

# REDUCING MYOCARDIAL REPERFUSION INJURY BY POSTCONDITIONING

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## REZUMAT

Fenomenul de postcondiționare constă în aplicarea unor scurte perioade de ocluzie coronariană, alternate cu reperfuzii imediat după reluarea perфуziei postinfarct miocardic. Postcondiționarea experimentală realizează o formă de "reperfuзie controlată" responsabilă de caracterul dual al protecției: (i) protecție vasculară prin efectul benefic asupra hemodinamicii coronariene cu reducerea disfuncției endoteliale și (ii) protecție antiinfarct similară celei realizate de precondiționarea ischemică clasică, utilizând, cel puțin parțial, căi comune de semnalizare intracelulară. Mecanismul complex al protecției presupune intervenția a 3 tipuri de factori: declanșatori, de tipul adenozeinei și opioidelor endogene, mediatori, cum sunt proteinkinaza C, canalele de potasiu mitocondriale ATP-dependente și respectiv, kinazele asociate cu supraviețuirea celulară și efectori finali, evenimentul central fiind reprezentat de inhibiția deschiderii porului de permeabilitate mitocondrială tranzitorie. Având în vedere avantajul aplicării protocoalelor de postcondiționare în condiții clinice, elucidarea mecanismelor intime de desfășurare în vederea identificării unor agenți farmacologici ce pot reproduce protecția oferită de postcondiționare (mimeticele postcondiționării) constituie o prioritate. Rolul major în cardioprotecție este deținut de activarea unei căi de semnalizare comune atât pre- cât și postcondiționării, reprezentată de activarea kinazelor protectoare cu inhibiția consecutivă a deschiderii porului de permeabilitate mitocondrială tranzitorie (PMT). Aceasta din urmă este considerată la ora actuală terapia de elecție a leziunilor letale induse de reperfuзia experimentală. Articolul de față rezumă datele referitoare la studiile privind fenomenul de postcondiționare, cu sublinierea celor mai recente descoperiri în privința mecanismelor cardioprotecției.

**Cuvinte cheie:** reperfuзia miocardică, infarct miocardic, cardioprotecție, postcondiționare, tranziția permeabilității mitocondriale

## ABSTRACT

The phenomenon of postconditioning is defined as a series of brief mechanical coronary artery occlusions and reperfusions applied at the very onset of reperfusion after myocardial infarction. The experimental design of postconditioning performs a particular type of "controlled reperfusion", which confers protection via a double mechanism: (i) vascular protection via the improvement of early reperfusion hemodynamics as it attenuates the damage to endothelial cells and (ii) anti-infarct protection similar to that provided by the classic ischemic preconditioning with the activation of, at least some, common pathways of intracellular signal transduction. The complex mechanism of protection include: triggers, such as adenosine and endogenous opioids, mediators such as protein kinase C, mitochondrial ATP-sensitive potassium channels and the pro-survival kinases and the end-effectors, with the inhibition of the permeability transition pore formation being the major event. The possibility to reduce infarction in clinical setting by using adjunct interventions amenable as late as the onset of reperfusion activated the interest for the elucidation of myocardial postconditioning signal transduction in order to develop "postconditioning-mimetics". Cardiac protection is mediated by the recruitment, at the very onset of reperfusion, of a signalling pathway common to both pre-and postconditioning phenomena and represented by the activation of pro-survival kinases which subsequently will inhibit the permeability transition pore (PTP) opening. The latter is considered nowadays the elective therapy for the experimental lethal reperfusion injury. The effective inhibition of PTP opening in the first minutes of reperfusion following an ischemic event appears as a promising future therapeutic approach that might efficiently accompany the revascularization procedures. This review focuses on current research into postconditioning-mediated cardioprotection, summarizing the postconditioning studies to date and highlighting the recent findings concerning the mechanisms of protection.

**Key Words:** Reperfusion injury, myocardial infarction, cardioprotection, postconditioning, mitochondrial permeability transition

## INTRODUCTION

Myocardial ischemia is a complex phenomenon in which reduction in blood flow elicits both reactive (biochemical, functional, structural) changes, as well as endogenous adaptive responses that under certain circumstances might be protective. From the latter group, the most powerful mechanism of endogenous cardioprotection, extensively studied for the past two decades, is the phenomenon of ischemic preconditioning (IPC). Initially described over 20 years ago by Murry et al, the phenomenon of classic

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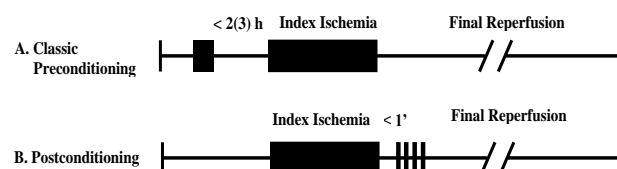
IPC consists of the application of brief episodes of non-lethal ischemia alternated with reperfusion that confer resistance against a subsequent lethal ischemic insult (referred to as index or test ischemia).<sup>1</sup> It has to be mentioned that IPC does not reduce the ultimate infarct size, but delays the development of infarction (so that with timely reperfusion the definite infarct size will be reduced).<sup>2</sup> Over the past 20 years this powerful protective phenomenon has generated a tremendous research activity which has led both to the partial elucidation of the signal transduction of IPC and to the discovery of several “preconditioning mimetic” agents able to simulate the biochemistry of preconditioning in the absence of ischemia (reviewed by Yellon et al).<sup>2</sup> Despite the fact that both ischemic and pharmacological preconditioning has been proven to be effective in reducing cell death in every species tested including humans, there are no preconditioning based therapies that are routinely used in clinical medicine to date.<sup>3</sup> The major reason is that, by definition, the preconditioning intervention should be applied before the onset of an acute coronary event, which obviously is difficult to predict.<sup>4</sup>

However, the last few years brought a shift in the paradigm of the preconditioning related cardioprotection: the protection is exerted mainly at reperfusion rather than during the prolonged ischemic period.<sup>4</sup> While for more than 15 years it was thought that IPC must be preventing some of the deleterious effects of the prolonged ischemia, several groups have recently established that the anti-infarct protection of IPC actually occurs early in reperfusion.<sup>5,6</sup> These findings together with the emergence of a diverse array of pharmacological agents (such as insulin, erythropoietin, glucagon-like peptide 1, atorvastatin, transforming growth factor- $\beta$ , cyclosporine) which given at the moment of post ischemic reperfusion could offer significant cardioprotection against cell death, renewed the interest of the scientific community for protective manoeuvres applicable at the time of myocardial reperfusion.

## CARDIOPROTECTION WITH POSTCONDITIONING

Restoration of blood flow remains the gold standard in the treatment of acute coronary syndromes, so obviously a more suitable approach to cardioprotection is to apply novel adjunctive protective strategies at the onset of reperfusion therapy.<sup>7</sup> Yet, reperfusion has been termed a “double-edged sword” since it is responsible per se for detrimental events (e.g., myocardial stunning,

ventricular arrhythmias) including the so-called lethal reperfusion injury.<sup>8</sup> For the last two decades the existence of lethal reperfusion injury as a separate entity was highly debated, with some investigators suggesting that reperfusion will simply accelerate the cellular death induced by prolonged ischemia, while others considered that reperfusion itself truly kills myocytes both by necrosis and apoptosis.<sup>9-12</sup> However, despite the clear benefits of revascularisation procedures (such as thrombolysis and coronary angioplasty), the need for enhancing cardioprotection by treatments targeting the reperfusion phase appears to be greater than ever. Indeed, as recently reported, only a third of the life-years lost by myocardial infarction are gained by reperfusion.<sup>13</sup> In this respect, the newly described phenomenon of postconditioning, whereby the brief ischemia–reperfusion cycles are applied at the very onset of reperfusion following the prolonged ischemic insult, conferred a protection comparable with that elicited by classic IPC.<sup>14</sup> (Fig. 1)



**Figure 1.** Timeframe for classic preconditioning and postconditioning related cardioprotection. Ischemic episodes are figured in black. In order to trigger protection the preconditioning episode should precede the index ischemia with 2 - 3 hours (according to species/experimental model), while the postconditioning protocol should be initiated within the 1<sup>st</sup> minute of the final reperfusion.

Thus, postconditioning demonstrated the existence of lethal reperfusion injury and represents a novel yet potent algorithm that may provide greater clinical potential in the future from two points of view: a higher number of clinical applications as compared to preconditioning (patients with evolving myocardial infarction receiving primary coronary angioplasty being mostly envisaged) and, more important, the possibility to develop agents for pharmacological postconditioning that can be virtually applied in all clinical situations associated with myocardial ischemia-reperfusion injury.<sup>7,15,16</sup> However, the design of “postconditioning mimetics” requires a thorough characterisation of the molecular mechanisms of postconditioning which represents nowadays an active research field currently involving many teams worldwide.

Several authors opined that postconditioning represents a form of modified reperfusion that has been known for several years to be beneficial to the ischemic myocardium.<sup>17-19</sup> Indeed, gradual, staged (or

“gentle”) reperfusion was reported long time ago to improve the postischemic contractile recovery and more recently to reduce the infarct size (but to increase neutrophil accumulation) in dog hearts subjected to left coronary artery ligation.<sup>20-22</sup>

Likewise, the rapid intermittent interruptions of blood flow characterizing postconditioning resulted in a limitation of infarct size similar in magnitude to that afforded by ischemic preconditioning in all animal models (reviewed by 7), with the exception of pig hearts.<sup>23,24</sup> However, a recent paper reported that postconditioning still elicits the antinecrotic protection in pigs but requires multiple ischemia/reperfusion cycles.<sup>25</sup>

At variance with the effects of “gentle” reperfusion, postconditioning was also associated with: decreased accumulation of neutrophils, reduced oxidative stress, reduced cardiac apoptosis and improved endothelial function.<sup>26</sup> This last issue, protection against endothelial dysfunction (together with the anti-inflammatory and antioxidant effects), makes postconditioning a particular attractive strategy in the clinical setting view the central role of endothelial dysfunction in the pathogenesis of atherosclerosis. Recently, Ma et al studied the arterial endothelial function non-invasively by echo Doppler in patients with acute myocardial infarction who underwent percutaneous coronary intervention, showing that the endothelium-dependent vasodilator function was improved in the postconditioned group.<sup>27</sup>

While the antinecrotic effect of experimental postconditioning has been shown to persist for 24 hours and 72 hours, no data is available concerning the long-term persistence of endothelial protection so far.<sup>28,29</sup>

Since all the above mentioned aspects of postconditioning related cardioprotection are also shared by ischemic preconditioning, one logical question that arose was if protection by postconditioning was additive to that of preconditioning.<sup>26</sup> Combination of both protocols did not result in cumulative antinecrotic protection in isolated rat hearts and in vivo canine hearts.<sup>30,31</sup> However, in one study performed in rabbit hearts in vivo, the combination of manoeuvres reduced infarct size significantly more than either intervention alone.<sup>32</sup>

Interestingly, in the same experimental model additive protection was reported between anaesthetic postconditioning with isoflurane and ischemic postconditioning.<sup>33</sup> It has been suggested that the duration of prolonged ischemia and/or species differences may be responsible for this discrepancy.<sup>34</sup>

### Characteristics of protection

Three observations are critical to the postconditioning related cardioprotection. The first one, concerning the timing, is that the time interval during postconditioning which is applied is critical to cardioprotection, i.e. the postconditioning protocol should be applied within the first minute of reperfusion. (Fig.1) The infarct sparing effect of postconditioning was lost if the intermittent blood flow interruption was applied after one minute of reperfusion in rat and rabbit hearts and after 10 minutes of reperfusion in rabbit hearts, respectively.<sup>32,35,36</sup> There is only one study in the in vivo rabbit heart that reported protection with a postconditioning protocol started at 1 minute of reperfusion.<sup>29</sup> These findings underline the importance of the first few minutes of reperfusion in the pathogenesis of the lethal reperfusion injury and the need for further studies aimed at elucidating the mechanisms beyond this short delay dependent protection.

The second observation related to the algorithm of protection, refers to the number and duration of ischemia-reperfusion cycles, with a more important contribution to cardioprotection for the duration as compared to the number of the episodes imposed. The 30 seconds protocol (i.e., 30 seconds reperfusion + 30 seconds ischemia cycle) was reported to be protective in the in vivo models of dogs and rabbits, whilst a 10 seconds protocol was efficient in rat hearts in situ.<sup>14,31,35</sup> In the rat experiment, 3 cycles of the previously mentioned postconditioning protocol were equally effective in reducing infarct size as were 6 cycles, observation that was recapitulated for the mouse heart when recovery of the left ventricular global function was used as endpoint of cardioprotection.<sup>35,37</sup> Yang et al initially reported that in rabbit hearts the 4-cycle algorithm reduced the infarct size to the same extent as did the 6-cycle one; however, a more recent paper from the same group attributed a better protection the latter protocol.<sup>32,38</sup>

However, postconditioning of the rabbit heart was associated with most inconsistency. The above mentioned 4 cycles postconditioning protocol described to elicit significant in vivo anti-infarct protection first, was not effective in the isolated rabbit heart model and moreover, could not be reproduced by another group which applied it in the same open-chest in vivo model.<sup>32,38,39</sup> Apparently, in isolated rabbit heart cycle duration is more important than the cycles’ number, since the 10 seconds cycles elicited a better protection when compared to the 30 seconds ones. Interestingly, Brownell et al reported in abstract form

that, using a postconditioning protocol of 9 episodes each of 15 to 60 seconds performed in isolated globally ischemic rabbit hearts, they were not able to reproduce the anti-infarct protection; this observation suggests a multiple cycles dependent loss of postconditioning effect that was previously reported for ischemic preconditioning as well.<sup>40,41</sup>

The third observation is related to the duration of the previous index ischemia. In a conscious rat model Bolli's group firstly demonstrated that cardioprotection afforded by postconditioning is limited to mild to moderate myocardial injury, i.e. prolonged ischemia of 30 minutes, but not of 45 and 60 minutes and suggested that its clinical potential may be restricted by this limited protective efficacy.<sup>42</sup> In this study the authors reported that extending the 10 seconds postconditioning algorithm from 6 to 20 cycles increased the cardioprotective potency of the protocol applied after 30 minutes of ischemia (albeit it did not reach the anti-necrotic protection elicited by ischemic preconditioning). In this setting, the further extension of the algorithm from 20 to 60 cycles reversed the infarct-sparing effect of postconditioning. Yet, they were not able to reproduce the anti-necrotic protection when applying a high number of postconditioning cycles (20 episodes of 10 seconds occlusion/10 seconds reperfusion after 45 and 60 minutes of ischemia, respectively). However, data concerning the effect of fewer cycles - reported by other groups to protect against 45 min ischemia and 60 min ischemia in other experimental models - are not presented in this paper.<sup>31,32,42</sup>

More recently, interesting data came out from Duncker's group: these authors showed that in the rat model postconditioning with three cycles of 30-s reperfusion and reocclusion induced a dichotomised effect: it increased the infarct size if followed an index ischemia of 15 and 30 minutes and decreased it for longer durations of the test ischemia (45 and 60 minutes, respectively).<sup>43</sup> These paradoxical effects of postconditioning were considered to be related to the divergent effects on Akt phosphorylation and superoxide production.

Despite these conflicting experimental data, a pioneering work of Ovize's group demonstrated that one can postcondition the human heart. The authors reported a reduction in creatine kinase (CK) release (a surrogate marker of infarct size) over the first 72 hours by 36% after performing a 4 episodes postconditioning protocol of 60 seconds inflation-deflation of the angioplasty balloon in patients with ongoing acute myocardial infarction.<sup>44</sup>

A very recent study, further addressed the potential clinical efficacy of postconditioning by performing a retrospective comparison of peak CK release in patients with single vessel occlusion who presented with ST-segment elevation myocardial infarction for primary angioplasty.<sup>45</sup> Peak CK release was significantly lower in the subset of patients receiving  $\geq 4$  balloon inflations versus the subset treated with 1-3 inflations or with direct stenting, an outcome consistent with the concept that stuttered reperfusion (or postconditioning) is associated with cardioprotection in the clinical setting.

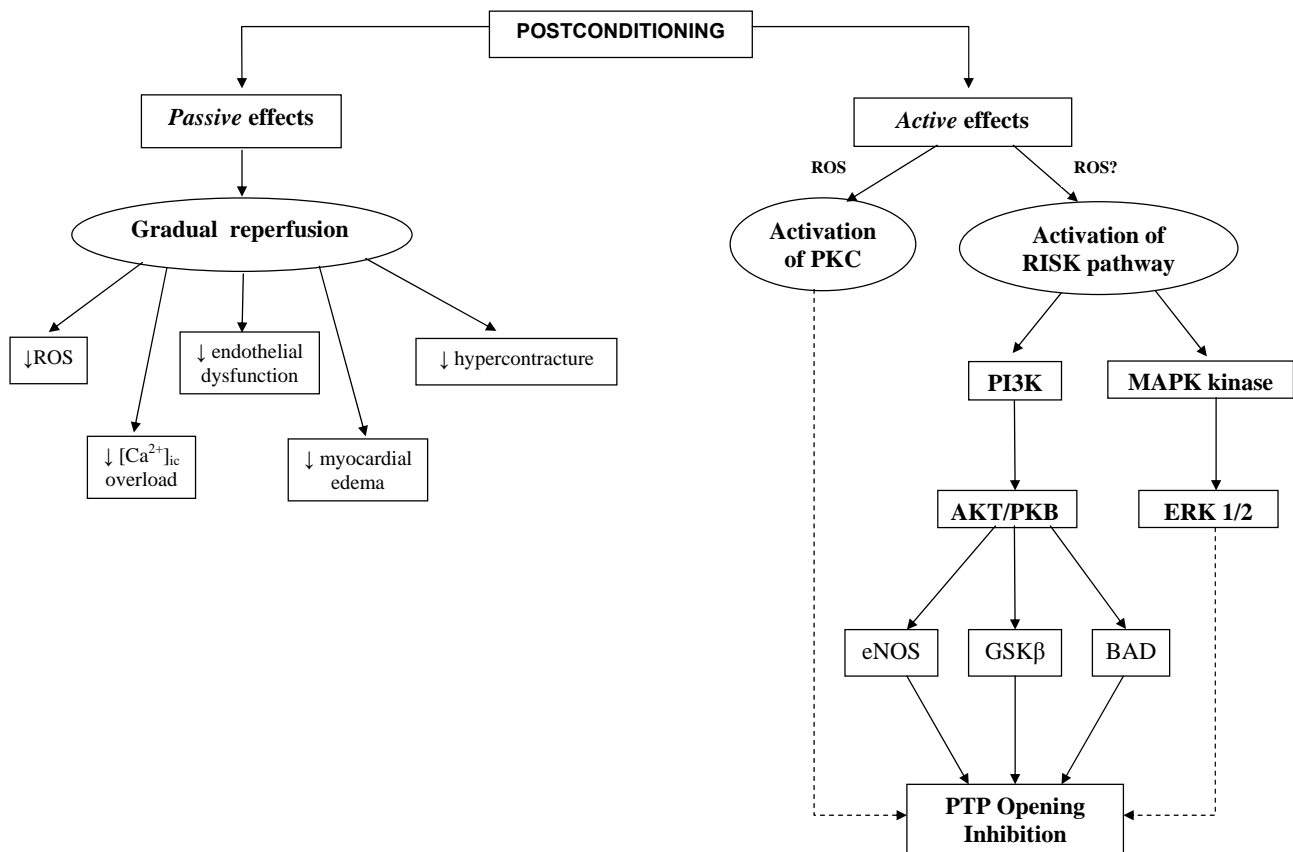
Generally, it has been empirically concluded that smaller species (rats and mice) require shorter postconditioning protocols (10-15 seconds) while larger species (rabbits, dogs, humans) need longer (20-30 seconds) cycles.<sup>34</sup> The current opinion is that when addressing the complexity of reperfusion injury a combination therapy between a mechanical manoeuvre and drug therapy should be envisaged.<sup>34</sup> Indeed, postconditioning may mimic the beneficial effects of shear stress on vasculature which are difficult to be reproduced by pharmacological intervention. In the light of this double protection, at cardiac and vascular level, the elucidation of the intimate molecular mechanisms of postconditioning definitely warrants further studies.

### **Mechanisms of protection**

Yellons's group firstly suggested that postconditioning related protection can be ascribed to 2 different aspects, a "active" and an "passive" one, but most probably they are intricated.<sup>19,34</sup>

The "active" effect, considered the most important, is related to the activation of a series of prosurvival kinases, they have termed the RISK (Reperfusion Injury Salvage Kinase) pathway.<sup>46</sup> These kinases are: (i) the phosphatidylinositol-3-OH kinase (PI3) which directly activates Akt or protein kinase B, PKB - the PI3-Akt pathway) and (ii) the mitogen activated protein kinase (MAPK) family members, the 42 and 44kDa extracellular signal-regulated kinases (Erk 1/2) i.e., the MAPK/ERK1/2 pathway. The major role of these kinases in the postconditioning elicited cardioprotection was demonstrated by the means of pharmacological approach: their inhibition at reperfusion abrogated the infarct size reduction of postconditioning.<sup>19</sup>

Potential downstream targets for the RISK mediated phosphorylation are: glycogen synthase kinase  $\beta$  (GSK $\beta$ ), endothelial NO synthase (eNOS) and the pro-apoptotic protein Bad.



**Figure 2.** Putative mechanisms of postconditioning related cardioprotection. Controlled reperfusion is associated with both passive and active effects, the latter being considered the major responsible for the inhibition of the mitochondrial permeability transition. (Modified from Ref. 19)

The current opinion is that RISK represents a common signalling pathway in cardioprotection which is early recruited during postischemic reperfusion and exhibits two important features: (i) it is upregulated by both ischemic pre- and postconditioning and (ii) it is acutely pharmacologically activated by agents such as insulin, erythropoietin, glucagons-like peptide.<sup>17</sup> However, the mechanisms through which IPC and postconditioning actually activates the RISK pathway and its downstream effectors are far from being elucidated.

The most appropriate approach in order to mechanistically understand the postconditioning protective sequence is to perform an analogy to the signal transduction of IPC reported to include triggers, mediators and end-effectors.<sup>3</sup> A comparison of their involvement in postconditioning vs. preconditioning has been recently published and clearly they share similar, yet not identical elements.<sup>34</sup> Moreover, the contrast between the redundancy of triggers and mediators and the paucity of direct evidences on the end-effectors remains evident for both cardioprotective mechanisms.

The “passive” effect of gradual reperfusion, thought to be ancillary, is responsible for the: (i) reduced

myocardial edema and ROS production (by limitation both coronary flow and O<sub>2</sub> supply), (ii) decreased cardiomyocytes hypercontracture (by attenuating the protons washout from the extracellular space; acidotic reperfusion may protect by decreasing the Na<sup>+</sup>/H<sup>+</sup> exchanger activation and by inhibiting mitochondrial permeability transition), (iii) delayed washout of autacoids such as adenosine which triggers protection by binding its specific receptors), (iv) diminished calcium overload (both cytosolic and mitochondrial) and (v) reduced endothelial dysfunction, effect which has been recently confirmed in clinical settings.<sup>19,23,35,38</sup>

Despite the “secondary” effect ascribed to the passive mechanisms, recently two groups independently underlined the importance of acidosis in triggering the postconditioning cardioprotection. The Japanese group of Kitakaze demonstrated in a canine model of myocardial infarction that prolonged transient acidosis during the early reperfusion phase was responsible for the protective effect of postconditioning since the administration of NaHCO<sub>3</sub> completely abolished its the infarct size-limiting effects.<sup>47</sup>

Furthermore, the phosphorylation of Akt and ERK in ischemic myocardium induced by postconditioning

was also blunted by the cotreatment of  $\text{NaHCO}_3$ . Similar results were provided by the American group of Downey in isolated rabbit hearts where 6 cycles of 10 seconds reocclusion/10 seconds reperfusion (but no 3 similar cycles) were followed by an anti-infarct protection equivalent to the one obtained by administering an hypercapnic buffer (pH 6.9) for the first 2 minutes of reperfusion.<sup>48</sup> Cardioprotection by both postconditioning and acidosis were blocked by concomitant administration of a ROS scavenger (N-2-mercaptopyrionyl glycine), or a PKC antagonist (chelerythrine) or a mitochondrial  $\text{K}(\text{ATP})$  channel closer (5-hydroxydecanoate). The authors concluded that the low pH during the postconditioning cycles prevents mitochondrial permeability transition pore opening, while the intermittent oxygen bursts allow mitochondria to produce small amounts of ROS, enough to activate PKC and its downstream effectors (mitoK ATP channels) and to put the heart into a protected state.<sup>48</sup>

### *Triggers of postconditioning*

The triggers can be classified, similarly to IPC, in:

- (i) triggers dependent on receptor occupancy and
- (ii) triggers independent on receptor occupancy.

Within the former group an indubitable role has been described for adenosine, as the infarct sparing effect of postconditioning was eliminated by non-selective and  $\text{A}_{2\text{A}}$  and  $\text{A}_3$  selective antagonists given before the postconditioning protocol.<sup>37</sup> Also, endogenous opioids have been incriminated as postconditioning triggers since naloxone and its peripheral acting derivative were reported to abrogate protection; apparently the  $\kappa$  and  $\delta$  opioid receptors are involved in mediating the infarct sparing effect.<sup>34</sup>

As concerning the involvement of reactive oxygen species (ROS), they display (as in the case of IPC) a dual role, being detrimental when highly released at reperfusion and cytoprotective when produced in small amounts during postconditioning's reperfusion cycles.

Kin et al firstly reported in rat hearts that postconditioning significantly reduced the in vivo superoxide anion generation associated with a reduction in plasma lipid peroxidation.<sup>35</sup>

The potential role of ROS as postconditioning triggers was recently confirmed by an elegant study coming from Pagliaro's group: these authors blocked the infarct size reduction of postconditioning by giving the ROS scavenger, N-acetylcysteine, during the postconditioning protocol (only the first 3 min of reperfusion) or for the whole (120 min) reperfusion

duration.

When the NAC administration was delayed and started after the postconditioning protocol (for the remaining 117 min of final reperfusion) the protective effect of postconditioning was not altered.<sup>48,49</sup>

The most recent work of the same group unraveled the role of bradykinin (BK) in triggering postconditioning (BK is also an important IPC-trigger).<sup>50</sup> In isolated rat hearts, they abolished the anti-infarct effect of postconditioning by giving B2 bradykinin (BK) receptors antagonists. When performing the reverse experiments, i.e. trying to mimic postconditioning by giving BK at reperfusion, only intermittent BK infusion (but not continuous administration of BK for 3 minutes) was able to trigger protection. Another important information brought by this paper was that neither intermittent re-oxygenation (5 cycles of 10 s oxygenated/hypoxic buffer) nor intermittent ROS generation with an exogenous system (purine/xanthine oxidase) were able to reproduce postconditioning. The authors concluded that intermittent autacoid accumulation and ROS compartmentalization play a pivotal role in triggering postconditioning. However, further studies are required to elucidate the origin and the species of free radicals involved in the postconditioning signal transduction.

Another potential trigger for postconditioning is the endogenous nitric oxide (NO). Yang et al reported the abrogation of the anti-infarct protection of postconditioning when the NO synthase inhibitor, L-NAME was administered at the very onset of reperfusion.<sup>32</sup> However, the involvement of NO in postconditioning signal transduction pathways appears to be much more complex and its role as a mediator remains to be determined.<sup>34</sup>

### *Mediators of postconditioning*

One of the most important steps in mediating the IPC-protection was the opening of ATP dependent potassium channels ( $\text{K}_{\text{ATP}}$ ). Cardiomyocytes contain two different types of  $\text{K}_{\text{ATP}}$  channels with a pharmacological distinct profile, sarcolemmal ( $\text{sarcK}_{\text{ATP}}$  the first incriminated IPC end-effector) and mitochondrial (mito $\text{K}_{\text{ATP}}$ ), whose opening is currently considered central to the IPC related cardioprotection.<sup>2</sup> Evidence for the involvement of mito $\text{K}_{\text{ATP}}$  in the postconditioning protection has been increasingly reported in rat, dog and rabbit models by using the pharmacological approach (infarct reduction by postconditioning was blocked by a selective mito $\text{K}_{\text{ATP}}$  channel inhibitor, 5-HD).<sup>27,31,33,48,50</sup>

Another mediator of postconditioning is protein kinase C (PKC). Despite the fact that it was extensively studied for its central role in IPC (phosphorylation of KATP channels and activation of several kinases), little information is available concerning its position in postconditioning signal transduction.

Zatta et al have recently reported in rat hearts that postconditioning increased PKC $\epsilon$  expression and non-selective blockade of PKC and of PKC $\epsilon$  reversed the anti-infarct protection of postconditioning.<sup>51</sup> Moreover, the level of PKC $\delta$  isoform and its translocation to mitochondria, which were proven to be deleterious in the setting of ischemia-reperfusion injury, were found to be reduced. Another recent report confirmed the role of PKC in postconditioning signal transduction of isolated rat hearts since the administration of the PKC inhibitor, chelerythrine, concomitant with the postconditioning protocol abolished the anti-infarct protection.<sup>49</sup>

Last but not least, central to the postconditioning related cardioprotection is, as previously mentioned, the activation of survival kinases, which are considered key mediators within its signal transduction. Tsang et al and Yang et al reported that pharmacological inhibition of PI3 kinase by the first group and of ERK1/2 by the second one blunted the postconditioning protection.<sup>30,38</sup> Yet, contradictory results have been reported in relation with the RISK pathway contribution to postconditioning in the rabbit heart. Darling et al reported no attenuation of the anti-infarct effect of postconditioning with a PI3 kinase inhibitor, while the protection was completely blunted when an ERK 1/2 antagonist was administered.<sup>52</sup>

On the other side, Schwartz and Lagranha have recently shown that, despite the fact that postconditioning increased the phosphorylation of Akt and ERK1/2 within the subendocardium postconditioned the pig heart, no infarct sparing effect was observed.<sup>53</sup> The complex interactions existing between the parallel cascades and with their upstream activators and downstream effectors make impossible to establish with certitude their position as mediators within the signal transduction of postconditioning.

However, as previously stated, it is evidently that activating the RISK pathway at the time of reperfusion induce cardioprotection through the phosphorylation of different downstream effectors. A number of investigators hypothesized that mitochondria play a central role in the cardioprotection induced by the RISK kinases recruitment via the inhibition of the phenomenon of mitochondrial permeability transition at the onset of reperfusion.<sup>52,53</sup>

### *End-effector(s?) of postconditioning*

Paraphrasing an old proverb saying that “all roads lead to Rome”, all the biochemical pathways responsible for the postconditioning protection appear to converge to mitochondria, particularly to the permeability transition pore (PTP).

Mitochondria have been classically recognized as the major ATP suppliers of the cells. However, in the last 2 decades an increasing body of experimental evidence showed that these organelles are playing an integral part in signal transduction and interorganelle communication of both physiological and pathophysiological events. One of the extensively investigated conditions belonging to the latter group is the myocardial ischemia and reperfusion injury.

The PTP is a high-conductance channel located in the inner mitochondrial membrane which stays closed during ischemia and opens in the first few minutes of reperfusion in the presence of matrix calcium overload together with an increased matrix pH, especially when accompanied by oxidative stress, elevated phosphate concentration and depleted adenine nucleotide. Opening of the PTP causes uncoupling of oxidative phosphorylation, massive swelling of mitochondria, rupture of the outer membrane and cell death through necrosis and/or apoptosis.<sup>56</sup>

Halestrap et al postulated that the extent of permeability transition may determine the type of cellular death in the settings of ischemia and reperfusion, suggesting that in the centre of the infarction, permanent PTP opening is followed by ATP depletion and cells death occurs through necrosis, while at the periphery, where ischemic insult is less severe, transient PTP opening may lead to apoptosis.<sup>57</sup>

Argaud et al were the first to demonstrate, albeit indirectly, that postconditioning protects the rabbit hearts through the inhibition of PTP opening.<sup>29</sup> In this landmark study postconditioning reduced the calcium-induced opening of the PTP in mitochondria isolated from the myocardium at risk. A possible link between the prosurvival kinases and the PTP was not investigated in this study.

However, several lines of evidence indirectly suggested that activation of the RISK pathway exerts its protection by inhibiting the PTP opening via multiple mechanisms that are believed to contribute each to cardioprotection through the phosphorylation of different substrates followed by the: (i) inhibition of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ , whose activation was reported to mediate PTP inhibition), (ii) the inactivation of pro-apoptotic proteins (e.g., Bad, able to elicit PTP opening), (iii) activation of

endothelial NO synthase (eNOS, thought to inhibit PTP opening via the NO release) and (iv) association between ERK1/2 and mitochondrial PKC $\epsilon$  (the latter reported to inhibit PTP opening).<sup>58</sup>

The direct evidence proving that activation of the PI3 kinase-Akt pathway (with insulin) inhibits the permeability transition in isolated cardiomyocytes came recently from Yellon's group.<sup>58</sup> The potential downstream effectors of Akt are mentioned, even if it is not evident how Akt phosphorylation couples with the pore inhibition.

More important, the same group reported the possibility to protect the human myocardium against the lethal hypoxia-reoxygenation injury by inhibiting the opening of PTP at the time of reoxygenation.<sup>59</sup> The experiments were conducted in two separate models, human atrial trabeculae and cardiomyocytes, isolated from the right atrial appendages harvested from patients undergoing coronary artery by-pass surgery. In atrial trabeculae subjected to 90 min of ischemia, the subsequent administration of the two classical pore inhibitors, cyclosporin A (CsA) and sangliferin A (Sf A) for 30 min resulted in a significant improvement of the force of contraction. Similarly, the human atrial cardiomyocytes loaded with a fluorescent probe (TMRM) that accumulates selectively in mitochondria according to the mitochondrial membrane potential were subjected to laser-induced oxidative stress in the presence and the absence of the pore inhibitors. The presence of either CsA or SfA significantly delayed the oxidative stress induced PTP opening. These authors have shown for the first time that, in human hearts, PTP inhibition, may become a viable target for cardioprotection in the clinical settings of reperfusion.

## CONCLUSIONS

Despite the fact that both phenomena of pre- and postconditioning are nowadays recognized as the most powerful mechanisms of endogenous cardioprotection, no ideal „recipe” able to elicit maximal cardioprotection is yet available. However, the discovery of postconditioning had a major contribution to the recognition of the controversial concept of lethal reperfusion injury and the resurgence of interest for developing strategies aimed at its limitation. Further elucidation of cellular mechanisms of postconditioning and specifically, the role of mitochondria within the signal transduction, could lead to the definition of new pharmacological interventions that might be used as standard adjunctive treatment in association with the

revascularization procedures (mainly, thrombolytic therapy) of ischemic myocardium.

The observation that cardiac protection provided by both preconditioning and postconditioning is mediated by the recruitment of a common signalling pathway at the very onset of reperfusion highlights the importance for further research targeting: (i) the direct activation of the pro-survival kinases and/or (ii) the permeability transition pore inhibition. An increasing body of evidence is pointing to the effective inhibition of PTP opening in the first minutes of reperfusion as the most potent therapy for the reperfusion injury. Future randomized clinical trials are warranted in order to confirm efficiency and to authorize this novel approach to cardioprotection.

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## REFERENCES

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-36.
2. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003;83:1113-51.
3. Kloner RA, Rezkalla SH. Preconditioning, postconditioning and their application to clinical cardiology. *Cardiovasc Res* 2006;70(2):297307.
4. Downey JM, Cohen MV. Reducing infarct size in the setting of acute myocardial infarction. *Progr Cardiovasc Dis* 2006;48(5):363-71.
5. Hausenloy DJ, Tsang A, Mocanu MM, et al. Ischemic preconditioning protects by activating pro-survival kinases at reperfusion. *Am J Physiol* 2005;288:H971-6.
6. Inseste J, Garcia-Dorado D, Hernando V, et al. Ischemic preconditioning prevents calpain-mediated impairment of Na/K-ATPase activity during early reperfusion. *Cardiovasc Res* 2006;70(2):364-73.
7. Yellon DM, Hausenloy DJ. Realizing the clinical potential of ischemic preconditioning and postconditioning. *Nature Clinical Practice Cardiovasc Med* 2005;2(11):568-75.
8. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Investig* 1985;76:1713-9.
9. Kloner RA. Does reperfusion injury exist in humans? *J Am Coll Cardiol* 1993;21(2):537-45.
10. Matsumura K, Jeremy RW, Schaper J et al. Progression of myocardial necrosis during reperfusion of the ischemic myocardium. *Circulation* 1998;97(8):795-804.
11. Vanden Hoek TL, Shao Z, Li C, et al. Reperfusion injury on cardiac myocytes after simulated ischemia. *Am J Physiol* 1996;270:H1334-41.
12. Gottlieb RA, Burleson KO, Kloner RA, et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Investig* 1994;4(4):1621-8.
13. van Domburg RT, Sonnenschein K, Nieuwlaet T et al. Sustained benefit 20 years after reperfusion therapy in acute myocardial infarction. *J Am Coll Cardiol* 2005;46:15-20.

14. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic preconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003;285(2):H579-88.
15. Garcia-Dorado D, Piper HM. Postconditioning: Reperfusion of "reperfusion injury" after hibernation. *Cardiovasc Res* 2006;69:1-3.
16. Yellon DM, Opie LH. Postconditioning for protection of the infarcting heart. *Lancet* 2006;367:456-57.
17. Heusch G. Postconditioning. Old wine in a new bottle? *J Am Coll Cardiol* 2004;44(5):1111-2.
18. Diaz RJ, Wilson GJ. Modifying the first minute of reperfusion: potential for myocardial salvage. *Cardiovasc Res* 2004;62:4-6.
19. Tsang A, Hausenloy DJ, Yellon DM. Myocardial postconditioning: reperfusion injury revisited. *Am J Physiol Heart Circ Physiol* 2005;289(1):H2-7.
20. Okamoto F, Allen BS, Buckberg GD, et al. Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. *J Thorac Cardiovasc Surg* 1986;92:613-20.
21. Hori M, Kitakaze M, Sato H, et al. Staged reperfusion attenuates myocardial stunning in dogs. Role of transient acidosis during early reperfusion. *Circulation* 1991;84:2135-45.
22. Sato H, Jordan JE, Zhao ZQ, et al. Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. *Ann Thorac Surg* 1997;64:1099-107.
23. Crisostomo PR, Wairiuko GM, Meijing Wang, et al. Preconditioning versus postconditioning: mechanisms and therapeutic potentials. *J Am Coll Surg* 2006;202(5):797-812.
24. Schwartz LM, Lagranha CJ. Ischemic postconditioning during reperfusion activates Akt and Erk without protecting against lethal myocardial ischemia-reperfusion injury in pigs. *Am J Physiol Heart Circ Physiol* 2006;290(3):H1011-8.
25. Iliodromitis EK, Georgiadis M, Cohen MV, et al. Protection from postconditioning depends on the number of short ischemic insults in anesthetized pigs. *Basic Res Cardiol* 2006 (in press)
26. Vinten-Johansen J, Zhao ZQ, Zatta AJ, et al. Postconditioning. A new link in nature's armor against myocardial ischemia-reperfusion injury. *Basic Res Cardiol* 2005;100:1-16.
27. Ma XJ, Zhang XH, Li CM, et al. Effect of postconditioning on coronary blood flow velocity and endothelial function in patients with acute myocardial infarction. *Scand Cardiovasc J* 2006;40(6):327-33.
28. Mykkytenko J, Reeves JG, Kin H, et al. Postconditioning reduces infarct size via mitochondrial KATP channel activation during 24 hours of reperfusion (Abstract). *J Mol Cell Cardiol* 2005;38:830.
29. Argaud L, Gateau-Roesch O, Raissy O, et al. Post-conditioning inhibits mitochondrial permeability transition. *Circulation* 2005;111:194-7.
30. Tsang A, Hausenloy DJ, Mocanu MM, et al. Postconditioning, a form of « modified reperfusion » protects the myocardium by activating the phosphatidylinositol 3 kinase-Akt pathway. *Circ Res* 2004;95:230-2.
31. Halkos ME, Kerendi F, Corvera JS, et al. Myocardial protection with postconditioning is not enhanced by ischemic preconditioning. *Ann Thorac Surg* 2004;78:961-9.
32. Yang XM, Proctor JB, Cui L, et al. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J Am Coll Cardiol* 2004;44:1103-10.
33. Chiari B, Bienengraeber MW, Pagel PS, et al. Isoflurane protects against myocardial infarction during early reperfusion by activation of phosphatidylinositol-3-kinase signal transduction: evidence for anesthetic induced postconditioning in rabbits. *Anesthesiology* 2005;102(1):102-9.
34. Zhao ZQ, Vinten-Johansen J. Postconditioning: Reduction of reperfusion induced injury. *Cardiovasc Res* 2006;70(2):200-11.
35. Kin H, Zhao ZQ, Sun HY, et al. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc Res* 2004;62:74-85.
36. Philipp SD, Downey JM, Cohen MV. Postconditioning must be initiated in less than 1 minute following reperfusion and is dependent on adenosine receptors and PI3-kinase (Abstract). *Circulation* 2004;110(Suppl III):804.
37. Kin H, Zatta AJ, Loyfe MT, et al. Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. *Cardiovasc Res* 2005;67:124-33.
38. Yang XM, Philipp S, Downey J, et al. Postconditioning's protection is not dependent on circulating blood factors or cells but involves adenosine receptors and regulates PI3-kinase and guanylyl cyclase activation. *Basic Res Cardiol* 2005;100(1):57-63.
39. Iliodromitis EK, Zoga A, Vrettou A, et al. The effectiveness of preconditioning and postconditioning on infarct size in hypercholesterolemic and normal anesthetized rabbits. *Atherosclerosis* 2006;188(2):356-62.
40. Brownell T, Schwemm MS, Ludwig PD, et al. Effect of ischemic postconditioning on left ventricular function and infarct size (Abstract). *Ann Emerg Med* 2004;44(4):S52.
41. Iliodromitis EK, Kremastinos DT, Katritsis DG, et al. Multiple cycles of preconditioning cause loss of protection in open-chest rabbits. *J Mol Cell Cardiol* 1997;29(3):915-20.
42. Tang XL, Sato H, Tiwari S, et al. Cardioprotection by postconditioning in conscious rats is limited to coronary occlusions < 45 minutes. *Am J Physiol Heart Circ Physiol* 2006;291(5):H2308-10.
43. Manintveld OC, Te Lintel Hekkert M, van den Bos EJ, et al. Cardiac effects of postconditioning depend critically on the duration of index ischemia. *Am J Physiol Heart Circ Physiol* 2007;292(3):H1551-60.
44. Staat P, Rioufol G, Piot C, et al. Postconditioning the human heart. *Circulation* 2005;112:2143-8.
45. Darling CE, Solari PB, Smith CS, et al. 'Postconditioning' the human heart: Multiple balloon inflations during primary angioplasty may confer cardioprotection. *Basic Res Cardiol* 2007;102(3):274-8.
46. Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischemia-reperfusion injury: targeting the RISK pathway. *Cardiovasc Res* 2004;61:448-60.
47. Fujita M, Asanuma H, Hirata A, et al. Prolonged transient acidosis during early reperfusion contributes to the cardioprotective effects of postconditioning. *Am J Physiol Heart Circ Physiol* 2007;292(4):H2004-8.
48. Cohen MV, Yang XM, Downey JM. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation* 2007;115(14):1895-903.
49. Penna C, Rastaldo R, Mancardi D, et al. Postconditioning induced cardioprotection requires signaling through a redox sensitive mechanism, mitochondrial ATP-sensitive K channel and protein kinase C activation. *Basic Res Cardiol* 2006;101(2):180-9.
50. Penna C, Mancardi D, Rastaldo R, et al. Intermittent activation of bradykinin B(2) receptors and mitochondrial K (ATP) channels trigger cardiac postconditioning through redox signaling. *Cardiovasc Res* 2007 Mar 12 (in press).
51. Zatta AJ, Kin H, Lee G, et al. Infarct sparing effect of myocardial postconditioning is dependent on protein kinase C. *Cardiovasc Res* 2006;70(2):315-24.
52. Darling CE, Jiang R, Maynard M, et al. Postconditioning via stuttering reperfusion limits myocardial infarct size in rabbit hearts: role of ERK 1/2. *Am J Physiol Heart Circ Physiol* 2005;289:H1618-26.
53. Schwartz LM, Lagranha CJ. Ischemic postconditioning during reperfusion activates Akt and ERK without protecting against lethal myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006;290(3):H1011-8.
54. Hausenloy DJ, Tsang A, Yellon DM. The Reperfusion Injury Salvage Kinase pathway: a common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med* 2005;15(2):69-74.

55. Gateau O, Argaud L, Ovize M. Mitochondrial permeability transition pore and postconditioning. *Cardiovasc Res* 2006;70(2):264-73.
56. Di Lisa F, Canton M, Menabo R, et al. Mitochondria and reperfusion injury – The role of permeability transition. *Basic Res Cardiol* 2003;98(4):235-41.
57. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion - a target for cardioprotection. *Cardiovasc Res* 2004;61:372-85.
58. Davidson SM, Hausenloy D, Duchon M, et al. Signalling via the RISK pathway links closure of the mitochondrial permeability transition pore to cardioprotection. *Int J Biochem Cell Biol* 2006;(38):414-9.
59. Shanmuganathan S, Hausenloy DJ, Duchon MR, et al. Mitochondrial permeability transition pore as a target for cardioprotection in the human heart. *Am J Physiol Heart Circ Physiol* 2005;289: H237-42.