Preconditioning and postconditioning: innate cardioprotection from ischemia-reperfusion injury

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Reperfusion itself, however, is not risk free. Numerous studies have shown that reperfusion has the potential to extend the degree of myocardial injury and its constituent cells (cardiomyocytes, vascular endothelial cells) beyond the injury that was present after the ischemic insult, the so-called “reperfusion injury” (6, 8, 46, 70–72). Cardiac surgeons have recognized reperfusion injury as a clinical entity for many years (7, 8). Increasing injury upon reperfusion likely occurs to the cardiomyocytes (necrosis and apoptosis), coronary vascular endothelium, and subcellular structures such as the mitochondrion. Indeed, the mitochondrial permeability transition pore may be the final effector of cell death by either necrosis or apoptosis (15, 22, 24). Some studies also suggest that this reperfusion injury is progressive and cumulative over time (89, 91). Therefore, the term “evolving” is a useful description of the infarction process. However, it is not clear yet how much of total postischemic injury can be allocated to ischemia and how much to reperfusion, although some broad axioms can be stated: since ischemia sets the stage for reperfusion injury, the greater the severity of ischemia, the greater the degree of reperfusion injury, and there is a duration of ischemia from which...
the myocardium is unsalvageable that is demonstrated by transmural involvement after permanent occlusion. However, it is not known what time boundaries this relates to. The “wavefront” time frame may not be applicable since the time line was developed in ischemic and reperfused myocardium, therefore incorporating both ischemic and reperfusion injuries. Some studies on surgical revascularization suggest significant recovery in patients exhibiting symptoms of evolving infarction well beyond the limit proposed by the wavefront model.

The majority of therapeutic approaches for treatment of acute evolving myocardial infarction have been designed to reduce the duration and severity of ischemia; newer generation approaches may target reperfusion injury. Numerous therapies have been advocated to reduce ischemic injury specifically, including drugs that are administered before the ischemic event (i.e., pharmaceutical preconditioning), hypothermia, and ischemic preconditioning. Indeed, the early studies by Maroko and colleagues (48) introduced the concept of myocardial salvage from ischemic injury. These studies approached the problem by attempting to lower the energy demands of the heart to reduce the overall severity of the energy supply/demand mismatch during ischemia. This approach was not effective in the absence of reperfusion. Ischemic preconditioning, introduced by Murry et al. (49) in 1986, has shown the greatest promise of any cardioprotective strategy and is to a great extent a “gold standard” (21) by which other strategies are compared. It has been thought that preconditioning intervenes during the ischemic period by numerous mechanisms reviewed below. These mechanisms were either triggered by the preconditioning stimulus itself (trigger phase) or mediated by largely molecular mechanisms that were set in motion after the stimulus (mediator phase). However, recent data suggest that preconditioning may also attenuate events that specifically occur during reperfusion (59).

On the other hand, various approaches have been taken to attenuate reperfusion injury, including the systemic or local infusion of adenosine, nitric oxide, oxygen radical scavengers, anti-inflammatory agents, and the filtration of inflammatory cells at the time of reperfusion. Recently, postconditioning, defined as brief intermittent cycles of ischemia alternating with reperfusion applied after the ischemic event (at the onset of reperfusion), has been introduced as a mechanical intervention to attenuate reperfusion injury specifically (87). This review compares and contrasts the cardioprotection phenotype and mechanisms of preconditioning and postconditioning and notes their similarities as well as their differences. The molecular mediators engaged by these two forms of “conditioning” are not discussed, but are reviewed in depth elsewhere (25, 69). Because there are significant clinical implications of these two strategies, their clinical applications are highlighted.

**THE PHYSIOLOGICAL END POINTS OF PROTECTION BY PRECONDITIONING AND POSTCONDITIONING**

**Infarct size (necrosis and apoptosis).** That ischemic preconditioning (IPC) and postconditioning (PoC) reduce infarct size acutely (26, 35, 50, 79, 85, 87) and long term (50) is well established. The total infarct area is likely composed of cardiomyocytes that have died by either necrosis or apoptosis (31; for a review on the effects of preconditioning on apoptosis, see Ref. 92). Both IPC (40) and PoC (60, 75, 80) have been shown to inhibit both forms of cell death. In the rat model, IPC has consistently demonstrated a robust reduction of infarct size (on the order of 50–80% reduction) independent of the animal model used. In contrast, PoC has demonstrated a less-substantive infarct size reduction in smaller animal models (mice, rats, rabbits ~30% reduction) than in larger animal models of coronary artery occlusion [~50% (23)–80% (26) reduction]. The reasons underlying this “species or size discrepancy” have not been determined but may relate to species differences in 1) myocardial metabolism and the supply/demand mismatch that defines the severity of ischemia, 2) the complement of endogenous antioxidants expressed in the animal model, 3) the role of inflammatory cells during reperfusion, 4) effectiveness of the PoC algorithm for each species, which is likely multifactoral, but may be related in part to the rate in which reperfusion injury events occur. Relevant to point number 4, for example, Iliodromitis et al. (26) found that the failure of PoC to reduce infarct size reported in the study by Schwartz and Lagrarna (57) in the pig model may be related to an insufficient number of reperfusion-occlusion cycles. However, cycles ranging from 30 to 60 s have been used in human studies with similar reductions in infarct size. Furthermore, Halkos et al. (23) found that IPC and PoC exerted similar reduction in infarct size in the canine model, whereas in the rat IPC exerts greater infarct reduction than does PoC. Halkos et al. (23) also showed that the cardioprotection by PoC was not additive to that of IPC in the canine model of left anterior descending coronary artery (LAD) occlusion and reperfusion. However, Yang et al. (77) found that infarct size reduction with IPC and PoC were additive if ischemic times were prolonged in the rabbit model. Whether such additive protection is unmasked with prolonged coronary occlusion in which each strategy is ineffective by itself, or whether this is a species-dependent effect, is not clear and warrants further investigation. The entire question of the optimal algorithm and durations of reperfusion and ischemic segments of the PoC stimulus is open.

**Contractile dysfunction.** In contrast to the consistent demonstration of infarct size reduction within a given species, the benefits of both IPC and PoC on postischemic contractile function in the absence of infarction (myocardial stunning) or in the presence of infarction is not clear. Kin et al. (34) reported that PoC did not alter global postischemic contractile function using a three-cycle (10 s each) algorithm, but global function (estimated grossly by +dP/dt) was significantly improved using a six-cycle algorithm. However, it must be stated that global function is influenced by a number of factors, paramount of which is infarct size. In a regionally ischemic model in which myocardial stunning was created by 10 min of either LAD or left circumflex coronary artery occlusion in a canine model, PoC did not improve regional wall motion in the area at risk or its time to recovery independent of whether the cycle length was 15, 30, or 60 s (10). The Vinten-Johansen and Zhao laboratory observed a similar lack of protection in stunned myocardium in a canine model of 10-min LAD occlusion and 3 h of reperfusion (Fig. 1). Whether altering the number of cycles or the duration of a fixed number of cycles can improve regional postischemic contractile function has not been answered at this time.

**Arrhythmias.** IPC has been reported to have antiarrhythmic effects in canine (23, 67) and rat (58) models of coronary artery occlusion-reperfusion. However, it has also been reported that
Preparation process immediately truncated
arrhythmias were initiating during the early period of reperfusion. If the
vulnerable to arrhythmias at the onset of reperfusion. If the
ventricular arrhythmias by 50% in the canine model, which is
reports support that PoC attenuates postischemic arrhythmias. IPC exacerbates the incidence of arrhythmias. In contrast, three
improved by postconditioning (3 30-s cycles) relative to control.

Protection of the coronary vascular endothelium and reduction of
the inflammatory response to reperfusion. An inflammatory-like response has been observed to occur at the onset of
reperfusion (17, 18, 68). The hallmarks of this response are 1) simultaneous activation of neutrophils (CD11/18 expression)
and coronary vascular endothelium (P-selectin expression) and the consequent recruitment of neutrophils to the reperfused
coronary vascular endothelium of the area at risk, 2) endothelial dysfunction (decreased endothelium-dependent vasodilation,
3) increased superoxide anion generation by coronary vascular endothelium (50), decreased endogenous nitric oxide
generation (42), and 4) release of proinflammatory cytokines into the interstitium and vascular space. These inflammatory-
like events have been indirectly correlated with the degree of myocardial necrosis in that attenuation of these events at
reperfusion is associated with a decrease in infarct size (2, 41, 64). IPC has been reported to attenuate postischemic coronary
vascular endothelial dysfunction in some studies (4, 12) but not
in others (4). Endothelial dysfunction assessed by vasodilator
responses to acetylcholine has been shown to be preserved by
PoC (87). It is not clear which of the physiological effects of
PoC are responsible for protection, since the inflammatory-like response is a complex and integrated cascade of events. PoC
could reduce superoxide generation that would damage endo-
theilum or limit neutrophil adherence. But at this point, it is
difficult to distinguish a cause and effect relation. This is a very
controversial area and one that will likely draw more investiga-
tive interest in the future. The implication in practice is that
the anti-inflammatory response of both IPC and PoC may be
amplified by coapplication of various anti-inflammatory and
other cardioprotective agents.

Prolonging tissue acidosis. Ischemia causes severe tissue acidosis, which is readily reversed by washout of hydrogen ions and metabolic byproducts at reperfusion. Indeed, this rapid reversal of tissue acidosis may contribute to reperfusion injury. Acidosis prevents opening of the mitochondrial permeability transition pore (mPTP; Ref. 22). In preconditioned
hearts, IPC decreases the degree of tissue acidosis achieved during index ischemia, and accelerates the normalization (de-
crease) in intracellular sodium during the early moments of
reperfusion (3). This accelerated normalization of tissue so-
dium is replicated by repetitive tissue acidosis applied before
index ischemia (47). Recently, Cohen and colleagues (9) found
that PoC prolonged tissue acidosis during reperfusion, which
may attenuate opening of the mPTP. They found that maintain-
ting tissue acidosis by perfusing the heart with hypercapnic buffer in isolated rabbit hearts subjected to 30 min of regional
ischemia recapitulated the infarct reduction of PoC, and infarct
sparing by PoC was reversed by perfusing with alkalotic buffer
during the PoC algorithm. In agreement with this “pH hypo-
thesis,” we found that in the in vivo canine model of ische-
emia-reperfusion postconditioning significantly prolonged tis-
eue acidosis during the early moments of reperfusion (Fig. 2).
How this mechanism of prolonging tissue acidosis and main-
taining the mPTP in a closed state interacts with other mecha-
nisms such as triggering by G protein-coupled receptors
(GPCR) ligands is not clear.

Receptor-dependent mechanisms of
preconditioning and postconditioning

Stimulation of any G protein-coupled receptor has been shown to
toggle preconditioning (84), and a similar involvement of
GPCR is being revealed for PoC. These GPCRs include ade-
sine, bradykinin, norepinephrine, and opioids and may include
protease-activated receptor type 2 (PAR2) at least for postcon-
ditioning. The involvement of these receptors must satisfy
several criteria: 1) the receptors must be present in the target
tissue (myocytes, endothelium, inflammatory cells) and active
during reperfusion and that the A2 receptor, rather than the A1
endogenously released adenosine also exerts cardioprotection
preconditioning stimulus exerts protection before the index
infarct size (30, 38). These data suggest that adenosine reduces
agonists (CGS-21680) at the onset of reperfusion also reduces
reperfusion did not abolish PoC cardioprotection (34, 55).
These results suggesting a role for the A2A receptor in PoC
reperfusion after IPC (59), which would make IPC and PoC
similar in their mechanisms. Blockade of adenosine recep-
tors at the onset of reperfusion with the non-specific antag-
onist 8-p-(sulphophenyl)theophylline at reperfusion abrogated
the infarct sparing effects of PoC; an A2A-selective blocker as
well as an A3-selective blocker also reversed the myocardial
salvage by PoC (34), but blockade of the A1 receptor at reper-
fusion did not abolish PoC cardioprotection (34, 55). These
results suggesting a role for the A2A receptor in PoC have
been supported by models of A1- and A2A-receptor
knockout mice (82). A role for the A2B receptor has been
reported by Philipp et al. (55) and Solenkova et al. (59).
Other GPCR have been implicated in the triggering of IPC
and PoC. Bradykinin is elevated during and after ischemia.
Bradykinin infused in rabbits or mice starting 5 min before
reperfusion reduces infarct size (5, 78) and the bradykinin
antagonist HOE-140 abrogates IPC (73), suggesting that en-
dogenous bradykinin mediates IPC. In addition, IPC is abro-
gated in B2-bradykinin receptor knockout mice (81). The role
of endogenous bradykinin in the triggering of PoC via activa-
tion of B2 receptors has recently been demonstrated (52).
Opioids have also been implicated in both IPC and PoC. Both
κ- and δ-opioid agonists reduce infarct size when administered
before ischemia or before reperfusion (20). The nonselective
opioid antagonist naloxone and the peripherally acting antag-
onist naloxone methiodide abrogate the effects of IPC, sug-
gesting that endogenous opioids, such as the enkephalins, are
involved in triggering of IPC. Both κ- and δ-agonists were
found to reverse the infarct reduction by IPC (74). Opioid
receptors have also been found to be involved in the triggering
of PoC (33) and, like IPC, may involve both the κ- and
δ-receptors. Hence the endogenous ligands that trigger IPC and
PoC seem to be similar. However, the question of whether
these ligands activate their cognate receptors and produce a
physiological event at different times (ischemia for IPC, reper-
fusion for PoC) is open to question, although preliminary data
suggest that reperfusion may be playing a greater role in
ligand-receptor responses.

It is fortunate that these GPCR ligands exert cardioprotec-
tion during both windows of opportunity, although different
receptor subtypes may be involved in each phase. Inducing
pre- and postconditioning by pharmacological mimetics that
operate through activation of GPCRs is of great clinical interest
currently. Agents may be administered before cardiac surgery
or angioplasty for an additive effect or may be administered
just before reperfusion to “pharmacologically postcondition”
the heart, assuming that exogenously delivered molecules have
the same access to target cells as do endogenously released
molecules. A summary of the GPCR ligands and other mech-
anisms involved in both IPC and PoC is shown in Fig. 3.

PRECONDITIONING AND POSTCONDITIONING
THE HUMAN HEART

Excised human myocardium demonstrates increased toler-
ance to ischemia by IPC. Furthermore, studies demonstrated
the phenomenon of IPC in the intact human heart in several
clinical presentations of ischemia-reperfusion, including prein-
farction angina, the warm-up phenomenon, percutaneous
coronary intervention (angioplasty), and cardiac surgery (63). In
the catheterization laboratory setting, repeated balloon infla-
tions during balloon angioplasty attenuated electrocardio-
graphic changes (ST-segment elevation; Ref. 13), lactate
release, and left ventricular segmental dysfunction (28) relative
to the first balloon inflation suggestive of a preconditioning-
like effect. Laskey (37) reported that repetitive 90-s balloon
occlusions during balloon angioplasty reduced ST-segment elevations,
increased the rate of ST-segment elevation resolution (a marker
of improved viability and perfusion) and increased flow veloc-
ity reserve in the infarct-related artery. Whether this alternating
ischemia-reperfusion induced by repeated balloon inflations
immediately after opening the coronary artery is precondition-
ing or postconditioning is arguable. Prodromal (preinfarction
angina, defined as anginal pain at rest experienced in the 24 h
before the onset of symptoms of myocardial infarction, re-
duced infarct size by 33% assessed by peak CK-MB release
(51). Some of this cardioprotection is likely a preconditioning
effect rather than recruitment of collateral vessels.

In cardiac surgery, cardiac motion is electively limited by
inducing fibrillation, or complete electromechanical arrest is
achieved by either global ischemia (aortic cross-clamping) or
chemical cardioplegia. Yellon et al. (83) and Alhulaifi et al. (1)
reported that preconditioning the heart by short periods of
aortic cross-clamping preceding elective ventricular fibrillation
conserved high energy phosphate (i.e., ATP) levels in myocar-
dial biopsy samples, suggestive of a cardioprotective effect.
However, a conservation of tissue ATP was not observed in a
follow-up study (29). The impact of these observations is
limited by the declining use of induced ventricular fibrillation
during aorto-coronary artery bypass in favor of some form of
chemical cardioplegia and by the hesitation of surgeons to
Ischemic Preconditioning (IPC)

Triggers
- GPCR: Ado, Cypoids, Bradykinin
- mKATP channels

Mitochondrial KATP channels have been implicated. Other G protein-coupled receptor (GPCR) ligands have been implicated. Other G protein-coupled receptor (GPCR) ligands have been implicated. Other G protein-coupled receptor (GPCR) ligands have been implicated.

Mediators
- MAPK kinases
- PI3 Kinase
- mKATP channels
- GSK-3β

Reperfusion

End Effectors
- mPTP

Cardiomyocyte
- Necrosis
- Apoptosis
- Swelling
- Cytoskeleton
- Endothelium

Protection

Postconditioning (PoC)

Triggers
- GPCR: A1, A2B, PAR2

Mediators
- MAPK kinases
- PI3 Kinase
- mKATP channels
- GSK-3β

Reperfusion

End Effectors
- mPTP

Cardiomyocyte
- Necrosis
- Apoptosis
- Swelling
- Cytoskeleton
- Endothelium

Protection

Fig. 3. A schematic diagram of triggers, mediators, end effectors, and end points in ischemic preconditioning and postconditioning (PoC). Both cardioprotective maneuvers share similarities in mechanisms, notably a division of participants into the classifications triggers, mediators and effectors. Notable exceptions are 1) obvious application of all three classifications of mechanisms at the onset of reperfusion in PoC; 2) adenosine (Ado) receptor subtype(s) active in IPC is the A₁ and/or the A₂B, while in PoC the A₂A and A₁ receptors have been implicated. Other G protein-coupled receptor (GPCR) ligands have been implicated in both IPC and PoC. Mitochondrial KATP channels have been implicated as both triggers and mediators in IPC, whereas in PoC this has been implicated in both IPC and PoC.

Cardiomyocyte
- Necrosis
- Apoptosis
- Swelling
- Cytoskeleton
- Endothelium

Protection

In summary, preconditioning has been shown to be cardioprotective in patients with coronary occlusive disease, but its clinical application is limited since it is not readily feasible to predict the onset of ischemia in non-surgical settings such as the catheterization laboratory. Whether sequential inflations and deflations of the angioplasty balloon in the catheterization laboratory at the onset of reperfusion are preconditioning or a form of postconditioning in acute PCI is arguable. However, the degree of infarct size reduction after angioplasty is markedly similar between the studies (~30%), and mechanisms may be similar to those revealed by experimental studies. Efficacy of preconditioning in the surgical arena using chemical cardioplegia is controversial, and surgeons are reticent to literally apply ischemia by cross-clamping the aorta. However, both preconditioning and postconditioning can be applied in off-pump surgery during elective occlusion of the target vessel.
The use of preconditioning mimetics in cardiac surgery is much more appealing. There are relatively few clinical studies on postconditioning, and these have been conducted in small populations of patients with acute myocardial infarction, with little experience in its application in cardiac surgery. Whether cardioprotection exerted by preconditioning or postconditioning is sustained in some experimental models of comorbidities including age, hyperlipidemia (16, 27, 86), diabetes, heart failure, and hypertension, salubrious effects of postconditioning have been reported in patients with multiple comorbidities. Resolving the discrepancies in efficacy of pre- and postconditioning between experimental models and patients with these comorbidities is open to further investigation.

THE FUTURE APPLICATION OF PRECONDITIONING AND POSTCONDITIONING

The strength of IPC and PoC is in their ubiquitous cardioprotection observed in all species tested, including humans, and the protection afforded to other organ systems. A limitation of both conventional IPC and PoC is the reliance on ischemia to induce the stimulus. However, IPC and PoC strategies can be “mimicked” by pharmacological agents given before the index ischemia or at reperfusion, respectively. IPC has the added limitation that it is applied before the ischemic event and therefore can be used only when the event is predictable (unless a chronic preconditioned phenotype can be induced). Whether for pre- or postconditioning, imposing ischemia is not part of the clinical mind-set and, therefore, is performed with great hesitation. Even surgical ischemia is avoided by continuous cardioplegia. In turn, the utility of PoC may be limited in patients in whom the onset of reperfusion is not predictable, for example, in patients with intermittent vasospasm. The utility of IPC and PoC in patients with incomplete occlusive disease has not been investigated. In addition, the duration of ischemia beyond which either IPC or PoC salvage myocardium has not been defined and cannot be predicted from the “wavefront phenomenon” in which unfettered reperfusion has undoubtedly contributed to the extent of infarction. A reduction in reperfusion injury would potentially turn a 4-h infarct into a 1-h infarct and divert the preoccupation of the interventionist from reducing the ischemic time to initiating reperfusion under controlled conditions. The reliance on myocardial ischemia can be overcome by inducing ischemia in other organs and thereby remotely conditioning the heart either before or after the index ischemia. The Holy Grail strategy would be to pharmacologically induce IPC or PoC. Indeed, this has been done for both protocols. The literature is replete with studies in which cardioprotective drugs have been administered either before the index ischemia or at the onset of reperfusion. Some of the drugs demonstrating myocardial salvage when administered at reperfusion include adenosine, nitric oxide, opioids, bradykinin, and erythropoietin, as well as drugs that activate PKC epsilon. In addition, the delivery of PoC mimetics to the target tissue specifically may improve efficacy that is otherwise limited by systemic hemodynamic side effects or other unwanted side effects of the agents. However, the strategy of reperfusion therapeutics to salvage myocardium (as opposed to simply restoring blood flow) has not yet been adopted into daily clinical practice.

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