

## Update: Acute Coronary Syndromes (IV)

## Protection Against Myocardial Ischemia-reperfusion Injury in Clinical Practice



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## ABSTRACT

Even when reperfusion therapy is applied as early as possible, survival and quality of life are compromised in a considerable number of patients with ST-segment elevation acute myocardial infarction. Some cell death following transient coronary occlusion occurs during reperfusion, due to poor handling of calcium in the sarcoplasmic reticulum-mitochondria system, calpain activation, oxidative stress, and mitochondrial failure, all promoted by rapid normalization of intracellular pH. Various clinical trials have shown that infarct size can be limited by nonpharmacological strategies—such as ischemic postconditioning and remote ischemic conditioning—or by drugs—such as cyclosporine, insulin, glucagon-like peptide-1 agonists, beta-blockers, or stimulation of cyclic guanosine monophosphate synthesis. However, some clinical studies have yielded negative results, largely due to a lack of consistent preclinical data or a poor design, especially delayed administration. Large-scale clinical trials are therefore necessary, particularly those with primary clinical variables and combined therapies that consider age, sex, and comorbidities, to convert protection against reperfusion injury into a standard treatment for patients with ST-segment elevation acute myocardial infarction.

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## Protección contra el daño miocárdico por isquemia-reperusión en la práctica clínica

## RESUMEN

Pese a recibir lo más tempranamente posible la terapia de reperusión, un amplio número de los pacientes que sufren infarto agudo de miocardio con elevación del segmento ST tienen infartos que comprometen su supervivencia y su calidad de vida. Parte de la muerte celular secundaria a una oclusión coronaria transitoria ocurre durante la reperusión, por mal manejo del calcio en el sistema retículo sarcoplasmático-mitochondria, activación de calpains, estrés oxidativo y fallo mitochondrial, favorecidos por la rápida normalización del pH intracelular. Varios ensayos clínicos han demostrado que se puede limitar el tamaño del infarto mediante estrategias no farmacológicas —como el poscondicionamiento isquémico y el condicionamiento isquémico remoto— o farmacológicas —como la estimulación de la síntesis de guanosina monofosfato cíclico, la insulina, los agonistas del péptido glucagonoide tipo 1, los bloqueadores beta o la ciclosporina. Diversos ensayos clínicos han dado resultados negativos, en la mayoría de los casos por falta de datos preclínicos consistentes o un diseño equivocado, en particular, administración tardía. Son necesarios, pues, ensayos clínicos grandes con variables clínicas primarias y terapias combinadas y que consideren edad, sexo y comorbilidades, para que la protección contra el daño por reperusión se convierta en un tratamiento estándar para los pacientes con infarto agudo de miocardio con elevación del segmento ST.

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## Palabras clave:

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Proteína G

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## INTRODUCTION

## Importance of the Problem. Contribution of Cell Death to the Social Impact of Ischemic Heart Disease

Ischemic heart disease is the leading cause of death worldwide and, unless previous trends are modified, will continue to be the

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most common cause of death in 2030.<sup>1</sup> The social impact of ischemic heart disease is considerable, not only because of the mortality it causes, but also because of its consequent morbidity, loss of quality of life, and high economic cost. This impact is largely due to a pathophysiological mechanism, namely, cardiomyocyte death. In ischemic heart disease, cardiomyocyte death almost always occurs in the context of severe and prolonged myocardial ischemic events, which are a consequence of thrombotic complications from atherosclerotic plaques in epicardial coronary arteries, known as acute coronary syndrome. Cardiomyocyte death is more significant when ischemia is caused by complete coronary occlusion, lacks well-developed collateral circulation, affects the majority of the left ventricular wall thickness, and shows ST-segment elevation on an electrocardiogram (ST-segment elevation acute myocardial infarction [STEMI]).

## Abbreviations

mPTP: mitochondrial permeability transition pore  
 PKG: protein kinase G  
 PostC: postconditioning  
 RIC: remote ischemic conditioning  
 STEMI: ST-segment elevation acute myocardial infarction

The cell death that occurs in acute coronary syndrome not only causes a direct loss of contractile activity, but can also cause geometric changes in the infarcted wall and adaptive changes in the remaining myocardium, which ultimately lead to general dysfunction and dilatation of the ventricle, a process called adverse remodeling.<sup>2,3</sup> Scarring and adverse remodeling cause heart failure and promote the appearance of potentially fatal ventricular arrhythmias,<sup>4</sup> so that cell death occurring during acute coronary syndrome eventually determines not only acute-phase mortality, but also long-time morbidity and mortality.<sup>5</sup>

Accordingly, reducing cell death during acute coronary syndrome, and in particular during STEMI, appears to be an obvious strategy for reducing the impact of ischemic heart disease on health and society.

## Prevention of Reperfusion Injury as a Strategy to Reduce the Impact of Ischemic Heart Disease

Ischemic heart disease has mixed genetic and environmental etiology whose pathological substrate is the atherosclerotic plaque. Coronary atherosclerosis develops in a clinically silent manner over years and only causes clinical symptoms when the vessel lumen is greatly narrowed, either due to atherosclerotic plaque growth or the development of intracoronary thrombosis due to plaque complication, resulting in a loss of endothelial continuity due to erosion, fissure, or endothelial rupture.<sup>6</sup> Thus, preventing the appearance of atherosclerosis coronary plaques or their progression is the first line of action against the disease.

Prevention of ischemic heart disease is not without difficulties. Population-wide interventions are expensive and cannot be particularly aggressive due to the risk of costly adverse events. The more aggressive interventions—aimed at avoiding plaque growth, reducing the risk of complications, or attenuating secondary thrombosis—should be limited to those individuals at high risk of the disease. Although much progress has been made in identifying risk factors and developing methods to calculate individual risk,<sup>7</sup> conventional risk factors for the disease are poorly controlled.<sup>8</sup> Moreover, genetic studies have identified multiple loci associated with the development of atherosclerosis disease, but this association is weak and its effect on the predictive value of conventional risk factors is limited, particularly when family history is taken into account. The difficulty in preventing ischemic heart disease is clear upon observing changes in its incidence in specific populations. Spain, for example, is far from controlling ischemic heart disease through preventive measures, given that the incidence of conventional risk factors has largely been stable and in some cases is predicted to increase, as is happening with type 2 diabetes mellitus, which has proliferated due to the growth in childhood obesity.<sup>9,10</sup>

The effectiveness of risk stratification and prevention strategies should be measured not only by their ability to reduce the appearance of clinical symptoms of ischemic heart disease, but more specifically by their ability to reduce the incidence of acute coronary syndrome and, in particular, acute myocardial infarction

(AMI), which causes most of the morbidity and mortality associated with the disease. However, extensive evidence indicates that it is difficult to predict the occurrence of AMI. In a recent large study, performed in 542 008 patients with a first myocardial infarction, approximately half had only one risk factor or none at all.<sup>11,12</sup> Moreover, analysis of distinct patient subgroups revealed that in-hospital mortality was inversely proportional to the number of classical risk factors present.<sup>11</sup> Taking both observations together, it can be estimated that most patients that die in hospital from their first AMI show a low risk profile, with 0-1 risk factors. Accordingly, although efforts to improve risk stratification and prevention of ischemic heart disease are clearly essential, no less essential is the need to improve the effectiveness of AMI treatment to improve prognosis. In many cases, this approach is the first and only option available to alter the course of the disease.

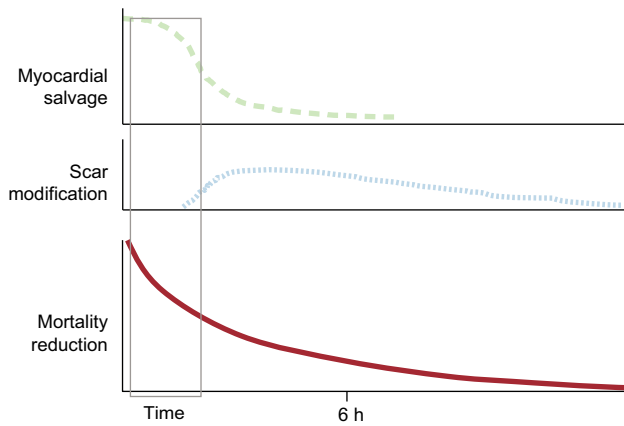
The prognosis of AMI largely depends on its extent, that is, the number of cells that die during the event.<sup>13</sup> The final necrotic extent is mostly due to the speed of the progression of ischemic injury (influenced by residual blood flow, whether through the lesion or through collateral circulation, and by temperature, among other factors) and the duration of the ischemia.<sup>14,15</sup> The most effective treatment to limit infarct size is early reperfusion.<sup>16–18</sup> However, the amount of myocardium salvaged by reperfusion rapidly decreases the longer it is delayed, and the window in which reperfusion effectively limits infarct size in patients with STEMI is short. After 3 h of ischemia, without collateral circulation and residual flow (TIMI [Thrombolysis in Myocardial Infarction] 0), the amount of salvaged myocardium is typically small or nonexistent.<sup>19</sup> Nonetheless, late reperfusion is also beneficial, and should generally be performed within 12 h of symptom onset.<sup>20</sup> In this case, however, the benefit is due to the positive effects of reperfusion on healing, with reperfusion limiting infarct expansion and secondary adverse remodeling (Figure 1).<sup>20</sup>

Because of the short reperfusion window for limiting infarct size, necrosis affecting most of the ventricular wall thickness—causing Q-waves and worsening prognosis—cannot be prevented in most patients, despite the availability of systems of rapid administration of reperfusion treatment (such as primary angioplasty or thrombolysis without emergency department referral, and optimal antiplatelet and anticoagulant agents, etc). The conventional outlook is that when patients with STEMI arrive at the catheterization laboratory, as it is no longer possible to reduce the ischemia duration or modify the collateral circulation or residual coronary flow (TIMI flow), the only remaining approach is to open the artery as much as possible and see if patients have arrived in time to save the myocardium. However, a wealth of preclinical information and a growing amount of clinical data indicate that the proportion of reperfusion-salvaged myocardium can be increased by applying treatments more or less at the time of reperfusion. The present article examines the current situation and the expected future of treatments aimed at reducing cell death in patients with STEMI that receive reperfusion treatment, and will not therefore analyze the effectiveness of these treatments in other clinical contexts, such as cardiac surgery or cardiopulmonary resuscitation.

## REPERFUSION INJURY

### Concept

The first laboratory experiments with transient coronary occlusion showed a paradoxical worsening during reperfusion of the functional changes associated with ischemia, particularly arrhythmias.<sup>21–23</sup> Studies in isolated and perfused hearts revealed an increase in cardiac enzyme release upon reperfusion following a



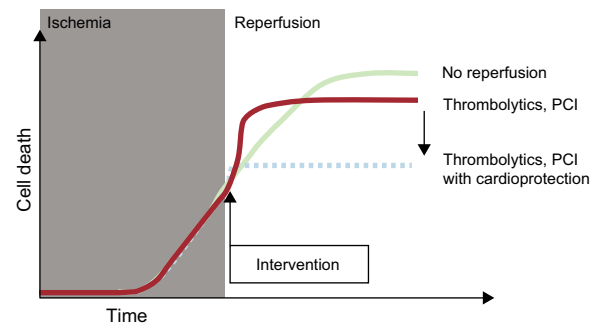
**Figure 1.** Reperfusion benefit is fundamentally due to infarct size limitation when the total ischemic time is short. With late reperfusion, the effect on healing, with reduced scar expansion and adverse remodeling, can be more substantial.

period of ischemia.<sup>24</sup> Afterward, it was verified that reperfusion was accompanied by transient contractile dysfunction (stunning), even in the absence of necrosis.<sup>25</sup> However, differences in the mechanisms and clinical relevance of these reperfusion-associated phenomena hampered initial acceptance of the concept of reperfusion injury in the scientific and medical community. The situation changed following the introduction of the concept of lethal reperfusion injury, with a precise operational definition: cell death that can be prevented by interventions applied at the time of reperfusion.<sup>26</sup> In this article, the expression “reperfusion injury” is always used according to this definition, whose use for STEMI is illustrated in Figure 2.<sup>27</sup>

### Pathophysiology of Ischemia-Reperfusion Injury

Occlusion of a coronary artery creates an ischemic zone (area at risk) whose lateral borders are sharply defined by a lack of communication between adjacent capillary beds<sup>28,29</sup> and in which residual blood flow, if any, is distributed through the subepicardium. Reperfusion is accompanied by extracellular and intracellular edema, due to endothelial permeability and the creation of an osmotic gradient between the extravascular and intravascular spaces.<sup>30</sup> The extracellular edema appears a few minutes after reperfusion and can contribute to changes in the mechanical function of the myocardium and last for several days in patients with STEMI, which is how it has been used to delimit the area at risk through magnetic resonance imaging.<sup>30</sup> In addition to edema, reperfusion is accompanied by the accumulation of platelets in the reperfused myocardium due to their adhesion (dependent on P-selectin) to activated endothelium.

An area of cell death is produced if reperfusion is not performed sufficiently early. Cell death rapidly occurs due to necrosis in the first few minutes of reperfusion.<sup>26</sup> In contrast to what was once thought, apoptosis plays no significant role in ischemia-reperfusion-induced cell death; in fact, the expression of proteins indispensable for the apoptotic program is silenced in adult cardiomyocytes.<sup>31</sup> The necrotic zone has a lateral extent that coincides with the lateral extension of the area at risk, and a transmural extent that extends toward the subepicardium as reperfusion is delayed (wavefront phenomenon).<sup>14</sup> When reperfusion is late and the infarction is extensive, the interior develops areas of severe microvascular injury with loss of the endothelial barrier, interstitial bleeding, and flow arrest (no-reflow areas).<sup>32</sup> No-flow areas are associated with extensive infarctions and poor



**Figure 2.** Illustration of the concept of reperfusion injury. During myocardial ischemia, cell death progresses. Reperfusion halts this process, but its benefit is limited by an increase in cell death during the first few minutes of reperfusion; this death can be avoided by using cardioprotective treatments at the time of flow restoration. PCI, percutaneous coronary intervention. Modified with the permission of Garcia-Dorado et al.<sup>27</sup>

prognosis, suggesting that they contribute to cell death. However, there is no definitive evidence to support this theory, and considerable data indicate that no-flow areas are produced in already necrotic zones.<sup>33</sup> What does seem likely is that areas without reperfusion hamper healing and favor scar expansion and subsequent adverse remodeling.<sup>34</sup>

### Ischemic Cell Damage

Reperfusion injury, as defined above, is not limited to STEMI, in which cell death is produced secondary to transient myocardial ischemia and can occur in diverse clinical situations, particularly during cardiac surgery or cardiopulmonary resuscitation. However, the mechanisms involved depend on the changes produced before reperfusion (such as concomitance of hypothermia, cardioplegic solutions, hypoxia with preserved blood flow, etc). This article focuses on the mechanisms of reperfusion injury that appear after a period of severe regional and normothermic ischemia.

The cell changes caused by ischemia have been reviewed in numerous publications.<sup>26,35–38</sup> Within seconds, regional ischemia causes oxidative phosphorylation arrest due to a lack of oxygen in the mitochondrial respiratory chain. Adenosine triphosphate (ATP) synthesis becomes restricted to the glycolytic pathway, which, with the Krebs cycle stopped, leads to an intracellular accumulation of lactic acid.<sup>26,38</sup> This buildup of lactic acid, together with the inability to eliminate CO<sub>2</sub> from the extracellular space due to blood flow arrest, causes intracellular pH to rapidly fall to almost 6.4 within minutes.<sup>39,40</sup> Acidosis and accumulation of inorganic phosphate rapidly halt contractile activity, even when the ATP concentration remains practically normal. In the following minutes, the ATP concentration continues to fall, reaching critical and almost zero levels and promoting the development of ischemic rigor.<sup>41</sup> Depletion of ATP is accelerated in the final phase by a reversal in the activity of mitochondrial ATPase (complex V of the respiratory chain), which consumes ATP to maintain the mitochondrial membrane potential.<sup>42</sup> Acidosis activates sarcolemmal Na<sup>+</sup>/H<sup>+</sup> exchangers, with a consequent influx of Na<sup>+</sup>, while the energy depletion stops Na<sup>+</sup> efflux through Na<sup>+</sup>/K<sup>+</sup> ATPase. Consequently, Na<sup>+</sup> accumulates in the interior of the cell, activating Na<sup>+</sup>/Ca<sup>2+</sup> exchangers in the reverse direction and increasing cytosolic Ca<sup>2+</sup>.<sup>26,38</sup> The cell becomes depolarized. At the initial stage of ischemia, mitochondria take up Ca<sup>2+</sup> due to the electronegativity of the mitochondrial matrix, but cease to do so when depolarized.<sup>43</sup> The sarcoplasmic reticulum cannot take

up  $\text{Ca}^{2+}$  from the cytosol either, as the transporter needs ATP to work.

### Reperfusion Injury

Restoration of myocardial blood flow leads to a recovery in respiratory activity, mitochondrial membrane potential, and ATP synthesis. The cell membrane potential is restored, which, in the presence of a high cytosolic concentration of  $\text{Na}^+$ , causes the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger to function in reverse, further exacerbating the cytosolic  $\text{Ca}^{2+}$  overload. The availability of ATP in the presence of increased  $\text{Ca}^{2+}$  activates uptake of  $\text{Ca}^{2+}$  by the sarcoplasmic reticulum, exceeding the threshold necessary to open ryanodine channels, which release  $\text{Ca}^{2+}$  into the cytosol. Cyclical repetition of this process causes  $\text{Ca}^{2+}$  oscillations that are propagated through the cell.<sup>26,38</sup> Ryanodine channels are physically connected to mitochondria, so that  $\text{Ca}^{2+}$  is specifically released into subcellular microdomains, allowing influx along the potential gradient into the mitochondrial matrix via the mitochondrial  $\text{Ca}^{2+}$  uniporter. The physicochemical characteristics of this transporter cause the  $\text{Ca}^{2+}$  oscillations to promote an increase in mitochondrial  $\text{Ca}^{2+}$ .  $\text{Ca}^{2+}$  oscillations and their effect on mitochondria depend, on one hand, on the activity of the ryanodine channels and the  $\text{Ca}^{2+}$  uniporter and, on the other, on the activity of the responsible transporter, which in turn is modulated by phospholamban.<sup>44–46</sup> During reperfusion, activation of protein kinase A, protein kinase G (PKG), and calcium- and calmodulin-dependent oxidation/phosphorylation of kinases modulate the magnitude of the  $\text{Ca}^{2+}$  oscillations and the degree of mitochondrial  $\text{Ca}^{2+}$  overload, through changes in the phosphorylation of ryanodine, phospholamban, and mitochondrial  $\text{Ca}^{2+}$  uniporter channels.<sup>47</sup>

Cytosolic  $\text{Ca}^{2+}$  oscillations have detrimental consequences for cells. First, the oscillations promote an excessive contractile activity, which can trigger hypercontracture.<sup>48</sup> Hypercontracture can disrupt cellular architecture and cause sarcolemmal rupture and cell death.<sup>49</sup> Second, the  $\text{Ca}^{2+}$  oscillations lead to cell membrane potential oscillations in the form of early depolarizations or afterdepolarizations, an important mechanism for reperfusion arrhythmias.<sup>50</sup> Third, the oscillations promote opening of the mitochondrial permeability transition pore (mPTP).

The mPTP is a high-permeability channel, of poorly understood molecular identity, that permits a direct connection between the mitochondrial matrix and intermembrane space.<sup>51</sup> When the channel opens, the mitochondrial membrane potential dissipates and ATP synthesis stops, resulting in edema and mitochondrial rupture and allowing the release of mitochondrial molecules to the cytosol, including  $\text{Ca}^{2+}$ . Mitochondrial permeabilization is considered to be an important mechanism of cell death during reperfusion. Permeabilization and hypercontracture secondary to  $\text{Ca}^{2+}$  overload and oscillations are interrelated, so that oscillations favor opening of the mPTP<sup>52</sup> and mitochondrial permeabilization promotes  $\text{Ca}^{2+}$  efflux into the cytosol, which promote further development of oscillations and hypercontracture.<sup>53</sup> Hypercontracture appears to play a more important role in the genesis of cell death after relatively brief ischemic events, whereas mPTP is the main mechanism of cell death during reperfusion after prolonged ischemia.<sup>54</sup>

Both hypercontracture and mPTP opening seem to be favored by 2 important phenomena that occur during the first few minutes of reperfusion. The first is the rapid normalization of intracellular pH, which permits contractile activity and mPTP opening, both inhibited by acidosis.<sup>55,56</sup> pH normalization also allows activation of calpains,  $\text{Ca}^{2+}$ -dependent proteases that are inhibited by an acidic pH. Calpain activation damages the cytoskeleton and

impedes the normal function of  $\text{Na}^+/\text{K}^+$  ATPases, which worsens the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  overload and closes the vicious circle.<sup>57–59</sup>

During reperfusion, reactive oxygen species are generated that play a crucial role in reperfusion injury, because they directly promote opening of the mPTP<sup>60</sup> and worsen changes in  $\text{Ca}^{2+}$  homeostasis, oxidizing calcium-dependent kinase and calmodulin.<sup>61</sup> Moreover, superoxide anion, which is a potent oxidant, dissociates nitric oxide (NO) synthase from tetrahydrobiopterin, resulting in superoxide anion production instead of NO and thereby decreasing the levels of NO and its downstream signaling pathways (cyclic guanosine monophosphate [cGMP] and PKG).<sup>62</sup> The reduction in PKG accelerates pH normalization (as PKG inhibits  $\text{Na}^+/\text{H}^+$  exchange) and promotes  $\text{Ca}^{2+}$  oscillations (acting on phospholamban); as explained above, both actions have negative consequences for cell survival.

Finally, cell death can be propagated between adjacent myocytes through gap junction-mediated intercellular communication,<sup>63–66</sup> which allows passage of  $\text{Na}^+$  from one cell to another.<sup>67</sup>  $\text{Na}^+$ , in turn, promotes  $\text{Ca}^{2+}$  influx into adjoining cells, which can cause death if the cells are sufficiently damaged (eg, if the Na pump function is altered or the cytoskeleton is damaged).<sup>67</sup> Intercellular communication through gap junctions also requires pH normalization, given that acidosis causes these channels to close.<sup>68</sup>

These mechanisms are summarized in Figure 3.

### Microvasculature and Blood Cells

Both myocardial ischemia and reperfusion actually occur in a tissue containing a considerable number of nonmyocyte cells, such as endothelial, fibroblastic, and blood cells. Activated platelets adhering to the reperfused myocardium negatively affect cardiomyocytes, by mechanisms independent of microvascular flow occlusion and probably related to the release of substances such as thrombin. Thus, activated platelets can worsen  $\text{Ca}^{2+}$  management and trigger other adverse events in cells.<sup>69–72</sup> Endothelial cell activation and microparticle and ribosomal RNA release also impair myocyte survival via massive activation of tumor necrosis factor alpha, among other factors.<sup>73</sup>

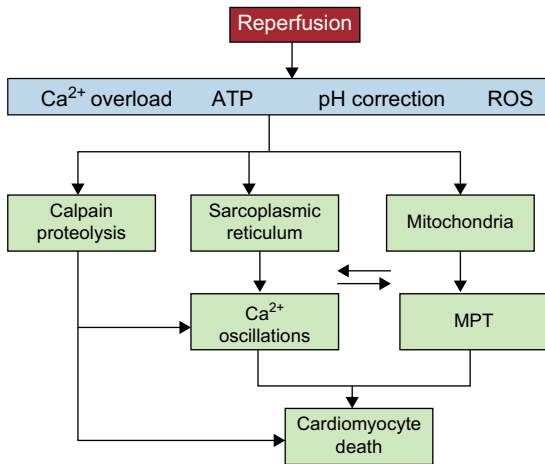
### THE MOST PROMISING STRATEGIES FOR LIMITING REPERFUSION INJURY

Demonstration of the existence of reperfusion injury used to be based on the use of pharmacological tools that interfered with the above described mechanisms. However, for reasons discussed below, none of these tools has been translated to clinical practice. Thus, drugs remain to be developed for many potentially useful targets for treating reperfusion injury.<sup>74</sup>

### Endogenous Cardioprotection

The first treatment successfully used in patients with STEMI was ischemic postconditioning (PostC).<sup>75,76</sup> In contrast with drug treatments, PostC reduces reperfusion injury by introducing brief episodes of ischemia, at the moment when reperfusion begins,<sup>77</sup> which has a protective effect without the need for external agents (endogenous cardioprotection).

PostC acts via mechanisms different from ischemic preconditioning, in which brief and repeated periods of ischemia immediately before a longer episode reduce the resulting cell death. Ischemic preconditioning provides a more robust protection, but is unsuitable for patients with STEMI. The protection afforded by PostC is not only lesser, but varies depending on conditions. Its effectiveness is weak or absent after brief ischemic



**Figure 3.** Diagram showing the main mechanisms of cardiomyocyte death during myocardial reperfusion. Reperfusion causes calcium oscillations that are dependent on the sarcoplasmic reticulum and mitochondrial permeabilization that is promoted by calcium overload and oxidative stress. Recovery from acidosis promotes both phenomena, which are intimately related due to physical contact between mitochondria and sarcoplasmic reticula and also the activation of calcium-dependent proteases (calpain) (see the detailed explanation in the text). ATP, adenosine triphosphate; MPT, mitochondrial permeability transition; ROS, reactive oxygen species.

episodes that cause small infarcts.<sup>78</sup> The protective effect of PostC is mainly due to its ability to delay the normalization of intracellular pH for a few minutes, by slowing metabolite washout secondary to flow interruptions, and to the reduction in oxidative damage, which preserves the NO-cGMP-PKG signaling pathway and inhibits Na<sup>+</sup>/H<sup>+</sup> exchange.<sup>79,80</sup>

Recently, a form of endogenous protection has been described that involves remote induction (generally in the extremities) of intermittent myocardial ischemia immediately before or in the first few minutes of reperfusion: remote ischemic conditioning (RIC).<sup>81–83</sup> One advantage of RIC over PostC is that it does not require manipulation of the coronary artery during the first few minutes of reperfusion, but the mechanisms by which it exerts its protective effect remain unclear.<sup>83,84</sup>

### Pharmacological Cardioprotection

Preservation of the PKG signaling pathway has many protective effects. As well as delaying pH normalization and reducing Ca<sup>2+</sup> oscillations, as described above, the PKG pathway seems to have direct mitochondrial effects that inhibit mitochondrial permeabilization.<sup>85</sup> In fact, there is ample evidence of the protective effects of treatments that normalize cGMP at the beginning of reperfusion, either through stimulation of particulate guanylate cyclase via natriuretic peptides<sup>86</sup> or through direct and NO-independent stimulation of soluble guanylate cyclase.<sup>87</sup> The usefulness of the stimulation of soluble guanylate cyclase via exogenous NO varies, probably because excess NO can cause oxidative damage.<sup>88</sup>

Another strategy that has been widely studied using both pharmacological interventions<sup>89,90</sup> and genetically modified models<sup>90</sup> involves inhibition of mPTP opening. Both investigational approaches focus on one of the proteins that participates in Ca<sup>2+</sup>-induced mPTP opening, cyclophilin D. Cyclosporine, a widely used immunosuppressor, acts through cyclophilin D to inhibit mPTP opening<sup>89,90</sup> in response to Ca<sup>2+</sup>. Preliminary results are available on other molecules that inhibit the mPTP. More recently,

dysfunction of complex I of the respiratory chain has been identified as a potentially useful target. Although various ways to prevent this dysfunction have been proposed, there are few results to date.

Preclinical studies are being undertaken involving other targets. The most prominent targets are related to insulin signaling and energy metabolism. For the former, the early use of solutions containing glucose, insulin, and potassium<sup>91–93</sup> and stable mimetics of glucagon-like peptide-1<sup>94</sup> have received particular attention and have achieved promising results. Other potentially protective ways of altering energy metabolism at the time of reperfusion include use of trimetazidine<sup>95</sup> and the addition of specific substrates.<sup>96</sup>

Finally, there are many other interventions directed at distinct reperfusion injury targets. However, their effects remain controversial. These interventions include targeting activation of leukocytes or complement factors (which occurs when most cell death has taken place) or caspase-dependent apoptosis (a type of cell death practically inexistent in adult cardiomyocytes).

### CLINICAL TRIALS

During the last decade, various clinical trials have tested different strategies for limiting infarct size and reducing reperfusion injury. Their target populations, sample sizes, designs, primary end points, and results have recently been re-viewed.<sup>97–100</sup> All of the studies lacked statistical power to detect effects on clinical course and were designed to detect effects on surrogate markers, specifically infarct size, measured or estimated by distinct methods. Some strategies, such as PostC, have been the subject of multiple studies, including meta-analyses, whereas only data from single studies are available for other interventions. Next, the results obtained with distinct cardioprotective interventions are assessed.

### Ischemic Conditioning

In general, studies based on ischemic conditioning maneuvers have been positive.<sup>101</sup>

#### Ischemic Postconditioning

Several studies have analyzed the efficacy of ischemic PostC, the first approach described as being able to limit reperfusion injury in patients. In the first trial, reocclusions were performed in the reperfused artery through four 1-min inflations of an angioplasty balloon, separated by 1-min periods with the balloon deflated and beginning within 1 min of artery opening. This protocol significantly reduced infarct size, measured as the area under the curve for creatine kinase.<sup>75</sup> Subsequent studies performed by other groups obtained varying results,<sup>76,102–105</sup> whose differences may be explained by the involvement of distinct factors, such as different levels of direct stent usage or differences in perioperative antiplatelet therapy.<sup>103</sup> Although the current data generally show that PostC reduces infarct size to a certain extent,<sup>106</sup> the use of this procedure has not appreciably expanded in recent years, probably due to the weak and variable protection that it appears to provide, its counterintuitive character (producing reocclusions), and fear of complications due to the repeated inflations of the angioplasty balloon. One limitation common to the different clinical assays of PostC is that this procedure cannot be performed in a double-blind manner. This is an important limitation that may introduce biases in patient exclusion, event allocation, and incompletely automated

determinations, such as in the case of infarct size evaluation via magnetic resonance imaging.<sup>107</sup>

### Remote Ischemic Conditioning

As with PostC, RIC was applied to patients soon after being described in laboratory studies and long before the mechanisms were understood.<sup>108</sup> RIC can be induced by inflating a blood pressure cuff placed on the arm to above systolic pressure for 5-min periods separated by 5-min intervals. The results described thus far are consistently positive and in agreement with the preclinical evidence. RIC has multiple advantages over PostC and is a strong candidate for becoming part of clinical practice recommendations. First, it is extremely safe and cheap; second, it can be applied in the ambulance and to all patients with STEMI that receive reperfusion treatment, not only those who undergo primary angioplasty; and, third, the protection afforded by RIC appears to go beyond the myocardial area at risk and STEMI. Studies are underway in other tissues,<sup>109</sup> and there is already solid evidence that it reduces myocardial injury in various types of surgery,<sup>110,111</sup> particularly coronary revascularization.<sup>112,113</sup> Although it is complicated to perform double-blind studies of RIC, it is possible, as the placebo treatment could involve cuffs automatically programmed to inflate to a low pressure.<sup>114</sup>

### Pharmacological Treatments

The results of pharmacological treatments to reduce infarct size in STEMI are as variable as the strategies and designs used. However, studies based on approaches with robust preclinical data and designs that faithfully reproduce the conditions of preclinical studies have generally been positive.<sup>108,115–119</sup>

#### Atrial Natriuretic Peptide

The cGMP/PKG signaling pathway is probably the pharmacological target for the prevention of myocardial reperfusion injury that has the most solid preclinical evidence. However, only 1 clinical trial has directly assessed this strategy in patients with STEMI.<sup>115</sup> The study showed limitation of the enzymatic infarct size and improvement in ventricular function in patients that received intravenous atrial natriuretic peptide at the time of reperfusion, thereby reproducing the beneficial effect of this treatment in animals.<sup>86</sup>

#### Glucose-insulin-potassium

Insulin administration also acts through the cGMP/PKG pathway (via Akt and PI3P), as well as by increasing glucose use.<sup>120,121</sup> Preclinical evidence showing the usefulness of insulin at the beginning of myocardial reperfusion is solid.<sup>91,120–123</sup> However, translation to the clinical setting gave negative results, easily explained by late treatment administration.<sup>124–126</sup> A recent randomized, double-blind study has found, however, that glucose-insulin-potassium can reduce infarct size and serious electrical complications when administered in the ambulance to patients with STEMI that will receive primary angioplasty.<sup>117</sup>

#### Glucagon-like Peptide-1

Stimulation of the glucagon-like peptide-1 pathway also acts through cGMP/PKG, among other pathways.<sup>127,128</sup> There is

solid evidence of the cardioprotective effect of increasing glucagon-like peptide-1-dependent signaling, either by using stable analogues of glucagon-like peptide-1 or inhibitors of its degradation.<sup>129,129</sup> Two clinical trials have evaluated the effect of a glucagon-like peptide-1 agonist, exenatide, in patients with STEMI who underwent primary angioplasty, and both studies have shown positive results.<sup>118,130</sup> Given its high safety profile, this strategy is especially promising.

#### Adenosine

Adenosine, a degradation product of adenosine monophosphate, has a potent vasodilatory action, among other effects. In addition to its effects on the vasculature and leukocytes, it directly acts on cardiomyocytes, increasing NO availability (via Akt) and, therefore, activating the cGMP/PKG pathway. This substance was used intravenously in the AMISTAD studies,<sup>131</sup> without conclusive results, although with evidence of protection at high doses in patients with previous infarctions.<sup>132</sup> Subsequently, its use was proposed in the form of an intracoronary injection before reperfusion.<sup>133</sup> The largest study performed to date is PROMISE, a double-blind study that included 200 patients with TIMI flow 0–1 and whose main end point was magnetic resonance imaging measurement of infarct size, with a pre-specified subanalysis according to ischemia duration. The results showed that adenosine fails to limit infarct size in all patients, but did achieve this goal in those receiving primary angioplasty within the first 3 h after the onset of pain.<sup>134</sup> The data also suggest an improvement in left ventricular ejection fraction during the first 6 months after the infarction (shown by magnetic resonance imaging). Because intracoronary adenosine is a safe and inexpensive treatment, its early use in reperfused patients could be easily adopted if later studies, based on clinical end points, confirm the benefits.

#### Cyclosporine

In a first pilot study, cyclosporine A limited infarct size (determined enzymatically and with magnetic resonance imaging) in 58 patients with STEMI undergoing primary angioplasty.<sup>119</sup> However, a recent study with a similar number of patients found no benefit.<sup>135</sup> A soon-to-be-completed international study with more than 1600 patients will presumably determine the usefulness of this treatment for improving the clinical course of patients with STEMI.<sup>136</sup>

#### Beta-blockers

Preclinical evidence on the use of beta-blockers for reducing infarct size is mixed. A recent study found that intravenous injection of metoprolol was protective in a pig model involving a 90-minute coronary occlusion.<sup>137</sup> This treatment was translated to patients in the METOCARD-CNIC study.<sup>138</sup> This blinded study aimed to determine the effect of intravenous metoprolol, administered immediately before primary angioplasty, on infarct size determined with magnetic resonance imaging; the approach showed protection via an increase in myocardial salvage without major complications.<sup>138,139</sup> Beta-blockers are already recommended for patients with STEMI. Therefore, the data from this study will advance the clinical use of beta-blockers, as long as they are confirmed by subsequent studies.

Some of the more promising strategies for combating reperfusion injury in patients with STEMI are shown in [Figure 4](#).

### Negative Studies and Problems With Translation

Cardioprotective therapy use in clinical practice has occasionally led to negative studies. In the overwhelming majority of cases, the negative results were clearly foreseeable from existing knowledge. The most common reasons for negative results are the following: a) use of treatments indicated by preclinical data to require administration before ischemia occurrence, such as  $\text{Na}^+/\text{H}^+$  exchanger inhibitors, which have been shown to be effective only when they are administered before ischemia<sup>140-142</sup> and failed when they were given at the time of reperfusion in clinical studies<sup>143</sup>; b) late treatment initiation, as multiple trials have begun study therapies after the first 30 min of reperfusion, when most of the reperfusion injury has already occurred, as in the case of studies with glucose-insulin-potassium<sup>124</sup> and erythropoietin,<sup>144</sup> and c) therapeutic approaches with clearly insufficient or contradictory preclinical evidence, such as that of mechanical ventricular assist during reperfusion,<sup>145</sup> protein kinase C-delta inhibition,<sup>146</sup> complement system inhibition,<sup>147</sup> or nicorandil.<sup>115</sup>

In addition to an inadequate poorly justified or risky design, other factors can hinder translation of experimental data to the clinical setting.<sup>100</sup> One very important factor is advanced age and comorbidities, which contrasts with the use of healthy animals and young people in laboratory studies. Another factor is the simultaneous use of therapies that may have a protective effect or interfere with that of the treatments.<sup>97</sup> Finally, the high variability in infarct size and residual flow or collateral circulation in patients with AMI hampers the detection of differences between

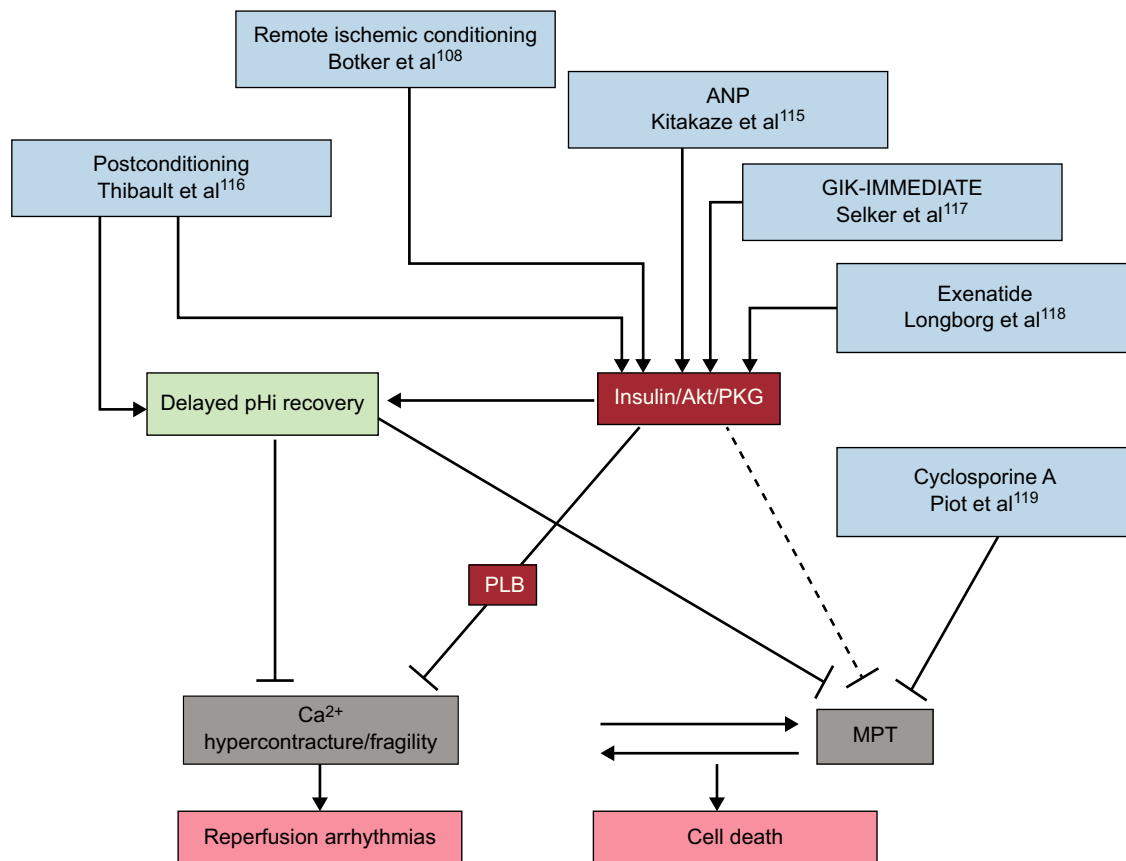
groups.<sup>97,100</sup> To avoid these problems, guidelines have been proposed for preclinical research and the design of clinical studies into protection against reperfusion injury.<sup>19</sup>

Finally, evidence that an intervention reduces infarct size in patients with STEMI is insufficient to justify its use in clinical practice. Studies must be performed with robust main clinical end points, which require a large number of patients with confirmed clinical benefit.

### PROTECTION AGAINST REPERFUSION INJURY IN CLINICAL PRACTICE, NOW AND IN THE IMMEDIATE FUTURE

All the therapies against reperfusion damage in STEMI described so far are currently experimental. However, protection against myocardial damage can be discussed in certain clinical practice contexts. First, preclinical studies have demonstrated that some treatments received by patients with STEMI have a cardioprotective effect. These treatments include morphine and its derivatives,<sup>148</sup> P2Y<sub>12</sub> inhibitors and other antiplatelet agents,<sup>149</sup> statins,<sup>150</sup> and beta-blockers.<sup>137</sup> Second, there are treatments that, due to their ease of use and safety, could be adopted before there is solid evidence of their real clinical usefulness. PostC is one such treatment. This treatment category could also encompass RIC.

Currently, studies are underway to evaluate treatments against reperfusion injury using surrogate markers, as well as other large studies that use clinical end points. The next frontier will probably have 2 fronts, involving, on one hand, personalization of antireperfusion therapy based on the clinical situation,



**Figure 4.** Some of the more promising strategies for combating reperfusion injury, by the mechanism of cell death interrupted. ANP, atrial natriuretic peptide; GIK, glucose-insulin-potassium; MPT, mitochondrial permeability transition; pHi, intracellular pH; PKG, protein kinase G; PLB, phospholamban.

patient characteristics, and comorbidities, and, on the other, a combination of distinct complementary strategies. Some of these studies are already being designed and are awaiting funding.

## CONCLUSIONS

Protection against myocardial ischemia-reperfusion injury is a promising strategy for ameliorating the consequences of coronary disease for individual and societal health. During the coming years, it will be necessary to focus on studying the molecular mechanisms of cell death during myocardial reperfusion and on developing new therapies to prevent cell death and to establish the best way to use these treatments in clinical practice. Given that these treatments would be used just once in most patients, their development is at a disadvantage compared with drugs that require more long-term use, and which are therefore much more profitable. To date, drugs used for this purpose have been used for other indications (repurposing). The task of adding to the arsenal of specifically designed drugs combating reperfusion injury must be actively encouraged. The role of public funding is particularly crucial for researching treatments without economic value, such as RIC. The available data raise the hope that, with the combined efforts of researchers, academics, public bodies, and industry, treatment of reperfusion injury will become a reality that improves survival and quality of life in patients with STEMI and limits the social impact of ischemic heart disease in the near future.

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## CONFLICTS OF INTERESTS

None declared.

## REFERENCES

- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 2006;3:e442.
- Sabbah HN, Goldstein S. Ventricular remodeling: consequences and therapy. *Eur Heart J.* 1993;14 (suppl C):24–9.
- Gajarsa JJ, Kloner RA. Left ventricular remodeling in the post-infarction heart: a review of cellular, molecular mechanisms, and therapeutic modalities. *Heart Fail Rev.* 2011;16:13–21.
- Gaudron P, Eilles C, Kugler I, Ertl G. Progressive left ventricular dysfunction and remodeling after myocardial infarction Potential mechanisms and early predictors. *Circulation.* 1993;87:755–63.
- Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol.* 2000;35:569–82.
- Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. *J Am Coll Cardiol.* 2005;46:937–54.
- Smith Jr SC. Current and future directions of cardiovascular risk prediction. *Am J Cardiol.* 2006;97:28A–32A.
- Scirica BM. Acute coronary syndrome: emerging tools for diagnosis and risk assessment. *J Am Coll Cardiol.* 2010;55:1403–15.
- Quelly SB, Lieberman LS. Global prevalence of overweight and obesity in preschoolers. *Anthropol Anz.* 2011;68:437–56.
- Franco M, Sanz B, Otero L, Dominguez-Vila A, Caballero B. Prevention of childhood obesity in Spain: a focus on policies outside the health sector SESPAS report 2010. *Gac Sanit.* 2010;24 Suppl 1:49–55.
- Canto JG, Kiefe CI, Rogers WJ, Peterson ED, Frederick PD, French WJ, et al. Number of coronary heart disease risk factors and mortality in patients with first myocardial infarction. *JAMA.* 2011;306:2120–7.
- Canto JG, Kiefe CI, Rogers WJ, Peterson ED, Frederick PD, French WJ, et al. Atherosclerotic risk factors and their association with hospital mortality among patients with first myocardial infarction (from the National Registry of Myocardial Infarction). *Am J Cardiol.* 2012;110:1256–61.
- Thompson PL, Fletcher EE, Katavatis V. Enzymatic indices of myocardial necrosis: influence on short- and long-term prognosis after myocardial infarction. *Circulation.* 1979;59:113–9.
- Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation.* 1977;56:786–94.
- Garcia-Dorado D, Garcia del Blanco B. Door-to-balloon time and mortality. *N Engl J Med.* 2014;370:179.
- Downey JM, Cohen MV. Reducing infarct size in the setting of acute myocardial infarction. *Prog Cardiovasc Dis.* 2006;48:363–71.
- Goel K, Pinto DS, Gibson CM. Association of time to reperfusion with left ventricular function and heart failure in patients with acute myocardial infarction treated with primary percutaneous coronary intervention: a systematic review. *Am Heart J.* 2013;165:451–67.
- ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Intravenous streptokinase given within 0–4 hours of onset of myocardial infarction reduced mortality in ISIS-2. *Lancet.* 1987;1:502.
- Hausenloy DJ, Bøtker HS, Condorelli G, Ferdinandy P, Garcia-Dorado D, Heusch G, et al. Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc Res.* 2013;98:7–27.
- Takemura G, Nakagawa M, Kanamori H, Minatoguchi S, Fujiwara H. Benefits of reperfusion beyond infarct size limitation. *Cardiovasc Res.* 2009;83:269–76.
- Tennant R, Wiggers CJ. The effect of coronary occlusion on myocardial contraction. *Am J Physiol.* 1935;112:351–61.
- Majidi M, Kosinski AS, Al Khatib SM, Lemmert ME, Smolders L, Van Weert A, et al. Reperfusion ventricular arrhythmia 'bursts' in TIMI 3 flow restoration with primary angioplasty for anterior ST-elevation myocardial infarction: a more precise definition of reperfusion arrhythmias. *Europace.* 2008;10:988–97.
- Lang TW, Corday E, Gold H, Meerbaum S, Rubins S, Costantini C, et al. Consequences of reperfusion after coronary occlusion Effects on hemodynamic and regional myocardial metabolic function. *Am J Cardiol.* 1974;33:69–81.
- Sakai K, Gebhard MM, Spieckermann PG, Bretschneider HJ. Enzyme release resulting from total ischemia and reperfusion in the isolated, perfused guinea pig heart. *J Mol Cell Cardiol.* 1975;7:827–40.
- Heusch G, Schulz R. Characterization of hibernating and stunned myocardium. *Eur Heart J.* 1997;18(18 Suppl D):D102–10.
- Piper HM, Garcia-Dorado D, Ovize M. A fresh look at reperfusion injury. *Cardiovasc Res.* 1998;38:291–300.
- Garcia-Dorado D, Piper HM. Postconditioning: reperfusion of «reperfusion injury» after hibernation. *Cardiovasc Res.* 2006;69:1–3.
- Sjoquist PO, Duker G, Almgren O. Distribution of the collateral blood flow at the lateral border of the ischemic myocardium after acute coronary occlusion in the pig and the dog. *Basic Res Cardiol.* 1984;79:164–75.
- Berry C, Balachandran KP, L'Allier PL, Lesperance J, Bonan R, Oldroyd KG. Importance of collateral circulation in coronary heart disease. *Eur Heart J.* 2007;28:278–91.
- Garcia-Dorado D, Andres-Villarreal M, Ruiz-Meana M, Inseste J, Barba I. Myocardial edema: a translational view. *J Mol Cell Cardiol.* 2012;52:931–9.
- Sanchis D, Llovera M, Ballester M, Comella JX. An alternative view of apoptosis in heart development and disease. *Cardiovasc Res.* 2008;77:448–51.
- Schwartz BG, Kloner RA. Coronary no reflow. *J Mol Cell Cardiol.* 2012;52:873–82.
- Barrabes JA, Garcia-Dorado D, Gonzalez MA, Ruiz-Meana M, Solares J, Puigfel Y, et al. Regional expansion during myocardial ischemia predicts ventricular fibrillation and coronary reocclusion. *Am J Physiol.* 1998;274:H1767–75.
- Reffelmann T, Hale SL, Dow JS, Kloner RA. No-reflow phenomenon persists long-term after ischemia/reperfusion in the rat and predicts infarct expansion. *Circulation.* 2003;108:2911–7.
- Garcia-Dorado D. Myocardial reperfusion injury: a new view. *Cardiovasc Res.* 2004;61:363–4.
- Garcia-Dorado D, Ruiz-Meana M, Piper HM. Lethal reperfusion injury in acute myocardial infarction: facts and unresolved issues. *Cardiovasc Res.* 2009;83:165–8.
- Piper HM, Meuter K, Schafer C. Cellular mechanisms of ischemia-reperfusion injury. *Ann Thorac Surg.* 2003;75:S644–8.
- Piper HM, Abdallah Y, Schafer C. The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovasc Res.* 2004;61:365–71.
- Hendriks M, Mubagwa K, Verdonck F, Overloop K, Van Hecke P, Vanstapel F, et al. New Na<sup>+</sup>-H<sup>+</sup> exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca<sup>2+</sup> overload in isolated perfused rabbit heart. *Circulation.* 1994;89:2787–98.
- Inseste J, Barba I, Hernandez V, Garcia-Dorado D. Delayed recovery of intracellular acidosis during reperfusion prevents calpain activation and determines protection in postconditioned myocardium. *Cardiovasc Res.* 2009;81:116–22.
- Bowers KC, Allshire AP, Cobbold PH. Bioluminescent measurement in single cardiomyocytes of sudden cytosolic ATP depletion coincident with rigor. *J Mol Cell Cardiol.* 1992;24:213–8.
- Grover GJ, Atwal KS, Sleph PG, Wang FL, Monshizadegan H, Monticello T, et al. Excessive ATP hydrolysis in ischemic myocardium by mitochondrial F1F0-ATPase: effect of selective pharmacological inhibition of mitochondrial ATPase hydrolase activity. *Am J Physiol Heart Circ Physiol.* 2004;287:H1747–55.



43. Ruiz-Meana M, Garcia-Dorado D, Miro-Casas E, Abellan A, Soler-Soler J. Mitochondrial Ca<sup>2+</sup> uptake during simulated ischemia does not affect permeability transition pore opening upon simulated reperfusion. *Cardiovasc Res.* 2006;71:715–24.
44. Piper HM, Kasseckert S, Abdallah Y. The sarcoplasmic reticulum as the primary target of reperfusion protection. *Cardiovasc Res.* 2006;70:170–3.
45. Piper HM, Abdallah Y, Kasseckert S, Schluter KD. Sarcoplasmic reticulum-mitochondrial interaction in the mechanism of acute reperfusion injury. *Viewpoint Cardiovasc Res.* 2008;77:234–6.
46. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. *Circ Res.* 2012;110:1646–60.
47. Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Gao Z, et al. CaMKII determines mitochondrial stress responses in heart. *Nature.* 2012;491:269–73.
48. Ruiz-Meana M, Garcia-Dorado D, Gonzalez MA, Barrabes JA, Soler-Soler J. Effect of osmotic stress on sarcolemmal integrity of isolated cardiomyocytes following transient metabolic inhibition. *Cardiovasc Res.* 1995;30:64–9.
49. Barrabes JA, Garcia-Dorado D, Ruiz-Meana M, Piper HM, Solares J, Gonzalez MA, et al. Myocardial segment shrinkage during coronary reperfusion in situ Relation to hypercontracture and myocardial necrosis. *Pflugers Arch.* 1996;431:519–26.
50. Priori SG, Mantica M, Napolitano C, Schwartz PJ. Early afterdepolarizations induced in vivo by reperfusion of ischemic myocardium. A possible mechanism for reperfusion arrhythmias. *Circulation.* 1990;81:1911–20.
51. Halestrap AP. What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol.* 2009;46:821–31.
52. Abdallah Y, Kasseckert SA, Iraqi W, Said M, Shahzad T, Erdogan A, et al. Interplay between Ca<sup>2+</sup> cycling and mitochondrial permeability transition pores promotes reperfusion-induced injury of cardiac myocytes. *J Cell Mol Med.* 2011;15:2478–85.
53. Ruiz-Meana M, Abellan A, Miro-Casas E, Garcia-Dorado D. Opening of mitochondrial permeability transition pore induces hypercontracture in Ca(2+) overloaded cardiac myocytes. *Basic Res Cardiol.* 2007;102:542–52.
54. Ruiz-Meana M, Inserte J, Fernandez-Sanz C, Hernandez V, Miro-Casas E, Barba I, et al. The role of mitochondrial permeability transition in reperfusion-induced cardiomyocyte death depends on the duration of ischemia. *Basic Res Cardiol.* 2011;106:1259–68.
55. Ruiz-Meana M, Pina P, Garcia-Dorado D, Rodriguez-Sinovas A, Barba I, Miró-Casas E, et al. Glycine protects cardiomyocytes against lethal reoxygenation injury by inhibiting mitochondrial permeability transition. *J Physiol.* 2004;558:873–82.
56. Griffiths EJ, Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J.* 1995;307:93–8.
57. Inserte J, Garcia-Dorado D, Ruiz-Meana M, Agullo L, Pina P, Soler-Soler J. Ischemic preconditioning attenuates calpain-mediated degradation of structural proteins through a protein kinase A-dependent mechanism. *Cardiovasc Res.* 2004;64:105–14.
58. Inserte J, Garcia-Dorado D, Hernandez V, Soler-Soler J. Calpain-mediated impairment of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity during early reperfusion contributes to cell death after myocardial ischemia. *Circ Res.* 2005;97:465–73.
59. Hernandez V, Inserte J, Sartorio CL, Parra VM, Poncelas-Nozal M, Garcia-Dorado D. Calpain translocation and activation as pharmacological targets during myocardial ischemia/reperfusion. *J Mol Cell Cardiol.* 2010;49:271–9.
60. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. *Cardiovasc Res.* 2004;61:372–85.
61. Erickson JR, He BJ, Grumbach JM, Anderson ME. CaMKII in the cardiovascular system: sensing redox states. *Physiol Rev.* 2011;91:889–915.
62. Inserte J, Hernandez V, Vilarrosa U, Abad E, Poncelas-Nozal M, Garcia-Dorado D. Activation of cGMP/protein kinase G pathway in postconditioned myocardium depends on reduced oxidative stress and preserved endothelial nitric oxide synthase coupling. *J Am Heart Assoc.* 2013;2:e005975.
63. Rodriguez-Sinovas A, Sanchez JA, Gonzalez-Loyola A, Barba I, Morente M, Aguilar R, et al. Effects of substitution of Cx43 by Cx32 on myocardial energy metabolism, tolerance to ischemia and preconditioning protection. *J Physiol.* 2010;588:1139–51.
64. Sanchez JA, Rodriguez-Sinovas A, Barba I, Miro-Casas E, Fernandez-Sanz C, Ruiz-Meana M, et al. Activation of RISK and SAFE pathways is not involved in the effects of Cx43 deficiency on tolerance to ischemia-reperfusion injury and preconditioning protection. *Basic Res Cardiol.* 2013;108:351.
65. Rodriguez-Sinovas A, Garcia-Dorado D, Ruiz-Meana M, Soler-Soler J. Enhanced effect of gap junction uncouplers on macroscopic electrical properties of reperfused myocardium. *J Physiol.* 2004;559:245–57.
66. Garcia-Dorado D, Rodriguez-Sinovas A, Ruiz-Meana M. Gap junction-mediated spread of cell injury and death during myocardial ischemia-reperfusion. *Cardiovasc Res.* 2004;61:386–401.
67. Ruiz-Meana M, Garcia-Dorado D, Hofstaetter B, Piper HM, Soler-Soler J. Propagation of cardiomyocyte hypercontracture by passage of Na<sup>(+)</sup> through gap junctions. *Circ Res.* 1999;85:280–7.
68. Liu S, Taffet S, Stoner L, Delmar M, Vallano ML, Jalife J. A structural basis for the unequal sensitivity of the major cardiac and liver gap junctions to intracellular acidification: the carboxyl tail length. *Biophys J.* 1993;64:1422–33.
69. Barrabes JA, Garcia-Dorado D, Mirabet M, Inserte J, Agullo L, Soriano B, et al. Antagonism of selectin function attenuates microvascular platelet deposition and platelet-mediated myocardial injury after transient ischemia. *J Am Coll Cardiol.* 2005;45:293–9.
70. Barrabes JA, Inserte J, Mirabet M, Quiroga A, Hernandez V, Figueras J, et al. Antagonism of P2Y<sub>12</sub> or GPIIb/IIIa receptors reduces platelet-mediated myocardial injury after ischaemia and reperfusion in isolated rat hearts. *Thromb Haemost.* 2010;104:128–35.
71. Mirabet M, Garcia-Dorado D, Inserte J, Barrabes JA, Lidon RM, Soriano B, et al. Platelets activated by transient coronary occlusion exacerbate ischemia-reperfusion injury in rat hearts. *Am J Physiol Heart Circ Physiol.* 2002;283:H1134–41.
72. Mirabet M, Garcia-Dorado D, Ruiz-Meana M, Barrabes JA, Soler-Soler J. Thrombin increases cardiomyocyte acute cell death after ischemia and reperfusion. *J Mol Cell Cardiol.* 2005;39:277–83.
73. Simsekilmaz S, Cabrera-Fuentes HA, Meiler S, Kostin S, Baumer Y, Liehn EA, et al. Role of extracellular RNA in atherosclerotic plaque formation in mice. *Circulation.* 2014;129:598–606.
74. Inserte J, Barrabes JA, Hernandez V, Garcia-Dorado D. Orphan targets for reperfusion injury. *Cardiovasc Res.* 2009;83:169–78.
75. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, et al. Postconditioning the human heart. *Circulation.* 2005;112:2143–8.
76. Thuny F, Lairez O, Roubille F, Mewton N, Rioufol G, Sportouch C, et al. Postconditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol.* 2012;59:2175–81.
77. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol.* 2003;285:H579–88.
78. Rodriguez-Sinovas A, Cabestrero A, Garcia DB, Inserte J, Garcia A, Garcia-Dorado D. Intracoronary acid infusion as an alternative to ischemic postconditioning in pigs. *Basic Res Cardiol.* 2009;104:761–71.
79. Inserte J, Ruiz-Meana M, Rodriguez-Sinovas A, Barba I, Garcia-Dorado D. Contribution of delayed intracellular pH recovery to ischemic postconditioning protection. *Antioxid Redox Signal.* 2011;14:923–39.
80. Inserte J, Barba I, Poncelas-Nozal M, Hernandez V, Agullo L, Ruiz-Meana M, et al. cGMP/PKG pathway mediates myocardial postconditioning protection in rat hearts by delaying normalization of intracellular acidosis during reperfusion. *J Mol Cell Cardiol.* 2011;50:903–9.
81. Andreaka G, Vertesaljai M, Szantho G, Font G, Piroth Z, Fontos G, et al. Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pigs. *Heart.* 2007;93:749–52.
82. Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, et al. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic preconditioning. *Am J Physiol Heart Circ Physiol.* 2007;292:H1883–90.
83. Hausenloy DJ, Iliodromitis EK, Andreadou I, Papalois A, Gritsopoulos G, Anastasiou-Nana M, et al. Investigating the signal transduction pathways underlying remote ischemic conditioning in the porcine heart. *Cardiovasc Drugs Ther.* 2012;26:87–93.
84. Shi W, Vinten-Johansen J. Endogenous cardioprotection by ischaemic postconditioning and remote conditioning. *Cardiovasc Res.* 2012;94:206–16.
85. Cohen MV, Downey JM. Ischemic postconditioning: from receptor to end-effector. *Antioxid Redox Signal.* 2011;14:821–31.
86. Padilla F, Garcia-Dorado D, Agullo L, Barrabes JA, Inserte J, Escalona N, et al. Intravenous administration of the natriuretic peptide urodilatin at low doses during coronary reperfusion limits infarct size in anesthetized pigs. *Cardiovasc Res.* 2001;51:592–600.
87. Bice JS, Keim Y, Stasch JP, Baxter GF. NO-independent stimulation or activation of soluble guanylyl cyclase during early reperfusion limits infarct size. *Cardiovasc Res.* 2014;101:220–8.
88. Ferdinandy P, Schulz R. Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. *Br J Pharmacol.* 2003;138:532–43.
89. Skyschally A, Schulz R, Heusch G, Cyclosporine A. at reperfusion reduces infarct size in pigs. *Cardiovasc Drugs Ther.* 2010;24:85–7.
90. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature.* 2005;434:658–62.
91. Abdallah Y, Gkatzoflia A, Gligorievski D, Kasseckert S, Euler G, Schluter KD, et al. Insulin protects cardiomyocytes against reoxygenation-induced hypercontracture by a survival pathway targeting SR Ca<sup>2+</sup> storage. *Cardiovasc Res.* 2006;70:346–53.
92. Jonassen AK, Aasum E, Riemersma RA, Mjos OD, Larsen TS. Glucose-insulin-potassium reduces infarct size when administered during reperfusion. *Cardiovasc Drugs Ther.* 2000;14:615–23.
93. Jonassen AK, Sack MN, Mjos OD, Yellon DM. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. *Circ Res.* 2001;89:1191–8.
94. Ravassa S, Zudaire A, Diez J. GLP-1 and cardioprotection: from bench to bedside. *Cardiovasc Res.* 2012;94:316–23.
95. Khan M, Meduru S, Mostafa M, Khan S, Hideg K, Kuppasamy P. Trimetazidine, administered at the onset of reperfusion, ameliorates myocardial dysfunction and injury by activation of p38 mitogen-activated protein kinase and Akt signaling. *J Pharmacol Exp Ther.* 2010;333:421–9.
96. Rodrigo R, Prieto JC, Castillo R. Cardioprotection against ischaemia/reperfusion by vitamins C and E plus n-3 fatty acids: molecular mechanisms and potential clinical applications. *Clin Sci (Lond).* 2013;124:1–15.

97. Dirksen MT, Laarman GJ, Simoons ML, Duncker DJ. Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. *Cardiovasc Res*. 2007;74:343–55.
98. Gerczuk PZ, Kloner RA. An update on cardioprotection: a review of the latest adjunctive therapies to limit myocardial infarction size in clinical trials. *J Am Coll Cardiol*. 2012;59:969–78.
99. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest*. 2013;123:92–100.
100. Rodriguez-Sinovas A, Abdallah Y, Piper HM, Garcia-Dorado D. Reperfusion injury as a therapeutic challenge in patients with acute myocardial infarction. *Heart Fail Rev*. 2007;12:207–16.
101. Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet*. 2013;381:166–75.
102. Darling CE, Solari PB, Smith CS, Furman MI, Przyklenk K. 'Postconditioning' the human heart: multiple balloon inflations during primary angioplasty may confer cardioprotection. *Basic Res Cardiol*. 2007;102:274–8.
103. Freixa X, Bellera N, Ortiz-Perez JT, Jimenez M, Pare C, Bosch X, et al. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur Heart J*. 2012;33:103–12.
104. Elzbieciak M, Wita K, Grabka M, Chmurawa J, Doruchowska A, Turski M, et al. Effect of postconditioning on infarction size, adverse left ventricular remodeling, and improvement in left ventricular systolic function in patients with first anterior ST-segment elevation myocardial infarction. *Pol Arch Med Wewn*. 2013;123:268–76.
105. Yang XC, Liu Y, Wang LF, Cui L, Wang T, Ge YG, et al. Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. *J Invasive Cardiol*. 2007;19:424–30.
106. Mewton N, Ivanès F, Cour M, Ovize M. Postconditioning. from experimental proof to clinical concept. *Dis Model Mech*. 2010;3:39–44.
107. Arai AE. Magnetic resonance imaging for area at risk, myocardial infarction, and myocardial salvage. *J Cardiovasc Pharmacol Ther*. 2011;16:313–20.
108. Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet*. 2010;375:727–34.
109. Candilio L, Malik A, Hausenloy DJ. Protection of organs other than the heart by remote ischemic conditioning. *J Cardiovasc Med (Hagerstown)*. 2013;14:193–205.
110. Zhong H, Gao Z, Chen M, Zhao J, Wang F, Li L, et al. Cardioprotective effect of remote ischemic postconditioning on children undergoing cardiac surgery: a randomized controlled trial. *Paediatr Anaesth*. 2013;23:726–33.
111. Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J, et al. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol*. 2006;47:2277–82.
112. Hausenloy DJ, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E, et al. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet*. 2007;370:575–9.
113. Munk K, Andersen NH, Schmidt MR, Nielsen SS, Terkelsen CJ, Sloth E, et al. Remote ischemic conditioning in patients with myocardial infarction treated with primary angioplasty: impact on left ventricular function assessed by comprehensive echocardiography and gated single-photon emission CT. *Circ Cardiovasc Imaging*. 2010;3:656–62.
114. Rahman IA, Mascaro JG, Steeds RP, Frenneaux MP, Nightingale P, Gosling P, et al. Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation*. 2010;122:S53–9.
115. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, et al. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet*. 2007;370:1483–93.
116. Thibault H, Piot C, Staat P, Bontemps L, Sportouch C, Rioufol G, et al. Long-term benefit of postconditioning. *Circulation*. 2008;117:1037–44.
117. Selker HP, Beshansky JR, Sheehan PR, Massaro JM, Griffith JL, D'Agostino RB, et al. Out-of-hospital administration of intravenous glucose-insulin-potassium in patients with suspected acute coronary syndromes: the IMMEDIATE randomized controlled trial. *JAMA*. 2012;307:1925–33.
118. Lonborg J, Kelbaek H, Vejlsstrup N, Botker HE, Kim WY, Holmvang L, et al. Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ Cardiovasc Interv*. 2012;5:288–95.
119. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med*. 2008;359:473–81.
120. Gao F, Gao E, Yue TL, Ohlstein EH, Lopez BL, Christopher TA, et al. Nitric oxide mediates the antiapoptotic effect of insulin in myocardial ischemia-reperfusion: the roles of PI3-kinase, Akt and endothelial nitric oxide synthase phosphorylation. *Circulation*. 2002;105:1497–502.
121. Opie LH, Bruyneel K, Owen P. Effects of glucose, insulin and potassium infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. *Circulation*. 1975;52:49–57.
122. Oates A, Nubani R, Smiley J, Kistler L, Hughey S, Theiss P, et al. Myocardial protection of insulin and potassium in a porcine ischemia-reperfusion model. *Surgery*. 2009;146:23–30.
123. Suranadi IW, Demaison L, Chate V, Peltier S, Richardson M, Leverve X. An increase in the redox state during reperfusion contributes to the cardioprotective effect of GIK solution. *J Appl Physiol*. 2012;113:775–84.
124. Diaz R, Paolasso EA, Piegas LS, Tajer CD, Moreno MG, Corvalán R, et al. ECLA (Estudios Cardiológicos Latinoamérica) Collaborative Group. Metabolic Modulation of Acute Myocardial Infarction. The ECLA Glucose-Insulin-Potassium Pilot Trial. *Circulation*. 1998;98:2227–34.
125. Ceremuzynski L, Budaj A, Czepliel A, Burzykowski T, Achremczyk P, Smielak-Korombel W, et al. Low-dose glucose-insulin-potassium is ineffective in acute myocardial infarction: results of a randomized multicenter Pol-GIK trial. *Cardiovasc Drugs Ther*. 1999;13:191–200.
126. Mehta SR, Yusuf S, Diaz R, Zhu J, Pais P, Xavier D, et al. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *JAMA*. 2005;293:437–46.
127. Timmers L, Henriques JP, De Kleijn DP, Devries JH, Kemperman H, Steendijk P, et al. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol*. 2009;53:501–10.
128. Ban K, Noyan-Ashraf MH, Hofer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*. 2008;117:2340–50.
129. Timmer JR, Van der Horst IC, Ottervanger JP, De Luca G, Van't Hof AW, Bilo HJ, et al. Glucose-insulin-potassium infusion as adjunctive therapy in myocardial infarction: current evidence and potential mechanisms. *Ital Heart J*. 2004;5:727–31.
130. Lonborg J, Vejlsstrup N, Kelbaek H, Botker HE, Kim WY, Mathiasen AB, et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J*. 2012;33:1491–9.
131. Mahaffey KW, Puma JA, Barbagelata NA, DiCarli MF, Leeser MA, Browne KF, et al. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial. *J Am Coll Cardiol*. 1999;34:1711–20.
132. Ross AM, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMIS-TAD-II). *J Am Coll Cardiol*. 2005;45:1775–80.
133. Lee CH, Low A, Tai BC, Co M, Chan MY, Lim J, et al. Pretreatment with intracoronary adenosine reduces the incidence of myonecrosis after non-urgent percutaneous coronary intervention: a prospective randomized study. *Eur Heart J*. 2007;28:19–25.
134. Garcia-Dorado D, Otaegui I, Rodríguez Palomares JF, Evangelista E, Pineda V, Ruiz Salmerón R, et al. Primary results of the PROMISE trial: myocardial protection with intracoronary adenosine given before reperfusion in patients with STEMI. *Eur Heart J*. 2014;34 Suppl 1:669.
135. Ghaffari S, Kazemi B, Toluay M, Sepehrvand N. The effect of prethrombolytic cyclosporine-A injection on clinical outcome of acute anterior ST-elevation myocardial infarction. *Cardiovasc Ther*. 2013;31:e34–9.
136. MITOCARE Study Group. Rationale and design of the MITOCARE Study: a phase II, multicenter, randomized, double-blind, placebo-controlled study to assess the safety and efficacy of TRO40303 for the reduction of reperfusion injury in patients undergoing percutaneous coronary intervention for acute myocardial infarction. *Cardiology*. 2012;123:201–7.
137. Ibanez B, Prat-Gonzalez S, Speidl WS, Vilahur G, Pinero A, Cimmino G, et al. Early metoprolol administration before coronary reperfusion results in increased myocardial salvage: analysis of ischemic myocardium at risk using cardiac magnetic resonance. *Circulation*. 2007;115:2909–16.
138. Ibanez B, Macaya C, Sánchez-Brunete V, Pizarro G, Fernández-Friera L, Mateos A, et al. Effect of early metoprolol on infarct size in ST-segment-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention: the Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) trial. *Circulation*. 2013;128:1495–503.
139. Ndrepepa G, Kastrati A. Intravenous beta-blockers in primary percutaneous coronary intervention: new hope for an old therapy. *Circulation*. 2013;128:1487–9.
140. Klein HH, Pich S, Bohle RM, Lindert-Heimberg S, Nebendahl K. Na(+)/H(+) exchange inhibitor cariporide attenuates cell injury predominantly during ischemia and not at onset of reperfusion in porcine hearts with low residual blood flow. *Circulation*. 2000;102:1977–82.
141. Rodríguez-Sinovas A, García-Dorado D, Padilla F, Inserte J, Barrabés JA, Ruiz-Meana M, et al. Pre-treatment with the Na<sup>+</sup>/H<sup>+</sup> exchange inhibitor cariporide delays cell-to-cell electrical uncoupling during myocardial ischemia. *Cardiovasc Res*. 2003;58:109–17.
142. Ruiz-Meana M, García-Dorado D, Pina P, Inserte J, Agullo L, Soler-Soler J. Cariporide preserves mitochondrial proton gradient and delays ATP depletion in cardiomyocytes during ischemic conditions. *Am J Physiol Heart Circ Physiol*. 2003;285:H999–1006.
143. Théroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) Investigators. *Circulation*. 2000;102:3032–8.

144. Ludman AJ, Yellon DM, Hasleton J, Ariti C, Babu GG, Boston-Griffiths E, et al. Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention: a randomised controlled clinical trial. *Heart*. 2011;97:1560–5.
145. Cyrus T, Mathews SJ, Lasala JM. Use of mechanical assist during high-risk PCI and STEMI with cardiogenic shock. *Catheter Cardiovasc Interv*. 2010;75 Suppl 1: S1–6.
146. Bates E, Bode C, Costa M, Gibson CM, Granger C, Green C, et al. Intracoronary KAI-9803 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction. *Circulation*. 2008;117: 886–96.
147. Mahaffey KW, Granger CB, Nicolau JC, Ruzyllo W, Weaver WD, Theroux P, et al. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction: the COMPLEMENT inhibition in myocardial infarction treated with thromboLYtics (COMPLY) trial. *Circulation*. 2003;108:1176–83.
148. Forster K, Kuno A, Solenkova N, Felix SB, Krieg T. The delta-opioid receptor agonist DADLE at reperfusion protects the heart through activation of pro-survival kinases via EGF receptor transactivation. *Am J Physiol Heart Circ Physiol*. 2007;293:H1604–8.
149. Yang XM, Liu Y, Cui L, Yang X, Liu Y, Tandon N, et al. Platelet P2Y<sub>12</sub> blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther*. 2013;18:251–62.
150. D'Annunzio V, Donato M, Erni L, Miksztowicz V, Buchholz B, Carrion CL, et al. Rosuvastatin given during reperfusion decreases infarct size and inhibits matrix metalloproteinase-2 activity in normocholesterolemic and hypercholesterolemic rabbits. *J Cardiovasc Pharmacol*. 2009;53:137–44.