, which, in the case of PerC and both direct and remote PostC, has allowed it to be successfully translated from bench to bedside in the span of a few years compared with IPC. As with many things, this strength is also its weakness; the need for ischaemia to trigger the cardioprotection is a liability related to the methods of inducing ischaemia. Before conditioning can realize its full therapeutic potential, many questions should to be answered regarding (i) what is the optimal algorithm experimentally and clinically, and can the optimal algorithm extend the threshold effect to longer durations of ischaemia; (ii) is there a real-time marker that can be used to signal that a conditioned state has been achieved; (iii) do the same molecular mechanisms of conditioning observed in animals drive the responses in man; (iv) is there an anti-inflammatory component to conditioning; (v) are the mechanisms common to all three forms of conditioning; (vi) is there an appropriate animal model that accurately reflects the human 'model' of multiple co-morbidities and metabolic syndrome? Further, would it not also be useful for the animal model to be treated with the drugs typically prescribed to patients with co-morbidities in order to accurately reflect the clinical? Clinically, one should ask (i) are there specific patient subtypes in which direct or remote conditioning is effective, or is cardioprotection exerted over all disease demographics; (ii) does conditioning of the heart during PCI or surgery protect other organs from multiorgan dysfunction; (iii) what is the duration of coronary artery occlusion (also taking into account the size of area at risk) beyond which the efficacy of conditioning is lost? Answering this latter question will have impact on how patients are triaged as salvageable or non-salvageable.

Both PerC and PostC have been rapidly translated to the clinical setting. This presents unprecedented opportunities for clinicians and basic scientists to work cooperatively and collaboratively, with each contributing to the unravelling of the mechanisms and potential of the conditioning response. However, the use of ischaemia to trigger cardioprotection has certain liabilities which reduce its enthusiasm for clinicians; fewer liabilities exist with remote ischaemia. Ischaemia will likely be replaced by pharmacological agents that exert cardioprotection, although these agents need to address the numerous and often redundant mechanisms involved in ischaemia–reperfusion injury to be effective. The development of pharmacological conditioning mimetics may therefore provide another option to protect the heart at other organs exposed to non-surgical and surgical ischaemia and reperfusion.

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