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Abstract	Since ischemic heart disease (IHD) is a major cause of mortality and heart failure, novel therapeutic strategies are expected to improve the clinical outcomes of patients with acute myocardial infarction. Brief episodes of ischemia/reperfusion performed at the onset of reperfusion can reduce infarct size; a phenomenon termed "ischemic postconditioning." Extensive research has determined that different autacoids (e.g., adenosine, bradykinin, opioid, etc.) and cytokines, their respective receptors, kinase signaling pathways, and mitochondrial modulation are involved in ischemic conditioning. Modification of these factors by pharmacological agents mimics the cardioprotection by ischemic postconditioning. Here, the potential mechanisms of ischemic postconditioning, the presence of comorbidities, and the possible extrapolation to the clinical setting are reviewed. In the near future, large, multicentered, randomized, placebo-controlled, clinical trials will be required to determine whether pharmacological and/or ischemic postconditioning can improve the clinical outcomes of patients with IHD.

Keywords (separated by '-')	Myocardial infarction - Ischemia - Ischemic postconditioning
Footnote Information	Bruno Buchholz and Martín Donato have contributed equally to this manuscript.

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Ischemic postconditioning: mechanisms, comorbidities, and clinical application

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Abstract Since ischemic heart disease (IHD) is a major cause of mortality and heart failure, novel therapeutic strategies are expected to improve the clinical outcomes of patients with acute myocardial infarction. Brief episodes of ischemia/reperfusion performed at the onset of reperfusion can reduce infarct size; a phenomenon termed "ischemic postconditioning." Extensive research has determined that different autacoids (e.g., adenosine, bradykinin, opioid, etc.) and cytokines, their respective receptors, kinase signaling pathways, and mitochondrial modulation are involved in ischemic conditioning. Modification of these factors by pharmacological agents mimics the cardioprotection by ischemic postconditioning. Here, the potential mechanisms of ischemic postconditioning, the presence of comorbidities, and the possible extrapolation to the clinical setting are reviewed. In the near future, large, multicentered, randomized, placebo-controlled, clinical trials will be required to determine whether pharmacological and/or ischemic postconditioning can improve the clinical outcomes of patients with IHD.

Keywords Myocardial infarction · Ischemia · Ischemic postconditioning

Introduction

Ischemic heart disease is still the first cause of death and heart failure in the whole world population despite advances in its treatment [1]. In the United States alone, nearly 1 million of acute myocardial infarctions occur annually, and approximately 29 % of them are ST-segment elevation myocardial infarctions [2]. For this reason, the development of new strategies to improve the outcome of patients with this pathology is transcendental. Nowadays, the most important therapeutic strategy, and the top choice for patients with acute myocardial infarction, is reperfusion. Paradoxically, reperfusion injury limits the beneficial effects of reperfusion.

Experimentally, several methods of myocardial protection proven to have a beneficial effect on infarct size have been described. However, only a few of these were extrapolated to clinical setting. Among these methods stands ischemic postconditioning, which consists of short periods of ischemia/reperfusion at the onset of reperfusion [3]. Given that this procedure needs to be performed at the beginning of reperfusion, it has caught the researchers' attention due to the fact it could have real clinical potential. This review will focus on describing some of the mechanisms involved in the protection provided by ischemic postconditioning, its association with comorbidities, and the possible extrapolation from bench-to-bedside.

Targets of ischemic postconditioning

Effect on infarct size

The pioneering study that showed the cardioprotective effects of ischemic postconditioning was performed in dogs

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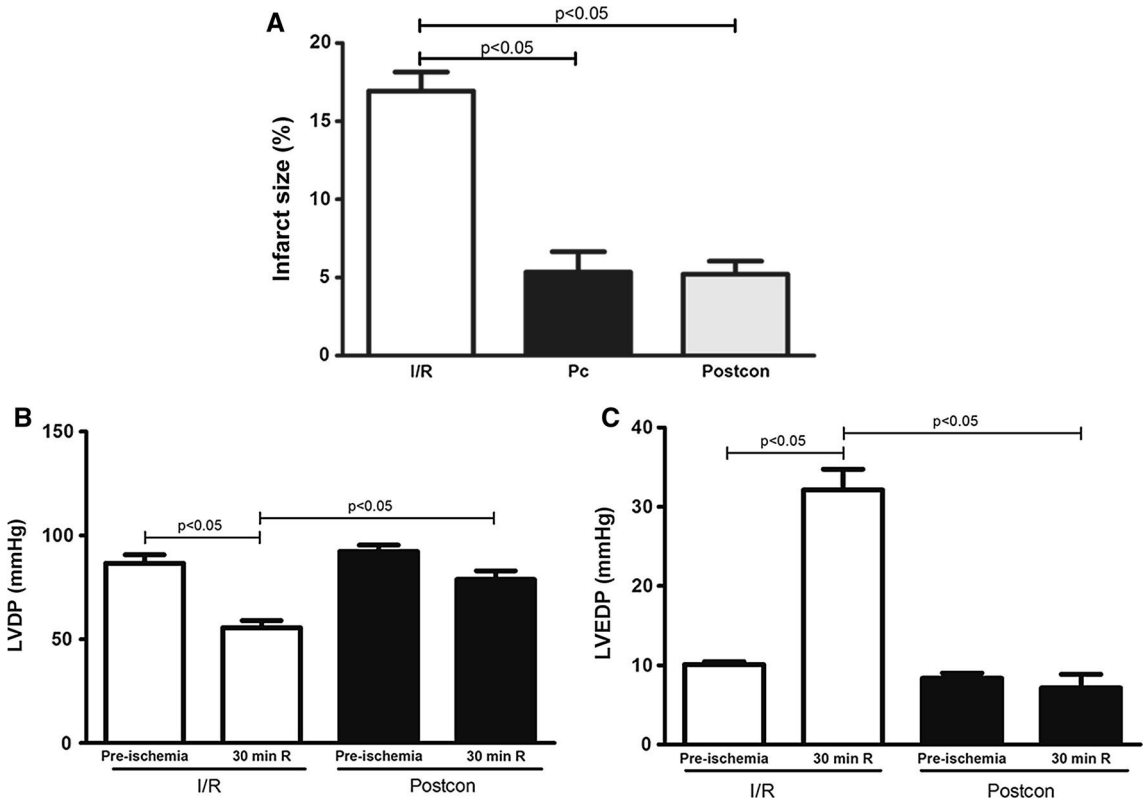

Fig. 1 **a** Shows the similar reduction of the infarct size produced by ischemic preconditioning and postconditioning. **b**, **c** Show the recovery of LVDP and LVEDP at 30 min of reperfusion in hearts subjected to an ischemic postconditioning protocol. Ischemic postconditioning improves the recovery of systolic ventricular function and attenuates the increase of diastolic stiffness

subjected to 1 h of coronary occlusion followed by 3 h of reperfusion [3]. Interestingly, in the same paper, the reduction of the infarct size observed was comparable with those obtained in animals subjected to an ischemic preconditioning protocol. According to the original paper of Zhao et al. [3], we have shown that ischemic postconditioning reduces the infarct size in isolated rabbit hearts [4]. This reduction was similar to the one found with ischemic preconditioning (Fig. 1a). These findings were also reproduced in our laboratory with isolated rat hearts, where ischemic postconditioning reduced the infarct size but in a lower proportion than ischemic preconditioning (data not shown). Also, other studies confirmed these findings in species like mice [5] and pigs [6]. An important variable to consider to obtain protection through ischemic postconditioning is the algorithm utilized. The postconditioning algorithm depends of the delay after which the first re-occlusion is established, the duration and number of re-occlusions and reperfusions [7]. In a rat model, we reduced the infarct size using a six-cycle algorithm of reperfusion/ischemia of 10 s each. Other researchers demonstrated that the beneficial effect on myocardial infarction is lost if the cycles are applied 60 s after the onset of reperfusion [8]. It is evident that the reduction of the infarct size depends on the "strength" of the stimuli. Short or sparse cycles are not able to reduce the infarct size; however, the protection appears when increasing the number of cycles. The algorithm also varies according to the species studied and its heart rate. Small animals with a high heart rate, like a rat or a mouse, need short periods of reperfusion/ischemia to reach protection. Bigger species, with lower heart rates, like dogs or pigs, need longer cycles. It is not clear why those differences exist, and interestingly the studies performed in humans show that the beneficial effect was reached using algorithms similar to those used on large animals [7]. As we have mentioned, in our experience we need to use short periods of reperfusion/ischemia in rats (6 cycles of 10 s each) and fewer but longer cycles in rabbits (2 cycles of 30 s each).

Even though it is not possible to identify an "ideal" postconditioning algorithm, it is clear that different factors, such as length of the ischemia, the type of algorithm used, and others like gender, age, and temperature, contribute and/or modify the results of different experimental studies.

Effect on the vascular endothelium

The vascular endothelium is damaged in the processes that involve ischemia/reperfusion injury. This endothelial injury is characterized by the reduction of the vasodilator

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response to acetylcholine, which is related with a lower synthesis of nitric oxide (NO) [9]. Also, under these circumstances, neutrophils are more prone to adhere to the vascular endothelium, in which the expression of adhesion molecules (P-selectine) and the generation of reactive oxygen species (ROS) are incremented. In this sense, Zhao et al. [3] demonstrated that post-ischemic endothelial dysfunction of coronary arteries, evaluated through the vasodilator response to acetylcholine, was significantly enhanced by ischemic postconditioning. Also, postconditioning decreases the P-selectine expression, the adhesion of neutrophils, and the accumulation of these inflammatory cells in the risk area [3]. These findings strongly suggest that ischemic postconditioning attenuates vascular dysfunction. However, it is not clear if this reduction of the inflammatory process is only the consequence of the lesser necrosis observed.

Something to consider when evaluating the damage by ischemia/reperfusion is the presence of the "no reflow" phenomenon. This entity defines a partial limitation to blood flow at the moment of reperfusion, despite the complete elimination of coronary occlusion [10]. The proposed mechanism to explain this phenomenon is multifactorial, and histological studies have demonstrated different degrees of vasospasms of the small vessels, endothelial injuries, formation of sarcolemmal bubbles in the endothelial cells, and the aggregation of neutrophils in capillaries [11]. In this sense, Zhao et al. [12] demonstrated that ischemic postconditioning reduces the "no reflow" area and improves coronary flow. However, in hypercholesterolemic conditions this beneficial effect is lost. The mechanism by which postconditioning improves the endothelial function seems to be related to an increase of neuronal and endothelial NO synthase activity and as a consequence an increase of NO bioavailability. Under hypercholesterolemia conditions, NO metabolism is severely damaged and postconditioning is incapable of favoring its synthesis [12].

Effect on apoptosis

AQ2 Besides reducing the area of necrosis, some studies suggest that ischemic postconditioning could attenuate the apoptosis that occurs during reperfusion [13]. In this sense, Tian et al. [14] demonstrated that ischemic postconditioning reduces the number of TUNEL positive cells through the activation of the JACK2-STAT3-Bcl-2 pathway. Similarly, Kin et al. [15] showed that ischemic postconditioning would reduce myocardial apoptosis, decreasing caspase-3 activity through the inhibition of NF-κB and TNFα [15]. In relation to this concept, Sun et al. [13] demonstrated, in a culture of neonatal myocytes subjected to 3 h of hypoxia followed by 6 h of re-oxygenation, that postconditioning attenuates the apoptosis rate inhibiting the JNKs/p-38 signals, reducing the liberation of TNFα and the caspase expression.

Interestingly, Penna et al. [16] demonstrated that postconditioning activates/inhibits or changes the levels of different kinases related to mitochondrial integrity. Postconditioning increases the phosphorylation of the mitochondrial isoform of GSK-3β. This effect is accompanied by a reduction in the release of cytochrome *c* from the mitochondria and in the activity of cytosolic caspase-3, suggesting an anti-apoptotic effect.

The presence of apoptosis in the areas adjacent to the infarct has been described by various authors [17, 18]. However, its contribution to the final infarct size continues to be controversial. Some studies indicate that this type of death, although present in the reperfused myocardium, only represents a small percentage of cellular death [19]. While in other studies, it has been observed that the interruption of the apoptotic mechanism, using caspase inhibitors, has contributed to attenuate the damage by ischemia/reperfusion [20]. However, given that the activity of caspases can also contribute to death by necrosis, these results do not discard the possibility that part of the reduction in the extension of the infarct is independent from apoptosis inhibition [21]. Finally, there only are a few studies that discriminate what type of cellular line (myocytes, fibroblasts, endothelial cells, etc.) is suffering a cellular death by apoptosis. Consequently, even though the attenuation of the apoptosis rate by ischemic postconditioning is an interesting finding, its importance in the context of myocardial infarction has to be taken with caution.

Effect on ventricular function

The study of different protection mechanisms (ischemic preconditioning and postconditioning) on the recovery of post-ischemic ventricular function (stunned myocardium) has been studied by different authors. Cohen et al. [22] described that in chronically instrumented rabbits, preconditioning reduces infarct size and improves ventricular function during reperfusion. However, this beneficial effect on ventricular function is only observed in 2 or 3 weeks after the beginning of reperfusion. This happens because there are areas of myocardial stunning adjacent to the infarct that do not allow an accurate ventricular function evaluation. This concept would also be valid for ischemic postconditioning. Thus, Penna et al. [23] found an improvement in the ventricular function of isolated rat hearts subjected to 10–30 min of global ischemia, is related to the reduction of infarct size by postconditioning. Using a longer period of ischemia with cardioplegia (90 min), Shinohara et al. [24] showed that ischemic postconditioning promotes an improvement in the recovery of

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ventricular function in pigs. There are only a few studies that evaluate the effect of ischemic postconditioning on a pure model of myocardial stunning without infarction. Sasaki et al. [25] showed, using an isolated rat heart subjected to 20 min of global ischemia, that ischemic postconditioning attenuates arrhythmias that occur during reperfusion but that it does not improve the recovery of ventricular function. In experiments performed in our laboratory (data not published) ischemic postconditioning, evaluated by using a model of stunning in isolated rabbit hearts, significantly improved the recovery of ventricular systolic and diastolic post-ischemic function (Fig. 1b, c).

As a consequence, there are few studies that evaluate ventricular function in a pure model of stunning without irreversible injury. Since their results are not conclusive, more studies would be necessary to elucidate the effects of ischemic postconditioning per se, on ventricular function.

Mechanisms of ischemic postconditioning

In 2003, the Vinten-Johansen group introduced the concept of ischemic postconditioning [3]. In this pioneering study, the proposed mechanisms for protection were initially attributed to a reduction in the deleterious effects of reperfusion injury. These were particularly related to oxidative stress, calcium overload, improvement of endothelial function, and the reduction of the inflammatory component. However, subsequent studies demonstrated that the protection is mediated by the activation of intracellular signals and, in many cases, shared with the ischemic preconditioning [26]. These pathways of intracellular signaling would include G-protein-coupled membrane receptors and would be activated by molecules liberated to the extracellular space at the beginning of reperfusion (Fig. 2) [27]. Our group showed experimental evidence demonstrating the participation as "triggers" of ischemic postconditioning to A_1 adenosine receptors (Fig. 3) [4]. This was shown by administering a selective blocker of these receptors (DPCPX). In a related research, Yang et al. [28] had shown that adenosine participates in the ischemic postconditioning mechanism. However, the subtype of receptor involved in postconditioning mechanisms is controversial, due to Kin et al. [29] showing the participation of A_{2A} and A_3 adenosine receptors in an in vivo ischemia/reperfusion model in rats. According to these authors, Philipp et al. [30] demonstrated that adenosine receptors A_{2b} would activate the protection mechanism of ischemic postconditioning. As we have mentioned, the topic is still controversial. Thus, another type of membrane receptors that could be involved in the postconditioning mechanism are the α-adrenergic receptors (α1-ARs). In this sense, we have recently showed that ischemic postconditioning decreases infarct size by activation of the α1-AR pathway, which could involve Akt and GSK-3β phosphorylation. The present results could indicate that after being phosphorylated, Akt may phosphorylate and inhibit GSK-3β [31].

On the other hand, the prolonging of an acidosis state during reperfusion also plays an important role in the ischemic postconditioning mechanism [32] (Fig. 2). In this sense, there is experimental evidence showing that a slower recovery of intracellular pH (pHi) at the onset of reperfusion could be relevant to prevention of the emergence and spread of hypercontracture [33] and the activation of Ca^{2+}-dependent proteases (calpains), among other things [32]. Both ischemic postconditioning and the reperfusion of the ischemic heart with a pH acid buffer delay the normalization of pHi during the first minutes of reperfusion and reduce cellular death [6, 8]. However, only postconditioning increases cGMP levels. The pharmacological inhibition of the cGMP/PKG signaling mechanism hastens the normalization of pHi during reperfusion and abolishes the postconditioning protection. The protection conferred by the perfusion with the acid buffer remains unchanged. This demonstrates that there is a relation between the cGMP/PKG signaling pathway and the regulation of pHi at the onset of reperfusion. Therefore, the activation of this pathway, using NO donors or with cGMP, attenuates injury by reperfusion. Conversely, administration of the NO synthase enzyme (L-NAME) or guanylate cyclase (GC) blocks the protective effect of postconditioning on infarct size [34, 35], demonstrating that the cGMP signaling pathway is also involved in the postconditioning mechanism.

Different studies have demonstrated that the PKG enzyme negatively modulates the Na^+–H^+ exchanger (NHE) [12, 13]. In isolated myocytes, the activation of PKG, as well as pre-treatment with a cGMP analogous, inhibits NHE producing acidification of the intracellular environment [14]. This strongly suggests that the cGMP/PKG pathway inhibits NHE contributing to prolong the acidosis state. This fact would occur during the performing of a postconditioning protocol.

On the other hand, it has been shown that the intracellular acidosis contribution to the cardioprotection conferred by postconditioning is also related to the activation of the Akt enzyme, the extracellular-regulated kinase signals (ERK) [36] and the prevention of the opening of the mitochondrial permeability transition pore (mPTP), during the early stage of reperfusion.

As it has been mentioned, the activation of Akt signaling pathway is part of the postconditioning mechanism, although this has not been completely clarified. Some authors have shown the activation of the protein p70S6K and the isoform eNOS, both "downstream" to the phosphorylation of Akt in postconditioned hearts (Fig. 2) [37].

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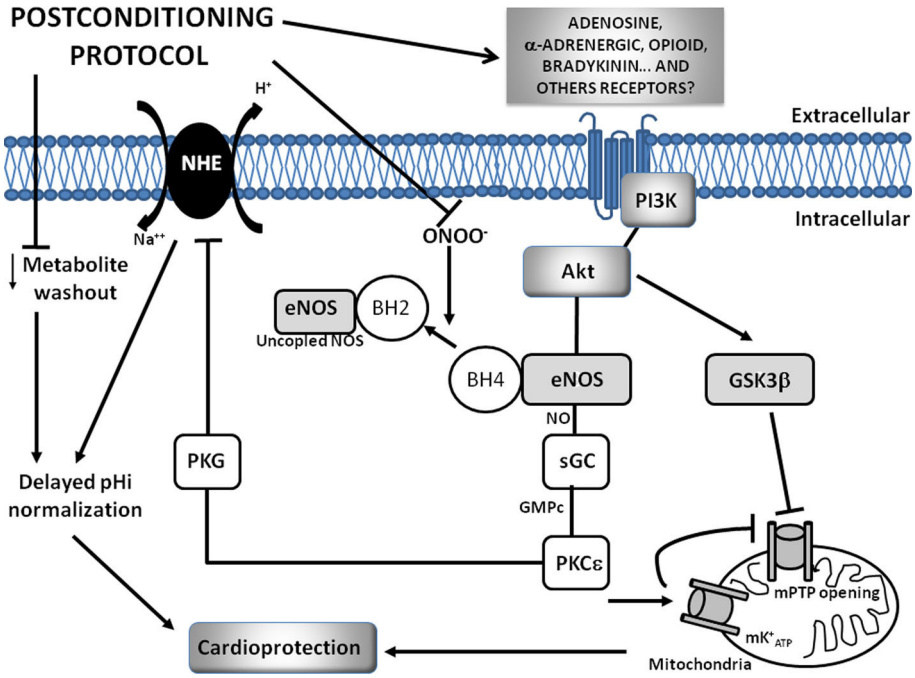

Fig. 2 Hypothetical scheme of the major pathways identified so far as contributing to postconditioning. Postconditioning may promote the accumulation or delay the washout of several autacoid mediators (extracellular cardioprotective ligands) whose participation in the mechanism is obligatory with obligatory participation in the mechanism. It is proposed that ligands such as adenosine, bradykinin, and opioids cause the activation of multiple kinases, including PI3K/Akt. There is also evidence that PKG activation may occur as part of the postconditioning mechanism and this may lie downstream of the Akt/NO/cGMP pathway. Activation of Akt also inhibits GSK-3β, thereby inhibiting the mPTP opening. A role of the mKATP channel opening is implied by some pharmacological studies, but how this mediates protection at reperfusion is unknown, although it is possible that mKATP-mediated inhibition of mPTP opening, as in the preconditioning model, plays a role. It is likely that other unidentified substrates and effector mechanisms play significant roles. *Blunt arrows* stand for inhibitory effects; *normal arrows* for activations

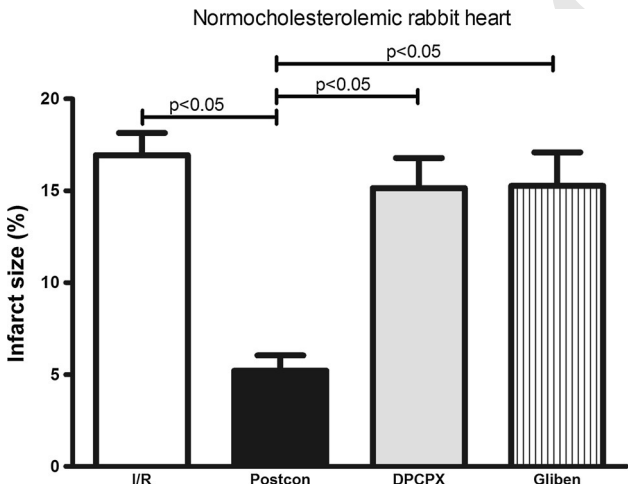

Fig. 3 The effect of different interventions on the infarct size in normocholesterolemic animal hearts. The infarct size is expressed as a percentage of the left ventricular area of the left ventricle. Ischemic postconditioning significantly reduces the infarct size, while the administration of DPCPX and glibenclamide abolish this effect

As we have mentioned, other studies involve protein kinase G (PKG) as a potential mediator of the protective effect [38]. This was demonstrated by researches that included inhibitors of eNOS, GC, PKG, and where the protective effect of ischemic postconditioning on the infarct size was abolished, confirming the participation of NO through the cGMP/PKG signaling pathway. The activation of the cGMP/PKG pathway by ischemic postconditioning has been proposed as part of the activation of the PI3K/Akt cascade (Fig. 2). However, a recent study suggests that the phosphorylation of the RISK kinases during reperfusion is not associated with a reduction in the infarct size [39]. This would question it is role in the protection provided by ischemic postconditioning. It is also known that at the beginning of reperfusion an increase the ROS formation, including the superoxide anion (O_2^-), take place. The formation of O_2^- occurs due to a reduced bioavailability of NO by an increase in the production of peroxynitrite ($ONOO^-$) and the oxidation of tetrahydrobiopterin (BH4). This is a necessary cofactor for NOS

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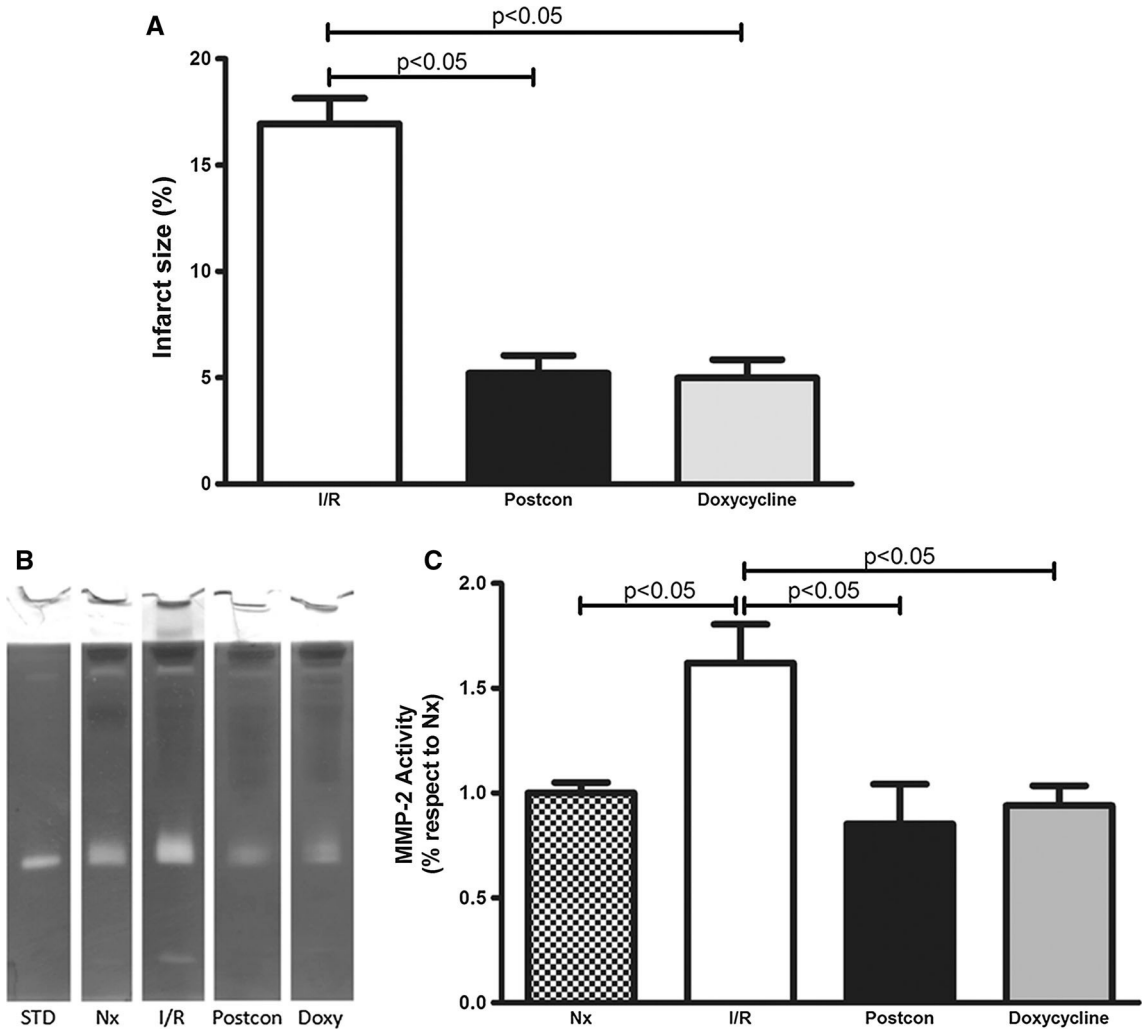

Fig. 4 **a** Shows the infarct size, expressed as a percentage of the left ventricular area of the left ventricle. The infarct area decreased significantly in the group with ischemic postconditioning and in the group treated with doxicycline (50 μM). **b** On the left we can see a zymogram, representative of the left ventricular gelatinolytic activity. On the right of the same, the densytometric analysis of the gelatinolytic activity of MMP-2 in samples taken in normoxic conditions (Nx) after 30 min of ischemic ischemia and 2 min of reperfusion (I/R) in hearts subjected to a protocol of ischemic postconditioning and treated with doxycycline, respectively, is plotted

coupling that decreases during ischemia/reperfusion. Since ischemic postconditioning prevents an abrupt reperfusion, it could reduce the "bursts" of ROS and increase the concentration of NO by the activation of the cGMP/PKG pathway. In relation to this, recently Inserte et al. [40] demonstrated that the activation of the PKG/cGMP pathway by ischemic postconditioning is independent of the PI3K/Akt cascade and dependent on a reduction in oxidative stress during reperfusion. These authors demonstrated that the attenuation in the production of O_2^- and/or $ONOO^-$, at the onset of reperfusion limits the oxidation of BH4 and reduces the decoupling of eNOS. Therefore, the levels of NO by activation of the cGMP/PKG pathway are increased (Fig. 2).

The $ONOO^-$ is recognized to play a key role in different cardiovascular pathologies such as ischemia/reperfusion injury [41]. Many enzymes are inactivated and decrease their function such as Na^+–K^+-ATPase upon exposure to $ONOO^-$ [42]. In contrast, the latent forms of matrix metalloproteinases (MMPs) are known to be activated by oxidant species including $ONOO^-$ [42]. During reperfusion, MMP-2 is activated intracellularly and is capable of cleaving troponin I [43] and the light chain of myosin I [44]. Also, Sung et al. [45] demonstrated that $ONOO^-$, through the activation of MMP-2, degrades α-actinin of the cytoskeleton. The relation between the MMP-2 activity and myocardial infarction was demonstrated by Giricz et al. [46]. These authors observed that the inhibition of MMP-2 reduces the infarct size in a similar way to the ischemic preconditioning. We also observe a beneficial effect of the doxycycline (inhibitor of MMPs) on the infarct size (Fig. 4a) and also demonstrated that ischemic

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postconditioning is capable of inhibiting the MMP-2 activity at the beginning of reperfusion in isolated rabbit hearts (Fig. 4c) [47]. This is in accordance with the results of Inserte et al. [40] who demonstrated a lower production of $ONOO^-$, the main activator of MMP-2. As a consequence, ischemic postconditioning could have beneficial effects attenuating the activity of MMP-2 and preventing the uncoupling of eNOS, increasing the levels of NO by the activation of the cGMP/PKG pathway.

The activation of PKG could also allow the opening of mitochondrial K^+ channels (mKATP), possibly through the phosphorylation of PKC-ε. In the same way as ischemic preconditioning [48], it has been proposed that there is a "link" between the opening of mKATP and the mPTP [49], suggesting that mKATP could be considered mediators of the cardioprotective effect of ischemic postconditioning. We show that the reduction in the infarct size induced by ischemic postconditioning depends on the opening of KATP channels [4] (Fig. 3). Additionally, Mykytenko et al. [50], using a 60-min coronary artery occlusion canine model followed by 24 h of reperfusion, demonstrated the participation of specific mitochondrial KATP channels.

The opening of mPTP is considered a key event in cellular death by ischemia/reperfusion [51]. This episode is favored by conditions like ischemia and reperfusion, including overproduction of ROS, ATP depletion, and more specifically, accumulation of Ca^{2+} in the mitochondrial matrix. After this last phenomenon, Ca^{2+} stimulates the interaction of cyclophilin D (CypD) with a mPTP component, which triggers a permeability transition [52]. However, the opening of the mPTP during reperfusion may be regulated by extramitochondrial activation/inhibition of several kinases, including the glycogen synthase kinase-3β (GSK3β) [53]. In this sense, Argaud et al. [54] showed that postconditioning inhibits this opening, a fact that was associated with the reduction in the infarct size. Gomez et al. [52] demonstrated that the inhibition of GSK3β by ischemic postconditioning is required in reducing the infarct size and most likely acts by preventing the opening of the mPTP at reperfusion independently of CypD.

Other authors [55] associated the closing of mPTP with the activation of PI3K and the signaling pathway of the RISK. However, as we have mentioned, the participation of RISK or its main role in the ischemic postconditioning mechanism should be re-considered [39].

Ischemic postconditioning and comorbidities

The phenotype of a patient with a high risk for myocardial infarction is: male, average age of 65-year old, and with a combination of comorbidities that include arterial hypertension, diabetes mellitus metabolic syndrome, hyperlipidemia and atherosclerosis, among others [56]. However, most studies performed in laboratory used young and healthy animals without any comorbidities. This is interesting, since it has been established that some of these comorbidities can modify the heart response to the different protective mechanisms [57].

The presence of left ventricular hypertrophy constitutes an independent risk factor that increases patient's comorbidity. In patients with ventricular hypertrophy, the risk of myocardial infarction, heart failure, sudden death, and other cardiovascular disorders are increased by sixfold [58]. However, there are only a few works that studied the effect of ischemic postconditioning in an animal model of ventricular hypertrophy. In relation to this, Penna et al. [59] demonstrated that the presence of hypertrophy in a model of rats, treated with nandrolone, increases the susceptibility of the heart to ischemia/reperfusion injury and abolishes the protective effect of ischemic postconditioning on the infarct size. On the contrary, Fantinelli and Mosca [60] have described that the presence of ventricular hypertrophy, in a SHR rat model, does not abolish the beneficial effects of ischemic postconditioning. These authors find protection, even with left ventricular hypertrophy, and demonstrate the participation of PKC in the postconditioning mechanism.

Hypercholesterolemia is a common finding in patients with cardiovascular diseases. Some studies have shown evidence that both the ischemic preconditioning and postconditioning are abolished in animals with hypercholesterolemia. Iliodromitis et al. [61] described that hypercholesterolemia abolishes the ischemic postconditioning (but not the preconditioning) protective effect. Kupai et al. [62] found similar results in a rat model fed during 12 weeks with a 2 % cholesterol enriched diet. Both authors used a prolonged length of time using this diet (12 weeks) and show the presence of atherosclerosis with several subintimal deposits of lipids in coronary arteries and a reduction of their lumen.

Our group demonstrated that ischemic postconditioning reduces the infarct size in normal and hypercholesterolemic rabbits (4 weeks of 1 % cholesterol enriched diet), through the activation of adenosine A_1 receptors and K_{ATP} channels (Fig. 5) [4]. It is important to note that in our experimental model, animals presented endothelial dysfunction evaluated through vasodilator response to acetylcholine, without atherosclerosis lesions.

The prevalence of obesity associated to diabetes mellitus (DM) has significantly increased, particularly in developed countries. As a consequence, the presence of these comorbidities is associated with a worse outcome in patients with coronary heart disease, increasing the risk of complications during revascularization procedures

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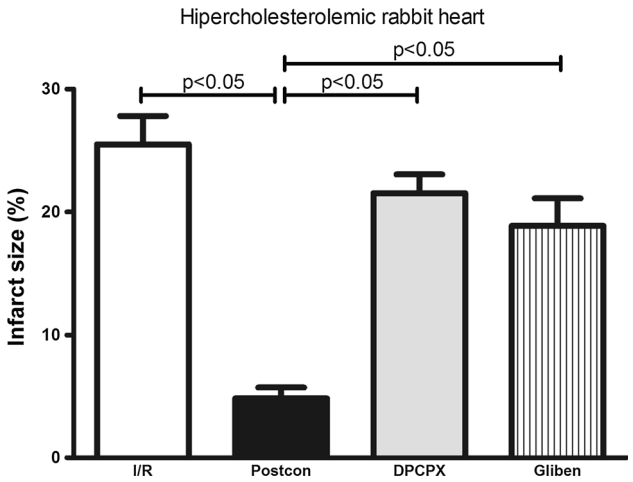

Fig. 5 The effect of different interventions over on the infarct size in hypercholesterolemic animals. The infarct size is expressed as a percentage of the left ventricular area of the left ventricle. Ischemic postconditioning significantly reduces the infarct size, while the administration of DPCPX and glibenclamide abolishes this effect

(angioplasty and CABG). Also, obesity and DM provoke damage per se in different cellular components, particularly at a mitochondrial level [63]. It is known that DM decreased the levels of BH4 and the uncoupling of NOS by increasing the ROS levels [64]. It should be mentioned that mitochondrial function is an important factor that participates in the adaptation of myocardium to ischemia. As a consequence, its alteration could modify the response to postconditioning. Keeping this in mind, it would not be surprising that in conditions of DM or obesity, the capacity of the myocardium to be postconditioned is abolished or diminished.

In relation to this concept, a number of studies have been carried out to determine the efficacy of ischemic postconditioning for protecting the myocardium in animal models of DM [65, 66]. The majority of those studies showed that DM interferes with the protective mechanisms of cardioprotective interventions [67]. Myocardial protection by postconditioning is achieved by activation of multiple protective signaling pathways that appear to converge, inhibiting the mPTP opening upon reperfusion via phosphorylation of glycogen synthase kinase-3β (GSK-3β) at Ser-9. DM-induced defects in the protective signaling may be different depending on the model and/or phase of DM [27, 57, 67, 68]. Thus, Przyklenk et al. [69] demonstrated a loss in efficacy of ischemic postconditioning in murine models of type-2 and type-1 DM, characterized by both an apparent inability to reduce infarct size and failed upregulation of ERK phosphorylation. Moreover, they provide novel evidence that the loss in efficacy of ischemic postconditioning does not reflect a permanent DM-associated defect in cardioprotective signaling. Rather, in the type-1 model, therapeutic control of insulin and blood glucose levels re-established the infarct-sparing effect of ischemic postconditioning. However, Oosterlinck et al. [65] showed that the cardioprotective effect of ischemic postconditioning was maintained in C57BL/6J mice after 10 weeks of myocardial infarction. Ischemic postconditioning also protected against adverse left ventricular remodeling in this model of type 2 DM.

As we have mentioned, ischemic heart disease in humans is a complex disorder caused by or associated with other systemic diseases and risk factors. Therefore, in this article we reviewed evidence that comorbidities accompanying coronary disease modify responses to ischemia/reperfusion and the cardioprotection conferred by postconditioning. We emphasize the importance of preclinical studies that examine cardioprotection, specifically in relation to complicated disease states, to maximize the likelihood of identifying rational approaches to therapeutic protection of the aged or diseased ischemic heart.

Clinical application of ischemic postconditioning

The main reason to study the intracellular mechanisms of different cardioprotective strategies is its application to humans of different ages and sex with coronary artery disease and concomitant risk factors. Different clinical studies on ischemic postconditioning have increased in recent years, but they were small studies with inconsistent results. In these studies, the authors used different end points to define a potential biological effect of ischemic postconditioning: (a) enzyme assessment of myocardial injury, (b) angiographic and invasive measures of coronary flow, (c) measures of left ventricular function, (d) measures of infarct size, and (e) adverse cardiac events during follow-up (Table 1).

Thus, Laskey et al. [70] described that ischemic postconditioning attenuated the elevation of the ST-segment and the plasmatic CPK peak, in patients undergoing to percutaneous angioplasty, compared with those that received a standard procedure. In the same way, Staat et al. [71] performed a study where they randomized 37 patients derived for primary percutaneous angioplasty. Those patients that achieved a TIMI grade flow 2–3 were randomized to receive a standard angioplasty procedure or an ischemic postconditioning protocol with four cycles of 1-min re-inflation followed by 1 min deflation of the angioplasty balloon. These authors demonstrated that the area under the CPK-MB curve was significantly reduced in those patients that received the ischemic postconditioning protocol. In concordance with these findings, Darling et al. [72] studied a more heterogeneous population of patients diagnosed with STEMI, TIMI flow 0–1, and with the lesion of a single blood vessel which were subjected to primary

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Table 1 Ischemic postconditioning in patients undergoing percutaneous coronary interventions or cardiac surgery

Reference	Delay (s)	Number of cycles	Ischemia/reperfusion duration time per cycle (s)	Intervention	Results
Laskey et al. [70]	180	2	90/180	Percutaneous coronary intervention	Improved extent of ST-segment resolution and coronary flow reserve and reduced peak serum creatine-kinase
Staat et al. [71]	60	4	60/60	Percutaneous coronary intervention	Reduced area under the curve of creatine-kinase and improved blush grade
Darling et al. [74]	30	6	25/25	Percutaneous coronary intervention	Reduced peak creatine-kinase release
Zhao et al. [82]	30	3	30/30	Percutaneous coronary intervention	Reduced area under the curve of creatine-kinase activity and increased ejection fraction
Luo et al. [83]	30	3	30/30	Cardiac surgery under cardioplegia	Reduced the postoperative peak creatine-kinase MB. The required inotropes and transcardiac release of lactate and neutrophil count during reperfusion were reduced
Thibault et al. [84]	60	4	60/60	Percutaneous coronary intervention	Reduced creatine-kinase and troponin I release. Left ventricular ejection fraction increase in 7 %
Luo et al. [85]	30	2	30/30	Cardiac surgery under cardioplegia	Decreased peaks of creatine-kinase MB and troponin I and transcardiac release of lactate
Ma et al. [86]	60	3	30/30	Percutaneous coronary intervention	Faster CTFC and improved WMSI. Decreased peaks of CK, CK-MB and MDA-reactive products. Endothelium-dependent vasodilation function was improved

Delay: time from the end of ischemia to the beginning of the posconditioning protocol

CTFC corrected TIMI frame count, *WMSI* wall motion score index, *MDA* malondialdehyde

angioplasty. They also found a lower liberation of CPK, although only in patients that received ≥4 cycles of "inflation"/"deflation" during angioplasty, compared with those that received between 1–3 "inflation"/"deflation." AQ5

Few studies evaluated the effect of ischemic postconditioning on the "no-reflow" phenomenon. It has been questioned if repeated insufflations of the balloon in the thrombotic occlusion site could not actually be responsible for microemboli or increment of no reflow [73]. In this sense, Mewton et al. [74] studied patients with an ST-elevation higher than 0.1 mV in two continuous derivations that were derived to the catheterization room for primary angioplasty. Infarct and no reflow areas were measured using magnetic resonance with gadolinium. This randomized study demonstrated that postconditioning with angioplasty (4 cycles of 1 min each), applied in patients with STEMI, achieves a significant reduction of no reflow. This protective effect was associated with a reduction on infarct size.

Among various phase II studies performed up to this date, nine have confirmed a significant reduction of creatine-kinase or troponin release in patients subjected to angioplasty plus ischemic postconditioning, in comparison to those that received conventional treatment [75, 76]. Also, one study demonstrated a sustained benefit 6 months after the infarct, evidenced through an improvement of the ejection fraction [77].

Wei et al. [78] performed a meta-analysis to investigate current evidence linking ischemic postconditioning to cardioprotection in patients receiving primary percutaneous coronary intervention (PCI). They analyzed thirteen studies comparing ischemic postconditioning with usual care in patients undergoing PCI. The authors concluded that ischemic postconditioning has a potent protective effect on the ischemic heart, particularly in patients with ST-elevation myocardial infarction. In a similar meta-analysis including ten randomized trials with 560 patients, it was observed that ischemic postconditioning performed during angioplasty reduces the myocardial enzyme levels and improves the ejection fraction in patients with STEMI. Such protective effects were more significant in young female individuals or when the direct-stenting techniques were used [79]. The direct-stenting technique has presented a lower microemboli incidence [75, 76, 80] mentioned that the utilization of this technique could eliminate the possible microembolization induced by ischemic postconditioning.

Even though the results are promising, we must be cautious because studies in patients present substantial dissimilarities regarding differences in the collateral circulation and in risk areas, given that these variables could

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skew the studies. As a consequence, all these findings have to be corroborated with more clinical studies with a higher amount of patients. Their results should answer some of the following questions: (a) Does the beneficial effect of ischemic postconditioning persist in patients with severe coronary artery disease and risk factors (arterial hypertension, hypercholesterolemia, obesity, diabetes mellitus, etc.)? (b) Does the postconditioning effect translate into an improvement of ventricular function, remodeling, and life expectancy of patients? (3) Is it a strategy that could be used in all patients with acute myocardial infarction?

Most likely, the results of the phase III study DANAMI-3 [81] "DANish Study of Optimal Acute Treatment of Patients With ST-elevation Myocardial Infarction"; (ClinicalTrials.gov Identifier: NCT01435408) that is taking place could answer some of these critical questions.

Conclusions

Despite the huge progress that has been achieved in the past decades regarding the knowledge of pathophysiological mechanisms that lead to lethal damage by ischemia/reperfusion, some results remain controversial and still a lot of the factors involved remain unknown.

Different pharmacological and mechanical interventions applied during early reperfusion have shown that it is possible to reduce the infarct size. Among those, ischemic postconditioning is a mechanical maneuver that, used during the first times instances of reperfusion, reduces the infarct size, attenuates endothelial dysfunction, and reduces the apoptosis rate. This way, the treatment of reperfusion's damage with an ischemic postconditioning protocol could be an opportunity to decrease cellular death, and consequentially improve the prognosis of patients with myocardium infarct subjected to reperfusion. However, an intense translational research effort, to take the cardioprotective treatment to clinical practice in patients with acute myocardial infarction, would be necessary.

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Author Query Form

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Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	Please check and confirm that the author names and their respective affiliations have been correctly identified and amend if necessary.	
AQ2	As References [13, 16], [28, 69], [77, 83], and [78, 84] are same, we have deleted the duplicate reference and renumbered accordingly. Please check and confirm.	
AQ3	This sentence 'percentage of the left ventricular area the area of the left ventricle' has been changed to 'percentage of the left ventricular area of the left ventricle' in Figures 3 and 4 captions.	
AQ4	'A2A y A3' has been changed to 'A2A and A3' in text 'However, the subtype of receptor…' Please check and confirm.	
AQ5	Please check and confirm the inserted opening quote in text 'They also found a lower liberation of CPK…'	