Myocardial postconditioning: reperfusion injury revisited

CORONARY HEART DISEASE is the leading cause of death in the Western world and represents one of the major burdens on healthcare systems today. Targeting those strategies that limit the damage sustained as a result of a lethal ischemic insult has been a major goal for many years. One of these strategies, termed ischemic preconditioning, in which brief nonlethal episodes of ischemia protect the myocardium before a subsequent prolonged ischemic insult, has received an enormous amount of interest over the past two decades. This phenomenon is recognized as the most powerful endogenous cardioprotective mechanism known, and many pharmacological preconditioning mimetics have been described since (45). However, ischemic preconditioning has its limitations in that the preconditioning mimetic must be applied before the index ischemic event, which in the clinical setting of acute myocardial infarction is unpredictable and impractical. Although preconditioning has given us invaluable information regarding cellular adaptation to stress, cell signaling, and viability, it in itself has failed to find a clinical niche. However, based on our understanding of the mechanisms associated with preconditioning, this has allowed us to revisit and focus on other cardioprotective strategies.

The prerequisite for rescuing viable myocardium and reducing the mortality and morbidity as a consequence of an acute myocardial infarction is the early restitution of coronary flow, i.e., reperfusion, after the ischemic event by such means as thrombolytic drugs, primary coronary angioplasty, or coronary bypass graft surgery. Because the onset of reperfusion is more predictable and is under the control of the operator, the period surrounding the reestablishment of coronary flow is a more attractive window of opportunity for protection from the consequences of both the ischemia and reperfusion-induced injury (32). Novel cardioprotective strategies, which protect the myocardium from the detrimental effects of ischemia-reperfusion injury, may therefore be applied as adjunctive therapy to current reperfusion strategies. However, reperfusion is not a completely benign process and can induce myocyte death, a phenomenon known as lethal reperfusion injury (8), which is now known to involve the processes of necrosis and apoptosis (11, 25). The concept of reperfusion-induced injury is controversial, being initially supported by most groups but then falling out of vogue (35). More recently, however, based on new hypotheses and a better understanding of cellular signaling, it seems that the concept has now returned full circle with the advent of pharmacological agents that have been shown to reduce myocardial cell death when given during the initial stages of reperfusion (44).

One such novel strategy targeting the reperfusion phase is the phenomenon known as ischemic postconditioning, which was first introduced in 2002 by Vinten-Johansen’s group (3). This phenomenon, in which the application of transient brief interruptions to reperfusion by ischemic episodes results in reduced myocardial injury, has led to renewed interest in the development of protective maneuvers to combat the effects of lethal reperfusion injury. More recent studies (40, 43) have demonstrated that ischemic postconditioning activates cellular prosurvival cascades such as the phosphatidylinositol 3-kinase (PI3K)-Akt and MEK 1/2-ERK pathways via downstream mediators such as endothelial nitric oxide (NO) synthase (eNOS) and p70S6K and that by inhibiting these pathways, the protective effects of ischemic postconditioning are abolished (see Fig. 1). However, ischemic postconditioning, as discussed below, is likely to represent a form of modified reperfusion that paradoxically has been known for a number of years to be beneficial to the ischemic myocardium.

MODIFIED REPERFUSION

The damaging effects of reperfusion are due to the many biochemical and physical perturbations that occur in the transition from ischemia to reperfusion. Immediate full-flow reperfusion leads to pressure overload and resultant myofibrillar stretching. This ultimately leads to myocardial edema, hypercontracture, and myocyte death (32). Other detrimental events are exposure to a sudden burst of free radicals, mitochondrial calcium overload, and increased endothelial dysfunction (6).

The beneficial effects of alterations to the conditions of reperfusion have been known since the mid-1980s. Studies from Buckberg and colleagues (28) in the dog model reported that contractile recovery was improved in those animals that were subjected to a gradual relief of the left anterior descending (LAD) artery occlusion compared with those with sudden relief. Work from Hori et al. (21) a few years later described the importance of pH during the early stages of reperfusion. They found that maintaining a more acidic pH by staged reperfusion led to improved myocardial contractility and that the administration of an alkaline solution during this maneuver abolished the improvement in recovery. Other studies have demonstrated that gradual reperfusion leads to the accumulation of those metabolites necessary to achieve improved postischemic functional recovery, namely, ATP, glutamate, aspartate, and adenosine nucleotides (33). Data that followed from Vinten-Johansen’s laboratory (34) reported that gradual reperfusion not only reduced endothelial dysfunction but also reduced infarct size in dogs subjected to left coronary artery ligation.

However, the concept of modified reperfusion as a cardioprotective intervention failed to make an impact. One potential reason for this may have been due to the controversy surrounding the very existence of lethal reperfusion injury, with some groups questioning the existence of reperfusion injury as a distinct entity, believing instead that reperfusion simply augmented the damaging effects of the ischemic insult (25). Others believed that the metabolic changes of reperfusion itself could induce cellular injury without an ischemic period (19, 41). More recently, however, it has been shown that a range of pharmacological agents given at the moment of reperfusion after an ischemic insult can significantly protect the myocardium, and this effect has been shown to involve protection against necrosis and apoptosis. Furthermore, during the early stages of reperfusion, there is an upregulation of prosurvival kinases [termed the reperfusion injury salvage kinase (RISK) pathway], which, taken together, has promoted a renewed interest in cardioprotective reperfusion strategies (18).
RISK PATHWAY

While the mechanisms involved in reperfusion injury are known to involve apoptosis and necrosis, among others, it is also realized that cells have an inherent program for survival after ischemia-reperfusion insults, via the recruitment of innate prosurvival kinase cascades. The PI3K-Akt and MEK 1/2-p42/44 kinases have been shown to be important components of these cell survival pathways (9) and have antiapoptotic effects. A reduction in reperfusion-induced injury can be obtained by the upregulation of these kinases (18). In this regard, work from our group and others has shown that this can be achieved using pharmacological agents such as insulin (22), insulin-like growth factor 1 (29), atorvastatin (4), bradykinin (5), urocortin (36), cardiotrophin 1 (7), transforming growth factor (TGF)-β1 (2), and opioids (13). Therefore, intervening at the time of reperfusion to attenuate the effects of lethal reperfusion injury provides an important strategy for cardioprotection. In this regard, the recently described phenomenon of ischemic postconditioning offers another potentially effective intervention for limiting myocardial injury that appears to have many similarities to that described above.

ISCHEMIC POSTCONDITIONING

In 2003, a phenomenon termed ischemic postconditioning, a cardioprotective maneuver that targets the reperfusion phase, was first published by Vinten-Johansen’s group (46). Their study described the application of several brief, transient cycles of alternating reperfusion-ischemia immediately after the sustained ischemic episode, which resulted in a reduction in myocardial injury. In the in vivo dog model, at the immediate onset of reperfusion after a 60-min occlusion of the LAD artery, postconditioning was achieved by allowing reflow for 30 s followed by 30-s reocclusion of the LAD artery repeated a total of three times before the remaining reperfusion phase (46). Ischemic postconditioning reduced infarct size by 44%, an effect comparable with ischemic preconditioning. However, in this study, the authors suggested that the mechanisms by which preconditioning and postconditioning conferred myocardial protection were likely to be different because preconditioning triggers protective pathways before ischemia, whereas postconditioning alters events after ischemia. Subsequent data from Yellon’s group (17) suggests that this may not be the case. They demonstrated that by inhibiting PI3K using LY-294002 or MEK1/2 using PD-98059 during the first 15 min of reperfusion the cardioprotective effects of ischemic preconditioning could be abrogated. This study, taken together with the key reports from Tsang et al. (40) and Yang et al. (43), which demonstrate the role of the PI3K-Akt and MEK1/2-ERK pathways in postconditioning (see Fig. 1), suggest that both preconditioning and postconditioning may share a common pathway.

The term postconditioning refers to the ischemic stimulus being applied after the lethal ischemic period rather than before it as in preconditioning. This use of ischemia after a prolonged ischemic period is a simple and logical progression. The facts that 1) brief ischemia before prolonged ischemia is protective, 2) many pharmacological agents have since been found to be preconditioning mimetics, and 3) many pharmacological agents have been shown to be protective when administered at early reperfusion would lead one to speculate that brief ischemia after a prolonged episode may protect the myocardium from injury if ischemic postconditioning is mediated by the same cellular signaling pathways as drugs given at reperfusion. However, this should not distract from the fact that intermittent
interruptions to reperfusion are physically modifying reperfusion, and, as discussed earlier, modified reperfusion is known to be beneficial.

Subsequent studies have shown postconditioning to be effective in other animal models, namely, in vivo rats (24), rabbits (43), isolated rat hearts (40), and neonatal rat cardiomyocytes (38) (see Table 1).

Further studies by Galagudza and colleagues (10) have shown that postconditioning has an antiarrhythmic effect on reperfusion-induced arrhythmias, but in this study “postconditioning” was achieved by a single episode of global ischemia for 2 min and therefore is likely to have been as a result of cessation of metabolites required for energy-dependent ventricular fibrillation rather than the mechanisms already discussed and as such should be interpreted with caution.

Mechanistic data were provided by subsequent work from Kin et al. (24), who demonstrated that ischemic postconditioning was effective in open-chest rats. In addition, postconditioned hearts showed less free radical generation and less oxidant-induced injury as demonstrated by malondialdehyde levels and dihydroethidium fluorescence for superoxide generation. The role of oxidant injury at reperfusion was further supported by data from Sun et al. (38) in rat cardiomyocytes exposed to hypoxic postconditioning. However, further studies are required to elucidate the source and exact role of free radicals that are reduced by postconditioning. Several interesting points were evident from the data of Kin et al. First, postconditioning was achieved using three or six cycles of 10-s interruptions to reperfusion compared with 30 s in the dog, suggesting that the duration of ischemia of the postconditioning protocol may be species dependent. This fact is further supported by work from our laboratory, which showed that postconditioning using six cycles of 30-s interruptions to reperfusion in isolated rat hearts failed to reduce infarct size (unpublished data). Furthermore, from the data of Kin et al., the limitation of infarct size from three or six cycles of postconditioning were not statistically different from one another, suggesting that it is not the quantity of cycles that is important but the duration. In this regard, Schwartz et al. (37) failed to demonstrate any infarct reduction in open-chest pigs using a postconditioning protocol of three cycles of 30-s reperfusion/reocclusion, the possible explanations being that in the pig species, the duration of interruption to reperfusion required is different from the dog in addition to the differences in coronary collateral flow that exist between these two species.

As discussed earlier, because several pharmacological agents have been shown to limit myocardial injury when administered at reperfusion by the activation of the prosurvival cellular kinase pathways PI3K-Akt and MEK1/2-ERK, one may speculate that ischemic postconditioning exerts its protective effects by the same mechanisms (18).

Postconditioning-induced protection is mediated via the RISK pathway. The first evidence that prosurvival kinase cascades mediated the protective effects of postconditioning was data from our group (40). Research had initially shown that ischemic preconditioning exerted its cardioprotective effect by activating the RISK pathway and that pharmacologically inhibiting these kinases at reperfusion abolished this effect (17). As a result, we hypothesized that postconditioning protected the myocardium by activating the same pathway. We showed that postconditioning’s protection in isolated rat hearts was abrogated in the presence of the PI3K inhibitors LY-294002 and wortmannin, and the protection was commensurate with the phosphorylation of Akt and its downstream targets, namely, p70S6K and eNOS, in accordance with the RISK pathway (see Fig. 1). Shortly afterward, Downey and colleagues (43) demonstrated that the protection afforded by postconditioning in rabbits was attenuated in the presence of PD-98059 (a MEK1/2-ERK inhibitor), 5-hydroxydecanoate [a mitochondrial ATP-sensitive K⁺ (Kₐ₅) channel blocker],

Table 1. Studies of ischemic postconditioning

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model</th>
<th>Postconditioning Protocol</th>
<th>End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhao et al. (46)</td>
<td>In vivo dog</td>
<td>3 Cycles of 30-s Rep/30-s Isch</td>
<td>Infarct size, oxidant injury, PMN accumulation, and endothelial function</td>
</tr>
<tr>
<td>Kin et al. (23, 24)</td>
<td>In vivo rat</td>
<td>3 Cycles of 10-s Rep/10-s Isch and 6 cycles of 10-s Rep/10-s Isch</td>
<td>Ventricular fibrillation, Infarct size and phosphorylation of Akt, eNOS, and p70S6K</td>
</tr>
<tr>
<td>Galagudza et al. (10)</td>
<td>In vitro rat</td>
<td>1 Cycle of 2-min Isch</td>
<td>Infarct size, oxidant injury, PMN accumulation, and superoxide generation</td>
</tr>
<tr>
<td>Tsang et al. (40)</td>
<td>In vitro rat</td>
<td>6 Cycles of 10-s Rep/10-s Isch</td>
<td>Infarct size, oxidant injury, PMN accumulation, and superoxide generation</td>
</tr>
<tr>
<td>Sun et al. (39)</td>
<td>In vivo rat liver</td>
<td>2-, 3-, 5-, and 7-min Rep separated by 2-min Isch</td>
<td>Apoptosis, oxidant injury and generation, and mitochondrial ultrastructure</td>
</tr>
<tr>
<td>Yang et al. (43)</td>
<td>In vivo rabbit</td>
<td>4 Cycles of 30-s Rep/30-s Isch and 6 cycles of 30-s Rep/30-s Isch</td>
<td>Infarct size</td>
</tr>
<tr>
<td>Halkos et al. (14)</td>
<td>In vivo dog</td>
<td>3 Cycles of 30-s Rep/30-s Isch</td>
<td>Infarct size, oxidant injury, CK levels, tissue edema, and superoxide generation</td>
</tr>
<tr>
<td>Schwartz (37)</td>
<td>In vivo pig</td>
<td>3 Cycles of 30-s Rep/30-s Isch</td>
<td>Infarct size (unable to show protection)</td>
</tr>
<tr>
<td>Philipp et al. (31)</td>
<td>In vivo rabbit</td>
<td>4 Cycles of 30-s Rep/30-s Isch</td>
<td>Infarct size</td>
</tr>
<tr>
<td>Yang et al. (42)</td>
<td>In vitro rabbit</td>
<td>6 Cycles of 10-s Rep/10-s Isch</td>
<td>Infarct size</td>
</tr>
<tr>
<td>Kin et al. (23, 24)</td>
<td>In vitro mouse</td>
<td>6 Cycles of 10-s Rep/10-s Isch</td>
<td>Endogenous adenosine levels</td>
</tr>
<tr>
<td>Pagliaro et al. (30)</td>
<td>In vitro rat</td>
<td>3 Cycles of 10-s Rep/10-s Isch</td>
<td>Infarct size and PMN accumulation</td>
</tr>
<tr>
<td>Sun et al. (39)</td>
<td>Isolated rat cardiomyocytes</td>
<td>3 Cycles of 5-min reoxygenation/5-min hypoxia</td>
<td>ROS generation, oxidant injury, and mitochondrial calcium levels</td>
</tr>
<tr>
<td>Argaud et al. (1)</td>
<td>In vivo rabbit mitoch</td>
<td>4 Cycles of 1-min Rep/1-min Isch</td>
<td>Infarct size, and mPTP calcium sensitivity</td>
</tr>
</tbody>
</table>

Rep, reperfusion; Isch, ischemia; PMN, polymorphonuclear neutrophils; eNOS, endothelial nitric oxide synthase; CK, creatine kinase; mPTP, mitochondrial permeability transition pore.
glibenclimide (nonselective K<sub>ATP</sub> blocker), and N-nitro-L-arginine methyl ester (L-NAME; antagonist of NOS), suggesting the involvement of another component of the RISK pathway, namely, the MEK 1/2-ERK cascade, and downstream effectors such as the production of NO and mitochondrial K<sub>ATP</sub> channel opening. However, in this study, it was not actually demonstrated that postconditioning led to the actual phosphorylation of ERK or eNOS. Interestingly, as in the data from Kin et al. (24), four or six cycles of 30-s reperfusion/reocclusion produced similar reductions in infarct size that is seen in Downey and colleagues’ study. Furthermore, when the index ischemia was extended from 30 to 45 min, the combined effects of one cycle of preconditioning (5-min ischemia/10-min reperfusion) plus four cycles of 30-s postconditioning resulted in an additive effect greater than either intervention alone. However, protocols using 30-min index ischemia in rats from our data (40) and 60-min index ischemia in dogs in a study by Halkos et al. (14) did not support this finding when pre- and postconditioning were combined. This may be explained by the different experimental protocols in these studies and that each intervention has a maximal protective effect that can only be exposed when the index ischemia is prolonged.

Further studies support the finding for the role of PI3K in the protection afforded by postconditioning. In this regard, data from Downey’s group (31, 42) found that postconditioning’s protective effect was abolished when wortmannin (the PI3K inhibitor) was infused just before the onset of reperfusion in their rabbit model.

**Upstream mediators of postconditioning.** While the involvement of the RISK pathway has been clearly demonstrated in postconditioning, how the RISK pathway is upregulated is unclear. Adenosine has been implicated as a potential upstream mediator by two recent studies. The first study from Vinten-Johansen’s laboratory, using a mouse and rat model, demonstrated that the levels of endogenous adenosine were reduced in the coronary effluent collected shortly after completion of the postconditioning protocol, suggesting that greater intravascular adenosine was being retained as a result of postconditioning (23). Furthermore, the infarct sparing effect of postconditioning was abolished by the presence of both 8-p-(sulfophenyl) theophylline (8-SPT) (a nonselective adenosine receptor antagonist) and ZM241385 (an A<sub>2a</sub> receptor antagonist). The second study from Downey’s laboratory also supported the inhibitory action of 8-SPT on postconditioning in rabbits (42). Taken together, these suggest that adenosine may have an important role in the activation of the RISK pathway. Other mechanisms by which postconditioning activates the RISK pathway need to be further investigated, for example, the possible role of PKC or ROS as upstream mediators.

**Downstream mediators of postconditioning.** Endothelial NO and p70S6 kinase have been shown to be downstream targets in postconditioning from our studies (40). Postconditioning has in addition, been shown to be blocked by L-NAME (a NOS inhibitor) and 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ) (a guanylyl cyclase antagonist) in both rabbits (42) and rats (30), implicating these as downstream components.

**Potential end-effector of postconditioning.** A number of studies have discovered a common finding with regard to the timing of postconditioning. The protection induced can only be taken advantage of if postconditioning is initiated at the onset of reperfusion and is lost if delayed by 1 min in the rat (24) and rabbit models (31). Therefore, this would suggest that the end-effector of protection must exert its actions during the initial stages of reperfusion. In this regard, pharmacologically inhibiting the mitochondrial permeability transition pore (mPTP) during the first few minutes of reperfusion (15, 16) has been shown to be cardioprotective and delaying this inhibition by 15 min abolishes this protection. Similarly, delaying the administration of insulin, which is known to activate the RISK pathway, until after the first 15 min of reperfusion abrogates its infarct-limiting effect (22). Therefore, one could speculate that the mPTP, which is known to regulate cell death during the first few minutes of reperfusion (12), is potentially the main candidate as the end-effector.

The importance of mitochondria in postconditioning was further demonstrated in a study using a rat model of hepatic ischemia-reperfusion injury that demonstrated that postconditioning of the liver resulted in a decrease in mitochondrial ultrastructural injury and apoptosis (39).

Evidence for mPTP involvement in postconditioning-induced protection has been strengthened by an important study from Ovize’s laboratory (1). Using isolated rat mitochondria, this group found that postconditioning reduced mitochondrial susceptibility to calcium overload compared with controls, an effect comparable with preconditioning and NIM811 (a cyclosporin analog-specific mPTP inhibitor). However, the mechanism by which postconditioning inhibits mPTP opening is still unclear, and further studies in this area are needed.

**CLINICAL IMPLICATIONS**

While the phenomenon of ischemic postconditioning is a novel and attractive strategy scientifically, in the clinical situation ischemic postconditioning may be difficult conceptually to introduce, i.e., reintroduction of ischemia at the time of reperfusion may lead to potential complications. For example, during primary angioplasty, repetitive inflations and deflations of the balloon may result in coronary plaque rupture with consequences for restenosis or embolic events. During coronary bypass surgery, interruptions to reperfusion via the newly grafted conduit may only lead to regional myocardial protection in the area supplied by that bypass. An alternative strategy during bypass surgery would be the repetitive clamping and unclamping of the ascending aorta to achieve ischemic postconditioning, a concept, however, that many cardiac surgeons would be unwilling to perform due to the high risk of disrupting atheromatous plaque debris and subsequent risk of stroke. The reintroduction of ischemia at the time of administration of thrombolytic drugs is also not feasible clinically.

However, the concept of “pharmacological postconditioning” by the administration of agents that activate the RISK pathway and mediate the protective effects of postconditioning is a more practical solution. Agents such as insulin (22), atorvastatin (4), bradykinin (5), TGF-β (2), and glucagon-like peptide1 (GLP-1) (27) could individually be used as adjuvants to current reperfusion strategies such as thrombolytics and primary percutaneous coronary intervention (PCI) to limit lethal reperfusion injury and could form the basis of much needed and important reperfusion strategies.

In conclusion, the novel phenomenon of postconditioning has attracted much attention over the past 2 years. Some of the mechanisms involved in this novel approach have been dis-
covered, most of which are shared by drugs that protect the myocardium when given at reperfusion, and, therefore, these may be considered as pharmacological postconditioning mimetics. The current evidence so far (see Table 1) suggests that postconditioning via upstream mediators, such as possibly adenosine, upregulates the prosurvival cascades of the RISK pathway, which then via downstream targets such as p70S6K, eNOS, and guanylyl cyclase, prevents the opening of the mPTP to exert its cardioprotective effect. Postconditioning, in addition to the above direct active effects on the myocyte, also reduces myocardial injury through the passive effects of modified reperfusion resulting in reduced endothelial dysfunction, myocardial stretch and hypercontracture, mitochondrial calcium overload, myocardial edema, and ROS, which has been known for a number of years to be beneficial (see Fig. 1). The comment by Heusch that postconditioning is possibly “old wine in a new bottle” is very apt (20) and indicative that we have probably just rediscovered an old phenomenon. However, the use of the term postconditioning has initiated a number of studies that might not have otherwise been undertaken had this phenomenon not been given such an appealing name. These important studies have allowed us to gain a greater understanding of cellular pathophysiology and in themselves created renewed interest that has enabled the concept of reperfusion injury to be revisited.

GRANTS

A. Tsang was supported by a project grant from the British Heart Foundation.

REFERENCES


42. Yang XM, Philipp S, Downey JM, and Cohen MV. Postconditioning’s protection is not dependent on circulating blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl cyclase activation. *Basic Res Cardiol* 100: 57–63, 2005.


Andrew Tsang
Derek J. Hausenloy
Derek M. Yellon
The Hatter Institute and Centre for Cardiology, University College London Hospitals, London, United Kingdom