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Effect and Role of Post-conditioning During Coronary Angioplasty in Patients Affected by ST-Elevation Acute Myocardial Infarction

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1. ABSTRACT

Background Reperfusion is the mainstay treatment for patients presenting with ST-elevation myocardial infarction (STEMI). Nevertheless, reperfusion itself may exacerbate myocardial injury, a process termed "reperfusion injury". Post-conditioning (PostC) has been suggested to reduce myocardial damage during primary percutaneous coronary intervention (PPCI), nevertheless clinical experience is limited.

Objectives We aimed to review all the known strategies to limit the reperfusion injury; moreover we explored the cardioprotective effect of mechanical postconditioning conducting a randomized trial aimed to evalutate infarct size (IS) at cardiac magnetic resonance (CMR) in STEMI patients treated by PPCI.

Methods A total of 78 patients with first STEMI (aged 59±12 years) referred for PPCI, were stratified for STEMI location and randomly assigned to conventional PPCI or PPCI with PostC. All patients, with occluded infarct related artery and no collateral circulation, received abciximab intravenously before PPCI. After reperfusion by effective direct stenting, control subjects underwent no further intervention, while in treated patients PostC was performed within 1 minute of reflow by 4 cycles of 1-minute inflation and 1-minute deflation of the angioplasty balloon. Primary end-point was IS reduction, expressed as percentage of left ventricle mass assessed by delayed enhancement on CMR at 30±10 days after index PPCI.

Results All baseline characteristics but diabetes (p=0.06) were balanced between groups. Postconditioning patients trended towards a larger IS compared to those treated by standard PPCI (20±12% vs 14±10%, p=0.054). After exclusion of diabetics, PostC group still showed a trend to larger IS (p=0.116). Major adverse events seem to be more frequent in PostC group irrespective to diabetes status (p=0.053 and p=0.080, respectively).

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Conclusions This prospective, randomized trial suggests that PostC did not have the expected cardioprotective effect and, on the contrary, it might harm STEMI patients treated by PPCI plus abciximab. (*Clinical Trial Registration*-unique identifier: NCT01004289).

RIASSUNTO

Razionale dello studio La terapia riperfusiva è la via principale per il trattamento di pazienti che si presentino con infarto miocardico con sopraslivellamento del tratto ST (ST-elevation myocardial infarction, STEMI). Tuttavia, la riperfusione di per sé può esacerbare il danno miocardico, un processo denominato "danno da riperfusione". Il post-conditioning (PostC) é un processo che sembra possa ridurre il danno miocardico da riperfusione durante angioplastica primaria (primary percutaneous coronary intervention, PPCI), ciò nonostante l'esperienza clinical è limitata.

Scopo dello studio Presentare e discutere tutte le strategie note in grado di limitare il danno riperfusivo; inoltre, valutare gli effetti cardioprotettivi del postconditioning ischemico meccanico mediante un trial clinico controllato randomizzato arruolante pazienti con STEMI e inviati a PPCI, con endpoint primario le dimensioni dell'infarto (infarct size, IS) finale alla risonanza magnetica cardiaca (cardiac magnetic resonance, CMR).

Metodi Un totale di 78 pazienti con primo STEMI (età 59±12 anni) inviati per PPCI, sono stati stratificati per sede dello STEMI e successivamente randomizzati a PPCI convenzionale o PPCI con PostC. Tutti i pazienti, con arteria responsabile dell'infarto occlusa e assenza di circolo collaterale, hanno ricevuto abciximab endovena prima della PPCI. Successivamente alla riperfusione, avvenuta con tecnica direct stenting, i soggetti di controllo non sono stati sottoposti ad ulteriori interventi, mentre i soggetti nel gruppo PostC hanno riveduto, entro un minuto dalla riperfusione, 4 cicli di 1 minuto di rigonfiaggio e 1 minuto di sgonfiaggio del pallone usato per l'angioplastica. L'endpoint primario oggetto dello studio, la riduzione dell'IS finale, veniva espresso come percentuale della massa ventricolare sinistra affetta, come possibile riconoscere ad una

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CMR con mezzo di contrasto eseguita a 30±10 giorni di distanza dalla procedura di PPCI indice.

Risultati Tutte le caratteristiche di base, ad eccezione del diabete (p=0.06), risultavano ben bilanciate tra i gruppi di trattamento. I pazienti nel gruppo postconditioning tendevano ad avere un IS maggiore quando paragonati a quelli sottoposti a PPCI convenzionale (20±12% vs 14±10%, p=0.054). Dopo esclusione dei pazienti diabetici, il gruppo di pazienti PostC sembrava ancora associato ad IS finali di maggiori dimensioni (p=0.116). Gli eventi avversi cardiovascolari maggiori sono risultati essere più frequenti nel gruppo PostC, indipendentemente dal loro status diabetico (p=0.053 e p=0.080, rispettivamente).

Conclusioni Questo trial clinico randomizzato prospettico suggerisce che il PostC non ha l'effetto cardioprotettivo atteso e, invece, potrebbe pure nuocere a pazienti affetti da STEMI e sottoposti a PPCI ed infuzione di abciximab. (Numero identificativo unico di registrazione del trial al sito *clinicaltrial.gov*: NCT01004289).

2. INTRODUCTION

2.1. Ischemic heart disease and the concept of myocardial reperfusion injury

Ischemic heart disease is one of the leading causes of morbidity and mortality in developed countries and its epidemiology is still expanding worldwide.[1] After an ST-Elevation Acute Myocardial Infarction (STEMI), timely and succesful myocardial reperfusion, by Primary Percutaneous Coronary Intervention (PPCI) or thrombolytic therapy, is the definitive therapy for reducing Infact Size (IS) and improve clinical outcome. Indeed, IS is a major determinant of prognosis after STEMI, and strategies that reduce IS are of utmost relevance.[2,3] However, the process of restoring blood flow to the ischemic myocardium may itself induce injury. This phenomenon, termed myocardial reperfusion injury, counteracts the benefit of early reperfusion and can paradoxically limit the beneficial effects of myocardial reperfusion strategies.

The lethal reperfusion injury is defined as the myocardial injury due to the restoration of coronary blood flow after an ischemic episode; this injury eventually culminates in the death of cardiac myocytes, by necrosis or apoptosis, that were viable before myocardial reperfusion.[4] This type of myocardial injury can increase infarct size (Figures 1 and 2), as suggested by studies conducted in animal models. In these studies, authors founded that lethal repefusion injury may account for up to 50% of the final IS; moreover, in these models a lot of strategies have been founded relating with less lethal reperfusion injury.
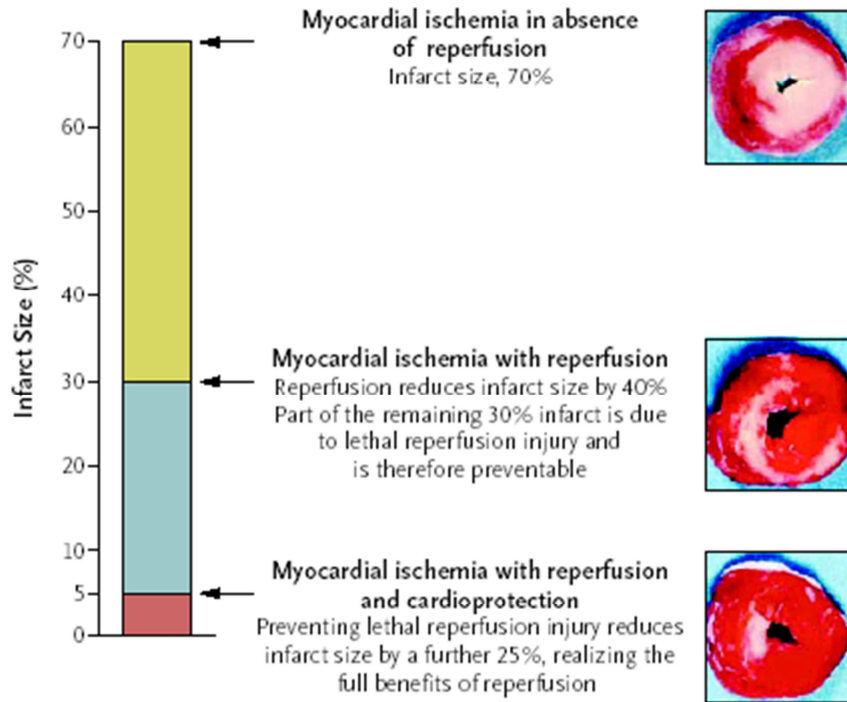
Figure 1. Infarct size and its determinants. Hypothetical scheme illustrating the final infarct size in absence of reperfusion (upper part), with early amd succesful muocardial reperfusion (mid), and after reperfusion with cardioprotection tecniques (lower). From Yellon et al.[16]

Notwithstanding, unlike animal models, clinical studies on the prognostic impact of these strategies had sometimes disappointing results.[5-10] Yet, recent clinical studies suggested a possible beneficial effect of some of these strategies, and especially of ischemic Post-Conditioning (PostC),[11] in which repetitive brief ischemic episodes applied immediately at the onset of reperfusion after a prolonged ischemic insult can exert cardioprotection, reduce IS and preserve endothelial function similarly to conventional preconditioning.[7,12,13] Unlike preconditioning, the experimental design of PostC allows direct application to clinical settings, especially during primary PPCIs. Inflation and deflation of the balloon after reopening the coronary artery can mimic repetitive coronary artery clamping in postconditioned animal models.[14,15]

In this thesis we analyzed, in the first part, the phenomenons underlying the myocardial reperfusion injury and the published preclinical and clinical studies on this issue and, in the second part, we report and discuss the results of a randomized trial,

designed and conducted in our Cardiac, Thoracic and Vascular Department, to

investigate the effect of PostC on IS, as assessed by cardiac magnetic resonance (CMR).
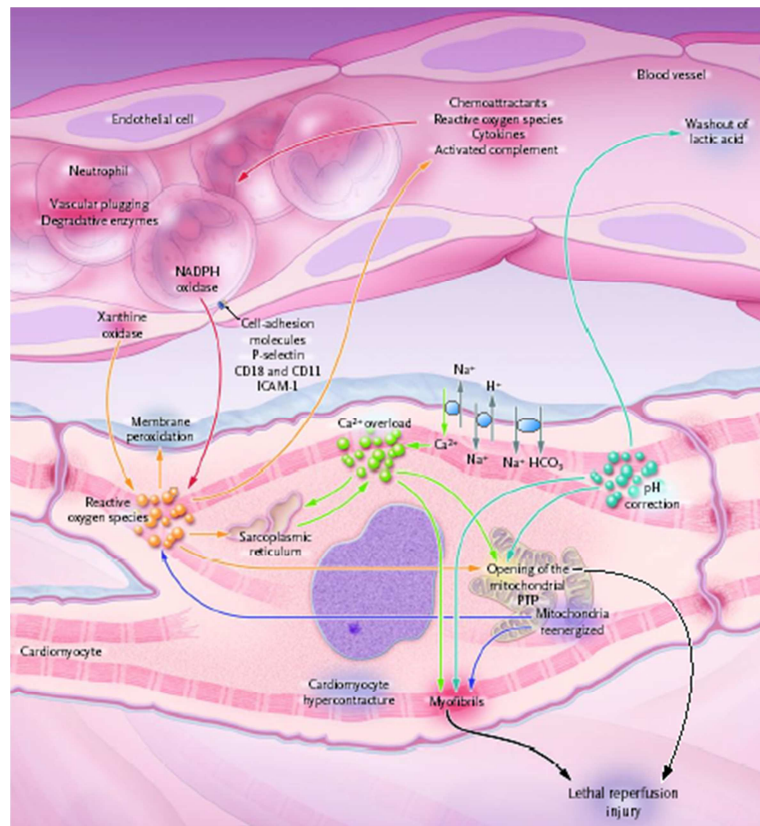
Figure 2. Mediators of lethal myocardium reperfusion injury. During reperfusion, the myocardium is subjected to intracellular Ca^{2+} overload (green), mitochondrial reoxigenation (purple), the generation of reactive oxigen species (ROS) (orange), restoration of physiologic pH (azure), and inflammation (red), all of which may interact with each other to determine cardiomyocyte death through the opening of the mitochondrial peremeability transition pore (m-PTP) and the induction of cardiomyocite ipercontracture. ROS are generated, during reperfusion by xanthine oxidase (mostly from endothelial cells) and the re-energyzed mitochondrial electron transport chain. A further source of ROS, several hours later, is NADPH oxidase (mainly from neutrophils). ROS mediate myocardial injury by inducing m-PTP opening, acting as neutrophils chemoactractants, mediating dysfunction of the sarcoplasmic reticulum and contributing to intracellular Ca^{2+} overload, damaging the cell membrane by lipid peroxidation, inducing enzyme denaturation, and causing direct oxidative damage to DNA. During reperfusion, cell is subjected to an important further influx of Ca^{2+} through the damaged cell membrane, the ROS-mediated disfunctionig sarcoplasmic reticulum and the reverse function of the Na^+-Ca^{2+} exchanger. The ATP generation by the reenergized electron transport chain in the setting of intracellular Ca^{2+} overload induces cell death by hypercontracture, a process that is facilitated by the fast and sudden restoration of physiologic pH during myocardial reperfusion. Moreover, the fast restoration of the mitochondrial membrane potential mediates the Ca^{2+} entry into mitochondria; this process, in conjunction with the loss of the inhibitory effect of the acidic pH on the m-PTP and the generation of ROS, determines the opening of the m-PTP that, eventually, induces cardiomyocyte death by uncoupling of the mitochondrial oxidative phosphorilation and inducing mitochondrial swelling. During reperfusion, the sudden washout of lactid acid and the function of the Na^+-H^+ and Na^+-HCO_3 transporters mediates the rapid restoration of physiologic pH, facilitating m-PTP opening and cardiomyocyte contracture. Several hours after reopening of the infarct related artery, neutrophils accumulates in the infarcted tisse in response to chemoactrants (i.e. ROS, cytokines, and the activated complement). Finally, the upregulation of cell-adhesion molecules as P-selectin, CD18, CD11, ICAM-1 facilitate the migration of neutrophils into the tissue, where they can further enhance cardiac cell death by vascular plugging, degradative enzymes release and ROS generation. From Yellon et al.[16]

2.2. Introduction to endpoints of reperfusion injury and experimental cardioprotection approaches

Myocardial ischemia occurs when coronary blood flow to myocardium is reduced, either in terms of absolute flow rate (low-flow or no-flow ischemia) or relative to increased tissue demand (demand ischemia). During ischemic conditions, at a cellular level, the oxigen supply to mytocondrya for the oxidative phosphorilation is inadequate.[17,18] The process of reperfusion, namely the re-admission of oxigen and metabolic substrates to the ischemic tissue, has been shown associated with complex and multiple biochemical, anatomical, and functional modifications in myocardium that could, eventually, determinate cell survival or death due to necrosis or apoptosis.

2.2.1. Clinical and experimental endpoints of injury

The myocite injury leading to myocardial infarction, the occurrence of arrhythmias, and the loss of myocardial contractility are all clinically relevant consequences of obstructive coronary artery disease, so they are frequently considered as endpoints in experimental and clinical studies. The following synoptic description of the major endpoints provides an introductory context for the studies reviewed subsequently

2.2.1.1. Irreversible myocite injury

After the onset of ischemia, myocardial ultasound changes occur rapidly. During the first minutes the alterations are reversible if a prompt and effective reperfusion of the ischemic tissue is provided; nevertheless, an ischemic severe condition lasting more than 20-30 minutes (without significant collateral flow to the ischemic area) results in

irreversible changes, that are characterized as coagulative necrosis (see fig. 3 and 4).[19-22]

The extent of area at risk, the duration of ischemia, the presence and extension of collateral blood flow or residual flow through the infarct-related artery, heart rate, and myocardial tissue temperature are all factors that have been shown in experimental models affecting the onset and extent of final infarct size.[20,23-25]
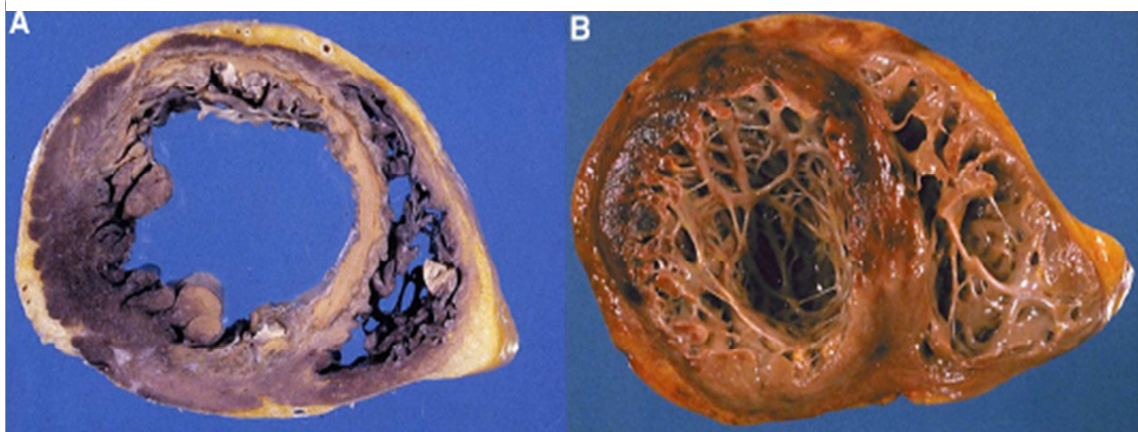
Figure 3. Gross features of AMI in the pre- and postrecanalization era: (A) white, anemic tranmural AMI with wall thinning and expansion; (B) hemorrhagic, red transmural AMI. Adapted from C. Basso et al.[22]
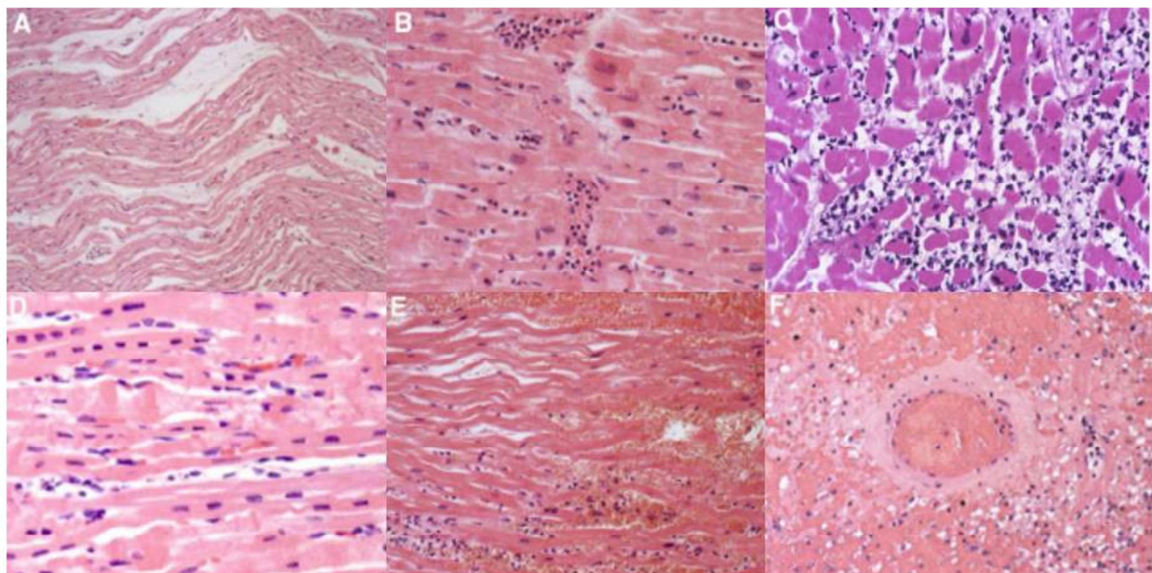
Figure 4. Histological features of AMI: (A) waviness of cardiomyocytes; (B) coagulative cardiomyocyte necrosis and neutrophil infiltrates; (C) macrophage infiltrates; (D) contraction band necrosis (reperfused AMI); (E) interstitial haemorrhage (reperfused AMI); (F) small vessel damage and massive interstizial haemorrhage (reperfused AMI). Hematoxylin and eosin stain. Adapted from C. Basso et al.[22]

In the absence of reperfusion, all the myocardial area at risk (extent of myocardial territory distal to the culprit coronary plaque) will undergo to necrosis, extending from the subendocardium towards the subepicardium, as to involve the full thickness of the ventricular wall, in a process termed "wavefront phenomenon of myocardial death" as first shown in 1970 by Reimer and Jennings et al. in a dog model of acute coronary artery occlusion.[20,26] All the strategies (PPCI, thrombolysis) aimed to reperfuse the acutely ischemic myocardium have the main goal of salvaging viable myocardium within the area at risk limiting the extension of necrosis; the concept of the absolute needing of a prompt and effective reperfusion therapy in patients affected by STEMI is commonly summarized with the axiom "time is muscle and muscle is life".[27] There is some uncertainty on the relevance, the precise timing, of apoptosis process in the reperfusion injury.[28-30] The totality of the mechanisms underlying the apoptosis process is still unknown, but is seems likely that a pivotal role is ruled out by the opening of mitochondrial Permeability Transition Pore (mPTP) during reperfusion phase, after a period of ischemia of sufficient duration.[32-34]

2.2.1.2. Contractyle dysfunction

It is well known that an early consequence of myocardial ischemia is a prompt reduction in term of myocardial contractility,[35] and this process can appear as an acute cardiac failure syndrome. Sudden occlusion of a coronary artery is followed by relevant physiological and metabolic changes that appear within seconds of the cessation of coronary flow and energy metabolism shifts from aerobic or mitochondrial metabolism to anaerobic glycolysis after only 8 seconds of reduced arterial flow.[36,37] This shift occurs as soon as the O_2 trapped in the tissue as oxyhemoglobin and oxymyoglobin is consumed. Simultaneously with the metabolism shift, effective contractions diminish and then cease, and the myocardium stretches rather than shortens with each systole.[38]

Usually, after complete early reperfusion and in absence of significant myocardial cell death, contractility recovers completely and myocardial global function come back to normality. However, this full recovery may take from minutes to several days to be complete. Braunwald and Kloner termed "myocardial stunning" the condition characterized by a "prolonged, postischemic dysfunction of viable tissue salvaged by reperfusion."[39] The length of time for function to fully recover is dependent on a plenty of parameters, including the duration of the index ischemic insult, the severity of ischemia during the original insult, and the completeness of the restore of the coronary arterial flow.[36]

The multifactorial pathway that conduces to myocardial stunning is complex and not fully elucidated, nevertheless a lot of hypothesis has been formulated. The oxiradical hypothesis is based on the results of a series of brilliant experiments of R. Bolli et al. that showing that 50% to 70% of the stunning effect is due to a burst of O_2-derived free radicals liberated during the first few minutes of reperfusion with arterial blood. These free radicals are short-lived and include superoxide anion and hydroxyl radical formed from superoxide via heavy metal–catalyzed reactions. This means that much of the stunning effect is a complication of reperfusion and therefore is a form of reperfusion injury. The evidence that free radicals cause stunning is very strong and began with the demonstration that much of the stunning effect could be prevented by pretreatment of the animals with intravenous infusion of 2 enzymes that scavenge O2-derived free radicals, SOD and catalase.[40-45]

Another pivotal role in myocardial stunning development is played by the altered calcium homeostasis, Ca^{2+} availability and the contractile apparatus sensitivity to Ca^{2+}. It is noteworthy that the oxyradical theory and calcium homeostasis alteration theory are not mutually exclusive: i.e. oxygen free radicals could damage membranes, allowing easier calcium overload during reperfusion that could then alter troponin,

contributing to reduced myofilament sensitivity to calcium; alternatively, oxygen radicals might contribute directly to disruption of troponin. Note that contractile recovery in experimental studies is a mixed endpoint, reflecting both possible loss of contractility due to irreversible injury as well as delayed recovery of viable "stunned" myocardium.

A further pattern of reduced contractility is myocardial "hibernation", a concept introduced by Diamond[46] and divulgated by Rahimtoola[47] to explain how myocardium subjected to reduced perfusion can remain alive and persistently acontractile. Rahimtoola defined hibernation as "a state of persistently impaired myocardial and Left Ventricular (LV) function at rest due to reduced coronary blood flow that can be partially or completely restored to normal if the myocardial oxygen supply/demand relationship is favorably altered, either by improving blood flow and/or by reducing demand."[47] The hibernation hypothesis proposes that the tissue has downregulated its metabolism in response to the reduced arterial flow and that this process allow the myocyte to survive in a situation in which flow was insufficient to maintain contraction. A fundamental aspect of hibernating myocardium is that reperfusion can restore fully contractile function.[38] The complex pathways underlying hibernating myocardium involve alteration of cellular metabolism, myocardial perfusion and subendocardial coronary flow reserve.[48,49]

2.2.1.3. Arrhythmias

Ischemia is a strong pro-arrhytmogenic triggers. In fact during ischemic insults, myocardial arrhythmias may occur, presenting as isolated ventricular premature beats, supraventricular or ventricular tachycardia, or ventricular fibrillation.[35,50,51] Arrhythmias in the early phase, after coronary artery occlusion (phase I arrhythmias), may determinate sudden death.[52] In some ischemia/reperfusion studies, the incidence,

severity and duration of arrhythmias has been used as endpoint; nevertheless it is noteworthy that arrhythmias may develop before the onset of irreversible injury. Reperfusion itself after periods of ischemia may also precipitate arrhythmias: the observation that ventricular fibrillation may occur within few seconds after restoration of blood flow to ischemic myocardium was originally made in the experimental laboratory in the 19th century by Cohnheim and Von Schulthess-Rechberg[53] and later confirmed in the early 20th century by Tennant and Wiggers.[35] In fact, it was found in subsequent experimental studies that ventricular fibrillation may occur more frequently after reperfusion than after coronary artery occlusion.[54] In the clinical scenario, reperfusion arrhythmias may occur during thrombolysis[55] and during, or soon after, PPCIs.[56]

2.2.2. Infarct size limitation: experimental approaches

2.2.2.1. Historical background

The experimental studies in the field of cardioprotection dates from the early 1970s, when E. Braunwald et al. first promoted the concept of therapeutic infarct size.[57] This research resulted in a huge and complex body of literature. The research during the 1970s and 1980s for agents and/or approaches that could limit the development of or prevent myocardial necrosis was largely unsuccessful. The following is a brief summary of the major historical developments as well as of the conceptual and technical obstacles to the successful development of infarct-limiting treatments.

Firstly, the experimental models of coronary artery occlusion provided an accurate description of the morphological changes associated with the development of necrosis,[19,58-64] but they provided few insights into the pathophysiology and underlying cell death molecular mechanisms. Thus, the earliest approaches to infarct size limitation

in the 1970s were focused on drugs aimed to reduce myocardial oxygen demand or vasodilators to increase oxygen and metabolic substrate delivery.[57,65] Therefore, drugs as β-adrenoceptor antagonists,[66-68] calcium-channel blockers,[69-71] and glyceryl trinitrate[72-74] were extensively investigated with no evidence of cardioprotection.

Secondly, the concept that reperfusion was necessary to limit the wave front of necrosis and salvage ischemic myocardium -a concept so clear to us now– was largely unknowledged until the late 1970s. In a plenty of studies, therapeutic agents were administered in animal models of AMI with permanent coronary occlusion, relying on the concept that myocardial tissue could be salvaged within a small but not unrelevant "border zone" between normal and ischemic tissue. In the early 1980s reperfusion with fibrinolytic agents spread out quickly and became an established primary approach in the therapy of patients affected by AMI; nevertheless, some experimental studies in the 1980s continued to use animal models with permanent coronary artery occlusion. The concept of the existence of an infarct border zone in evolving myocardial infarction was probably uncorrect or of negligible significance.[75]

Thirdly, the recognition that reperfusion of the infarct-related coronary artery was associated with specific patterns of injury, termed reperfusion injury, identified myocardial contractyle disfuncion in the form of stunning and reperfusion arrhythmias as possible therapeutic targets. However, the concept of irreversible reperfusion injury resulted very controversial: several mechanisms associated with the pathophysiology of reperfusion, including the generation of reactive oxygen species, intracellular calcium overload, the rapid restoration of pH and inflammation, became the basis of experimental studies in which drugs were administered as adjuncts to reperfusion. In this setting studies seeked for a benefit, as adjunct to reperfusion, of superoxide dismutase,[76-79] adenosine and adenosine receptor agonists,[80] nonsteroidal anti-inflammatory drugs,[70,81-84] and antineutrophil antisera.[85] The resulting literature was

characterized by poor experimental reproducibility or consistency of interpretation with regard to pharmacological infarct limitation.

To recapitulate briefly, during the 1970s and 1980s the relatively late recognition of reperfusion as an essential requirement and the limited understanding of appropriate molecular targets limited the development of effective experimental and clinical studies. From the late 1980s, the recognition of ischemic preconditioning (PreC) was the most significant development in the search for rational approaches to cardioprotection. Finally, nowadays a burst of research effort has identified a number of molecular pathways associated to cell death and cytoprotection that constitutes the basis of all the contemporary experimental and clinical studies.

2.2.2.2. The reperfusion injury paradigm of irreversible injury

It is well knowns that a permanent ischemic insult determines cell death by coagulative necrosis (section 2.2.1.1.). On the contraty, until recently, a lot of discordant opinions existed about the role of reperfusion (Fig. 5, A and B). In fact the previous predominant concept was that cell death occurred mostly during the ischemic phase, basically as a consequence of the depletion of high-energy phosphates and its numerous effects. The authors supporting this older hypothesis considered any cells dying during reperfusion as already irreversibly injured by the ischemic phase.

In the last 15 years, researchers have better understood the previous evidentiated alterations in mitochondrial structure, and especially the pivotal role of mytochondria in determining cell survival during and after cellular stresses.[86] Mitochondrial dysfunction affects cell viability through many possible mechanisms: loss of ATP synthesis and increase of ATP hydrolysis, impairment in ionic homeostasis (especially of calcium), formation of reactive oxygen species and release of proapoptotic proteins.[87-91]
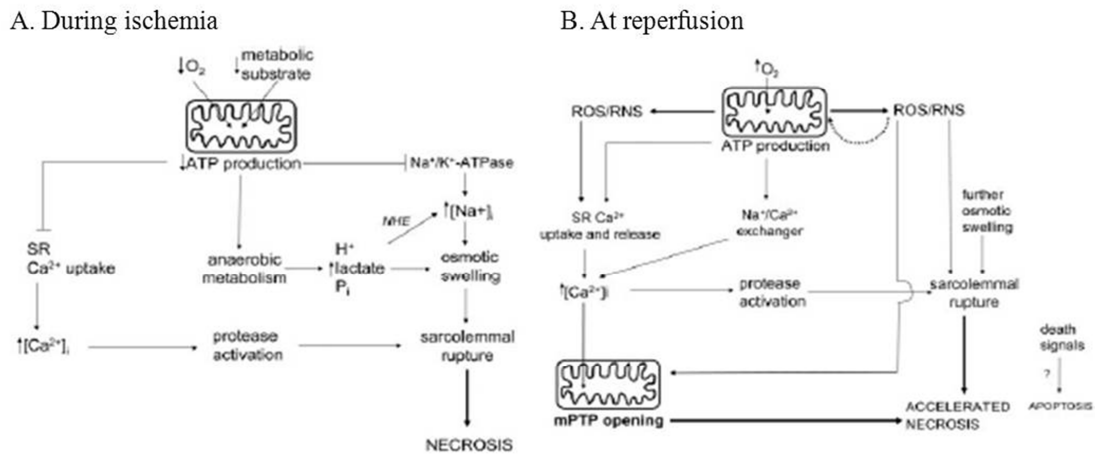
Figure 5. Major cellular effects of ischemia and reperfusion leading to irreversible injury. A, during ischemia, reduced availability of molecular oxygen and metabolic substrates results in a deficit of high energy phosphates. Sarcoplasmic reticulum (SR) Ca^{2+} uptake mechanisms are altered leading to intracellular Ca^{2+} accumulation. Anaerobic metabolism is associated with intracellular accumulation of inorganic phosphate, lactate, and H$^+$. Activation of the sodium-hydrogen exchanger (NHE) by intracellular acidosis leads to accumulation of intracellular Na$^+$, exacerbated by inhibition of the sodium pump due to ATP depletion. Increasing intracellular concentrations of solutes results in osmotic swelling that may be sufficient to cause sarcolemmal fragility or disruption, further exacerbated by the activation of Ca^{2+}-dependent proteases and phospholipases. The process of irreversible injury is time-dependent and, in permanent ischemia, will result in the pathological features of necrosis. B, at reperfusion, cell death occurs predominantly by necrosis although some apoptosis may occur. The sudden reintroduction of molecular oxygen causes re-energization of mitochondria and reactivation of the electron transport chain with massive production of ROS, which may stimulate further ROS production (ROS-induced ROS release) and generation of RNS in the presence of NO. ROS/RNS cause oxidative and nitrosative damage to cellular structures, including the SR leading to Ca^{2+} release. Moreover, under conditions of restored ATP production, the activity of the Na$^+$/Ca^{2+} exchanger is restored, leading to the extrusion of Na$^+$ in exchange for Ca^{2+}, and SR Ca^{2+} release is further accentuated by restoration of ATP leading to cytosolic Ca^{2+} overload. The combined effects of Ca^{2+} accumulation in the mitochondrial matrix, ROS/RNS, and increasing intracellular pH due to H$^+$ washout favor the opening of the mPTP. Opening of the mPTP is associated predominantly with necrotic cell death, most likely in those cells that have already sustained injury during ischemia. Some cells display hallmarks of apoptosis after reperfusion. The mechanisms leading to activation of the apoptotic program are unclear and could be related to either mitochondrial or extracellular death signals. The precise rate of injury or mode of cell death during reperfusion will be determined by the severity of changes during ischemia as well as by the extent of sarcolemmal fragility and disruption, which may be further exarcebated during reperfusion by osmotic swelling and protease acitivity. Adapted from Ferdinandy et al.[118]

This series of mechanisms explains why mitochondria are involved in both necrosis and apoptosis processes following post-ischemic reperfusion. Recently a pivotal role in the cell response to stresses seems to be played by the mitochondrial permeability transition pore (mPTP). The mPTP is a voltage-dependent, high-conductance multimeric channel located in the inner mitochondrial membrane. In the fully open state, the apparent pore diameter allows passive nonspecific diffusion of

solutes with molecular masses up to about 1.5 kDa.[92,93]. A relevant characteristic of the mPTP is that it can be partially inhibited by cyclosporin A that binds cyclophillin-D, a key-component of mPTP. Since the effect of cyclosporin A can be relieved by increasing the calcium load,[93] the effect of cyclosporin A is preferably described as ''desensitization'' of the mPTP to calcium. mPTP is in a closed state during normal physiological conditions, when the membrane is impermeable to most solute. Under stressing conditions, the mPTP opening (by formation of the an open pore in the inner membrane) results in major modifications of mitochondrial function and structure that eventually jeopardize the maintenance of cell viability. The immediate consequence of mPTP opening is the collapse of mitochondrial membrane potential; as a consequence oxygen consumption is initially increased and ATP produced by glycolysis is hydrolyzed by the reverse operation of ATPase leading to ATP depletion.[94-97] These events may be followed by subsequent changes making the outcome of the cell quite unpredictable. The mPTP opening allows the efflux and then the hydrolysis of pyridine nucleotides resulting in a reduction of oxidative metabolism and oxygen consumption.[34] Moreover, oxidative stress might be exacerbated, since NAD(P)H is essential for the maintenance of both mitochondrial and cytosolic antioxidant defenses.

These functional alteration in mPTP reflects on a morphologic point of view as a wide range of structural changes of mitochondria: cristae remodeling, matrix swelling and outer membrane alterations. Eventually these alterations can result in the rupture of the outer mitochondrial membrane and release of proteins from the intermembrane space. An important member of this group of protein is cytochrome c (normally sequestered within internal mitochondrial membrane cristae), which after binding Apaf-1 in the cytosol causes the activation of caspase 9, triggering the apoptotic cascade.[91,98,99] Moreover, it has been proposed that structural changes caused by PTP

opening might prompt the removal of damaged mitochondria by means of autophagy, a process termed "mitoptosis".[100,101]

Currently, the consensus of experts is that conditions during the early phase of reperfusion, but not during ischemia, may favor the opening of mPTP and that inhibition (or desensitization) of pore opening during the early phases of reperfusion might protect cardiomyocites. In the current concept of reperfusion injury the opening of mPTP at reperfusion is the fundamental determinant of cell fate. mPTP opening can cause cell death either by necrosis or apoptosis,[34,102] depending on the extent and duration of mPTP opening.[97] As a direct consequences of this new concept, it is clear that manipulation of the conditions that inhibit mPTP opening during the early phase of reperfusion may offer the potential to limit cell death: ischemic preconditioning, pharmacological treatments mimicking preconditioning, ischemic postconditioning, and selected drugs administered at reperfusion might protect through a common pathway of attenuating mPTP opening.

In summary, the present view of reperfusion process is that it is absolutely necessary to salvage the ischemic myocardium; nevertheless, reperfusion itself has the potential to cause further irreversible myocyte injury in a way closely related to the extent of mPTP opening in the early reperfusion phase. Recent knowledge do not detracts the fundamental proven therapeutic value of reperfusion, but it has highlighted and revisited the reperfusion phase, better evaluating the mechanisms of reperfusion injury and suggesting potential therapeutic targets to realize the full benefits of reperfusion in patients affected by acute myocardial infarction.

2.2.2.3. Cardioprotection through preconditioning

Although transient episodes of myocardial ischemia can induce the reversible injury of stunned myocardium (see 2.2.1.2.), they can also protect significantly the heart

from extensive necrosis. In 1986 Murry et al. first described, in one of the most quoted and influential articles in cardiac literature, the concept of ischemic preconditioning (PreC).[103] In a study involving anesthetized dogs subjected to 40 minutes of circumflex coronary artery occlusion and reperfusion they demonstrated a profound limitation of myocardial IS when the dogs received 4 brief episodes of 5 minutes of ischemia separated by 5 minutes of reperfusion just before the 40-minute occlusion. Notably, this cardioprotective effects of ischemic PreC were independent of changes in transmural myocardial blood flow. Within the control animals group analysis of the extent of necrosis within the risk zone as a function of coronary collateral flow showed an inverse relationship: the lower the coronary collateral flow the greater the percentage of the risk zone that went on to develop necrosis. In the ischemic PreC animals group this relationship was altered: even in dogs with low coronary collateral flow, the extent of necrosis was reduced markedly with the PreC stimulus. Moreover, the PreC phenomenon can be seen in models of extremely low coronary collateral blood flow, such as the rabbit, and therefore is not due at all to recruitment of coronary collateral flow.[104] In fact, ischemic PreC subsequently was shown to be widely reproducibly and consistent in a variety of species (rat, rabbit, pig and mouse hearts) and in different PreC protocols and experimental preparations.[105] Ischemic PreC have been shown cardioprotective in all mammalian hearts tested thus far, with maximal effects in large-animal hearts, in which heart rates (and so metabolism) are lower.

Preconditioning protection can be obtained by periods of ischemia as short as 3 to 5 minutes followed by 5 minutes of reperfusion; a single episode of transient ischemia is sufficient to induce PreC,[106,107] although laboratories often use multiple repetitive episodes of brief ischemia to induce the phenomenon.[108]

The cardioprotective effect of PreC is transient: if the duration between PreC and the more prolonged ischemic episode to induce necrosis is extended beyond 60

minutes, the benefit of ischemic PreC is lost.[42,103] However, if the duration between PreC stimulis and the more prolonged ischemic episode to induce necrosis is further extended to 24 to 96 hours, however, then the protective effect partially returns and IS is somewhat reduced, although not to as markedly as when the long-standing occlusion episodes occurs shortly after PreC stimulus.[108,109] To distinguish this late-onset, long-lasting phenomenon from the the "classic" or "early" PreC, it was called originally "the Second Window Of Protection (SWOP)" but now it is best termed "delayed" or "late preconditioning". A schematic representation of the temporal nature of the windows of PreC is shown in Fig. 6.
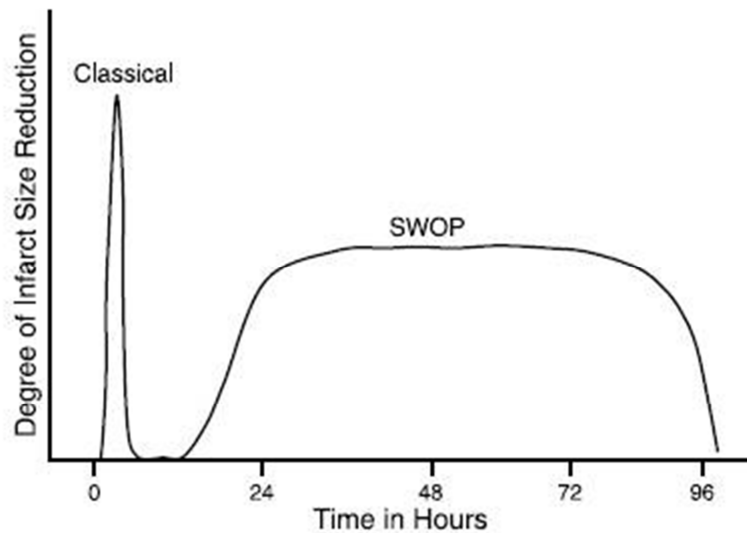
Fig. 6. Schematic representation of the temporal nature of the two windows of preconditioning. Modified from Sumeray MS and Yellon DM.[110]

Currently, the biology of classic PreC is thought as a phenomenon that can delay but does not prevent myocardial cell death during the long-lasting episode of ischemia: if the duration of the ischemic PreC is excessive or reperfusion is not eventually restored, PreC will not work.[103] Noteworthy, there are two phenomenon that could limit the beneficial effects of PreC: the energy demand in the preconditioned tissues must be diminished and too frequent and too close PreC induce tachyphylaxis.[106,111,112] Originally the definition of ischemic PreC was referred to

limitation of IS, but subsequently some researchers extended it to describe all the effects

of brief ischemia also on cardiac function and arrhythmias, although these latter effects

have not been as consistent as effects on IS.[113,114] The mechanism(s) that underlies the

potent but short-lasting protective effect seen in classic PreC is undoubtedly complex

and not fully understood, involving second messenger pathways (Fig. 7).[115-117]
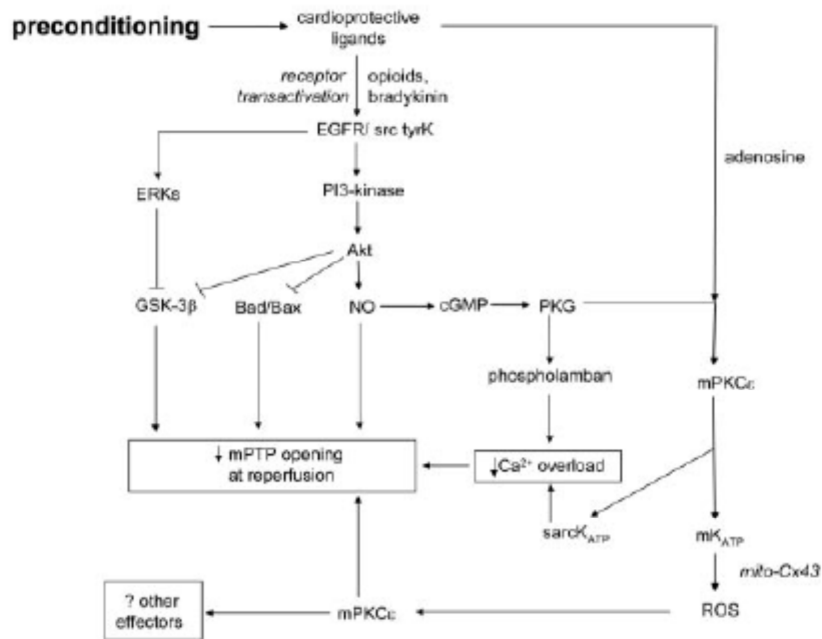
Fig. 7. Schematic representation of the major pathways of classic ischemic preconditioning. Classic PreC promotes the accumulation of various cardioprotective ligands for G-protein-coupled receptors, especially adenosine, bradykinin and opioid peptides. Evidence exists for the participation of receptor tyrosine kinase activity, possibly through transactivation, although adenosine may couple directly to PKC. The activation of numerous other protein kinases has been implicated, including the PI3K/Akt cassette, which is thought to be proximal in the signaling pathway. Akt phosphorylates a number of substrates including proapototic members of the Bcl-2 protein family and GSK-3β (causing inactivation) and eNOS (causing activation). NO generated from eNOS leads to activation of PKG via elevation of intracellular cGMP. Substrates for PKG may include the SR regulatory protein phospholamban, which promotes SR Ca^{2+} uptake, thereby reducing cytosolic Ca^{2+} overload. Recent evidence suggests that PKG is the final cytosolic signal transduction component and leads to activation of mitochondrial pools of PKCε. Downstream consequences of PKCε activation include activation of mitochondrial K$_{ATP}$, opening of which promotes ROS formation and further PKCε activation. Inhibition of mPTP opening can occur as a result of PKCε activation. Sarcolemmal K$_{ATP}$ and mitochondrial connexin-43 have also been implicated in the mechanism of classical PreC. The latter is an essential component of the classical PreC mechanism. The generation of ROS and RNS appears to be a consequence of mitochondrial K$_{ATP}$ opening and an obligatory part of the signaling cascade. It is likely that ROS/RNS signal the activation of distal kinases which may include p38 MAPK, PKC, and JAK/STAT. Although inhibition of mPTP opening in early reperfusion appears to be an important mechanistic feature of PreC, it is possible that other distal proteins may serve as effector mechanisms. From Ferdinandy et al.[118]

Definition of the role of an endogenous mediator in PreC, late PreC or PostC

processes ideally requires satisfaction of all of the following criteria: abolition of the

protective/beneficial effect by specific receptor blockade or by inhibition of the mediator's production; absence of the effect in animals, tissues, or cells with genetic disruption of the mediator's production or its receptor(s); induction of a pharmacological effect by exogenous administration of the mediator at the time of reperfusion. Increased production or maintenance of extracellular concentrations of the mediator(s) as a direct effect of the studied process might be added to this list although in practice this is the most difficult criterion to satisfy experimentally.

The fully preconditioned myocardium shows a smaller adenine nucleotide pool, an excess in intracellular glucose, a creatine phosphate overshoot, and stunning.[52,136] Moreover, during a second episode of ischemia it reacts differently than not-preconditioned myocardium: it trends to utilize high-energy phosphates and accumulate lactate and H^+ much more slowly.[106,111,119,120] This scenario of slowed anaerobic glycolysis (principal pathway of ATP synthesis during ischemia) despite slowed depletion of high energy phosphate (HEP) has been interpretated by hypothesizing a reduced energy demand induced by ischemic preconditioned tissue.[106,111,121,122] Notably, since it is known that low level of intracellular HEP and high tissue level of lactate and H^+ are associated with ischemic cell death, it has been theorized that preconditioned tissue, via an energy demand reduction, dies more slowly.[106,111]

The alterations occurring during the PreC ischemic episodes as well as the changes that persist within the preconditioned tissue mediating the PreC response have not been thoroughly estabilished and are a field of intense research.[123,124]

In classic PreC a pivotal role is thought to be played by protein kinases. It is probable that multiple signal transduction pathways converge on mitochondria, either preserving ATP synthesis or preventing the onset of mPTP formation after reperfusion or both. The most important protein kinases identified to play essential roles in classic PreC are protein kinase C (PKC),[125-127] p38 mitogenactivated protein kinase

(MAPK),[116,128-130] phosphatidylinositol 3-kinase (PI3K) and its substrate kinase Akt,[131-133] p42/p44 MAPK/ERK,[134,135] the JAK/STAT pathway,[136,137] and receptor tyrosine kinases of the Src family.[138-140] Recently interest has focused on glycogen synthase-3β (GSK-3β), a distal kinase inactivated by phosphorylation by other kinases including Akt and p42/p44 MAPK/ERK.[141,142] Evidence supporting involvement of these protein kinases has relied mostly on the demonstration that they are translocated, phosphorylated, or display increased activity in preconditioned myocardium when compared to virgin myocardium and/or that pharmacological agents inhibiting their activation abolish or blunt the PreC induced cardioprotection.

The upstream agents triggering the activation of these kinases and the multistep cascade sequence of their activation are not fully elucidated. However, regarding the protection offered by PreC against cell death, it seems highly likely that adenosine, opioid peptides, prostaglandins, and bradykinin released or accumulating in the preconditioned ischemic myocardium, bind to G-protein-coupled receptors, namely adenosine A_1 and A_3, kinin B_2, opioid δ_1 and EP_3, respectively.[143] Among species, and according to the PreC protocol applied, the relative importance of these triggers may vary widely.[144] However, the pharmacological blockade of individual receptors abrogate or blunts the cardioprotective effect of PreC whereas a "pharmacological preconditioning", by transient preischemic activation of any of the receptors with exogenous triggers or synthetic agents induces beneficial effects usually quantitatively similar to that seen with ischemic PreC.

An another pivotal mediator of PreC phenomenon is the K_{ATP} channel. The K_{ATP} channel, expressed in high concentration in the sarcolemma, opens during hypoxia and/or whenever intracellular ATP declines substantially (hence during ischemic episodes of sufficient duration). This effect of ischemia can be abolished by pretreatment of the myocardium before the PreC episode of ischemia with inhibitors

(glibenclamide and 5-hydroxydecanoate) of the K_{ATP} channel, whereas pharmacological openers of K_{ATP} confer protection similar to that obtained with ischemic PreC, all findings supporting the concept that the K_{ATP} channel is an essential mediator of the PreC protection. The sarcolemmal K_{ATP} channel opening mediates an increased K^+ influx (increasing osmotic load) and a shortening of action potential duration (potentially arrhythmogenic). Moreover, a K_{ATP} channel is present also in the mitochondria.[145] This mitochondrial channel is opened quite specifically by diazoxide and is blocked with low concentrations of 5-hydroxydecanoate compared with the quantities required to block the sarcolemmal K_{ATP} channel.[146] At mitochondria level, K^+ flux across the inner mitochondrial membrane affects mitochondrial membrane potential, volume regulation, Ca^{2+} homeostasis, and energy production. In dog model, pretreatment with diazoxide pharmacologically preconditions the heart and limit IS as well as ischemic PreC.[147] All these findings support the concept of a pivotal role of mitochondrial K_{ATP} channel in the protection conferred by PreC, with triggers exerting their effect by opening the mitochondrial K_{ATP} channel.[146,148]

The current view of signal transduction in classic PreC is characterized by a multistep pathway. Adenosine, acting on A_1 or A_3 receptor, couples directly to PKC via phospholipase C and diacylglycerol formation.[149-151] Bradykinin and opioids trigger a complex signal transduction pathway involving transactivation of receptor tyrosine kinase and subsequent phosphorylation (activation) of PI3K/Akt. Akt phosphorylates, in its turn, eNOS resulting in NO production, activation of soluble guanylyl cyclase, cGMP accumulation, and activation of cGMP-dependent protein kinase (PKG). ROS/RNS play a critical role in the signal transduction pathway, leading to activation of PKC.[152] PKG appears to be the terminal cytosolic step in the signal transduction cascade, phosphorylating an unknown target at the mitochondrial outer membrane.[153,154] The opening of mitochondrial K_{ATP} channel is both PKG- and PKCε-dependent.[153] It is

highly likely that PKG is the terminal cytosolic component of the trigger pathway; it transmits the cardioprotective signal from cytosol to inner mitochondrial membrane by a pathway that includes PKCε. PKG phosphorylates an unknown target at the mitochondrial outer membrane that induces a subsequent activation of a PKCε pool within the intermembrane space.[153,154] Moreover, PKG seems to inhibit mPTP opening through a mechanism involving activation of two mitochondrial pools of PKCε.[154] PKCε1 promotes the opening of mitochondrial K_{ATP} channel, leading to modest increase in matrix H_2O_2. H_2O_2 promotes further PKCε1 activation and activates PKCε2, which inhibits mPTP formation.

Connexin-43 is another protein implicated in classic PreC. Connexin-43 forms the multimeric hemichannel structure of gap junctions in myocardium and appears to be obligatory for classic PreC, as experiments conducted in connexin-43 heterozygous knockout mice display no PreC response.[155-158] Moreover, Connexin-43, is also expressed in the mitochondrial inner membrane of cardiomyocyte (and mitochondrial content of Connexin-43 during ischemic PreC is increased),[159] its transport being mediated by heat shock protein 90 and the translocator of the outer mitochondrial membrane.[160] Loss of connexin-43 reduces ROS formation secondary to diazoxide, leading to a loss of pharmacological PreC-induced protection.[161]

2.2.2.4. Cardioprotection through late preconditioning

Late precondioning involves multiple signaling pathways that are related with the intensity, duration and characteristics of the index stimulus.[162] The mechansims underlying late PreC have been less elucidated than that for early PreC. It is highly probable that the phenomenon share some common pathways with classic PreC with the adjunct of some multiple mechanisms of protein synthesis/modification and genetic adaptation responsible for the long lasting effects. In the pioneristic study of Marber et

al.[109] the ischemia/reperfusion stress have been found, in rabbit hearts, causing a de novo synthesis of a series of protein as the 72-kDa heat shock protein (HSP72), with a clear association between HSP72 level and infarct size reduction after ischemic PreC. Subsequently, Hoshida et al. focused their attention on the precise time course of induction of the intracellular inducible antioxidant SOD.[163] It is weel recognized that exist a conserved stress response in eukariotes involving the induction of cytoprotective factors such as SOD and HSPs, although the regulatory pathways of these factors in mammalians is less clear.

An important autacoid factor also in late PreC is adenosine. In a study on rabbit hearts, the pharmacological blockade of adenosine receptors during ischemic PreC abolished the development of late protection 24 hours later.[164] On the other hand the administration of selective A1 receptor agonist to naive rabbits resulted in cardioprotection 24 to 72 hours later, mimicking the effect of late PreC.[164-166]

Also NO, bradikinin, cytokine, selective opioid δ receptor agonists, and ROS have been recognosized as relevant triggers in late PreC.[162,167]

The downstream pathways that link triggers to transcriptional regulation of protein involved in the late PreC beneficial effects is complex and poorly elucidated. The available literature have shown the involvement, in a wide time window ranging between minutes to hours after the index stimulus, of the JAK/STAT signaling pathway,[168] PKC, especially PKCε,[169-171] Src and Lck tyrosine kinases, probably downsteam of PKC,[172]; p38 MAPK,[148,173,174] PI3K and p70s6 kinase/mammalian target of rapamycin[175] and p42/p44 MAPK/ERK.[148]

The synoptic scheme of late PreC involves the interaction of NO and superoxide to form peroxynitrite anion, which activates PKCε, which in turn activates Src and Lck tyrosine kinases. The phosphorylation (activation) of the transcription factor NF-κB is made by both both PKC and tyrosine kinases. NF-κB induces the expression of myocyte

protective genes as inducible NOS (iNOS) and cyclooxygenase-2 (COX-2). The NO production derived by iNOS in turn regulates the activation of COX-2 in the preconditioned myocardium, resulting in a improved generation of prostanoid, essential for the cardioprotective effect.[176] A schematic representation of these processes is shown in Fig. 8.
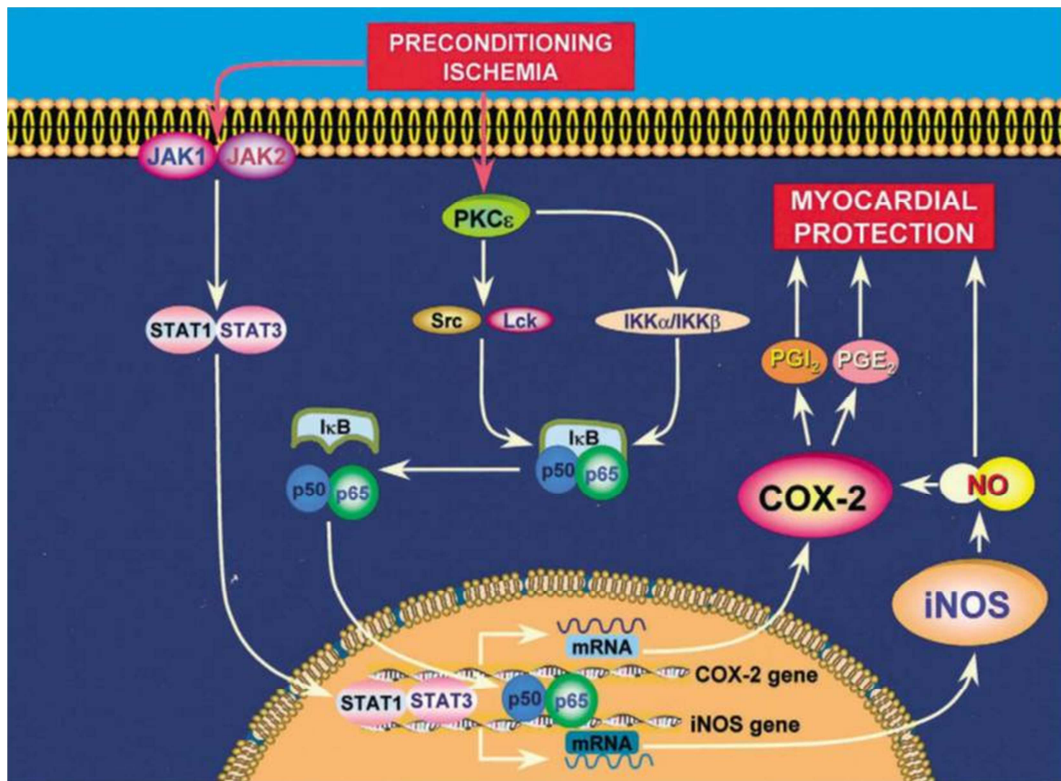
Fig. 8. Schematic representation of our current understanding of the cellular mechanisms whereby COX-2 is upregulated by ischemic preconditioning and participates in cardioprotection. A sublethal ischemic stress (ischemic preconditioning) activates a complex signal transduction cascade that includes PKC (specifically, the ε isoform), PTKs (specifically, Src and/ or Lck kinases), and probably other as yet unknown kinases, leading to phosphorylation of IkBa and mobilization of the transcription factor NF-kB. In addition, ischemic preconditioning activates the non-receptor tyrosine kinases JAK1 and JAK2 with subsequent tyrosine phosphorylation and activation of the transcription factors STAT1 and STAT3. Other, as yet unknown, transcription factors are most likely involved as well. The promoter of both the iNOS and the COX-2 genes contains cognate sequences for NF-kB and STAT1/STAT3. Binding of NF-kB and STAT1/STAT3 to these promoters results in a coordinated transcriptional activation of the iNOS and COX-2 genes with synthesis of new iNOS and COX-2 proteins. The activity of newly-synthetized COX-2 protein requires iNOS-dependent NO generation whereas the activity of iNOS does not require COX-2-dependent prostanoid generation. Thus, COX-2 is downstream of iNOS in the pathophysiological cascade of late preconditioning. iNOS-derived NO can protect the myocardium from recurrent ischemia both via direct actions and via activation of COX-2-dependent synthesis of cardioprotective prostanoids. Among the products of COX-2, PGE and/ or PGI appear to be the most likely effectors of cytoprotection. A similar upregulation of COX-2 can be elicited pharmacologically by δ-opioid receptor agonists but not by adenosine A or A receptor agonists. Adapted from Bolli et al.[176]

2.2.2.5. Cardioprotection through postconditioning

The term "postcondioning" was first introduced by Na et al. regarding the prevention of arrhythmias;[177] Zhao et al. were the first to demonstrate, in their pioneristic study in 2003, the application of PostC to reduce lethal reperfusion injury in dog hearts.[11] In this landmark study 3 cycles of 30-s reperfusion and 30-s reocclusion of the left anterior descending artery were applied at the onset of reperfusion after a sustained 1-hour occlusion, resulting in marked limitation (48%) of infarct size (see also Fig. 9).
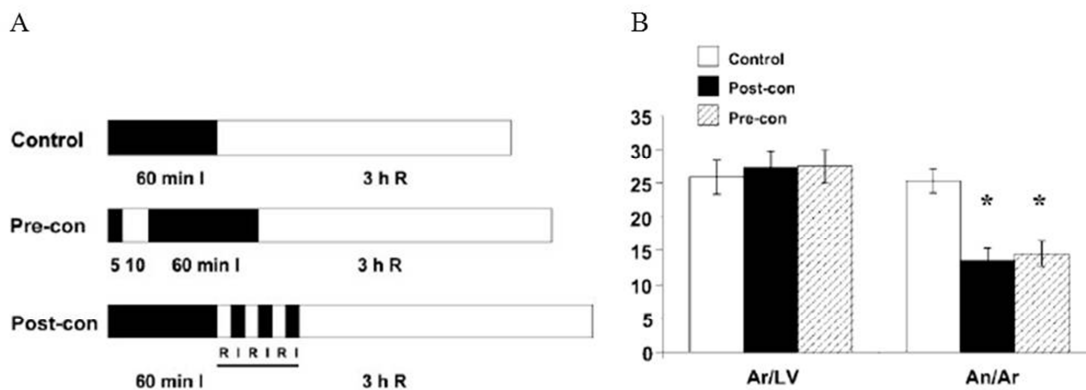
Fig. 9. A. Experimental protocol used to determine the effect of ischemic PostC (Post-con) on myocardium after ischemia (I) and reperfusion (R). B. Bar graph showing determination of infarct size by triphenyltetrazolium chloride (TTC) staining. Post-con significantly reduced area of necrosis (AN)-to-area at risk (AR) ratio by 48% compared with the control group, showing equivalent cardioprotection to that of Pre-con, *P< 0.05 vs. control. Values are group means ± SE. Adapted from Zhao et al.[11]

In their study they found also that tissue edema in the area at risk was similarly reduced in PreC and PostC group compared with controls, as well as polymorphonuclear neutrophil (PMN) accumulation, adherence of PMN to postischemic endothelium, plasma malondialdehyde (a product of lipid peroxidation) levels. Moreover, endothelial function, expressed as maximal vasodilatation after acetylcholine was significant greater in PostC and PreC when compared with controls.

The current mechanisms thought to be involved in PostC invokes the activation of signal transduction cascades by autacoid triggers accumulating in the extracellular space (during PostC process) and acting on cell surface receptors or other molecular

targets. A schematic representation of signalling mechanisms in PostC is shown in Fig. 10.
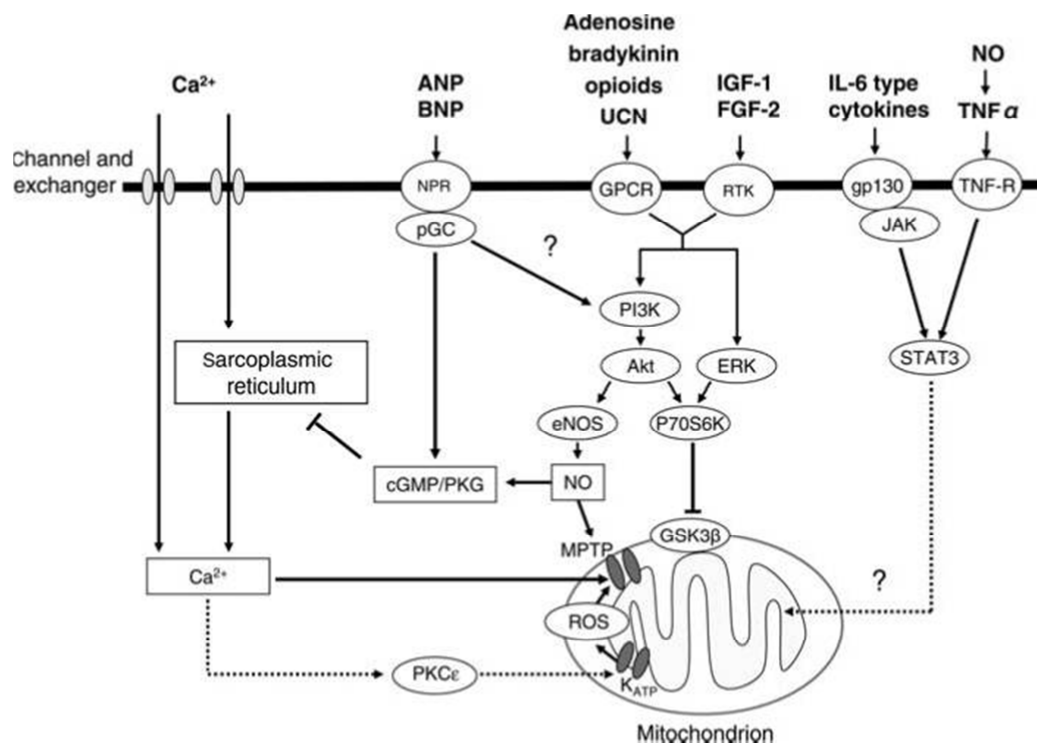
Fig. 10. Signalling mechanisms in postconditioning (PostC). Several extracellular factors produced endogenously are known to play an essential role in ischaemic PostC (adenosine, bradykinin, and opioid peptides). However, other additional autacoids could play a role, since their exogenous administration at reperfusion mimics the effect of ischaemic PostC. These include natriuretic peptides (ANP and BNP), peptide growth factors (IGF-1 and FGF-2), and TNF-α. After binding to cell surface receptors, these autacoids promote the activation of kinase signalling pathways. The precise sequence of elements in these pathways and the extent of interaction between different pathways are unclear. However, evidence from some models implicates the activation of PI3K/Akt and p42/p44 ERKs. This pathway, known as the RISK pathway, is proposed to result in inhibition of mPTP opening at reperfusion, via distal components of the cascade which include NO and inhibition of GSK3ß. The extent to which cGMP accumulation and PKG activation contribute to ischaemic PostC is not clearly defined at present, but several pieces of evidence support the hypothesis that the activation of cGMP/PKG, either by NO or by other factors such as natriuretic peptides, is protective during reperfusion by attenuating Ca^{2+} cycling which may be a stimulus for mPTP opening. Furthermore, it has been proposed that the activation of an intramitochondrial pool of PKC1 might cause opening of the mitochondrial K_{ATP} channel (mitoK_{ATP}), resulting in a slight increase in reactive oxygen species (ROS) formation which eventually causes mPTP inhibition. An alternative pathway, the so-called SAFE pathway, has been proposed to play a role in ischaemic PostC. The major components of the SAFE pathway are TNF- α, the kinase JAK which phosphorylates the transcription factor STAT3. It is proposed that after translocation to the nucleus, STAT3 controls the transcription of factors that confer cardioprotection. Also a mitochondrial localization of STAT3 has been suggested; however, both actions of STAT3 need to be finally proven. eNOS stand for endothelial nitric oxide synthase; GPCR, G-protein coupled receptor; GSK3β, glycogen synthase kinase-3β; MPTP, mitochondrial permeability transition pore; ERK, p42/p44 extracellular regulated kinase; NPR, natriuretic peptide receptor; pGC, particulate guanylyl cyclase; PKG, cGMP-dependent protein kinase; RTK, receptor tyrosine kinase; SR, sarcloplasmic reticulum; TNF-R, TNF receptor; ?, unclear at present. From Ovize et al.[178]

2.2.2.5.1. Autacoids factors and receptor-mediated mechanisms

A variety of autacoid factors, acting in an autocrine–paracrine fashion, have been shown playing a role in the PostC process. These includes 3 groups of substances thought to be involved in triggering the PostC pathways: autacoid factors (i.e. adenosine, bradykinin, opioids) activating via receptor-mediated mechanisms the PostC effects; locally produced substances (i.e. ROS, NO, reactive nitrogen species, hidrogen sulphide (H_2S), calcitonin gene-related peptide, epoxyeicoesatrienoic acids); finally, some naturally occurring substances (i.e. natriuretic peptides, erythropoietin, adrenomedullin, urocortins, adipocytokines as apelin, visfatin, and leptin, insulin, peptide growth factors as transforming growth factor-β, and insulin-like growth factors) with proven pharmacological PostC effect but with little experimental evidence of an effective role in native ischemic PostC.

Analyzing the first group of substances, probably the most important in inducing the PostC cardioprotection, adenosine has been found playing a pivotal role, as highlighted by studies in which pharmacological selective adenosine receptor antagonism during reperfusion abrogated the effects of ischemic PostC.[179-181]

Adenosine levels augment during ischemia, whereas they decrease during reperfusion.[182] Postconditioning, delays the adenosine washout during early phases of reperfusion and enhances its myocardial interstitial accumulation.[179] In the myocardium tissue and coronary vasculatory bed have been identified multiple adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3). Studies with selective adenosine receptor ligands suggested that A_1 receptor activation seems not involved in rabbit or mouse myocardium.[179,183,184] Activation of A_2 receptor subtypes (A_{2A} in mouse)[179] or A_{2B} in rabbit[181] as well as A3 receptor in mouse[179], may play a role. These observations, likely to be associated with a resurgence of interest in the application of adenosine and adenosine receptor agonist as adjunct to reperfusion, suggest that a general role of

adenosine receptor activation is fundamental, but the relative contribution of different adenosine receptor subtype may be species- and model-dependant.

Ischaemic PostC, as for adenosine, maintained myocardial opioid peptide concentrations during early reperfusion.[185] Opioid receptor activation seems to be involved in the effect of ischaemic PostC in rodents as evidentiated by a variety of study.[185-190] Normally, opioids peptides as met-enkephalins, leu-enkephalins, dynorphins, and three major opioid receptors (μ-, δ-, and κ- subtypes) are present in myocardium. In rat models, non-selective opioid receptor antagonist naloxone or selective of μ-, δ-, or κ-opioid receptors antagonists abrogated PostC induced effects.[185,186] On the other hand, morphine (non-selective agonist);[185,187,188] selective δ-opioid,[187,189,190] or κ-opioid receptor agonists[190] induced effective pharmacological PostC, suggesting that opioid receptor activation is important in morphine-induced PostC.

Bradykinin, a peptidic substance of the kinin family, is naturally produced by vascular and cardiac endothelium from precursor kininogens. Two bradykinin receptor subtypes are usually expressed in the cardiovascular tissues: B_2, constitutively expressed, and B_1, up-regulated under ischemic/hypoxic and inflammatory stresses.[191] The admistration of B_2 selective receptor antagonists abolished PostC effects,[192] and PostC cardioprotection was not evokable in B_2 receptors knockout mice; in B_1 receptor knockout mice a partial attenuation of PostC effect was evident as well.[193] Finally, thiese observations were corroborated by the findings that bradikinin administration at reperfusion redeuced IS in mouse, rat, and rabbit models.[192-196]

Summing up these findings, during early reperfusion ischaemic PostC enhances, delaying their washout, the concentration of a numerous endogenous autacoid factors that, by activation of their specific receptors, contribute to the reduction in lethal reperfusion injury. The blockage of the effect of any of the endogenous autacoids with

specific antagonist abolish ischaemic PostC induced cardioprotection, whereas an exogenous administration of each autacoid is capable of inducing PostC-like protection.

2.2.2.5.2. Ionic homeostasis

During ischaemia a progressive decrease in intra- and extra-cellular pH usually occurs within few minutes via an accumulation (increased production/reduced washout) of H^+ (see Fig. 11A). Reperfusion suddenly removes extracellular H^+ and corrects intracellular acidosis (see Fig. 11B), mainly through the the sarcolemmal Na^+-bicarbonate co-transporte and Na^+/H^+ exchanger (NHE1) activity, resulting in intracellular Na^+ accumulation. The latter determines, via the reverse-mode activity of the sarcolemmal Na^+/Ca^{2+} exchanger (NCE), an overload of cytosolic Ca^{2+}.[197,198] The rapid correction of intracellular acidosis is thought to be a fundamental determinant of reperfusion injury, permitting intracellular Na^+ and Ca^{2+} overload[199] and activating systems as the opening of the mPTP and the calpain-mediated proteolysis.[200] The time course of correction of the tissue pH and of the intracellular Ca^{2+} concentration seems to determine between cell death (recovery of pH occurs first) and survival (recovery of Ca^{2+} occurs first).[198]
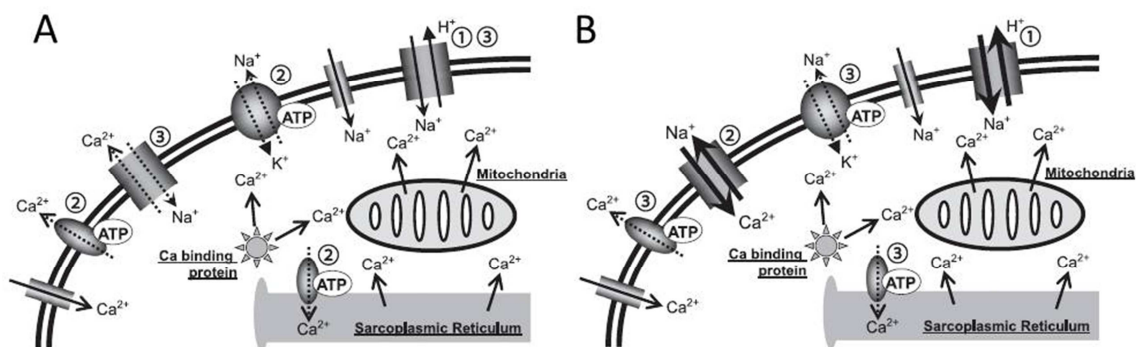
Fig. 11. Ion exchanges during ischemia (A) and reperfusion (B). During ischemia (A): 1) excretion of H^+ due to pH lowering, 2) deactivation due to loss of ATP, and 3) reduction of Na^+/Ca^{2+} exchange due to lowered extracellular pH and intracellular accumulation of Na^+. During reperfusion (B): 1) robust excretion of H^+ due to prompt recovery of extracellular pH, 2) "reverse mode" excretion of accumulated Na^+ and Ca^{2+} influx in turn, and 3) reexcretion of Ca^{2+} followed by recovery of ATP synthesis. Modified from Sanada et al.[201]

Thus, acidosis during early phases of reperfusion is protective, through vary mechanisms: low pH inhibits contractile activity and hypercontracture, reduces gap-junction communication (limiting the spreading of cell death),[4] prevents opening of the mPTP,[200] and the calpain activation with the subsequent calpain-mediated proteolysis.

Heusch first postulated that the maintenance of acidosis is essential for obtaining the effect of PostC.[202] The group of Cohen et al. found, in isolated rabbit heart, that reperfusion with acidic solution were capable of an equivalent protection as PostC, and that protection was strictly related to the inhibition of mPTP opening.[203,204] The pH recovery delay duration is important: acidic infusion for less than 2 minutes was found ineffective, whereas infusion prolongation beyond 3 minutes resulted in progressive loss of benefit.[204] Subsequent studies corroborated this finding, observing a close correlation between delay in pH recovery and the magnitude of myocardial salvage.[205,206]

There are only rare studies about Ca^{2+} homeostasis and handling during postonconditioning process, although its well-known importance in mediating the lethal reperfusion injury. Authors found in isolated cardiomyocytes that hypoxic PostC was able to reduce the cytosolic and mitochondrial Ca^{2+} accumulation.[207] On the contrary, mitochondria isolated from postconditioned rabbit hearts contained more total (free plus bound) Ca^{2+} when compared with nonpostconditioned counterparts.[208] This discrepancy might be partially explained by ionic[207] vs. total[208] mitochondrial calcium measurements.

Nevertheless, PostC may interfere with subsequent Ca^{2+}-dependent calpain-mediated proteolysis. Calpains, ubiquitous cytosolic Ca^{2+}-dependent proteases, act on a large variety of substrates including structural myofibrillar and sarcolemmal proteins.[209] Activation of calpains enhances the processes conducting to sarcolemmal fragility and cell rupture during reperfusion,[209] to detachment of the Na^+ pump,[209] to activation of the pro-apoptotic Bad/Bax pathway and to cleavage of the anti-apoptotic protein Bid.[210]

Recently,the group of Inserte et al.[205] demonstrated that calpain system is an important effector of PostC's cardioprotection (see also Fig. 12).
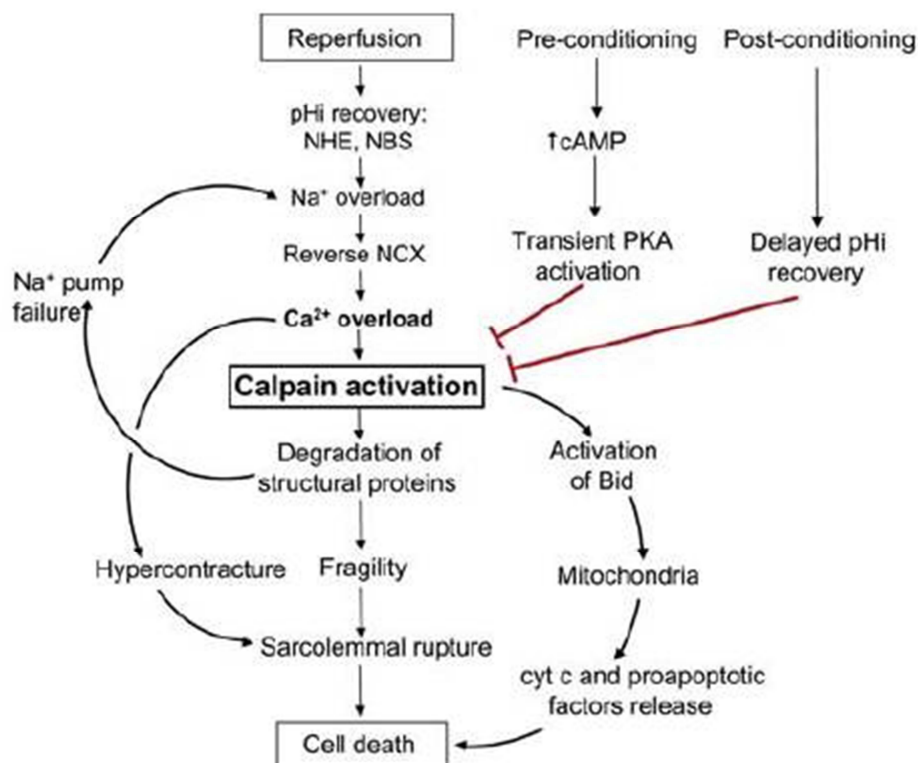
Fig. 12. Schematic diagram showing the proposed mechanisms by which calpain participates in reperfusion induced cell death and in the cardioprotective effects of pre-conditioning and post-conditioning. From Inserte et al.[211]

Calpain activity is strictly dependant on intracellular pH, being maximal at pH close to 7.2 and almost absent at pH of 6.4.[212] During reperfusion, the abrupt correction of intracellular acidosis activates the calpain-mediated proteolysis, as demonstrated by the close correlation in the rat model between the myocardial calpain acitivity during early reperfusion and IS. The finding that transient acidosis at the early phases of reperfusion effectively limit calpain-mediated proteolysis and reperfusion injury suggests that calpain, despite the increased cytosolic Ca^{2+} concentration, is not activated during ischaemia, and that low pH during the ischaemic phase is responsible for this effect. Thus, authors proposed the calpain translocation and activation processes as

possible pharmacological targets, as recently shown in rat hearts submitted to transient coronary occlusion.[213]

2.2.2.5.3. Protein kinase signal transduction

Multiple protein kinase cascades have been identified as involved in the transduction of the PostC signal. One of the most important is the Reperfusion Injury Salvage Kinase (RISK) pathway, first introduced in 2002 by Yellon et al.[214] They demonstrated that the p42/p44 (ERK 1/2) mitogen activated protein kinase (MAPK)-dependent signalling pathway represented a relevant survival mechanism against reperfusion injury, and suggested that the heart possessed prosurvival 'Reperfusion Injury Salvage Kinase' (RISK) pathways. Yellon et al. subsequently demonstrated an IS reduction up to 40-50% through the pharmacological activation (using a wide range of agents, including G-protein coupled receptor agonists and natriuretic peptides, but also pharmacological agents such as statins) of pro-survival kinases, such as PI3 kinase-Akt and ERK1/2, at the immediate onset of myocardial reperfusion.[215] Subsequent experiments confirmed the role for Akt and ERK1/2 in the setting of simulated ischaemia/reperfusion injury in non-diseased animal hearts, in post-infarct remodelling models and in ex-vivo studies using human atrial muscle.[216-219] The Glycogen Synthase Kinase-3β (GSK-3β) is a protein kinase linked to the regulation of multiple cellular functions (i.e. glycogen metabolism, gene expression, and cellular survival) and it is considered by some authors as a component of the RISK pathway or as a specific downstream target of the RISK pathway. GSK-3β inhibition by phosphorylation confers cardioprotection through its potential mitochondrial effects (involving the inhibition of mPTP opening)[220] and the control of mitochondrial adenine nucleotide transport through the outer mitochondrial membrane.[221] However, there is conflicting information regarding the role of GSK-3β, as well as the role of other member of MAPK family like

JNK and p38MAPK, as important mediators of PostC.[222-225] Taken together, these findings suggest that the RISK pathway is fundamental to obtain the PostC induced myocardial protection, even if it is highly probable that additional and independent cardioprotective pathways exist, each one solicitable and available in the adequate setting.[226]

An alternative cardioprotective pathway, termed "Survivor Activating Factor Enhancement" (SAFE), is the JAK-STAT. The SAFE pathway usually conveys different extracellular stress signals from cellular membrane cytokine receptors to the nucleus, where they regulate the transcription of a variety of proteins involved in multiple cellular processes, including those involved in cardioprotection.[227-229] Agents inhibiting the SAFE pathway at the onset of myocardial reperfusion, or its genetic ablation, can abolish the cardioprotective effects of PostC.[230,231]

Both the RISK and the SAFE pathways seem to converge on the mitochondria which seems to be the target for the protection offered by PostC.[232]

Other studies have suggested also a role for sphingosine kinase (SPhK), a protein that formes sphingosine-1-phosphate (S1P), and regulate multiple cell function like mitosis, apoptosis, survival and cytoskeletal rearrangement.[233] SPhK1 knockout mice had larger myocardial infarcts and were resistant to PostC cardioprotection.[234] Moreover, the S1P generated by SPhK in early reperfusion may enhance other components of the RISK pathways via the S1P-receptor.

Currently, the mechanisms involving protein kinases C and G in ischaemic PostC cardioprotection are unclear and limited. Agents non-specific PKC inhibiting were found to abolish the effects of PostC in perfused rat hearts, suggesting a precise role of PKC.[235] Another mediator of cardioprotection seems to be protein kinase G (PKG),[236] but its role in ischaemic PostC induced IS limiting is unclear. Finally,

protection by PostC have been found sensitive to numerous pharmacological agents, including inhibitors of the NO-sGC-cGMP-PKG pathway.[180,237]

In summary, during the early phase of reperfusion ischaemic PostC seems to activate multiple signalling pathways, including protein kinase and/or phosphatase involved in different cell functions. The relative contribute of each signalling pathway for the final cardioprotective effect is still unclear and might be species-, model- and/or PostC protocol-related.

2.2.2.5.4. Role of mitochondria

Mitochondria have assumed a central role in research since they act as both a main target of processes triggered by ischaemia (i.e. intracellular Ca^{2+} overload and ROS formation) and as central site for determining the preservation of cell viability. Strategies aimed at protecting mitochondria against ischaemia/reperfusion lethal injury have focused their attention especially on the mPTP.[238-240]

The mPTP is a voltage- and Ca^{2+}-dependent, high conductance channel expressed in the inner mitochondrial membrane. Under normal physiological conditions, the mitochondrial inner membrane is impermeable to almost all metabolites and ions, and the mPTP is in a closed conformation. Under some stress conditions, the mPTP opens and allows the equilibration of molecules with molecular mass up to 1500 Da.[31,92,241-244] Osmotic force of matrix proteins results in matrix swelling, leading to further rupture of the outer mitochondrial membrane and release in the cytosol of pyridine nucleotides and proapoptotic factors like cytochrome c (see also figure 13). In addition, collapse of the mitochondrial membrane potential ($\Delta_{\Psi m}$) results in the ATP synthase to behave as an ATPase and accelerate energy depletion secondary to the ischemic insult. The mPTP opening is facilitated by binding of Cyclophilin D (Cyp-D), a mitochondrial matrix protein, to the inner mitochondrial membrane in a process

modulated by both Ca^{2+} and inorganic phosphate.[245] It's noteworthy that Cyp-D binding

to the inner mitochondrial membrane is a process inhibited by CsA and also other

molecules interacting with Cyp-D that are usually described as mPTP inhibitors.
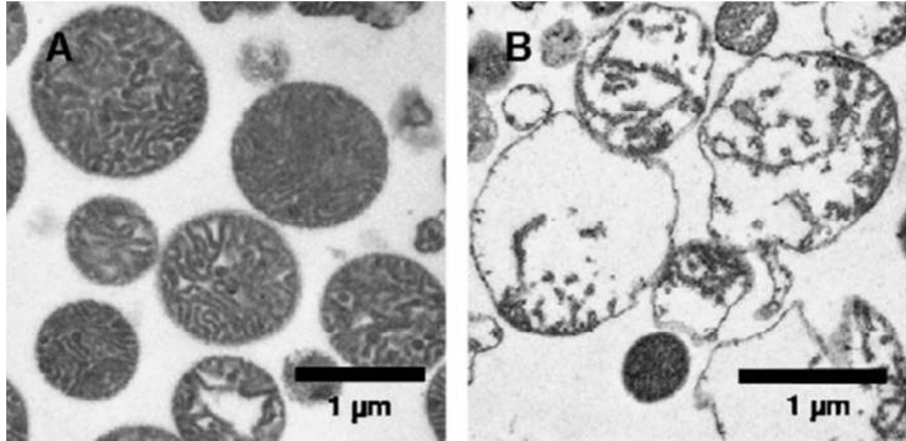
Fig. 13. Isolated mitochondria before and after Ca^{2+}-induced mPTP opening. Electron microscopy confirmed the integrity and purity of isolated mitochondria before Ca^{2+}-induced mPTP opening (A). Following Ca^{2+}-induced mPTP opening, mitochondria appeared swollen with disappearance of membrane integrity (B). Adapted from Argaud et al.[246]

The mPTP opening induce inhibition of electron flow and increased ROS

generation, a process that estabilish a vicious cycle of injury at the onset of reperfusion

since that ROS itself favours the opening of mPTP.[247,248] Opening of the mPTP is

favoured by decreased inner membrane potential, low adenine nucleotides, matrix Ca^{2+}

accumulation, inorganic phosphate, oxidative stress and alkalinization, and inhibited by

elevated values of $\Delta\Psi_m$, adenine nucleotides and matrix cations like H^+, Mg^{2+}, Mn^{2+},

Sr^{2+}.[245]

During ischaemia, intracellular acidosis, together with high levels of Mg^{2+} and

ADP, overrides the mPTP-opening promoting conditions ($\Delta\Psi_m$ decrease, high levels of

Ca^{2+} and of inorganic phosphate). Viceversa, during reperfusion, the abrupt

normalization of pH, along with a burst in ROS generation and the presence of high

mitochondrial concentrations of Ca^{2+} and of inorganic phosphate determinates optimal

conditions for mPTP opening despite the antagonizing effect of $\Delta\Psi_m$.

Protein kinase and phosphatase signalling pathways activated by PostC have been assumed to regulate the probability of mPTP opening. Nevertheless, information regarding the processes occurring between cytosol pathways and mPTP opening modulation in the inner mitochondrial membrane is still limited, but it is thought to be involved the translocation of cytosolic proteins into mitochondria or by phosphorylation/dephosphorylation of critical proteins in the outer mitochondrial membrane.[249]

Moreover, additional processes seems to be involved in mPTP opening modulation. Oxidative stress might be an important modulator of mPTP opening as evidentiated by studies in which the administration of N-acetyl-cystein during the initial 3-minute of reperfusion abolished the PostC protection.[235] This early ROS formation would then stimulate protective mechanisms including the mK_{ATP} channel activation and PKC, as evidentiated by experiments with selective inhibitors.[192,235] In particular, mK_{ATP} channel activation might reduce the susceptibility to mPTP opening, mediating PostC cardioprotective effect.[192,216,235,250]

Taken together, these finding suggest a pivotal role of mitochondria as end effectors of multiple protective pathways. It is highly likely that disparities exist between the processes through which the various conditioning stimuli (PreC, late PreC, PostC) modulate mitochondrial function. Nowadays, the only characterized finding of these possible differences is mitochondrial connexin 43, that is causally involved in ischaemic PreC,[251,161] whereas is not essential for ischaemic PostC.[252]

2.3. Animal models and human studies of cardioprotective strategies

In this paragrraph we discuss the main findings on how PreC and PostC strategies may impact the subsequent development of reperfusion injury. Nevertheless, it is noteworthy that the experimental characterizations of the cardioprotective effects have been mainly observed in healthy juvenile animals or myocardial tissue from such animals.

2.3.1. Classic preconditioning

There are multiple PreC stimuli that can elicite the PreC cardioprotection: ischemia,[103] hypoxia,[253] rapid cardiac pacing,[254,255] thermal stress, and various pharmacological receptor-dependent and independent agents (pharmacological PreC).[115-117] Apart from the original endpoint of IS limitation,[104] in which PreC have been proved as protective in all species tested so far (including humans),[117,256] a lot of other effects of PreC have been investigated. To this regard, ischemic PreC have been found reducing the apoptosis process,[257] ameliorating the LV remodelling and improving functional recovery in rabbits[258] and humans,[259] and protecting against arrhythmias in several species (mice, rats, rabbits, and dogs) but not in pigs.[116]

To note, the PreC protocol (combinations, number and durations of ischemic and reperfusion episodes) used is really important in inducing the cardioprotective effects, with a critical low threshold of ischemia (only 1 or 2 min of ischemia with subsequent reperfusion before the index ischemia has no protective effect)[144,260] required to trigger the adaptive mechanism and a rapid saturation (in dogs 1, 6, or 12 5-min PreC cycles offered similar protection).[261] Withing this "therapeutic" window, ischemic PreC

cardioprotection seems to be a graded phenomenon associated with the intensity of the PreC stimulus: in anesthetized rabbits two cycles of 10 minutes of occlusion of a major epicardial branch of the left coronary artery each followed by 30 minutes of reperfusion before a 45-minute coronary occlusion and 2 hours of reperfusion result in greater IS limitation reduction than a single cycle of PreC.[262] The duration of intermittent reperfusion also is relevant for the ischemic PreC cardioprotective effect, as evidentiated in experimental studies with rats in which protection was still evident when the reperfusion period was shortened to 1 minute, but no protection was evident with reperfusion period of 30 seconds.[263]

Preconditioning of the heart may even be elicited by brief episodes of ischemia and reperfusion in other organs, a phenomenon termed remote ischemic PreC.[264] In anesthetized dogs Przyklenk et al. demonstrated that four cycles of 5-minute occlusion/reperfusion of the left circumflex coronary artery reduced infarct size after 1 hour of sustained left anterior descending coronary artery occlusion and 4.5 hours of reperfusion.[265] In contrast, Nakano et al. found that preconditioning one myocardial zone does not precondition the whole rabbit heart in the Langerdorff model ex vivo.[266] An IS reduction have been evokable by prior occlusion and reperfusion of a mesenteric or renal artery in rats, renal artery occlusion/reperfusion in rabbits, or stenosis of the femoral artery plus electrical stimulation of the gastrocnemius muscle in rabbits.[264]

The mediator(s) of remote PreC effect have still not been elucidated, with evidences involving an unknown humoral substance[264] as well the incolvement of neuronal pathways.[267]

Cheung et al. have evidentiated in children undergoing congenital heart defects reparation that remote ischemic PreC induced by four 5-min cycles of lower limb ischemia/reperfusion using a blood pressure cuff could determine low levels of troponin I (and inotropic stimulation was less required) postoperatively compared with non

precontioned patient group, suggesting a possible cardioprotective effect of remote PreC also in humans.[268] Finally, Loukogeorgakis et al. found that limb ischemia/reperfusion induced remote ischemic PreC influenced flow-mediated arm dilatation. To note, it seems that remote ischemic PreC in humans has two phases of protection against endothelial rerfusion injury: an early (short) and late (prolonged) phase.[269] Recently, the same group evidentiated that remote ischemic precondtioning protects the brain against hypothermic cyrculatory arrest-induced injury, resulting in accelerated recovery of neurological function and suggested that remote ischemic precondtioning might be neuroprotective in patients undergoing surgery with hypothermic cyrculatory arrest and improve long-term outcomes.[270]

2.3.2. Late preconditioning

The late-onset, long-lasting phenomenon of late PreC is less powerful than classic PreC.[165] It have been found protective not only against ischemic injury, but also against other endpoints as myocardial stunning[271,272] and reperfusion-induced arrhythmias.[273] The late PreC protective effects have been estabilished in isolated rat cardiomyocytes[274] and human myocardial atrial tissue.[275]

A lot of substance (adenosine receptor agonists, NO donor compounds, and prostacyclin derivatives) have been found capable of invoking pharmacological late PreC protection against many reperfusion injury endpoints, including reduction of early morphological changes, infarct size, post-ischemic myocardial dysfunction and arrhythmias.[80,167,276,277] To note, the induced protection is time- and dose-dependent, with optimal effects 24 to 48 h after treatment.[278] Nevertheless, the findings of late PreC as a naturally occurring phenomenon in humans is still lacking.

2.3.3. Postconditioning

Postconditioning have been found effective in reducing infarct size in a variety of animal isolated and in vivo models (rat, rabbit, mice, dogs, pigs) and in humans.[7,11-13,180,181,216,217,252,279-299] Nevertheless, the only studies that used gadolinium delayed enhancement-CMR to assess PostC cardioprotective effects showed a modest[15] or lack[6,300] of IS reduction in humans. In preclinical studies the cardioprotective effect on IS reduction achievable by ischemic PostC is similar[11,287,301] to or slightly smaller[281] than that obtained by ischemic PreC. The PostC induced cardioprotection is not influenced by ischemic PreC.[287] Regarding the other reperfusion injury endpoints, PostC in isolated rat hearts reduces cardiomyocyte apoptosis and reperfusion-induced arrhythmias, whereas it does not protect against myocardial stunning in mouse isolated hearts or rabbit and dog hearts in vivo.[179,271,286,303]

Similar to classical PreC and late PreC, the protocol used to induce ischemic PostC is important, with the brief cycles of ischemia/reperfusion that must be applied immediately after the long-lasting ischemic insult.[202,304-307] However, recently Roubille et al. found in an in vivo mouse model of myocardial ischemia/reperfusion injury, that delaying the intervention of postconditioning to 30 minutes does not abrogate the cardioprotective effect of PostC, providing evidences that the time window of protection afforded by postconditioning may be larger than initially reported. Nevertheless, they found a linear correlation between IS and delay of the PostC manouvres, indicating that the cardioprotective effect of delayed PostC was progressively smoothed when the delay interval time increased.[308,309] The cardioprotection evoked is related with the number and duration of PostC ischemia/reperfusione episodes, as evidentiated in experiments with pigs.[288,310] However, as PreC, the effect quickly saturates, as shown by experiments in wich increasing the number of ischemia/reperfusion episodes does not further decrease IS in multiple species.[216,217,281] A more detailed description of clinical

studies regarding the PostC effect in patients affected by STEMI, including a discussion with the results presented in this thesis, is provided in the section 3.3. and in Table 11.

Noteworthy, a "remote postconditioning" phenomenon also exists. Experiments in rat evidentiated that the occlusion and release of the renal artery 1 minute before coronary artery reperfusion provided reduction of final myocardial IS.[311] Similarly, in pig model, Andreka et al. found a 26% reduction in IS by four 5-minute cycles of blood pressure cuff inflation applied to the lower limb immediately after reperfusion.[312] Finally, Loukogeorgakis et al. demonstrated in humans that remote PostC can be induced by transient limb ischemia and is as effective as remote PreC in preventing endothelial ischemia/reperfusion injury as assessed by arm flow-mediated dilation.[313]

Various pharmacological agents can substitues ischemic trigger during the first minutes of reperfusion to limit IS such as some inhalational anesthetics (i.e. isoflurane) applied only during the initial minutes of reperfusion.[285,314,315] In a recent small study of 58 patients Piot et al. demonstrated that administration of an intravenous bolus of 2.5 mg of cyclosporine per kilogram of body weight immediately after PPCI was associated with smaller IS, as assessed by CMR 5 days after infarction.[316]

2.4. Effects of comorbidities and co-treatments on postconditioning strategies

Most experimental studies on cardioprotection have been undertaken in young and healthy animals. However, ischaemic heart disease in humans is a complex disorder caused by or associated with known cardiovascular risk factors including age, smoking, obesity, hyperlipidaemia, diabetes and hypertension, or pre-existing diseases (e.g. heart failure). In addition, patients with coronary artery disease (CAD) vulnerable to myocardial infarction may be on various pharmacological treatments. All these factors may be responsible of the different results obtained in animals and patients (see also Table 2). Many of the signalling pathways described in the previous paragraphs may be influenced by confounders, co-morbidities, and co-treatments. In fact it is known that age reduces the expression of protein kinases and STAT3, atherosclerosis may alter NO/ROS balance, and bradykinin is increased by treatment with ACE inhibitors. Thus, it is relevant to discuss the effects of these entities on PostC cardioprotection.

Hypercholesterolaemia, well known independent risk factor for the development of CAD, is associated with increased severity of myocardial reperfusion injury and it interferes, independently of the development of coronary atherosclerosis, with cardioprotection cellular mechanisms.[118,317] However, informations are still controversial about the effect (and mechanisms) of PostC in hyperlipidaemia. Iliodromitis et al. showed that PostC-induced cardioprotection was abrogated in rabbits with experimental hyperlipidemia and/or atherosclerosis.[318] Similarly, also in isolated hearts of cholesterol-fed rats there was no evidence of PostC-induced IS reduction.[319] A lot of hypothetical mechanisms have been proposed regarding the mechanisms by which hyperlipidemia alters reperfusion injury severity and PostC cardioprotection:

accumulation/redistribution of tissue/membrane cholesterol,[320-322] altered gene expression (i.e. heat shock proteins),[323] decrease in cardiac NO bioavailability due to increased nitrosative stress,[317,324-327] inactivation of matrix metalloproteinases,[328] enhanced apoptotic cell death via the caspase-1 cascade.[329] Moreover, the most frequently used antihyperlipidemic drugs, the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), although showing cardioprotective effects and decreasing cardiovascular mortality in large patient populations,[330] may influence the PostC cellular mechanisms, as evidentiated by studies in which statins attenuate PostC IS limiting effects in rat hearts.[331]

Diabetes mellitus is a wordwide expanding condition.[332] Both type 1 and type 2 diabetic patients are more susceptible to CAD in all its forms, including ST-elevation acute myocardial infarction, and postinfarct complications.[333-336] Little is known regarding the interaction of diabetes with reperfusion injury and PostC cardioprotection. Experiments in both diabetic and obese mice have shown a resistance to PostC induced cardioprotection, probably because of an insufficient activation of the RISK pathway[337,338] or by other mechanisms, including hyperglycemia-induced alterations of oxidative/nitrosative stress[339] or impaired Akt phosphorylation.[304] Consistently with these findings, in pre-diabetic rats with metabolic syndrome, pharmacological posconditioning (with sevoflurane or cyclosporine A) failed to be cardioprotective.[340] Moreover, many antidiabetic drugs may influence reperfusion injury and PostC cardioprotective mechanisms.[341,342] For example, sulfonylureas and glinides enhance insulin secretion through the inhibition of the pancreatic β-cell membrane K_{ATP} channel. This channel is also important in coronary smooth muscle cells (control of coronary blood flow at rest and in hypoxia), in myocardial cells (stress response pathway), and in cardioprotective mechanisms (as evidentiated in the dedicated section 2.2.2.5.).[343]

Hypertension may be another diffuse CAD risk factors able to alterate reperfusion jury and PostC protection mechanisms.[118] Hypertension promotes the development of atherosclerosis in the coronary artery tree[344] and LV hypertrophy may alter coronary vasculature structure (reduced cross-sectional density of endomyocardial capillaries) and function (reduced vasodilatation), regardless the presence of detectable coronary atherosclerosis.[345] Pressure overload LV hypertrophy, even in the early phase, have been associated with structural, metabolical and biochemical changes that may enhance reperfusion injury.[346] Experimental studies on PostC effect in systemic arterial hypertension had conflicting results: in a study of Penna et al. ischemic PostC failed to reduce IS with spontaneously hypertensive rats,[347] but in other studies with rats characterized by pressure overload-induced LV hypertrophy, PostC still reduced IS.

Finally, aging may determines some further alterations in cardiomyocite metabolic and biochemical pathways. Ageing, per se, affects cardiomyocytes at different levels: at DNA level it may be observed mutations and telomere shortening (associated with limited regenerative capacity and increased mortality); at protein expression level the aged myocardium is associated with a shift from fatty acid to carbohydrate metabolism, changes in intracellular matrix and signal transduction component, changes in handling of cellular waste; finally the aged cardiomyocytes have increased oxidative stress and decreased mitochondrial oxidative defense that contribute to greater susceptibility to apoptosis and necrosis.[348] Increased apoptosis and necrosis reduce the number of cardiomyocytes and result in the development of hypertrophy in the remaining cardiomyocytes. All of these changes decrease contractile function, resulting in decreased left ventricular systolic and diastolic function and decreased peak cardiac output.[349] The overall increased oxidative stress in aged cardiomyocites determines protein, lipid, and DNA oxidation, potentially contributing

to contractile impairment,[349] reduced tolerance to ischemic injury,[349] and loss or reduction of PostC cardioprotective properties.[350]

     A summary of the study attempting to reduce lethal reperfusion injury in patients with ST-elevation acute myocardial infarction is provided in Table 1.

Table 1. Previous Attempts (other than postconditioning) to Reduce Reperfusion Injury in Patients with Acute Myocardial Infarction.

Cardioprotective Strategy and Trial	No. of Patients	Period of Ischemia *Hr*	Timing of Intervention	Notes and details of study	Results
Antioxidants					
Flaherty et al.[351]	120	≤4 (92% of pts)	Before PCI	IV bolus of superoxide dismutase (10 mg/kg of body weight) followed by a 60-min inufsion of 0.2 mg/kh/min	No difference in recovery of LVEF 4-6 wks after PCI
Downey (EMIP-FR)[352]	19725	≤6 (83% of pts)	≤15 min after thrombolysis	IV infusion of trimetazidine	No difference in 35-day mortality
Guan et al.[353]	38	4.5	Before PCI	Oral allopurinol	Improved LVEF and less oxidative stress
Tsujita al.[354]	101	3.5	Before PCI	IV edaravone	Reduced IS, less oxidative stress and reperfusion arrhythmias, improved short-term clinical outcomes
Reduction of intracelllar Ca^{2+} overload and Na^+-H^+ exchange inhibitors					
Boden et al.[355]	874	≤6 (85% of pts)	After thrombolysis	Oral diltiazem 36-96 hr after onset of infarct symptoms	No effect on death, nonfatal myocardial infarction, or recurrent ischemia but reduction in nonfatal cardiac events, including myocardial revascularization
Théroux et al.[356]	3439	3	Before PCI	Na^+-H^+ exchange inhibitors cariporide	No effect on IS or clinical outcomes
Zeymer et al.[357]	1389	3	During thrombolysis, before PCI	Na^+-H^+ exchange inhibitors eniporide	No effect on IS or clinical outcomes
Bär et al.[358]	387	3.5	Before PCI	IV MCC-135	No effect on IS or LVEF measured at SPECT at either 7 days or 30 days
Jang et al.[359] (EVOLVE)	500	3.3	Before PCI	IV MCC-135	No effect on IS or clinical outcomes
Antiinflammatory agent					
Baran et al.[360]	394	3.5	Before or during thrombolysis	Anti-CD18 antibody	No effect on IS, coronary blood flow, or ST-segment resolution
Faxon et al.[361]	420	3.8	Before PCI	Anti-CD11 and anti-CD18 antibody	No effect on IS measured on SPECT at 5-9 days and no effect on TIMI flow or clinical events

Tanguay et al.[362]	598	≤6	During thrombolysis	P-selectin antagonist	No effect on IS measured on SPECT or LVEF at 30 days or on ST-segment resolution or clinical outcomes
Mertens et al.[363]	88	≤6	During thrombolysis	P-selectin antagonist	Prematurely discontinued but no effect on myocardial blood flow, LVEF, or ST-segment resolution
Mahaffey et al.[364]	943	2.7	During thrombolysis	Pexelizumab (Alexion) (an anti-C5 complement antibody)	No difference in CK-MB-measured IS or 90-day composite end point of death, cardiac failure, or stroke
Granger et al.[365]	960	3.2	Before PCI	Pexelizumab	No difference in CK-MB-measured IS or 90-day composite end point of death, cardiac failure, or stroke
Armstrong et al.[366]	5745	3.2	Before PCI	Pexelizumab	No difference in 30-day mortality or 90-day composite end point of death or cardiac failure
Adenosine					
Ross et al.[367] and Kloner et al.[368]	2118	3.3	15 min after PCI	IV adenosine	An 11% reduction in IS but no effect on clinical outcomes; however, subgroup analysis revealed improved clinical outcomes in patients receiving adenosine ≤3.3 hr after onset of chest pain
Metabolic modulation (glucose, insuline, and potassium)					
Mehta et al.[369]	20201	3.9	Both before and after reperfusion	IV glucose, insulin, and potassiun given during thrombolysis or PCI	No effect on mortality, cardiac arrest, cardiogenic shock, or reinfarction at 30-days
Beshanky and Selker et al.[370]	15450		Before reperfusion	IV glucose, insulin, and potassiun given in ambulance	No results available
Magnesium					
Woods et al.[371]	2316		During thrombolysis	IV magnesium	Reduced mortality and cardiac failure with magnesium treatment
ISIS-4.[372]	4319		During thrombolysis	IV magnesium	No effect on mortality
Santoro et al.[373]	150	3.3	Before PCI	IV magnesium	No effect on infarct zone wall-motion score or LVEF
Antman et al.[374]	6213	3.8	Before PCI or before or during thrombolysis	IV magnesium	No effect 30-day mortality

Nicorandil					
Ono et al.[375]	58	5.6	Before PCI	IV nicorandil	Improved microcirculation and clinical outcomes in short term
Ishii et al.[376]	360	4.8	Before PCI	IV nicorandil	Improved myocardial reperfusion and fewer deaths and less cardiac failure after 2.4-yr follow-up
Kitakaze et al.[377]	545		Before PCI	IV nicorandil	No effect on mortality, IS, LVEF, or myocardial reperfusion
Therapeutic hypothermia					
Dixon et al.[378]	42	3.5	Before PCI	Endovascular cooling to 34.7 °C for fist 3 hr of reperfusion	Non significant reduxtion in adverse cardiac events and IS
O'Neill.[379]	400	≤6	Before PCI	Endovascular cooling to 34.7 °C for fist 3 hr of reperfusion	No difference in adverse cardiac events and IS, although patients with anterior acute MI sufficiently cooled before PCI may benefit
Ly et al.[380]	12	3	During	Noninvasive surface cooling to 34.5 °C	Safe and feasible
Atrial natriuretic peptide					
Kitakaze et al.[377]	569		Before PCI	IV infusion	Reduced IS by 15%, improved LVEF by 15%, and improved myocardial reperfusion, but no effect on mortality; reduced composite end point of cardiac death and cardiac failure
Protein Kinase C-delta inhibitor (KAI-9803)					
Roe et al.[381]	150		Before PCI	Intracoronary bolus of KAI-9803	Reduced IS and improved ST-segment resolution
Glucagon-like peptide 1					
Nikolaidis et al.[382]	21	6.3	3 hr after PCI	IV glucagon-like peptide 1 given to patients with poor LVEF	Improved LVEF from 29% to 39%
Darbepoetin alfa (a long acting erythropoietin analogue)					
Lipsic et al.[383]	22	3.3	Before PCI	IV bolus of darbepoietin alfa	Mobilized endothelial progenitor cells but no effect on left ventricular function
Atorvastatin					
Patti et al.[384]	171		Before PCI	High-dose atorvastatin administered 12 hr before PCI	Reduced myocardial injury during PCI

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Mitochondrial PTP inhibition					
Piot et al.[316]	58	5 (≤12)	Before PCI	IV bolus of cyclosporine (2.5 mg per kg of body weight)	Reduced IS by 40% (CK-AUC)

PCI stands for Percutaneous Coronary Intervention; LVEF, Left Ventricular Ejection Fraction; IS, infarct size; SPECT, Single-Photon Emission Tomography; TIMI, Thrombolysis In Myocardial Infarction; CK-AUC, Creatinine Kinase-Area Under the Curve. Modified from Yellon et al.[16]

Table 2. Major Differences between Animal Models and Clinical Studies of Patients with STEMI.

Characteristics	Animal models	Clinical studies	Comments
Subjects	Most studies use a homogenous group of healthy, realtively young animals, free of coexisting illness.	Studies use heterogenous, middle-aged patient populations with coexisting illness such as diabetes, hypertensione, and dyslipidemia, all of which may influence cardioprotection	Encourage the use of older animals with coexisting illness such as diabetes, hyperlipidemia, atherosclerosis, and hypertension to ensure cardioprotection.
Medications	In most studies, the animales are receiving no other medication.	Patients may be taking different medications that may influence cardioprotection.	Ensure that patients are not receiving medication that could interfere with cardioprotection.
Period of acute myocardial ischemia	Beneficial effects with cardioprotection are observed after relatively short periods of ishcemia, ranging from 30 to 60 min. The animals are subjected to the same duration and severity of ischemia.	Most patients present with longer periods of ischemia, ranging from 3 to 12 hr. Both the duration and severity of ischemia vary between patients whithin the same study; these factors may influence cardioprotection.	Consider selecting certain patient groups such as those presenting early (<3 hr) after symptoms onset or those with an anterior myocardial infarction. Alternatively, use more clinically relevant animal moders such as human-sized pig subjected to a long period of ischemia.
Reperfusion time	Most studies assess cardioprotection after relatively short periods of reperfusion, ranging from 120 min to 3 days.	Much longer periods of reperfusion occur in patients, permitting time for the effects of infarct healing and left ventricular remodelling	Encourage the use of a longer period of reperfusion in studies in animals.
Infarction model	In most studies acute coronary occlusion is mechanically indeuced in healthy coronary arteries.	An acute myocardial infarction is an acute inflammatory condition. In most patients with this condition, acute coronary occlusion is due to thrombus formation at a site of a ruptured coronary atherosclerotic plaque.	Consider using more clinically relevant animal models such as animals with atherosclerotic hearts.
Intervention	Many of the intervention administered at the time of myocardial reperfusion have not shown conclusive cardioprotection.	If interventions have not shown conclusive cardioprotection in experimental studies, they are also unlikely to be cardioprotective in the clinical setting.	In the clinical setting, use only interventions rigorously shown in experimental studies to be conclusively cardioprotective.
Timing of intervention	The timing of intervention relatively to the period of ischemia and the onset of myocardial reperfusion is similar in all animals.	The timing of the intervention relatively to the period of ischemia and the onset of myocardial reperfusion varies between patients. The timing of the intervention should be guided by the studies in animals.	Consider selecting certain patient groups, such as those presenting after a specific time. In clinical studies, ensure that the intervention is administered before myocardial reperfusion.
Infarct size	Varies from 30% to 60% of the total volume of myocardium at risk, providing a greater scope for cardioprotection.	Infarct size of 13% to 16% expressed as a percentage of left ventricular colume (using SPECT) appear to be the normal range, which may limit the scope for cardioprotection.	Encourage the use of more accurate measurement of infarct size using delayed-enhancement cardiac magnetic resonance imaging, which can express infarct size as a apercentage of the ischemic risk area.
End-points for cardioprotection	Most studies use recovery of left ventricular function or myocardial infarct size as the measured end points.	The clinically relevant end points are outcomes such as short-term and long-term effects on illness and death.	Consider more robust end points in studies in animals, such as long-term effects on left ventricular function and death.

From Yellon et al.[16]

3. THE POSTCONDITIONING DURING CORONARY ANGIOPLASTY IN ACUTE MYOCARDIAL INFARCTION (POST-AMI) POST-AMI TRIAL

3.1. Design and Methodologies of the POST-AMI Trial

The prognosis of STEMI has significantly improved but it still represents a major cause of mortality and morbidity in industrialized countries.[1] Although reperfusion remains the definitive treatment for ischemic myocardium, restoring blood flow to myocardium carries the potential to exacerbate the ischemia-related injury (i.e. reperfusion injury). Experimental studies have demonstrated that the extent and amount of the reperfusion injury is related to both ischemic time and duration of reperfusion.[385] Despite major therapeutic advances in STEMI treatment, adjunctive therapy to reduce reperfusion injury and, ultimately, IS are lacking in humans.

Early strategies to attenuate reperfusion injury applied concepts derived from cardiac surgery and from the observation that reperfusion damage could be modified by

slowing the initiation of reflow.[386] This "gentle" or "ramped" reperfusion reduced the IS, restored the post-ischemic contractile function, reduced the edema in the risk area, and avoided the blood flow defects characterized as "no reflow" response.[386]

Postconditioning (PostC) strategy could modify reperfusion-induced adverse events. It derived from the simple application of preconditioning (PreC), by moving the "preconditioning stimulus" at the beginning of reperfusion. Whereas PreC is triggered by brief episodes of ischemia-reperfusion occurring before a prolonged coronary artery occlusion, PostC is a comparable sequence of reversible ischemia-reperfusion that is applied after the prolonged ischemic insult. According to experimental data, the protection of myocardium by PostC is as potent as that provided by PreC.[7]

Unlike PreC, the experimental design of PostC allows direct application to clinical settings, especially during Primary Percutaneous Coronary Intervention (PPCI).[7] Inflation and deflation of the balloon after reopening the coronary artery can mimic repetitive coronary artery clamping in postconditioned animal models. As reported by Staat et al.,[7] a simple procedure that any interventional cardiologist can apply, could reduce the enzymatic IS by 36%, a value closed to that reported in the preconditioned human models by Ottani et al. and Kloner and al.[387,388]

In this trial, we intended to evaluate the usefulness of PostC in limiting IS and microvascular damage during the early and late phases after STEMI.

3.1.1. Study objectives

The primary objective was to evaluate whether PostC induced by brief episodes of ischemia–reperfusion performed during the first minutes of reperfusion obtained by PPCI, compared to PPCI without additional intervention, reduces IS estimated by CMR at 30±10 days after the index STEMI. The initial hypothesis was that PostC would reduce the IS by at least 25 %.

Secondary objectives included investigating whether PostC improves microvascular obstruction observed at CMR, ST-segment elevation resolution, persistent ST-segment elevation, angiographic myocardial blush grade <2 and non sustained/sustained ventricular tachycardia in the 48 hours following PPCI. Further secondary end-points were enzymatic IS reduction, left ventricular (LV) remodeling and LV function at CMR performed at 6±1 months, and the reduction of major adverse cardiac events at 30 days and 6 months.

3.1.2. Study design

This was a single center, prospective, randomized, open label, controlled trial. Patient recruitment continued until 78 patients with STEMI were randomized. Patients were randomly assigned, after having been stratified by STEMI location, to PostC arm (PPCI and stenting followed by brief episodes of ischemia–reperfusion performed during the first minutes of reperfusion) or non-PostC arm (PPCI and stenting without additional intervention). The study was been approved by the local institutional review board. The study took place at the Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, a tertiary care centre with high experience in PPCI and with 24-hour access to emergency cardiac surgery.

3.1.3. Patient selection and randomization

Patients with STEMI admitted or referred to the Coronary Care Unit (CCU) of the Division of Cardiology of Padua, after successful PPCI and eligible for CMR were enrolled prospectively. Informed consent was obtained from each patient before enrollment in the study, according to the approved protocol.

3.1.3.1. Inclusion and exclusion criteria

Inclusion criteria for the study were: a) clinical evidence of myocardial infarction defined by the presence of ischemic chest pain lasting more than 30 minutes, with a time interval from the onset of symptoms less than 6 hours before hospital admission, associated with typical ST-segment elevation (≥ 0.1 mV in two or more contiguous leads) on the 12-lead ECG; b) angiographic-detected culprit lesion with stenosis diameter $\geq 70\%$ and TIMI flow grade ≤ 1.

Exclusion criteria were: previous STEMI, or previous myocardial revascularization, previous heart valve replacement, previous heart transplant. Other exclusion criteria were: cardiogenic shock or persistent hypotension (systolic blood pressure <100 mmHg), rescue angioplasty after thrombolytic therapy, evidence of coronary collaterals (Rentrop grade>0) in the risk area, advanced atrioventricular block, significant bradycardia, absence of sinus rhythm, inability to lay flat (due to severe cardiac heart failure/respiratory insufficiency), history or clinical evidence of bronchospastic lung disease, pregnancy, known existence of a life-threatening disease with a life expectancy <6 months and inability to give informed consent. In addition, exclusion criteria were any contraindication to undergo CMR, such as implanted metallic objects (cardiac pacemakers and/or implantable cardioverter defibrillator, implanted insulin pumps or any other type of electronic devices, cerebral clips, aneurysm clips) or any other contraindication to CMR (such as claustrophobia). Patients with newly placed intracoronary stents were included.

3.1.3.2. Randomization

After informed consent, eligible patients were randomized 1:1, after being stratified by STEMI location, to a strategy of PostC after stenting of the infarct-related artery or to standard PPCI with stenting of the infarct-related artery without additional procedure. Randomization was performed in the catheterization laboratory before coronary angiography by a computer-generated random sequence. Randomization outcome was recorded in a dedicated Case Report Form.

3.1.4. Treatment

3.1.4.1. Medication

Before PPCI, the patient was treated by the following medical therapy:

- aspirin (300 mg bolus if not already taken, followed by 160 mg/die);

- intravenous heparin 70 mg/Kg (maximum 4000 U)

- clopidogrel (300 or 600 mg loading dose, followed by 75 mg/die)

- glycoprotein IIb/IIIa inhibitor (abciximab was administered intravenously before PPCI in all patients).

Additional standard treatment consisted in nitroglycerin intravenously for correct evaluation of vessel size before stenting. Standard therapies after PPCI included β-blockers, lipid-lowering agents, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, according to current international guidelines.[389]

3.1.4.2. Postconditioning protocol

In the control group no additional intervention was performed during the first 8 minutes. In the PostCvgroup, the angioplasty balloon, positioned into the implanted stent, was re-inflated 4 times for 1 minute with low pressure (6 atm) inflations, each separated by 1 minute of reflow, within 1 minute from the reflow after direct stenting. This sequence of 4 short episodes of ischemia-reperfusion resembles the experience of Staat P. et al.[7] At minute 8, coronary angiography was performed in both groups to assess coronary patency and to estimate the myocardial perfusion by Myocardial Blush Grade (MBG). The PPCI procedure was then completed according to the physician judgment with respect to patient clinical status.

3.1.5. Clinical data and definitions

3.1.5.1. Baseline demographic and clinical characteristics

Baseline characteristics included age, sex, time from the symptom onset, time of admission, history of coronary artery bypass grafting, previous percutaneous coronary intervention, stroke and myocardial infarction, positive family history for cardiovascular diseases, existence of diabetes mellitus, hypertension, smoking status, heart rate, systolic and diastolic blood pressure, weight, height, and the findings of cardiac and pulmonary auscultation. Major Adverse Cardiac Events (MACE) were defined as the combination of death, re-infarction, re-hospitalization for heart failure or repeat revascularization and were recorded at 30 days and at 6 months. Follow-up information were obtained from hospital records as well as by telephone interviews. An independent

clinical events committee**,** whose members were unaware of treatment assignments**,** adjudicated all the events.

3.1.5.2. Coronary angiography

Coronary angiograms were obtained before and after PPCI procedure. TIMI flow grades were estimated, as previously described.[9] The evaluation of MBG was performed as described by van' t Hof et al.: 0=no myocardial blush; 1=minimal myocardial blush or contrast density; 2=moderate myocardial blush or contrast density, but less than that obtained during angiography of a counter lateral or ipsilateral non–infarct-related coronary artery; and 3=normal myocardial blush or contrast density, comparable with that obtained during angiography of a counter lateral or ipsilateral non–infarct-related coronary artery.[10] Persisting myocardial blush ("staining")**,** suggesting leakage of contrast medium into the extravascular space**,** was graded 0. Distal embolization was defined as new circumscribed filling defects and/or abrupt cut-off of the vessel distally to the target lesion. TIMI flow grade, the MBG and distal embolization was evaluated before and after the PPCI procedure. Coronary angiograms were analyzed by two interventional cardiologists on spliced films, blinded to the type of procedure. Angioplasty of the target lesion were performed and intraprocedural drugs and devices were used as clinically indicated.

3.1.5.3. Electrocardiography

Standard 12-lead ECGs were acquired at the time of presentation, at 30-60 minutes, and after 3-6-9-12 hours from the end of the procedure. Mean time interval

between pre- and post-intervention were registered. The magnitude of ST-segment elevation were measured 60 milliseconds from the J point. ST-segment score was calculated as the sum of ST-segment elevation >0.1 mV in leads V_1-V_6 and I, aVL in anterior infarction and in leads I, II, III, aVF, V_5, and V_6 in non-anterior infarction. ST-segments elevation of the first post-intervention ECG was compared to those of the ECG at presentation. The percentage ST-segment elevation resolution was categorized as complete (>70%), partial (30%-70%), or absent (<30%).[390] Furthermore, analysis of persistent ST-segment elevation at 24 and 48 hours after the procedure were performed. Two observers blinded to study randomization and angiographic findings analyzed all the ECG recordings.

3.1.5.4. Cardiac Magnetic Resonance

After successful PPCI, patients were be scheduled for CMR including contrast-enhancement scanning at 30±10 days after the STEMI and 6±1 months later.

3.1.5.4.1. CMR acquisition protocol.

Cardiac magnetic resonance was performed on a 1.5-T clinical scanner Achieva (Philips Medical System, Best, the Netherlands) using a phased-array cardiac receiver coil. Baseline scan was scheduled at 30±10 days after the AMI, period in which IS has been demonstrated to be more stable,[391] and 6±1 months later. Electrocardiogram-gated breath-hold cine imaging was performed to determine left ventricular function, using a segmented steady-state free-precession pulse sequence (TrueFISP) in multiple short-axis views every 8 mm by encompassing the left ventricle from base to apex; vertical and horizontal long-axis views were also acquired. Typical in-plane resolution was 1.6 x 1.9 mm2, with slice thickness 8 mm; TE=1.75 ms, TR =3.5 ms, flip angle 60°, matrix

= 256 x 256, slice thickness 8 mm, gap 2 mm. We evaluated also the risk area (identified with myocardial edema) applying a breath-hold, black-blood, T2-weighted triple inversion recovery sequence (TR 2 x R-to-R interval; TE= 65 ms; TI = 140 ms) in 3 (basal, midventricular, and apical) short-axis slices (slice thickness 15 mm; gap 5 mm; field of view 34 to 38 cm; matrix 256x256; 1 = number of excitations).[392] Rest first-pass myocardial perfusion was performed during administration of a gadolinium-based contrast agent (Multihance, 0.05 mmol/kg, Bracco, Milano, Italia) at a rate of 4.0 ml/s, using a single-shot saturation recovery gradient-echo pulse sequence. Three short-axis slices were obtained per heartbeat, every 10 mm, covering the infarct area as seen during cine imaging (90° pre-pulse, TR/TE/FA 2,5ms/1,3ms/20°, slice thickness 10 mm, matrix 128x256, NEX 1). Immediately after first-pass perfusion, an additional 0.1 mmol/kg gadolinium-based contrast agent was administered (cumulative dose 0.2 mmol/kg). Late Gadolinium Enhancement (LGE) images were acquired 10 to 15 min after the second contrast administration,[393] using a 2-dimensional segmented inversion recovery gradient-echo pulse sequence, with slice position identical to the cine images, including long axis views. Sequence parameters were as follows: TR = 450 ms, TE = 1.31 ms, flip angle = 15°, slice thickness 8 mm, gap 2 mm. The inversion time was set to null the signal of viable myocardium and typically ranged from 250 to 300 ms.

3.1.5.4.2. CMR analysis.

All CMR data were analyzed on a off-line dedicated workstation using dedicated software (Philips Medical System, Best, the Netherlands). Cine, first-pass perfusion, and LGE images acquired during the same imaging session were matched by using slice position. Registration of follow-up to baseline cine and LGE images were achieved by consensus of 2 observers using anatomic landmarks, such as papillary muscles and right ventricular insertion sites. On all short-axis cine slices, the endocardial and epicardial

borders were outlined manually on end-diastolic and end-systolic images. Left ventricular ejection fraction, end-diastolic and end-systolic volumes, and left ventricle mass were calculated from the short axis views. Each short axis were divided in 12 equiangular segments, starting at the posterior septal insertion of the right ventricle.[394] First-pass perfusion was evaluated qualitatively. Microvascular Obstruction (MO) was identified and quantified by the presence of an hypoenhancement region, with decreased signal intensity, both from first pass and delayed post-contrast images.[395,396] Infarct size was evaluated as region of hyperintensity on LGE sequences. Finally, both MO and IS were quantified by manually drawing short-axis slices. The MO was included in the infarcted area. Both MO and IS were expressed in grams (assuming 1.05 g/ml as the specific gravity of the myocardium) and as percentage of left ventricle mass. The same drawing and expression were used for myocardial edema (risk area) on T2-weighted images. For analysis of segmental function and transmural extent of infarction, the 2 most basal and 2 most distal slices were excluded, because segmental evaluation at these levels is not considered to be reliable due to the left ventricular outflow tract and partial volume effect respectively. All CMR studies were supervised by 1 operator, and all images were analyzed by 2 experienced observers blinded to the patient data.

3.1.5.5. Laboratory data

After angioplasty procedure and opening of the coronary artery, blood samples were taken at baseline (in the catheterization laboratory, after diagnostic coronary angiogram), every 6 hours during day 1 and day 2, every 12 hours during day 3, once a day from day 4 to day 8. Three different tubes were filled in at each blood sample: a tube with lithium heparine, a tube with EDTA and a tube without additives.

Cardiac Troponin I (cTnI) was measured by means of the most recent third-generation cTnI assay (Dimension RxL, Siemens Diagnostic) which shows no cross

reactivity with skeletal TnI. This assay is a "sandwich" immunometric assays, being 99th percentile of the cTnI level in a reference population 0.07 µg/L and the 10% CV cut-off 0.15 µg/L. Peak as well as 72 or 96 hours post diagnosis values of cTnI were used as surrogated markers of IS. Moreover, additional markers (such as C-Reactive Protein or CD40 Ligand, in order to evaluate the contribution of inflammation; and TNF-alfa or E-selectins or Asymetric DiMethyl Arginine, in order to evaluate the microvascular damage) or others were measured in order to clarify pathophysiology of PostC.

3.1.6. End point assessment

3.1.6.1. Primary end point

The primary end point was the reduction of IS, estimated by CMR, at 30±10 days after the STEMI. The IS in the control group was expected to be 15% ± 5%. We hypothesized at least a 25% reduction of the IS in the experimental group compared to controls.

3.1.6.2. Secondary end points

The secondary end points were the impact of PostC on: 1) incidence of severe MO at CMR; 2) extent of ST-segment elevation resolution; 3) incidence of persistent ST-segment elevation; 4) incidence of MBG <2 at angiography; 5) incidence of non sustained/sustained ventricular tachycardia in the 48 hours following PPCI at 24-hour ECG monitoring; 6) enzymatic IS as measured by cardiac enzymatic markers (cTnI) considering the Area Under the Curve (AUC); 7) left ventricular remodeling[397] and left ventricular function at CMR performed at 6±1 months; 8) reduction of MACE at 30 days and 6 months.

3.1.7. Statistical analysis

3.1.7.1. Statistical methods

Primary analyses were performed according to the intention-to-treat principle for the whole population. An analysis per protocol was also performed. The results were summarized by treatment group reporting the mean and standard deviation or the median, range and interquartile range for the quantitative variables, the count and the percentage in each category for the categorical variables.

Differences between groups were assessed by the 2-tailed Wilcoxon rank sum test in case of quantitative variables, by the χ^2 or Fisher exact test for proportions. A conventional general linear model procedure, non parametric ANCOVA, was used to assess the difference between groups in relation to IS, assessed at contrast enhanced CMR. The Cox regression was used to evaluate differences in MACE between the two treatment groups. Statistical significance was considered as a 2-tailed $p < 0.05$. All the analysis were conducted with SAS 9.1.3, SAS Institute Inc., Cary, NC, USA.

3.1.7.2. Calculation of sample size

Sample size for the primary end point was based on an expected IS of 15% in the control group and assuming a 25% reduction in the experimental group with an equal standard deviation of 5%. Assuming that IS was not normally distributed and using the Wilcoxon rank sum test, 33 patients per group were required to detect the 5% absolute reduction with 2-sided 5% alpha level and 80% power (nQuery Advisor 6.01). Assuming a drop-out rate of 15%, the total calculated sample size consisted of 78 patients randomized equally in the two groups.

3.1.8. Study records

Clinical data were prospectively collected by research nurses or by the physician involved in the study. Independent study monitors employed by University of Padua verified 100% of the data in the Case Report Forms (CRF) provided for data recording. Case report forms were numbered and were used in ascending numerical order. All data were recorded in a dedicated database. The investigators ensured that patient anonymity was maintened. On CRFs or other documents, patients were not be identifiable by their names but by the CRF code. Log of patient codes, names, and addresses were kept separately. The data for all patients with primary end-point events were reviewed by an independent adjudication committee blinded to the treatment assignments. Events were adjudicated separately by 2 members, and in case of disagreement, the opinion of a third member was obtained in order to take a final decision by consensus. The committee was also responsible for the adjudication of all clinical events according to the Academic Research Consortium.[398]

3.2. Results

3.2.1. Study group and treatment

From April 2007 to July 2009, 453 patients with STEMI were admitted to our centre and screened for eligibility. As shown in Fig. 14, 78 patients were randomized and stratified by STEMI location (42% anterior). Seventy-five subjects completed the study protocol for primary analysis. Baseline clinical and angiographic characteristics with the exception of diabetes (p=0.056), were well balanced between groups, as shown in Table 3 and 4.
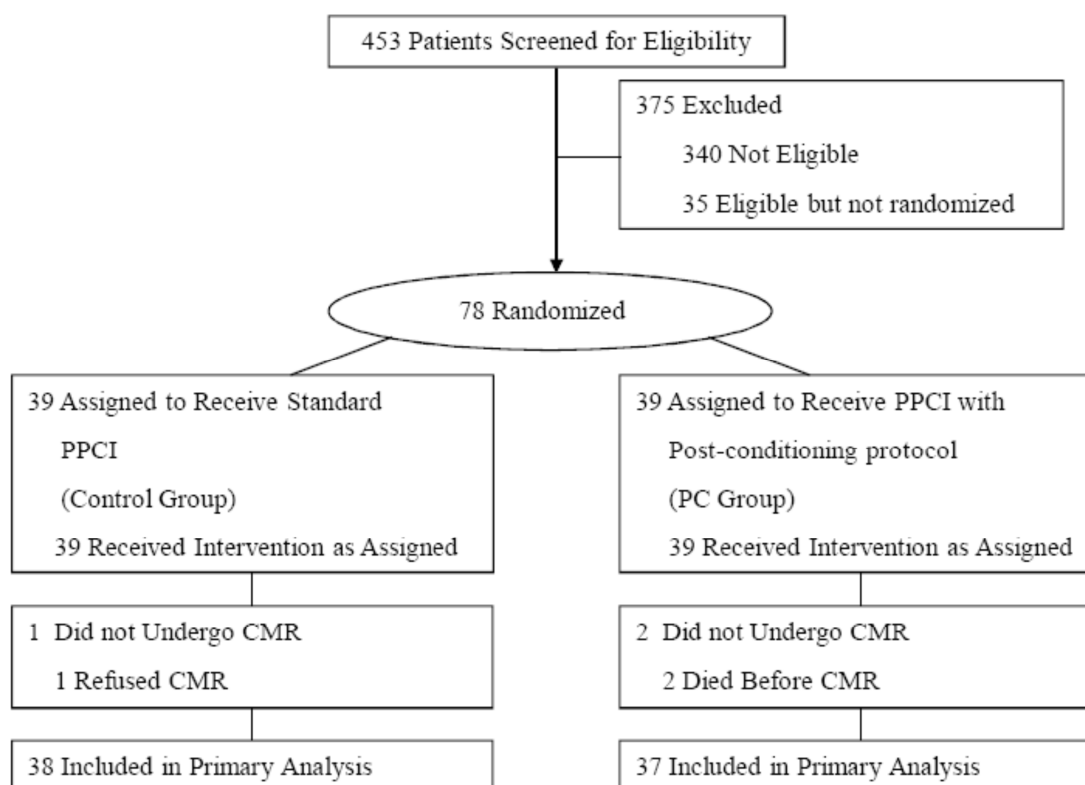
Fig. 14. Patient flow chart. PPCI indicates Primary Percutaneous Coronary Intervention; PC, PostConditioning; CMR, Cardiac Magnetic Resonance.

Table 3. Baseline Clinical Characteristics. Risk factors and former comorbidities.

Characteristics	Post-C (n=39)	Control (n=39)	P
Age, years	59.6±12.7	59.55±12.0	0.992
Male	33 (84.6)	33 (84.6)	1.000
Caucasian race	38 (97.4)	38 (97.4)	1.000
Family history of CAD	19 (48.7)	15 (38.5)	0.361
Hypercholesterolemia	20 (51.3)	19 (48.7)	1.000
Diabetes mellitus	7 (17.9)	1 (2.6)	0.056
Insulin treated	2 (5.1)	0 (0)	
Non-insulin treated	5 (12.8)	1 (2.6)	
Smoker	26 (66.7)	30 (76.9)	0.314
Former smoker	3 (7.7)	9 (23.1)	
Current smoker	23 (59.0)	21 (53.8)	
Hypertension	23 (59.0)	19 (48.7)	0.364
Body Mass Index, kg/m^2	26.8±3.3	27.5±3.1	0.263
Height, meters	1.72±0.10	1.73±0.09	0.699
Weight, kg	79.5±13.7	82.7±12.3	0.329
Former unstable angina	16 (41.0)	12 (30.8)	0.345
Former stable angina	0 (0)	1 (2.6)	1.000
Former CVA	3 (7.7)	2 (5.1)	1.000
Former PCI	1 (2.6)	0 (0)	1.000
Former CABG	0 (0)	0 (0)	-
History of CHF	1 (2.6)	1 (2.6)	1.000
History of peripheral vascular disease	1 (2.6)	0 (0)	1.000

Data are presented as n (%) or mean±SD unless otherwise indicated. CAD indicates Coronary Artery Disease; CVA, Cerebro-Vascular Accident; PCI, Percutaneous Coronary Interventions; CHF, Congestive Heart Failure.

Table 4. Baseline Clinical and Angiographic Characteristics.

Characteristics	PostC (n=39)	Control (n=39)	*P*
Interval Times, minutes			
Symptoms'onset-Hospital admission	116.82±81.44	109.31±74.04	0.791
Arrival in Cath Lab	61.85±32.46	58.87±43.46	0.261
Sheat positioning	15.72±12.82	12.44±9.26	0.403
1st angiography	7.49±9.33	5.49±5.51	0.474
Balloon	9.95±6.68	8.13±3.53	0.345
Total ischemic time	211.82±84.67	194.23±79.87	0.274
AMI location			
Anterior	16 (41.0)	17 (43.6)	0.819
Lateral	9 (23.1)	11 (28.2)	0.604
Inferior	22 (56.4)	21 (53.9)	0.820
Systolic Blood Pressure, mmHg	139.9±23.1	138.0±27.6	0.637
Diastolic Blood Pressure, mmHg	89.0±14.2	86.4±15.0	0.378
Heart Rate, bpm	76.49±15.03	76.64±18.87	0.920
Killip class			0.329
1	35 (89.7)	32 (82.1)	
2	4 (10.3)	7 (17.9)	
TIMI Risk Score	2.7±1.6	2.7±1.7	0.945
TIMI Risk Index	20.6±9.5	20.4±8.5	0.996
IRA			0.389
Left Descending Artery	16 (41.0)	17 (43.6)	
Left Circumflex Artery	7 (17.9)	3 (7.7)	
Right Coronary Artery	16 (41.0)	19 (48.7)	

Data are presented as n (%) or mean±SD unless otherwise indicated. AMI indicates Acute Myocardial Infarction; TIMI, Thrombolysis In Myocardial Infarction; IRA, Infarct Related Artery.

Table 5. Therapy at home, at admission and at discharge.

Characteristics	PostC (n=39)	Control (n=39)	*P*
Treatment at home, before admission			
Aspirin	3 (7.7)	6 (15.4)	0.481
Clopidogrel	0 (0)	0 (0)	-
Beta-blockers	4 (10.3)	3 (7.7)	1.000
Calcium-channel-blockers	3 (7.7)	3 (7.7)	1.000
ACE-inhibitors	4 (10.3)	3 (7.7)	1.000
AT II Receptor Antagonists	2 (5.1)	4 (10.3)	0.675
Vasodilators	1 (2.6)	1 (2.6)	1.000
Long-acting nitrates	0 (0)	1 (2.6)	1.000
Short-acting nitrates	0 (0)	0 (0)	-
Diuretics	3 (7.7)	3 (7.7)	1.000
Lipid-lowering agents	3 (7.7)	1 (2.6)	0.615
Digitalis or derivates	0 (0)	0 (0)	-
Anti-arrhythmic agent	1 (2.6)	0 (0)	1.000
Treatment at admission			
Aspirin	38 (97.4)	38 (97.4)	1.000
Clopidogrel	38 (97.4)	39 (100)	1.000
Beta-blockers	13 (33.3)	8 (20.5)	0.202
Calcium-channel-blockers	3 (7.7)	4 (10.3)	1.000
ACE-inhibitors	9 (23.1)	3 (7.7)	0.597
AT II Receptor Antagonists	2 (5.1)	4 (10.3)	0.675
Vasodilators	1 (2.6)	1 (2.6)	1.000
Long-acting nitrates	0 (0)	1 (2.6)	1.000
Short-acting nitrates	15 (38.5)	16 (41.0)	0.817

Diuretics	4 (10.3)	5 (12.8)	1.000
Lipid-lowering agents	6 (15.4)	3 (7.7)	0.481
Digitalis or derivates	0 (0)	0 (0)	-
Anti-arrhythmic agents	1 (2.6)	6 (15.4)	0.108
Inotropic agents	0 (0)	4 (10.3)	0.115
IIb/IIIa Rec Blockers	38 (97.4)	39 (100)	1.000
Treatment at discharge[a]			
Aspirin	37 (97.4)	39 (100)	0.494
Clopidogrel	38 (100)	39 (100)	-
Beta-blockers	34 (89.5)	34 (87.2)	1.000
Calcium-channel-blockers	0 (0)	1 (2.6)	1.000
ACE-inhibitors	33 (86.8)	29 (74.4)	0.167
AT II Receptor Antagonists	0 (0)	1 (2.6)	1.000
Vasodilators	0 (0)	0 (0)	-
Long-acting nitrates	1 (2.6)	0 (0)	0.494
Short-acting nitrates	0 (0)	0 (0)	-
Diuretics	6 (15.8)	8 (20.5)	0.591
Lipid-lowering agents	38 (100)	39 (100)	-
Digitalis or derivates	0 (0)	0 (0)	-
Anti-arrhythmic agents	1 (2.6)	4 (10.3)	0.358

Data are presented as n (%). ACE indicates Angiotensin-Converting Enzyme; AT II, Angiotensin II. a In the postconditioning group one patient died before discharge, therefore percentages in this group refer to 38 patients.

Table 6. Procedural Characteristics.

Characteristics	PostC (n=39)	Control (n=39)	*P*
Nb. of treated vessel during index PPCI			0.615
1	36 (92.3)	38 (97.4)	
2	3 (7.7)	1 (2.6)	
Stent type			1.000
Bare-Metal Stent	38 (97.4)	38 (97.4)	
Bare-Metal and Drug-Eluting Stents	1 (2.6)	0 (0)	
Drug-Eluting Stent	0 (0)	1 (2.6)	
IRA - Total nb. stent	1.72±0.94	1.44±0.55	0.113
Post-procedural TIMI 3 flow	38 (97.4)	38 (97.4)	1.000
Post-procedural MBG			0.352
0/1	17 (43.6)	13 (33.3)	
2/3	22 (56.4)	26 (66.7)	
LVEF, %	58.74±11.82	58.82±9.41	0.992
LVEDP, mmHg	20.53±7.38	21.57±6.75	0.427

Data are presented as n (%) or mean±SD unless otherwise indicated. PPCI indicates Primary Percutaneous Coronary Intervention; IRA, Infarct Related Artery; TIMI, Thrombolysis In Myocardial Infarction; MBG, Myocardial Blush Grade; LVEF, Left Ventricle Ejection Fraction; LVEDP, Left Ventricle End-Diastolic Pressure.

Table 7. Quantitative Coronary Analysis.

Characteristics	PostC (n=39)	Control (n=39)	*P*
Pre-Procedural			
Proximal RVD, mm	3.11±0.52	3.09±0.45	0.810
Distal RVD, mm	2.95±0.51	2.96±0.39	0.963
MLD, mm	0.94±0.24	0.91±0.44	0.614
Lesion Length, mm	24.72±13.33	23.03±10.00	0.745
Diameter Stenosis, %	66.90±10.49	70.57±15.27	0.149
Area Stenosis, %	82.13±14.41	81.40±19.95	0.215
Post-Procedural			
RVD, mm	3.15±0.37	3.25±0.30	0.347
MLD, mm	2.71±0.48	2.77±0.31	0.450
Lesion Length, mm	9.95±10.95	9.17±8.03	0.910
Diameter Stenosis, %	14.46±7.35	14.36±5.72	0.940
Area Stenosis, %	18.17±11.83	19.83±12.71	0.822

Data are presented as mean±SD. RVD indicates Reference Vessel Diameter; MLD, Minimal Lumen Diameter.

Table 8. Blood analysis.

Characteristics	PostC (n=39)	Control (n=39)	*P*
Haemoglobin, g/L	140.14±11.58	136.72±13.30	0.355
Haematocrit, %	41.59±3.28	40.82±3.45	0.387
White Blood Cells, x 10^9/L	11.11±3.19	11.05±3.52	0.909
Platelet, n	214.94±43.06	197.46±45.00	0.219
K^+, mEq/L	3.89±0.39	3.97±0.37	0.401
Na^+, mEq, L	138.97±2.65	139.05±2.74	0.952
BUN, mg/dL	5.85±2.01	7.20±4.73	0.091
Creatinine, µmol/L	76.67±16.52	99.69±108.74	0.096
Glucose, mmol/L	6.83±2.10	6.83±1.82	0.960
Total bilirubin, mmol/L	13.86±8.08	15.58±13.23	0.857
ALP, U/L	71.95±23.56	68.89±22.16	0.899
ALT, U/L	55.77±32.51	52.87±28.95	0.916
AST, U/L	294.87±272.747	227.54±193.92	0.337
ESV, mm/h	20.79±14.77	20.30±13.05	0.962
CRP,	13.34±21.03	11.77±11.17	0.259
Total-cholesterol, mmol/L	5.22±0.88	4.94±0.96	0.269
LDL-cholesterol, mmol/L	3.55±0.92	3.36±0.88	0.387
HDL-cholesterol, mmol/L	1.22±0.41	1.13±0.26	0.436
Triglycerides, mmol/L	1.47±0.86	1.54±1.03	0.920
Lp (a)	390.61±420.40	296.56±424.82	0.255

Data are presented as mean±SD. BUN indicates Blood Urea Nitrogen; ALP, Alkaline Phosphatase; ALT, Alanine Transaminase; AST, Aspartate Transaminase; ESR, Erithrosedimentation Rate; CRP, C-Reactive Protein; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; Lp(a), lipoprotein(a).

Table 9. Variables at first Transthoracic Echocardiography.

Characteristics	PostC (n=39)	Control (n=39)	*P*
LV Ejection Fraction, %	49.4±7.4	49.9±7.0	0.869
LV End-Diastolic Volume Index, ml/m^2	60.33±9.66	60.69±12.07	0.830
LV End-Systolic Volume Index, ml/m^2	30.45±8.65	31.05±9.90	0.869
LV End-Diastolic Diameter, mm	50.54±5.26	51.69±4.80	0.273
LV End-Systolic Diameter, mm	34.58±6.48	34.08±5.57	0.985
LV Inferior Wall Thickness, mm	11.95±1.29	12.00±0.93	0.963
LV Interventricular septum, mm	12.16±1.44	12.14±1.05	0.987
LV Mass Index, g/m^2	88.56±29.93	81.82±22.40	0.490
E velocity, cm/s	62.79±11.26	64.82±14.56	0.668
A velocity, cm/s	70.88±16.45	70.36±20.84	0.947
E/A ratio	0.90±0.21	0.95±0.41	0.945
TEI index	0.51±0.35	0.47±0.13	0.956
Deceleration Time, ms	176.91±32.09	207.50±54.64	0.051
WMSI	1.73±0.31	1.70±0.33	0.622
IVRT, ms	83.44±20.87	71.56±17.86	0.108
Mitral Regurgitation			0.156
0+/4	23 (59.0)	22 (56.4)	
1+/4	12 (30.8)	17 (43.6)	
1-2+/4	1 (2.6)	0 (0)	
2+/4	3 (7.7)	0 (0)	
Admission-TTE interval time, hours	32.24±34.15	24.99±18.20	0.529

Data are presented as n (%) or mean±SD. LV indicates Left Ventricle; WMSI, Wall Motion Score Index; IVRT, Iso-Volumetric Relaxation time; TEE, Transthoracic Echocardiography.

There were no differences between groups with regard to medical therapy, at home, administered in emergency room and at discharge (see Table 5). Procedural characteristics are reported in Table 6. TIMI flow grade was comparable between the two treatment groups. Quantitative coronary analysis is reported in Table 7. The AUC (arbitrary units) and peak value of serum TnI release during the first 8 days of reperfusion was similar between groups, averaging 3697.4±2968.3 in PostC group vs 3183.8±2182.4 in control group (p=0.734). Peak Troponin I in PostC group was 112.40±97.78 µg/dL, compared to 89.18±66.02 µg/dL in control group (p=0.469). Detailed blood analysis are reported in Table 8. Transthoracic echocardiogram was performed at 28.6±27.4 hours after hospital admission. All examined variables were not different between the two groups and with a mean LVEF of 49.4±7.4 in PostC and 49.9±7.0 in controls (see Table 9).

3.2.2. Infarct size and other secondary end-points

Infarct size assessed at first CMR (28.0±16.4 days after index PPCI) and calculated as % of LV mass (20.2±11.9 vs 14.3±9.9) or grams (19.19±11.30 vs. 13.57±9.46) trended to be higher in PostC group compared to controls (*p*=0.056, for both, see Table 10 and Fig. 15). There was no evidence of benefit of PostC in different subgroups of STEMI patients (Fig. 16). Also the MO trended to be more frequent in PostC group (13.5% vs 2.6%, *p*=0.200). Electrocardiographic (i.e. ST-segment resolution) and angiographic (i.e. MBG<2) indexes of perfusion were comparable between groups (Table 10). No differences were observed regarding the incidence of non sustained/sustained ventricular tachycardia in the 48 hours following PPCI at 24-hour ECG monitoring (*p*=0.314). At CMR performed at 6±1 months, LV remodelling and LV function were not statistically different (Table 10).

The event-free survival curves are shown in Fig. 17. In the PostC group there was 1 sudden cardiac death after 16 days from index STEMI, 2 patients underwent repeat revascularization (at 1 and 3 months), 2 patients were re-hospitalized because of acute heart failure (at 2 and 4.2 months). In the control group 1 patient died because of cerebrovascular event. After exclusion of diabetic patients, MACE rate trended to be higher in PostC group when compared to controls (16.7% vs 2.6%, p=0.080).
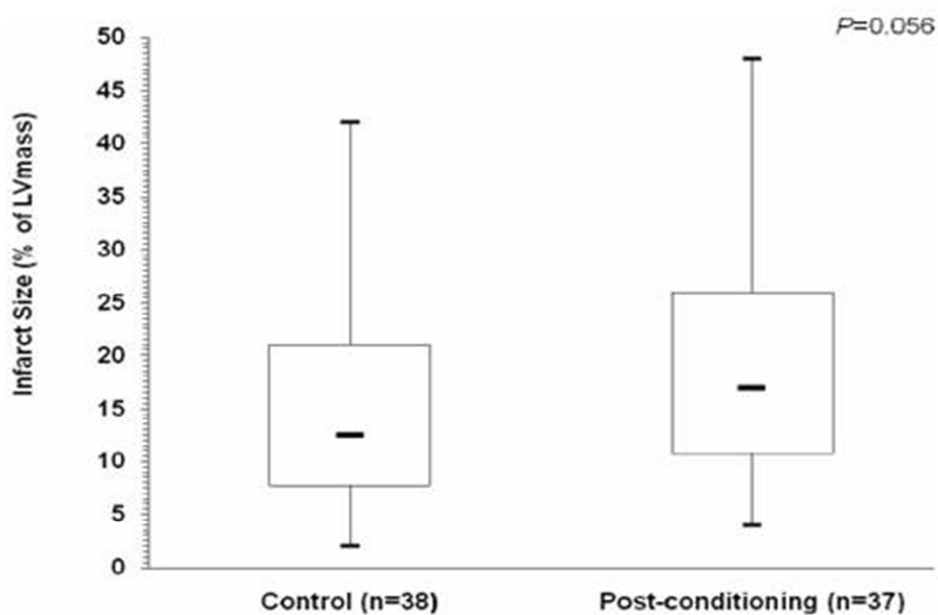

Figure 15. Box-plot of infarct size, expressed as % of left ventricle mass at LGE CMR. Infarct size did not differ significantly between patients in the two groups but trended to be higher in PostC group vs. control group.

Table 10. Primary and Secondary End Points

Endpoint	PostC (n=39)	Control (n=39)	*P*
Primary endpoint			
Infarct size on LGE CMR, % of LV mass	20.2±11.9	14.3±9.9	0.056
Infarct size on LGE CMR, grams	19.2±11.3	13.6±9.5	0.056
Time to CMR, days	29.1±17.1	27.1±16.1	0.659
Secondary endpoints			
ST-segment resolution			1.000
Complete (≥70%)	22 (56.4)	21 (53.8)	
Partial (30%≤x<70%)	13 (33.3)	13 (33.3)	
Absent (<30%)	4 (10.3)	5 (12.8)	
NSVT/SVT	26 (66.7)	30 (76.9)	0.314
Angiographic MBG<2	17 (43.6)	13 (33.3)	0.352
MO, on CMR	5 (13.5)[a]	1 (2.6)[b]	0.200
LV remodelling (Delta EDV≥20%)	10 (31.3)[a]	8 (23.5)[b]	0.482
F.Up-Delta EDV, ml/m^2	11.6±23.3[a]	8.8±20.2[b]	0.672
F.Up-Delta LVEF, %	2.8±7.0[a]	3.4±6.8[b]	0.537
Troponin I, AUC	3697.4±2968.3	3183.8±2182.4	0.734
Peak Troponin I, μg/dL	112.4±97.8	89.2±66.0	0.469
MACE at 30 days	3 (8.2)	0 (0.0)	0.111
MACE at 6 months	6 (16.2)	1 (2.6)	0.053
MACE at 6 months (without diabetics)	5 (16.7)	1 (2.6)	0.080

Data are presented as n (%) or mean±SD unless otherwise indicated. LV indicates Left Ventricle; CMR, Cardiac Magnetic Resonance; LGE, Late Gadolinium Enhancement; NSVT/SVT, Non-Sustained Ventricular Tachicardia/Sustained Ventricular Tachicardia in the 48 hours following PA; MBG, Myocardial Blush Grade; MVO, Microvascular Obstruction; EDV, End-Diastolic Volume; LVEF, Left Ventricle Ejection Fraction; AUC, Area Under Curve; MACE, Major Adverse Cardiac Events. a In the postconditioning group 2 patients died before 1st CMR, therefore data refer to 37 patients. b In the control group 1 patient refused 1st CMR, thus data refer to 38 patients.

Infarct Size (% of LV)
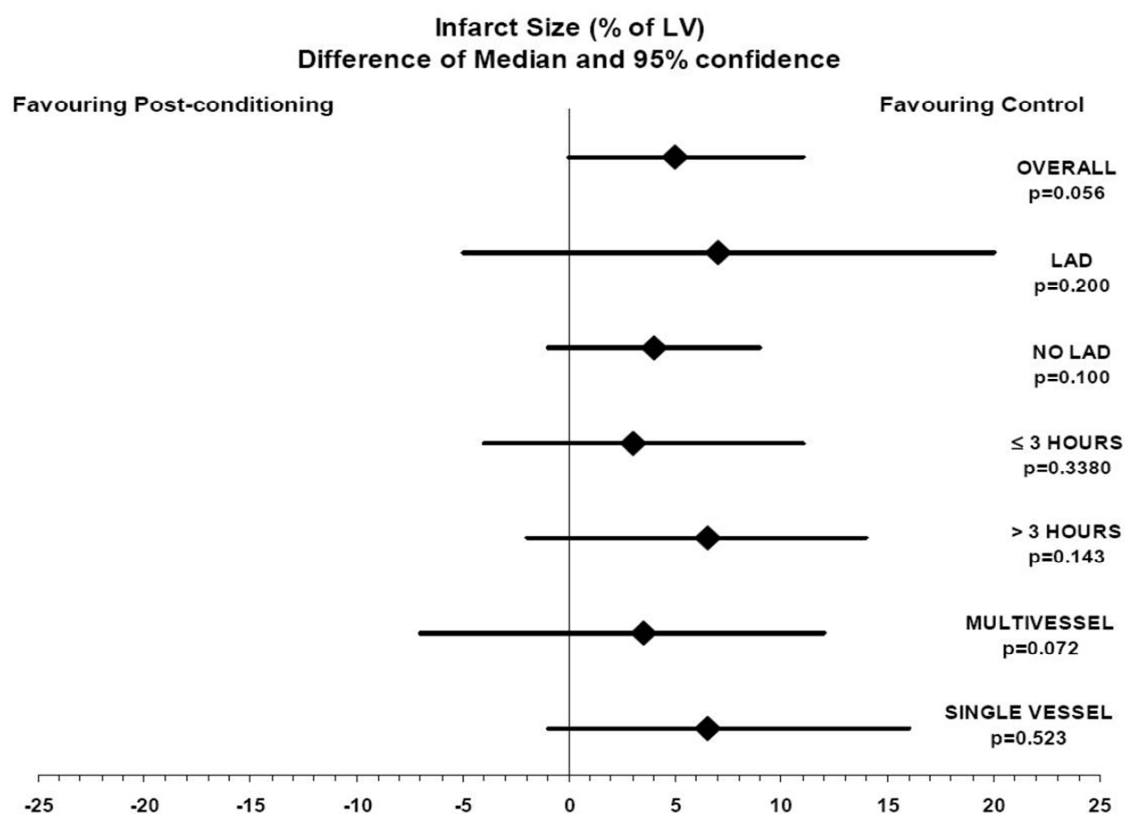**Difference of Median and 95% confidence**

Figure 16. Infarct size, expressed as % of left ventricle mass at DE-CMR, analyzed in different subgroup; see text for description. LV indicates left ventricle; LAD, left descending artery; MVD, multivessel disease; SVD, single vessel disease.
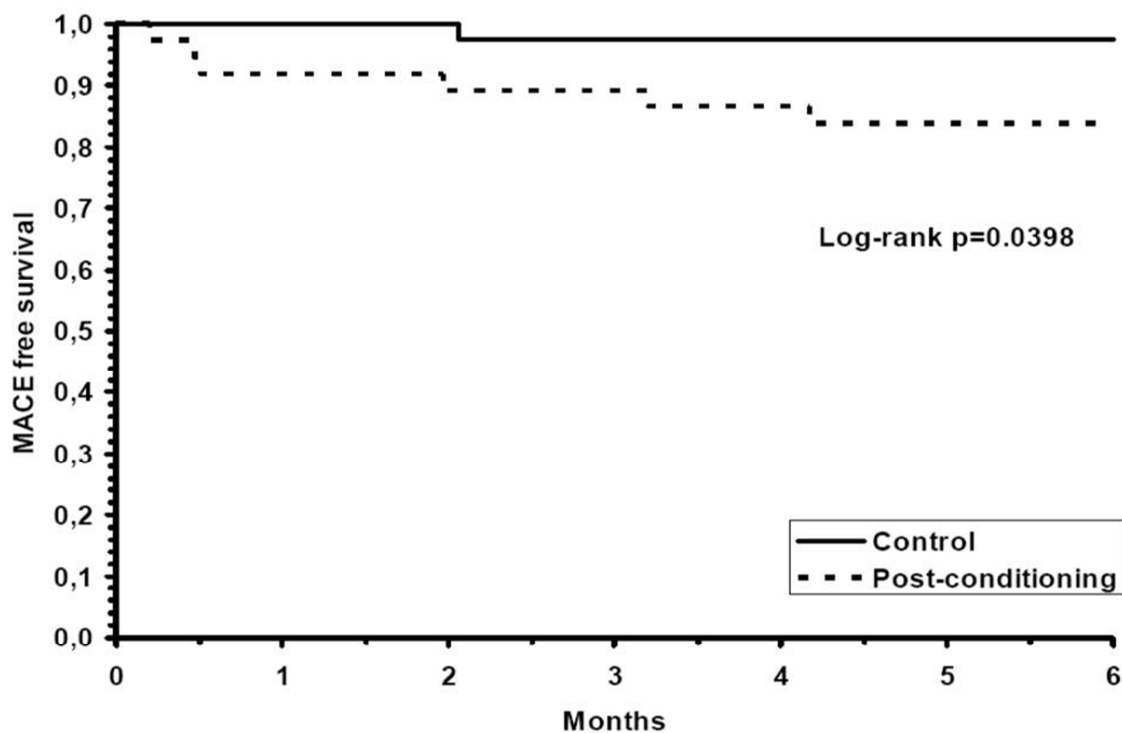

Figure 17. Kaplan-Meier MACE free survival curves. Post-conditioning group patients had a higher incidence of events at follow-up.

3.3. Discussion

This prospective randomized study evaluating the effect of PostC on IS in STEMI patients treated by PPCI with direct stenting and intravenous abciximab administration showed that IS assessed by CMR did not significantly differ but trended to be larger in PostC group compared to controls both in overall population and subgroups analyses. Although not statistically different, also the MO rate at CMR trended to be more frequent in PostC group compared to controls. Moreover, other indices exploring the effect of PostC on myocardial and microvascular injury, left ventricular function and outcome (secondary end-points) failed to show any benefit and were consistent with the primary end-point result. These observations are somehow in contrast with earlier clinical experiences looking at the impact of PostC on IS (See also Table 11 and Section 2.3.3).[6,7,12-15,295-297,300] In small studies of less than 30 patients PostC during PPCI seem to protect the human heart during STEMI,[7,12] showing a 36% IS reduction as determined by cardiac biomarkers and improved coronary flow reserve and ST resolution. Similarly, Yang et al. reported a 27% reduction in IS in 41 STEMI patients treated by PPCI plus PostC as assesses by SPECT.[297] Finally, Thibault et al. confirmed and extended these preliminary results showing a stable favorable effect of PostC on the final IS assessed by SPECT at 6 months after the initial treatment.[295] However, SPECT is a relatively gross measurement of IS compared to CMR, which has proven to be superior to SPECT with regard to detection and quantification of MI.[399] Moreover, all the previous studies did not stratify the randomization for STEMI location nor related IS to the myocardial area at risk. These adjustments, although not definitive, might be important to reduce bias in particular when studying small population.[400]

Table 11. Postconditioning randomized control trials.

RCT	N. of patients (PC/C)	Inclusion criteria	Coll.Circ. to IRA	Abciximab	PostC protocol	IS Assessment Method	Other EP evaluated	Δ (PC-C) ischemic time (min)	IS RRR	Other EPs
Laskey et al.[12]	10/7	STEMI; ≤12 h	-	-	90 s x 2	CK (peak)	ST segment shift; Distal coronary velocity	+55	CK (peak): -4%	Improved ST segment resolution in PC †
Staat et al.[7]	14/16	STEMI; ≤6 h	-	NA	60 s x 4	CK-AUC (72 h)	CK (peak); MBG	-13	CK-AUC (72 h): 36%†; CK (peak): 35%*	Better MBG *
Ma et al.[13]	47/47	1st STEMI; ≤12 h	+	NA	30 s x 3	CK, CK-MB (peak)	CTFC; MDA; endothelial function; WMSI before and 8 wks after PPCI	-31	CK (peak): 27%; CK-MB (peak): 32%	Faster CFTC; Better ΔWMSI *; Lower MDA-reactive products *; Improved endothelial-dependant vasodilation *
Yang et al.[297]	23/18	1st STEMI; ≤12 h	-	-	30 s x 3	SPECT at 1 week; CK-AUC (72 h)	ST shift at 2 h ECG; TTE at day 1 and 7	-48	1w-SPECT: 27%*; CK-AUC (72 h): 27%*	Similar ST-segment resolution, TIMI 3, MBG, LVEF, cardiac events in both groups
Thibault et al.[295]	17/21	1st STEMI; ≤6 h	-	NA	60 s x 4	CK-AUC and TnI-AUC (72 h); SPECT at 6 mos	LV-EDV, LVEF, WMSI, LV strain rate at 1-year TTE	-14	6m-SPECT: 39%*; CK-AUC: 40%†; TnI-AUC: 47%*	Improved LVEF (Δ 7%) *; WMSI (Δ -0.2) *; Strain rate in AAR (Δ 0.6, †).
Laskey et al.[296]	12/12	1st STEMI; ≤6 h	-	+	90 s x 2	CK (peak)	ST-segment elevation resolution; CFVR	+6	CK (peak): 18%*	Improved ST segment resolution in PC *; better CFVR in PC † ; similar MBG 2/3 (p=0.6) and in-H LVEF.
Lømborg et al.[15]	43/43	1st STEMI; ≤12 h	+	+	30 s x 4	DE-CMR at 3 mos	LVEF; TnT; clinical events (NYHA class; angina pectoris CCS class; MI, TVR or Non-TVR; D) at 3 mos.	-14	3m-CMR-IS: % of LV mass: 18%*, % of total AAR: 19%†	Similar LVEF (p=0.60);better NYHA class *; similar CCS class (p=0.36) and rates of MI (p=0.31), TVR (p=0.32), CABG (p=0.32), D (p=0.15)

Sörensson et al. [6]	38/38	1st STEMI; ≤6 h	+	+	60 s x 4	DE-CMR at 6-9 days	LVEF; TnT-AUC and CK-MB-AUC (48 h); TIMI flow after PPCI	-20	6-9d-CMR-IS: % of AAR: -7%. CK-AUC: -7%; TnT-AUC: -12%	Similar LVEF (*p*=NS) and TIMI 3 flow after PPCI (*p*=1.0)
Garcia et al.[299]	22/21	1st STEMI; ≤12 h	-	- (UFH or bivalirudin)	30 s x 4	CK and CK-MB (peak)	MPG; LVEF; Long-term clinical event (D/re-H d/t HF)	+6	CK (peak): 11%	Better LVEF in PC (Δ 9%) * amd MBG (PC 2.5±0.5 vs C 2.1±0.6) *; similar re-H d/t HP (PC 2/22 vs C 4/21, *p*=0.2) and D rates (PC 1/22 vs C 0/21, *p*=0.9)
Freixa et al.[300]	39/40	1st STEMI; ≤12 h	-	+	60 s x 4	DE-CMR within 1st week	IS at 6m-DE-CMR; LVEF at 1 week and 6 mos; MBG; biomarkers (peak); ST-segment resolution at 90 min; MO.	-4	1w-CMR-IS: IS (% of LV mass): -24% Myocardial salvage index (% of AAR): -38.8%	Similar IS (% of LV mass) (PC 21.8 vs C 18.7, p=NS) and LVEF (47.5% vs 50.3%, p=NS) at 6m-DE-CMR; Similar MBG (PC 58% vs C 53%, p=NS) Higher peak of TnI (PC 299±72 vs C 148±24, p=0.05); similar CK and CK-MB peak; Similar ECG resolution

PC indicates postconditioning; C, control; IRA, infarct related artery; IS, infarct size; EP, Endpoint; RRR, relative risk reduction; STEMI, ST-elevation myocardial infarction; CK, creatinine phosphokinase; NA, not available; AUC, area under the curve; MBG, myocardial blush grade; MDA, malondialdehyde; WMSI, wall motion score index; PPCI, primary percutaneous coronary intervention; CFTF, corrected TIMI frame count; SPECT, single photon emission tomography; TTE, Trans-thoracic Echocardiogram; LV-EDV, left ventricle ejection fraction; LVEF, left ventricle ejection fraction; AAR, area at risk; CFVR, coronary flow velocity reserve; DE-CMR, delayed-enhancement cardiac magnetic resonance; TnT, Troponin T; NYHA, New York Heart Association; CCS, Canadian Cardiovascular Society, MI, myocardial infarction; TVR, target vessel revascularization; D, death; IS, infarct size; CABG, coronary artery bypass graft; UFH, unfractionated heparin; re-H d/t HF, rehospitalization due to heart failure; MO, microvascular obstruction. * p <0.05; † p <0.01.

Our data seem also to differ from the apparently more positive results of PostC on IS assessed by CMR, reported by Lønborg et al.[15] However, the absolute IS by CMR (3 months post STEMI, manual delineation) in their study (the primary end point) did not differ significantly between groups and the potential benefit in IS reduction was inferred only after use of the infarct endocardial surface area to estimate the myocardial area at risk, in order to estimate myocardial salvage. Although the authors found a good correlation between this parameter and CMR edema imaging, these data remain unpublished. Moreover, even T2-weighted CMR for assessment of post STEMI myocardial edema is subject to a number of technical limitations[401] such as the variable temporal course of resolution of myocardial edema and/or the possible effect of PostC on edema. In a third randomized trial on PostC for STEMI, Sörensson et al.[6] did not find significant differences between control and PostC groups in IS by CMR performed 6-9 days post STEMI. In this case the IS was quantified by automatic CMR algorithm and related to the myocardial area at risk determined by left ventriculography. Finally, more recently Freixa et al.[300] found that PostC during PPCI not only did not reduce IS at both early and late follow-up but also might have a potential harmful effect. Thus, collectively, the infarct data from the randomized trials of PostC for STEMI do not appear to differ greatly. Moreover, although clinical benefit associated with PostC was suggested by NYHA class status after 3 months, in the Lønborg et al.[15] study, there was no significant difference in CCS anginal status. Overall MACE rates in the Lønborg et al.[15] study did not differ, but the only two deaths occurred in the PostC group. This clinical data are also somewhat consistent with the two deaths occurred in the PostC group in our study.

It should be acknowledged, however, that in all these studies, inclusion/exclusion criteria differed substantially: time from symptom onset >6 hours;[12,13,15,297,300] presence of collateral flow to infarct zone;[6,13,15] thrombolytic therapy

was not always an exclusion criteria[6,7,13,15,296,299,300] and abciximab was used at discretion of the operator or was an exclusion criteria.[6,7,12,13,15,295-297,299,300] Moreover, PostC protocol was different across the studies, being 90 s x 2 (duration of balloon inflation x number of inflations),[12,296] 30 s x 3,[13,297] 30 s x 4[15,299] and 60 s x 4[6,7,295,300] in other studies. On this regard, original observations suggested that brief cycles of reperfusion/reocclusion are recommended in small animal models, while longer periods (60 s) may be more effective in larger species.[298] Thus, also in agreement with the first human experience showing a significant effect on IS by PostC[7] we preferred a PostC protocol of 60 s x 4. Infarct size evaluation also was different among studies, both in term of method of assessment and of timing adopted for measurements: during the first 72 hours,[7,12,13,296,299] at 1 week,[6,297,300] at 3 months[15] or at 6 months.[295] To reduce possible confounders, we enrolled STEMI patients with symptoms onset of less than 6 hours, with occluded infarct related artery, and absence of collaterals. Moreover, we stratified the randomization by STEMI location and calculated absolute final IS on LGE images at 30±10 days and also at 6±1 months, in order to avoid confounding relations with myocardium at risk (edema) on T2 weighted images. The 1 month interval has been proven to be more reliable in terms of accuracy on IS assessment than during the acute phase.[402] By doing so, we avoided not only the major contribution of edema to final IS quantification but also minimized dropouts of patients and kept the ability to detect small MI, considering, as already pointed out, that the quantification of myocardial edema is not yet as robust as IS quantification.[401,403] For this reason we evaluated the area at risk on T2 weighted images (edema) at 30±10 days only to identify any potential episodes of myocardial ischemia after the index STEMI.

To note, all the baseline characteristics resulted well balanced between our study groups except for the diabetic status that trended to be more frequent in PostC group. Nevertheless, after exclusion of diabetics, IS still trended to be larger in PostC group

compared to controls. Different to previous studies where abciximab use was not specified or utilized at discretion of the operator (ranging from 0 to 79%) all our patients received intravenous abciximab during PPCI. It is possible that abciximab administration has influenced our results and explains partially some dissimilarities with previous studies. No differences in peak and AUC cTnI, used as surrogate of IS, were observed between the two groups. The 30-day and at 6-month MACE rates were not significantly different with a trend towards a worse outcome in PostC patients, irrespective to the presence of diabetes.

3.3.1. Study limitations

The observed standard deviation for the primary endpoint assessment was higher than expected, thus the power of our study resulted of 73% instead of 80%. The unfavorable trend of PostC towards worse outcomes compared to controls might result significant in a larger cohort.

Although the interventionalist cardiologists were not blinded to the treatment protocol assigned, the investigators evaluating the primary and secondary end points were unaware of the assigned study group. Thrombectomy was not performed in our study, due to the fact that the importance of thrombus aspiration during PPCI in STEMI patients was acknowledged after both the trial design approval and the beginning of the study. However, since a potential concern with PostC protocol might lie in the absence of thrombectomy as well as in the potential for periods of stasis to increase platelet activation, aggregation, and/or microembolization we used abciximab in all patients. Thus, although we cannot theoretically exclude microembolization during PostC, the PPCI efficacy was high in both groups (97.4% post-procedural TIMI 3 flow). Finally, although thrombectomy could be performed prior to PostC, the few minutes required for its performance during early reperfusion might reduces its potential efficacy.

The CMR at 30±10 days may have underestimated IS and MO rate; however, at this time point IS have been demonstrated to be more stable and the protocol included a second CMR at 6±1 months, in order to confirm the first CMR results. Area at risk was not evaluated because we preferred to stratify the randomization for STEMI location. On the other hand, the emerging role of CMR quantification of myocardial salvage remains to be established, moreover it is unknown if cardioprotective strategies specifically targeting edema would improve outcomes.

An important limitation, however, is the extreme complexity of the numerous physiological and biochemical mechanisms responsible for the beneficial effects of PostC in experimental models.[404]. Extrapolation of experimental studies to the clinical setting is further complicated by the fact that the optimal window for coupling of PostC to beneficial responses may be substantially shorter than the mean duration of ischemia in typical STEMI patients. In addition, underlying microvascular disease, which is common in STEMI patients (e.g., those with diabetes or left ventricle hypertrophy), may blunt PostC responses.[405] Microvascular injury associated with prolonged periods of ischemia might also be included in this category. The results of our trial call attention to the need to better understand the mechanisms of PostC and the potential conditions under which it may benefit STEMI patients, as well as to potential adverse effects of the treatment.

4. Conclusion and perspectives

This prospective randomized study evaluating the effect of PostC in STEMI patients showed that PostC did not have any cardioprotective effect and might even harm patients treated by PPCI with direct stenting and intravenous abciximab administration.

The precise multifaceted signaling pathways involved in PostC, including autacoids triggers, protein kinases, end-effectors, need to be further clarified especially evaluating older, clinical relevant (i.e. pigs) animal models with coexisting illness (such as diabetes mellitus, hyperlipidemia, hypertension, atherosclerosis) to confirm that cardioprotection is still possible in these settings. The relevance of confounding factors, such as CAD risk factors and drug treatment, on reperfusion injury and PostC-induced pathways need to be better defined.

From a clinical point of view, it would be relevant to define the optimal protective protocol (delay after which the first re-occlusion should be estabilished, duration and number of each reocclusion, duration of the interspersed reperfusion) for a given duration of the index ischemia. Moreover, it could be necessary to consider the enrolling of only some specific patients groups, such as those with short symptoms'onset-hospital admission time. Taken together, these findings suggest that future larger, multicenter, controlled randomized trials and patient level metanalysis will be necessary to better clarify the effect of ischemic PostC on IS and on clinical end points.

5. Acknowledgements

6. References

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