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Lesões de Isquemia-Reperfusão Miocárdica

Myocardial Ischemia-Reperfusion Injury

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TÍTULO MONOGRAFIA

Lesões de isquemia-reperfusão miocárdica

ORIENTADOR

Prof. Dr. Manuel Joaquim Lopes Vaz da Silva

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
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Faculdade de Medicina da Universidade do Porto, 20/03/2019

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Título: Lesões de Isquemia-Reperfusão Miocárdica

Myocardial Ischemia-Reperfusion Injury

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Resumo:

A Síndrome Coronária Aguda, nomeadamente o Enfarte Agudo do Miocárdio, é, nos dias que correm, não só responsável por afetar um elevado número de doentes como por causar incapacidade e morte dos mesmos. Na Europa, segundo o European Society of Cardiology Atlas - *Cardiovascular Disease Statistics 2017*, a doença coronária foi a principal causa de morte, responsável por 1,7 milhões de mortes/ano (20% de todas as mortes). O tratamento desta patologia passa por impor um limite à isquemia o mais precocemente possível. Atualmente, são reconhecidas como principais meios terapêuticos a Intervenção Coronária Percutânea e a trombólise.

O desafio atual envolvendo a terapêutica do enfarte é atuar no passo seguinte: evitar ou controlar a lesão que é causada pela própria reperfusão, na qual também há morte de cardiomiócitos (em alguns casos responsável por cerca de 50% do total da necrose final).

Nesta revisão, serão apresentados os mecanismos fisiopatológicos da lesão de isquemia-reperfusão, abordadas as intervenções clínicas e terapêuticas com resultados mais promissores até à data, do ponto de vista de uma perspetiva evolucional e descritos os fármacos que mais recentemente têm sido estudados e outros que se encontram ainda numa fase pré-clínica e que poderão ser possíveis ferramentas em áreas de investigação clínica futura.

Palavras-Chave: Síndrome Coronária Aguda; Lesão de Isquemia-Reperfusão; Enfarte Agudo do Miocárdio; Cardioproteção; Intervenção Coronária Percutânea.

Abstract:

Nowadays, Acute Coronary Syndrome, namely Acute Myocardial Infarction, is not only responsible for affecting a large number of patients, but also for causing their disability and death. According to the European Society of Cardiology Atlas - *Cardiovascular Disease Statistics 2017,* coronary heart disease was the leading cause of death, accounting for 1.7 million deaths / year (20% of all deaths). The treatment for this pathology involves limiting ischemia as early as possible. Percutaneous Coronary Intervention and thrombolysis are currently recognized as the main therapeutic means used for that purpose.

The current challenge around infarct therapy is to act on the next step: avoid or control the injury that is caused by the reperfusion itself, in which there is also death of cardiomyocytes (in some cases responsible for about 50% of the total final necrosis).

In this review, we will present the pathophysiological mechanisms of ischemia-reperfusion injury, the clinical and therapeutic interventions with the most promising results to date, from an evolutionary perspective, the drugs that have been studied more recently and others that are still at a pre-clinical stage and that may be possible tools in future clinical research areas.

Keywords: Acute Coronary Syndrome; Myocardial Ischemia-Reperfusion Injury; Acute Myocardial Infarction; Cardioprotection; Percutaneous Coronary Intervention.

List of Abbreviations:

ACS	Acute Coronary Syndrome
AMI	Acute Myocardial Infarction
ANP	Atrial Natriuretic Peptide
AAR	Area at Risk
CMR	Cardiac Magnetic Ressonance
CK	Creatine Kinase
CK-MB	Creatine Kinase-Muscle/Brain
CsA	Ciclosporine A
DAMPS	Damage associated molecular patterns
ERK	Extracellular signal-regulated kinases
GLP1	Glucagon-like peptide-1
GLP1-R	GLP1 receptor
cGMP	Cyclic guanosine monophosphate
IC	Ischemic Conditioning
IPC	Ischemic Preconditioning
IPost	Ischemic Postconditioning
IRI	Ischemia-Reperfusion Injury
LV	Left Ventricle
LVEF	Left Ventricle Ejection Fraction
mPTP	Mitochondrial Permeability Transition Pore
mPTPi	Mitochondrial Permeability Transition Pore inhibitor
MEK	Mitogen-activated protein kinase kinase
MVO	Microvascular Obstruction
NO	Nitrous Oxide
PCI	Percutaneous Coronary Intervention
PI3K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
PKG	Protein Kinase G
RIC	Remote Ischemic Conditioning
ROS	Reactive oxygen species
RISK	Reperfusion Injury Signalling Kinase
SAFE	Survival Activating Factor Enhancement
SR	Sarcoplasmatic Reticulum
STEMI	ST elevation myocardial infarction
TnI	Troponin I

List of figures:

List of tables

Index

Introduction

Given the prevalence of acute coronary syndrome (ACS) and its impact in the worldwide morbidity and mortality, it is important to understand that the damage caused to the myocardium during acute myocardial infarction (AMI) is the result of both ischemia and subsequent reperfusion: ischemia-reperfusion injury (IRI).

After an AMI, the objective is to reduce myocardial necrosis, minimize myocardial IRI, recover viable myocardium, and limit the area at risk for infarction. For that, the best treatment is the adequate timely execution (especially in the first two hours after the onset of symptoms)[1, 2] of primary percutaneous coronary intervention (PCI).[3] As *Yellon and Hausenloy* state, the myocardial reperfusion process, can paradoxically cause injury and induce the death of cardiomyocytes the so called "myocardial ischemia-reperfusion injury" (IRI) and it is knowns that this injury can contribute up to 50% of the final AMI size.[4] As such, and according to *Cung* et al, the mortality and morbidity following AMI remain significant with 7% mortality and 22% death at one year, respectively.[5]

It is accepted that therapies to limit ischemia are currently a great success, so it is time to focus efforts on therapies to reduce myocardial ischemia-reperfusion injury (IRI), developing a strategy capable of promoting cardioprotection against this damage. Another concern to keep in mind is the timing; this subject will be addressed ahead in this review.

As stated earlier, early reperfusion using PCI is the most effective way to limit injury/necrosis. Until reperfusion therapy is instituted, the cellular death due to ischemia increases, reaching the total area at risk (AAR) (Figure 1).

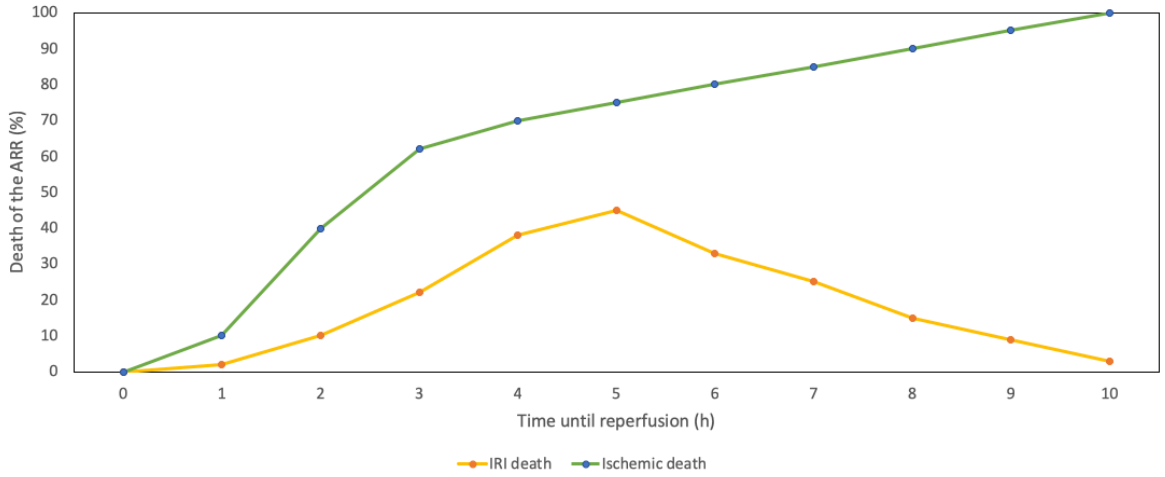

Figure 1 Proportion of myocardial death of the area at risk attributable to ischemia and myocardial ischemia-reperfusion injury (IRI) as a function of time, in hours (h). Taken and adapted from *Bainey, Armstrong* (2014).[1, 2]

Two hours after the onset of symptoms, progression of the ischemic lesion is increasing, and cell death due to IRI is relatively low.

In the first two hours, the myocytes are dying mainly because of ischemia. After this period, and up to 8 hours after the onset of symptoms, reperfusion therapy, although strictly necessary to reduce ischemic injury, produces a greater reperfusion injury, and, as previously said, the relative weight of this type of injury in the area of the end infarction can reach 50%.[4]

In this period of time (2 to 8 hours after the onset of symptoms) myocytes die because of two causes: ischemia and reperfusion.

It is important to understand that when reperfusion is initiated promptly within the first 2 hours, we have the best results of myocardial recovery. This way, the ischemic lesion is minimized, although there is still room for reperfusion injury. Since we are minimizing the ischemic lesion, we are already doing our best to globally reduce IRI. The longer the ischemic time, the greater the chance that reperfusion injury will limit myocardial salvage, thus accentuating myocardial necrosis. So, we have a potential window for cardioprotection that begins after the first 2 hours until 8 to 12 hours after the onset of symptoms and this will be the best time window for intervention at myocardial IRI level, maximizing the results of reperfusion therapy and protecting more cardiomyocytes.[1] In the practical field, ideally, cardioprotective measures should be attempted before PCI, this way therapeutic concentrations

at the time of reperfusion will be achieved. What happens is that this is difficult sometimes because we don't want to delay life-saving procedures like PCI.

Currently, since there is no targeted-therapy for primarily treating IRI following ischemia, the main treatment is supportive. This is an active area of investigation in Cardiology and one of the biggest needs, considering the dimension of AMI worldwide and its devastating effects.

In this review, we aim to describe the actual known and promising therapies to reduce IRI based on the cellular and molecular mechanisms involved in its pathophysiology, focusing in the most relevant cardioprotection mechanisms.

Methods

The literature research was performed in Pubmed, Scopus and Web of Science databases, without date restrictions, with the terms "myocardial ischemia-reperfusion injury" (OR) "cardioprotection". Review articles and experimental articles written in English were selected after reading the abstract. Other articles were then added from the reference lists of studies included in the reviews. There was no restriction on the type of study.

Acute Myocardial Infarction impact: Epidemiology

In Europe, over the last three decades, there has been a reduction in mortality by ischemic heart disease, but this is still the most common cause of death worldwide and its prevalence is increasing. Ischemic heart disease accounts for almost 1.8 million annual deaths, or 20% of all deaths in Europe, although with large variations between countries.[6]

Despite the decline in acute and long-term death associated with ST elevation myocardial infarction (STEMI), associated with the event of reperfusion, mortality remains substantial. The in-hospital mortality rates of unselected patients with STEMI in national

European registries vary between 4–12%[7], and as said before, this is still the single most common cause of death worldwide. Morbidity following STEMI, remains significant, even in PCI era, with 22% heart failure hospitalization at 1 year in patients presenting with an anterior STEMI.[5] The patients live longer, but with larger amounts of comorbidities.[8]

Since the treatment for IRI is primarily supportive, we aren't able to avoid it or treat it properly yet. Myocardial IRI is still a leading cause for events in the field of cardiovascular disorders and a considerable contributor to the comorbidities and deaths associated with coronary occlusion. It is the result of the combination of substances accumulating during the lack of perfusion from the coronary arteries and those which are release on reperfusion.[9]

Infarct size measured by Cardiac Magnetic Resonance (CMR) imaging is nowadays the recommended technique for measuring infarct size in STEMI trials (and every day clinic when needed), and MI size acutely and chronically should be assessed, as well LVEF, as a functional parameter, since it is the one of the best option because of its association with long-term mortality and morbidity after STEMI.[10]

Myocardial ischemia-reperfusion injury: Pathophysiology

It is crucial to get to know the mechanisms on which myocardial IRI is based; only running this route we are able of think of strategies to target ischemic and reperfusion injury that can reduce infarct size and improve functional parameters of the heart, leading to improvement of the cardiac function on a long-term analyses and less comorbidities and deaths.[10]

Coronary Circulation and Microvascular Obstruction

Myocardial IRI is a complex process, which starts on circulation and have repercussion on a cellular level. When there is a thrombus causing ischemia, there are platelets being

activated, aggregated and adhering to the atheromatous plaque, causing the thrombus (rich in platelets and fibrin) to clot the artery. This way, ischemia is installed, and what happens when we perform reperfusion is a combination of phenomena that end up causing myocardial IRI and its consequences (Figure 2).

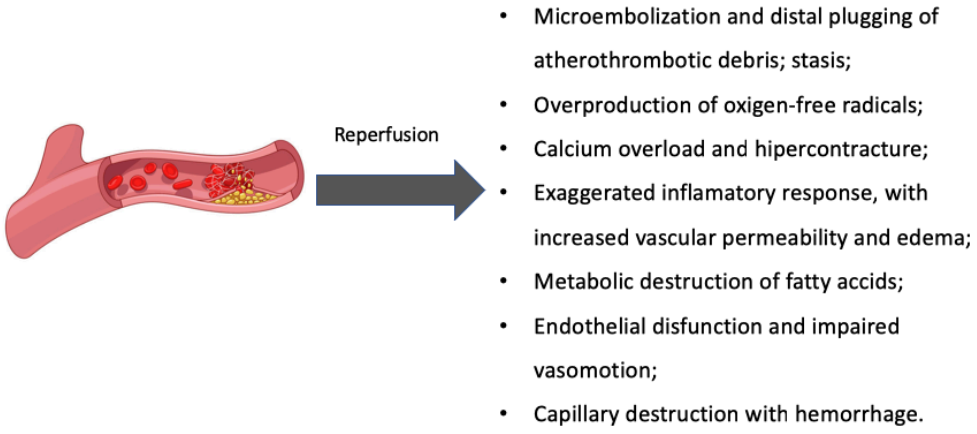

Figure 2 Pathophysiologic events leading to myocardial reperfusion injury after reperfusion of an occluded coronary artery by a thrombus. Taken and adapted from *Bainey, Armstrong* (2014).[1]

The reperfused myocardium have contraction bands, karyolysis (dissolution of the cell nucleus), swelling and disruption of the mitochondria, and rupture of the membrane structure of the myocytes, accompanied by microvascular destruction/microembolization-obstruction (MVO) (Table 1), hemorrhage on the interstitium, and inflammation.[11, 12] This is what happens on the coronary circulation and on a cellular level after the myocardial IRI.

Even if the blood supply is efficiently and rapidly reestablished to the epicardial artery, if MVO occurs, the cardiomyocytes will stay unproperly reperfused. Myocardial infarction and MVO are parallel phenomena which result from mechanisms with the same *rationale*: first there is a deficit in energy and then there is an accumulation of reactive oxygen species (ROS) during reperfusion; this way, irreversible damage to the myocardium and the coronary microcirculation are closely related.[13] MVO has inclusively the potential for causing more cardiomyocyte necrosis and it is associated with worse outcomes in patients with AMI.[14, 15]

During IRI, MVO is an important predisposing factor that determines infarct size; it is also an independent predictor of morbidity/mortality.[16]

On the clinical setting, MVO is seen in 10% to 30% of patients with reperfused STEMI[17, 18] even if successful blood supply to the epicardial coronary arteries is restored; MVO is assessed either by CMR imaging or by angiography. In this last technique what we see if MVO occurs is a slow or no reflow of the contrast, that's why MVO can also be called no-reflow phenomenon.

Table 1 Mechanisms contributing to MVO.

1	Embolization of small debris from the ruptured culprit atherosclerotic lesion, with physical obstruction of the coronary microcirculation.[14]
2	Platelet and platelet/leukocyte aggregates that are released from the site of the culprit lesion, form in the coronary microcirculation, or arrive with the blood flow, where they form as part of the general inflammatory status associated with STEMI.[19]
3	Intense vasoconstriction induced by soluble vasoconstrictor substances released from the culprit lesion.[20][21]
4	Extravascular coronary microvascular compression due to edema in the surrounding myocardium.[22]
5	Primary physical destruction of the capillary endothelium.[23]
6	High intramyocardial pressure, with a predominant contribution from edema, might be the principal cause of MVO in the endocardial layer.[24]

Taken and adapted from *Borja Ibáñez* et al (2015).[10]

Intracellular and molecular mechanisms

Myocardial IRI encompasses a complexity of processes that involve and lead to a reduction in adenosine triphosphate (ATP) in the cell, raise of hydrogen (H^+) ions, calcium (Ca^{2+}) oscillations and reactive oxygen species (ROS) (Figure 3). All these phenomena combined act to the direction of causing cellular damage and cardiomyocyte death.[9]

At first, when ischemia is installed, since there is a lack of oxygen, oxidative phosphorylation in the mitochondria stops and this automatically leads to a decrease in ATP levels. In response to this insult, the affected cardiomyocytes start anaerobic glycolysis and only by this way, they are able to produce ATP; on the other hand, this alternative way of producing ATP leads to the accumulation of protons and lactate, causing intracellular acidosis.

There is a raise in intracellular H^+ which activates the Na^+/H^+ exchanger in the plasma membrane; this exchanger puts H^+ out of the cell in exchange for Na^+, whose levels in the cytoplasm increase. The Na^+/K^+-ATPase has its activity diminished due to the excess of H^+ and insufficient ATP during ischemia, and this pump' inhibition also increases the Na^+ levels inside the cell. This raise in Na^+ levels inside the cardiomyocytes activate the Na^+/Ca^{2+} exchanger[25] in the Sarcoplasmatic Reticulum (SR) and this leads also to a raise in levels of intracellular Ca^{2+} (Figure 3).

Next, when myocardium is reperfused, oxidative phosphorylation in the mitochondria is restored as well as ATP synthesis. Since anaerobic glycolysis is no more needed because there is oxygen again, pH inside the cardiomyocyte returns to normal, and this activates a Ca^{2+} dependent protease, called calpain, whose function is to break the cytoskeleton and the sarcolemma of the cell[26], causing its death.

The raise in ATP levels during reperfusion concomitantly with high Ca^{2+} levels activates SR, which causes uptake of Ca^{2+}; this causes saturation of ryanodine channels, that in response release Ca^{2+} to the cytoplasm. The repetition of this process in a cyclic way leads to Ca^{2+} oscillations that ultimately cause hypercontraction of the myofibrils in a chaotic way[27] and also leads mitochondrial permeability transition pore (mPTP) to open (Figure 3).[28]

The aperture of mPTP leads mitochondrial matrix to swell and this causes rupture of the outer membrane of the mitochondria and release of contents such as cytochrome c, that was accumulated in the mitochondrial intermembrane space, into the cytoplasm. Cytochrome *c* activates the caspase cascade leading to programmed cell death. The Ca^{2+} oscillations also promote the activation of xanthine oxidases responsible for ROS production; these molecules cause more injury to the membrane because they directly open mPTP[29]; this way they contribute to cell death during reperfusion[30] (Figure 3). ROS is also produced by NADPH oxidase and mitochondrial electron transport chain reactions in injured cardiomyocytes, endothelial cells and neutrophils in the ischemic zone, that we've talked before.[31]

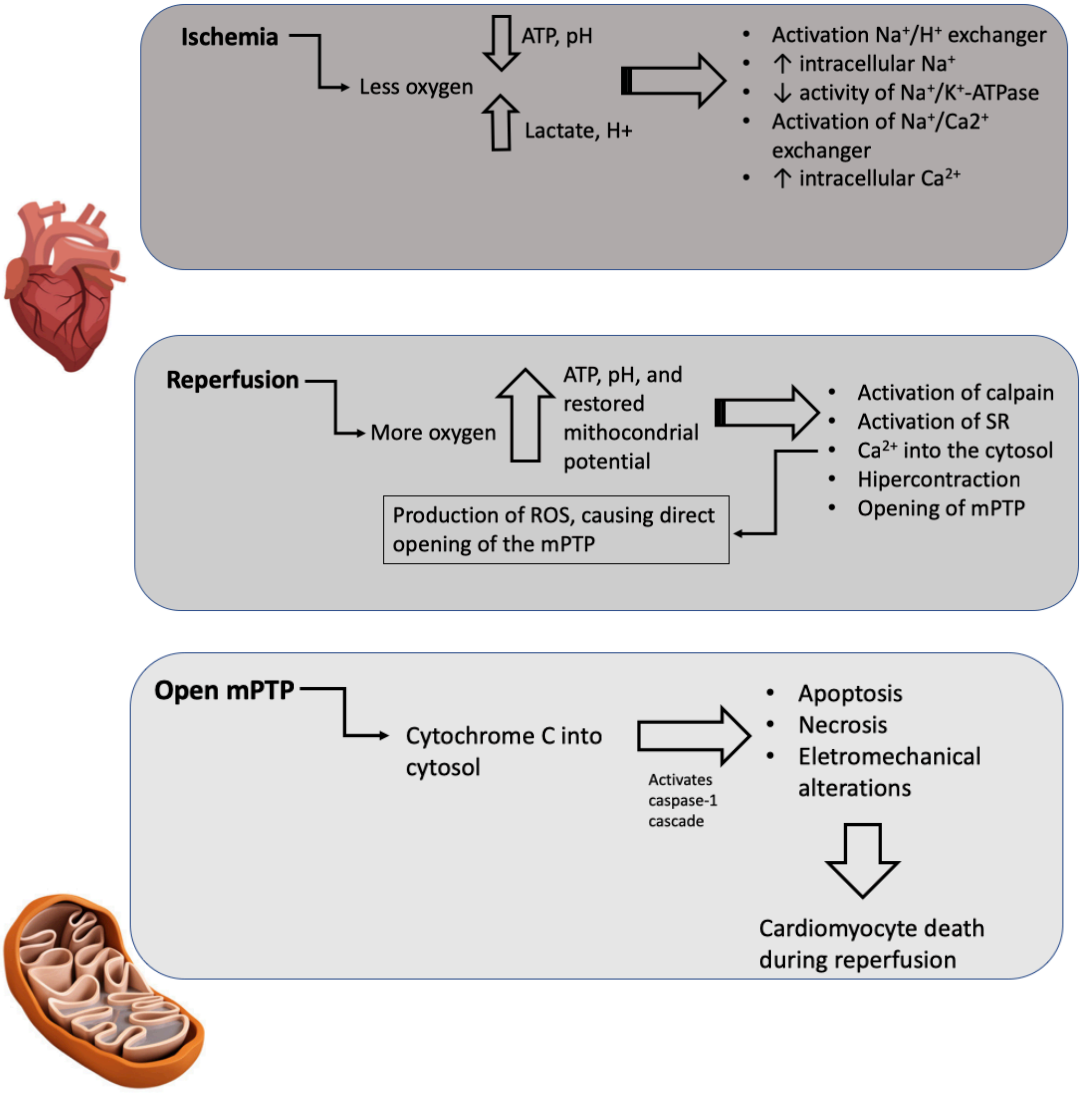

Figure 3 The main intracellular events of myocardial IRI, including mechanisms culminating in the opening of the mPTP. mPTP: mitochondrial permeability transition pore; ROS: reactive oxygen species; SR: sarcoplasmatic reticulum. Taken and adapted from *Xianchi* et al (2016).[9]

The high Ca^{2+} levels and the dissociation that occurs in the oxidative chain lead to necrosis; cytochrome-C activates the caspase cascade, and this leads to apoptosis; in addition, Ca^{2+} in excess causes stimulation of apoptotic factors to be released through the external mitochondrial membrane.

The normal pH after reperfusion is also more favorable to mPTP opening, causing more cell death.[32] Summarizing , the main stimuli for the opening of mPTP in myocardial reperfusion are alkalization of pH[32], Ca^{2+} overload[33] and ROS activity[34].

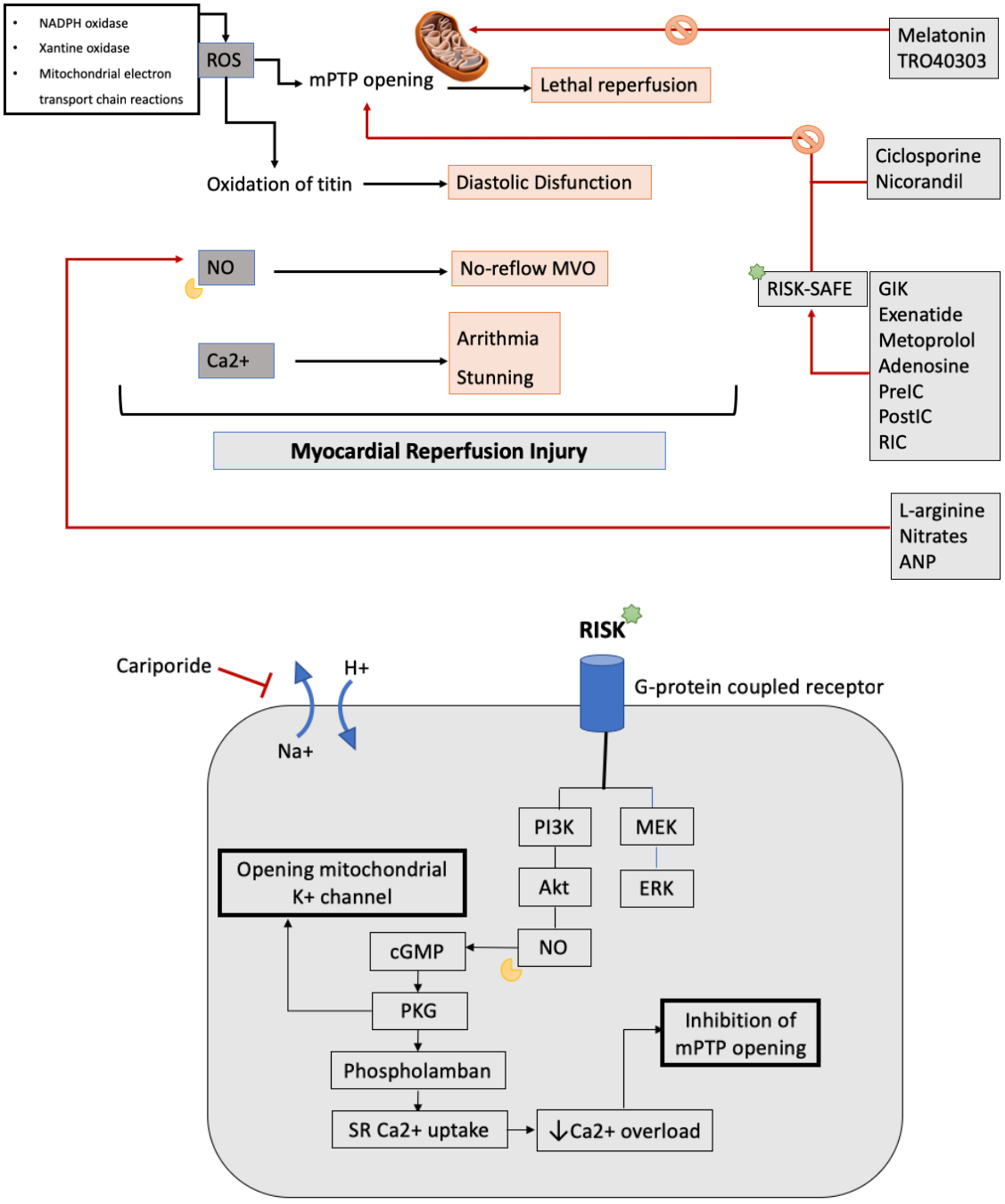

Figure 4 Stakeholders in myocardial reperfusion injury, its clinical implications and pharmacological approaches to prevent it at intracellular level. ANP: atrial natriuretic peptide; GIK: Glucose/Insulin/Potassium; mPTP: mitochondrial permeability transition pore; MVO: microvascular obstruction; NO: nitric oxide; PreIC: ischemic preconditioning; PostIC: ischemic postconditioning; RIC: remote ischemic conditioning; RISK: Reperfusion injury salvage kinase pathway; ROS: reactive oxygen species; SAFE: Survivor activating factor enhancement pathway; SR, sarcoplasmatic reticulum. Taken and adapted from *Jaime González-Montero* et al, 2018.[35]

The RISK pathway is a combination of two parallel cascades, PI3K-Akt and MEK1-ERK1/2, which target mainly the mitochondria, that meets a group of pro-survival protein kinases. When these are activated, specifically at the time of reperfusion, they confer cardioprotection[36, 37] because they cause mitochondrial K+ channel[38] opening and mPTP[39]

closure (Figure 4). This pathway is therefore considered a universal signaling cascade shared by most cardioprotective therapies.

The RISK pathway can be stimulated during ischemic conditioning, but also by insulin, bradykinin, adenosine or statins.[4, 40]

In addition to the RISK pathway, other signaling cascades mediate cardioprotective effect:[41] the Survivor Activating Factor Enhancement (SAFE) and the NO/cGMP/PKG pathway.[42] The three of them inhibit mPTP opening.

Synthesizing, the intracellular targets to reach myocardial IRI can include pro-survival signaling pathways (e.g., the RISK, SAFE, and NO-cGMP-PKG cascades), cell death pathways (e.g., necrosis, apoptosis), and cellular organelles (e.g., mitochondria, sarcoplasmic reticulum).[43]

All these interactions are summarized in Figure 4.

Inflammation

During AMI, the ischemia causes the formation of inflammasomes, which are large protein complexes, released to the cytoplasm in reaction to DAMPS (damage associated molecular patterns, that include, for example ATP and RNA). They cause activation of pro-inflammatory cytokines, caspase-1 (inflammasomes contain pro-caspase 1) dependent death of cardiomyocytes (pyroptosis, characterized by apoptosis and necrosis - highly inflammatory form of programmed cell death), reviewed in van Hout et al, 2016)[44] and death of the cardiomyocytes through Toll-like receptors. They also recruit leucocytes into the infarct area and mediate mitochondrial disfunction by calcium overload and ROS production,[45] molecules already talked in the context of myocardial IRI.

Therapeutic approach

Cardioprotective measures targeting IRI have not yet been successful and remain one of the biggest clinical needs in the whole field of Cardiology.

Administering a cardioprotective measure before reperfusion by PCI can be a challenge, because the reperfusion should never be delayed. This way we would increase the ischemic lesion.

Cardioprotective treatments for IRI should be applied as early as possible, during reperfusion because most cell death occurs during the first minutes of reflow.

They can be classified into 4 different categories,[43] according to their main mechanisms, time scheduled and potential targets:

1 - Protective modality; 2 - Time of application; 3 - Cellular target; 4 - Intracellular target (Figure 5).

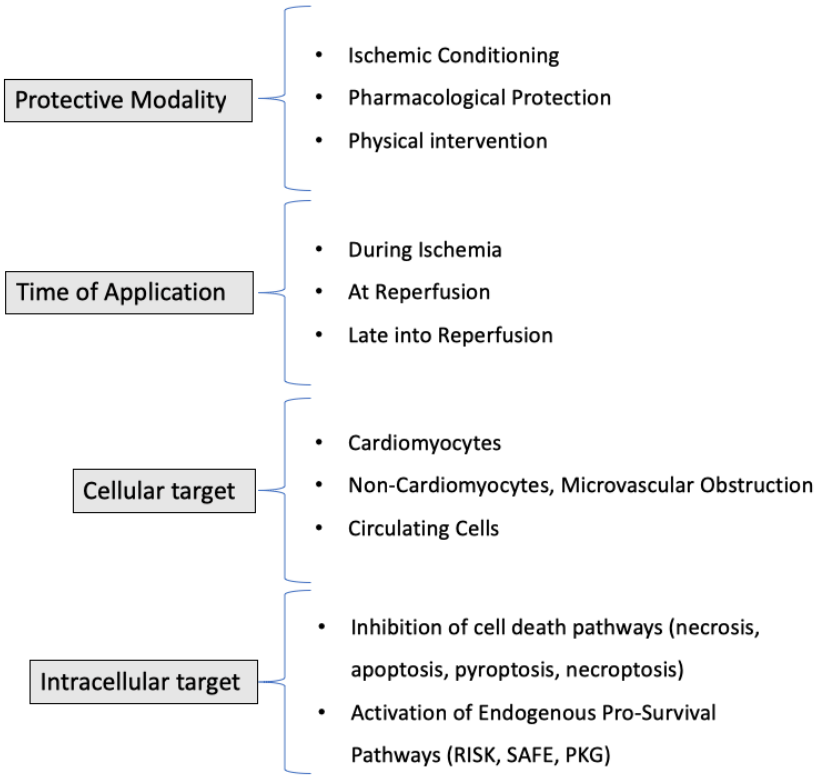

Figure 5 The 4 main strategies to control the negative implications of myocardial IRI. Herein, the strategies related to ischemic conditioning (pre, post and remote conditioning) and the main drugs used according to their mechanism of action/pathophysiological pathway will be addressed, whether used alone or in combination (multitarget strategy). Taken and adapted from *Davidson* et al (2019).[43]

Protective modality *(Ischemic conditioning and hypothermia)*

IPC (ischemic preconditioning) is a strong form of *in vivo* protection against myocardial ischemic injury. A period of ischemia prior to a cardiac surgery, for example, protects the heart from more prolonged episodes of ischemia, reducing the infarct size and reperfusion-induced arrhythmias; although the obvious advantages, these are limited when we talk about acute myocardial infarction (AMI) because of the unexpected character of this disease.

The most recent meta-analysis on protective modalities used CMR imaging data to evaluate ischemic post-conditioning (IPost) and its effects on 1069 patient with STEMI infarction treated with thrombolysis; the study showed that local IPost and remote IPost improve myocardial salvage and decreases myocardial edema in STEMI patients without affecting final infarct size, left ventricular volume or microvascular obstruction.[50]

Thus, these days, and according to the data in Table 2 (where we can find the main mechanisms involved in ischemic conditioning and hypothermia, its consequences and studies) neither IPC, IPost or RIC have a place in the prevention of IRI, unless the protocols and timing of application were modified.

Table 2 The main mechanisms involved in ischemic conditioning and hypothermia, its consequences and outcomes.

Protective modality	Mechanisms/ Consequences	Outcomes	References
Ischemic pre-conditioning (IPC)	Brief coronary occlusion releases ligands for adenosine, bradykinin, opioid, and sphingosine. These four receptors act in an additive fashion. Blocking a single receptor subtype only raises the ischemic threshold for protection.[36] Delays in recovery of pH, prevents uncoupling of nitric oxide synthases (NOS) and subsequent generation of ROS, and increases PKG, RISK and SAFE signaling in reperfused cardiomyocytes.[46]	Studies showed that PKC and K_{ATP} in the mitochondria could be stimulated by diazoxide (pharmacological preconditioning). These are also in the IPC signalling pathway, which is not fully understood.[36]	*Hausenloy* et al (2016)[36] *Inserte* et al (2013)[46]

Post-conditioning (IPost)	Brief cycles of ischemia/reflow soon after the damaging prolonged ischemia followed by reperfusion.[47]	After reperfusion had already taken place failed to reduce MI size.[48] *Favaretto* et al (2014)[49] showed that CMR evaluation of postconditioning effect on infarct size led to the disappearance of the presumed benefit on reduction of infarct size. The same was observed in *Lou* et al (2018).[50]	*Kin* et al (2004)[48] *Favaretto* et al (2014)[49] *Lou* et al (2018)[50]
Remote ischemic conditioning (RIC)	Brief episodes of non-lethal ischemia and reperfusion to another organ or tissue (e.g., lower limb).[51] Share with IPC preservation of PKG[52], action on mitochondrial function and activation of the RISK and SAFE pathways.[41, 53]	The infarct-size-limiting effects of RIC have been shown to be comparable to the effects of ischemic post-conditioning.[54]	*Haunsenloy* et al (2008)[51] *Gritsopoulos* et al (2009)[54] *Alburquerque-Bejar* et al (2015)[52] *Kleinbongard* et al (2017)[53] *Heusch* (2015)[41]
Physical intervention: Hypothermia (may also reach ischemic myocardium when applied before reperfusion); Neural stimulation	Limitation of metabolic disorders, mitochondrial dysfunction, ROS production, inflammatory mediators and effectors, ionic disorders, including Ca^{2+}, apparently through the inhibition of Na+/ Ca^{2+} exchanger.	120 patients with STEMI: PCI + EV infusion of cooled saline or only PCI - 38% decrease in infarct size, by CMR imaging.[55] In 2014, *Erlinge* et al were unable to find differences for the same primary outcome, but found incidence of heart failure at 45 days.[56]	*Gotberg* et al (2010)[55] *Erlinge* et al (2014)[56]

CMR: cardiac magnetic resonance; IPC: ischemic preconditioning; IPost: ischemic postconditioning; PCI: percutaneous coronary intervention; PKC: protein kinase C; PKG: protein kinase G; RIC: remote ischemic conditioning; RISK: Reperfusion injury salvage kinase pathway; ROS: reactive oxygen species; SAFE: Survivor activating factor enhancement pathway; STEMI: ST elevation myocardial infarction.

Drugs

When talking about potential drugs to myocardial IRI, it is important, on a first approach to keep in mind that, in patients with STEMI, if these drugs are given after coronary artery occlusion, they may not be able to reach the area of ischemia, where they would be able to do their function. However, since we have potent antiplatelet agents, there is already a considerable percentage of AMI that was partially reperfused prior to PCI. At present, primary PCI is performed in a large number of patients with STEMI in many centers, which provides a unique opportunity to institute intracoronary therapy to reduce / control IRI (even investigational drugs - phase I clinical trials), thus exceeding the difficulty of the drug reaching its target.

The main drugs tested for efficacy and safety and the main signaling pathways in the attempt to avoid/control myocardial IRI in experimental animal models (Table 3) and in clinical trials are shown in Figure 4 and Tables 4 (a-d).

Table 2 Main animal experimental studies testing for drugs attempting to avoid/control myocardial IRI and its main signaling pathways.

Study	Drug (mechanism of action)	Protocol/Characteristics	Results/outcomes
***Yamada* et al (2000)[57]**	SM-20550 (Na^+/H^+ exchanger inhibitor: by inhibiting the Na^+/H^+ exchanger, consequently inhibits the Na^+/ Ca^{2+}, that favors the calcium oscillations that lead to injury)	Rabbits were administrated SM-20550 or nicorandil before and after ischemia.	IV SM-20550 pre-ischemia reduced the infarct size by 30–70%. IV nicorandil pre-ischemia reduced the infarct size by 33%. IV SM-20550 post-ischemia reduced the infarct size by 20–40%. IV nicorandil post-ischemia did not reduce the infarct size.
***Padilla* et al (2001)[58]**	Urodilatin (NO-cGMP-PKG cascade activator: activation of PKG, opening of mKATP, and stimulation of downstream kinases)	Isolated rat hearts (n=62) receiving different Urodilatin concentrations during initial reperfusion.	At 0.05 microM, Urodilatin increased myocardial cGMP to 111% of values in normoxic hearts, improved functional recovery (P=0.01) and reduced peak LDH released by 40% (P=0.02).
***Yang* et al (2006)[59]**	ANP (NO-cGMP-PKG cascade activator)	Isolated rabbit hearts: 20-min infusion of ANP (0.1 microM) starting 5 min before 2 h of reperfusion.	ANP infusion decreased infarction from 31.5±2.4% of the risk zone in untreated hearts to 12.5±2.0% (P<0.001).
***Ibanez* et al (2011)[60]**	Metoprolol (Beta-blocker acts through the RISK pathway)	Reperfused anterior AMI was induced in 30 male Yorkshire Albino pigs: Pre-reperfusion-metoprolol, post-reperfusion-metoprolol or placebo.	The intravenous administration of metoprolol before coronary reperfusion results in larger myocardial salvage than its oral administration initiated early after reperfusion.

AMI: acute myocardial infarction; ANP: atrial natriuretic peptide; cGMP: Cyclic guanosine monophosphate; LDH: lactate dehydrogenase; NO: nitric oxide; PKG: protein kinase G; RISK: Reperfusion injury salvage kinase pathway.

Considering Na^+/H^+ exchange inhibitors, they consequently inhibit the Na+/ Ca2+ exchanger mechanism inhibiting also the calcium oscillations that lead to myocardial IRI. SM-20550 (when compared with nicorandil) was beneficial when given in post-ischemia period. Although this fact, this study was done in rabbits and not yet transfered to humans (as for example with cariporide – Table 4a).

GIK (glucose/insulin/potassium), insulin administration acts through the cGMP/PKG signaling pathway, as well as by increasing glucose use[61]. This complex was previously studied in 2012, but promising results need to be confirmed in a prospective trial powered to detect differences in infarct size (see Table 4a).

GLP-1 *(*Glucagon-like peptide-1) is an insulinotropic incretin, and as an analogue: exenatide. This analogue was extensively studied, showing, at first, some promising results but later in 2018, on ExSTRESS trial[62], this drug showed no significant difference considering beneficial effect related with its use no reduction in MI size by CMR in patients submitted to PCI and/or by troponin plasma concentration and LVEF improvement in patients scheduled for CABG) (Table 4a).

Table 4a Main clinical studies in humans testing for drugs attempting to avoid/control myocardial IRI and its main signaling pathways: drugs affecting Na^+/H^+ Exchanger/ RISK pathway (GIK and GLP-1 receptor).

Study	Drug (mechanism of action)	Protocol	Results/outcomes
***Rupprecht* et al (2000)[63]**	Cariporide (Na^+/H^+ exchanger inhibitor)	100 patients: Placebo (n=51) or a 40-mg IV bolus cariporide (n=49) before reperfusion.	Improved LV function (higher ejection fraction (50% versus 40%; P<0.05); lower end-systolic volume; improvements in the percentage of chords with hypokinesis and the severity of hypokinesis in the border zone of infarct region. CK, CK-MB, or LDH release was significantly reduced.
***Selker* et al (2012)[64]** **IMMEDIATE Randomized Controlled Trial**	GIK (Glucose/insulin/potassium acts through the RISK pathway) (GIK solution was 30% glucose (300 g/L), 50 U/L of regular insulin, and 80 mEq of KCl/L)	911 patients (mean age, 63.6 years; 71.0% men) with high probability of ACS: IV GIK solution (n = 411) or identical-appearing 5% glucose placebo (n = 460) out-of-hospital for 12 hours. Aims: evaluating whether GIK will reduce progression of unstable angina pectoris to AMI, mortality, cardiac arrest, development of HF, and infarct size in patients with suspected ACS.	No significant difference in the rate of progression to MI among patients who received GIK (n=200; 48.7%) vs those who received placebo (n=242; 52.6%) (odds ratio [OR], 0.88; 95% CI, 0.66-1.13; P = 0.28). The composite of cardiac arrest or in-hospital mortality occurred in 4.4% with GIK vs 8.7% with placebo (OR, 0.48; 95% CI, 0.27– 0.85; P=.01) Among the relatively small subgroup of patients who underwent imaging at 30 days, infarct size was reduced. Among patients with STEMI, 30-day mortality was 4.9% with GIK vs 7.7% with placebo (HR, 0.63; 95% CI, 0.27–1.49; P=0.29).
***Lønborg* et al (2012)[65]**	Exenatide (Analogue of Glucagon-like peptide-1 (GLP-1) that acts through the RISK pathway)	172 patients with STEMI and TIMI flow 0/1 were randomly assigned to exenatide or placebo (saline) IV: exenatide (0.12 µg/min, IV) 15 minutes before PCI and exenatide (0.043 µg/min, iv) for 6 hours after PCI.	23% reduction in infarct size at 90 days (estimated by CMR); 15% increase in myocardial salvation index (calculated from myocardial area at risk, measured in the acute phase, and final infarct size measured 90+21 days after PCI by CMR).
***Woo* et al (2013)[66]**	Exenatide (Analogue of Glucagon-like peptide-1 (GLP-1))	58 patients with STEMI and TIMI flow 0: exenatide or placebo (saline) SC. Infarct size was assessed by CK-MB and troponin I for 72 hours and by performing CMR imaging at 1 month after infarction.	40% reduction in infarct size (quantification of CK and troponin I). CMR: the absolute mass of delayed hyperenhancement was significantly reduced in the exenatide group as compared with the control group (12.8±11.7 versus 26.4±11.6 g; *P*<0.01).

***Roos* et al (2016)[67]**	Exenatide (Analogue of Glucagon-like peptide-1 (GLP-1))	191 patients: IV exenatide or placebo initiated prior to PCI using 10μg/h for 30min followed by 0.84μg/h for 72h.	91 patients' study with no effect of IV exenatide on MI size on CMR at 1 month over AAR acutely (T2 CMR).
***Besch* et al (2018)[62]** **ExSTRESS trial**	Exenatide (Analogue of Glucagon-like peptide-1 (GLP-1)	Elective CABG patients: IV exenatide (1-h bolus of 0.05 μg min−1 followed by a constant infusion of 0.025 μg min−1) or IV insulin therapy for blood glucose control (target range 100–139 mg dl−1) during the first 48 h after surgical incision. Primary outcome was the highest value of plasma concentration of TnI measured between 12 and 24 h after ICU admission.	Primary outcome did not significantly differ between the two groups; The highest troponin value measured during the first 72 h did not differ significantly; Proportion of patients with LVEF <50% in the control and in the exenatide groups did not differ; Postoperative IV exenatide did not provide any additional cardioprotective effect compared to IV insulin in low-risk patients undergoing scheduled CABG surgery.

ACS: Acute coronary syndrome; AMI: acute myocardial infarction; ARR: area at risk; CABG: coronary artery bypass graft; CK: creatine kinase; CK-MB: creatine kinase-muscle/brain; CMR: cardiac magnetic resonance; HF: heart failure; LDH: lactate dehydrogenase; LV: left ventricle; LVEF: left ventricle ejection fraction; PCI: percutaneous coronary intervention; RISK: Reperfusion injury salvage kinase pathway; TIMI: thrombolysis in myocardial infarction; TnI: troponin I.

cGMP activates PKG, which is responsible for phosphorylation of intracellular proteins, vasorelaxation, endothelial permeability, cell differentiation and proliferation. Through all these actions it is capable of controlling vascular tone, relaxing the smooth muscle cells around the vessels and thus, promoting cardioprotection. Considering drugs that target NO/cGMP pathway, they include ANP, nitroglycerine, nitrates and PDE5 inhibitors. Beneficial cardioprotetive effects of PDE5 inhibitors (vardenafil and sildenafil) at reperfusion, was mediated by activation of PKG, opening of mitochondrial KATP (mitoKATP), and stimulating downstream kinases.(68) However, for example adenosine, acting in the same pathophysiological pathway, did not reduce acute MI size and did not change de incidence of non-fatal MI or all-cause mortality. Also, sodium nitrite did not reduce AMI size (Table 4b).

Adenosine receptor agonists act through many ways and its cardioprotective effect was suggested in some experimental models, through several mechanisms including potent vasodilation, effects on leukocytes and increases in NO availability via activation of Akt/cGMP/PKG signaling pathway.[69] Although there is no benefit confirmed or controversy about its efficacy adenosine, namely by intracoronary route is safe and inexpensive and its early use in reperfused patients could be easily feasible (incidence of heart failure was

significantly lower and the incidence of coronary no-reflow was reduced; there was no difference in heart failure incidence in the intravenous adenosine group but most RCTs in this subgroup were from the thrombolysis era) (Table 4b).

Table 4b Main clinical studies in humans testing for drugs attempting to avoid/control myocardial IRI and its main signaling pathways: Adenosine/ nitrate (purinergic P1 receptor agonist/ NO donor targeting cGMP/PKG cardioprotective pathway).

Study	Drug (mechanism of action)	Protocol	Results/outcomes
***Garcia-Dorado* et al (2014)[70]**	Adenosine (ADO) (Purinergic P1 receptor agonist - as an adjunct to reperfusion in STEMI patients) (NO donor targeting cGMP/PKG cardioprotective pathway)	201 patients with STEMI and PCI: 4.5mg adenosine over 2 minutes distal to the lesion immediately before thrombectomy and direct stenting or saline immediately prior to reperfusion.	Failed to demonstrate that intracoronary administration of ADO prior to PCI limits infarct size; in patients receiving early PCI, ADO might enhance myocardial salvage and has a favorable effect on LVEF evolution.
***Bulluck* et al (2016)[71]**	Adenosine (purinergic P1 receptor agonist - as an adjunct to reperfusion in STEMI patients)	Meta-analysis: 13 RCTs (4273 STEMI patients) divided into 2 subgroups: intracoronary adenosine versus control (8 RCTs) and intravenous adenosine versus control (5 RCTs). Aim: determine the impact on major clinical endpoint including all-cause mortality, non-fatal myocardial infarction, and heart failure.	Intracoronary adenosine: incidence of heart failure was significantly lower (risk ratio [RR] 0.44 [95% CI 0.25–0.78], P = 0.005) and the incidence of coronary no-reflow was reduced (RR for TIMI flowb3 postreperfusion 0.68 [95% CI 0.47–0.99], P = 0.04). There was no difference in heart failure incidence in the intravenous adenosine group but most RCTs in this subgroup were from the thrombolysis era. There was no difference in non-fatal MI or all-cause mortality in both subgroups.
***Siddiqi* et al (2014)[72]** **NIAMI**	Nitrite (NO donor targeting cGMP/PKG cardioprotective pathway)	229 patients presenting with acute STEMI: IV infusion of 70 µmol sodium nitrite (n=118) or matching placebo (n=111) over 5 min immediately before PCI.	229 patient study with no effect of IV nitrite (70 µmol) on AMI size (by CMR 6–8 days).
***Jones* et al (2015)[73]**	Nitrite (NO donor targeting cGMP/PKG cardioprotective pathway)	Patients undergoing PCI (n=80): Intracoronary (10 mL) sodium nitrite (1.8 µmol) or NaCl (placebo) before balloon inflation.	80 patient study with no effect of IC nitrite (1.8 µmol) on AMI size (by total CK) In patients with TIMI <1 there was a reduction in MI size.

AMI: acute myocardial infarction; cGMP: Cyclic guanosine monophosphate; CK: creatine kinase; CMR: cardiac magnetic resonance; LVEF: left ventricle ejection fraction; NO: nitric oxide; PCI: percutaneous coronary intervention; PKG: protein kinase G; RCT: randomized controlled trial; STEMI: ST elevation myocardial infarction; TIMI: thrombolysis in myocardial infarction.

The mitochondrial permeability transition pore inhibitor (mPTPi) cyclosporine-A, is the first pharmacologic agent shown to limit infarct size by inhibiting the opening of the mPTP, an event also seen in post-conditioning. However, CsA administration may not protect heart from reperfusion injury in clinical patients with myocardial infarction, in terms of LVEF improvement, incidence of death, heart failure, and adverse LV remodelling

reduction[5],[74],[75],[76] (Table 4c). As well, the indirect mPTP inhibitor TRO40303 a drug binding to an unclear molecular target in the outer mitochondrial membrane (Table 4c) did not show significant results.

Table 4c Main clinical studies in humans testing for drugs attempting to avoid/control myocardial IRI and its main signaling pathways: Mitochondrial permeability transition pore (mPTP inhibitors).

Study	Drug (mechanism of action)	Protocol	Results/outcomes
***Piot* et al (2008)[74]**	Cyclosporine-A (mPTP direct inhibitor)	Cyclosporine A (2,5 mg/kg, iv) 10 minutes before PCI.	44% reduction in infarct size (CK AUC at 72h); 28% reduction in infarct size at 6 months (CMR).
***Song* et al (2015)[75]**	Cyclosporine –A (mPTP direct inhibitor)	Meta-analysis: 5 randomized controlled blind trials. Cardiac injury following reperfusion was quantified: infarct size, LVEF, TnI, CK, and CK-MB.	Infarct size (SMD: −0.41; 95% CI: −0.81, 0.01; *P*=0.058), LVEF (SMD: 0.20; 95% CI: −0.02, 0.42; *P*=0.079), TnI (SMD: −0.21; 95% CI: −0.49, 0.07; *P*=0.149), CK (SMD: −0.32; 95% CI: −0.98, 0.35; *P*=0.352), and CKMB (SMD: −0.06; 95% CI: −0.35, 0.23; *P*=0.689). There is no significant difference on cardiac function and injury with or without CsA treatment.
***Cung* et al (2015)[5]** **CIRCUS**	Cyclosporine-A (mPTP direct inhibitor)	970 patients with an acute anterior STEMI + PCI within 12 hours after symptom onset: bolus injection of cyclosporine (administered IV at a dose of 2.5 mg/kg) or matching placebo before coronary recanalization.	970 patients' study with no effect of IV cyclosporine-A on one-year clinical endpoints (death, heart failure, and adverse LV remodelling).
***Latini* et al (2016)[76]** **CYCLE**	Cyclosporine-A (mPTP direct inhibitor)	410 patients with large STEMI within 6 h of symptom onset, TIMI flow grade 0 to 1 in the infarct-related artery, and PCI, to 2.5 mg/kg intravenous CsA (n = 207) or control (n = 203) groups.	410 patients' study with no effect of IV cyclosporine-A on ST-segment resolution. ST-segment resolution ≥70% was found in 52.0% of CsA patients and 49.0% of controls (p = 0.55). The 2 groups did not differ in LV ejection fraction on day 4 and at 6 months.
***Atar* et al (2015)[77]** **MITOCARE clinical trial**	TRO40303 (Indirect mPTP inhibitors, that binds to an unclear molecular target in the outer mitochondrial membrane)	Patients with STEMI: TRO40303 (*n*=83) or placebo (*n*=80) via IV bolus injection prior to PCI. Primary endpoint was infarct size expressed as AUC for CK and for TnI over 3 days. Secondary endpoints included measures of infarct size using CMR and safety outcomes.	Infarct size, as measured by CK and TnI AUCs at 3 days was not significantly different between treatment groups. There were no significant differences in the CMR-assessed myocardial salvage index (mean 52 vs.58% with placebo, p=0.1), mean CMR-assessed infarct size (21.9 g vs. 20.0 g, or 17 vs.15% of LV mass), LVEF (46 vs. 48%) or in the mean 30-day echocardiographic LVEF (51.5 vs. 52.2%) between TRO40303 and placebo. The study did not show any effect of TRO40303 in limiting reperfusion injury of the ischemic myocardium.
***Gibson* et al (2015)[78]** **EMBRACE STEMI**	MTP-131 (Mitochondrial peptide targeting cardiolipin)	MTP-131 vs. placebo infused at a rate of 0.05 mg/kg/h for 1 h among first-time anterior STEMI subjects undergoing PCI	118 patient study with no effect of IV MTP-131 infusion on acute MI size (by 72 h AUC CK-MB)

AUC: area under curve; CK: creatine kinase; CK-MB: creatine kinase isoenzyme-muscle/brain; CMR: cardiac magnetic resonance; CsA: cyclosporine A; HF: heart failure; LV: left ventricle; LVEF: left ventricle ejection fraction; PCI: percutaneous coronary intervention; SMD: standard mean difference; STEMI: ST elevation myocardial infarction; TIMI: thrombolysis in myocardial infarction; TnI: troponin I.

Beta-blockers act reducing energy demands from cardiomyocyte. Beta-1 adrenergic receptors blockade on neutrophils decrease neutrophil–platelet aggregate formation during reperfusion and reduce MVO. The beneficial effect of β-blockers on LV remodeling, reinfarction, life-threatening arrhythmias, and most importantly, mortality are well documented.[10] According to *Heusch* (2016)[15], brain natriuretic peptide, exenatide, metoprolol, and esmolol are the only drugs capable and unquestioned to reduce infarct size in patients with reperfused AMI, although there is no information about them considering coronary circulation and more studies are necessary in the context of efficacy and safety on IRI).

Chen et al (2005)[79] has showed that metoprolol use was associated with significantly decreased risk of reinfarction and ventricular fibrillation but increased risk of cardiogenic shock. Other authors demonstrated that metoprolol reduced infarct size by CMR imaging and also increased left ventricular ejection fraction[80-82] (Table 4d) with no excess of adverse events during the first 24 hours after STEMI. However, *Roolvink* et al[83] (Table 4d) showed that early IV metoprolol before PCI was not associated with a reduction in infarct size and/or improvement on LVEF.

Other drugs include glycoprotein IIb/IIIa inhibitors, also have a major effect on platelets itself and interaction between platelets and leukocytes. By acting on these, these drugs reduce thrombotic events implicates in myocardial IRI.[10] Intracoronary Abciximab reduced significantly the Infarct size at 30 days (Table 4d) and persist as one of useful therapeutical approach for IRI. Delcasertib, a PKC inhibitor which prevents apoptotic cell death, failed as a cardioprotective agent in STEMI patients (Table 4d).

The most recent cardioprotective targets, which will not be developed herein, include complex regulatory mechanisms that involve lncRNA and miRNA, and there is also space for

working on inhibiting the formation of the inflammasome as well as on neutrophils and platelets that are recruited.[84-86]

Recent evidence showed a promising role of melatonin, which has anti-inflammatory and anti-oxidative properties in the prevention of IRI. In addition to this , it also has benefits on the regulation of blood lipid profile, platelet function, and microcirculation.[87]

Table 4d Main clinical studies in humans testing for drugs attempting to avoid/control myocardial IRI and its main signaling pathways: Beta-blockers, GpIIb/IIIa inhibitors and PKC inhibitors.

Study	Drug (mechanism of action)	Protocol	Results/outcomes
***Chen* et al (2005)[79]** **COMMIT clinical trial**	Metoprolol and clopidogrel in patients with AMI (Beta-blocker acts through the RISK pathway) (Clopidogrel: antiplatelet P2Y12 inhibitor)	2×2 factorial design (metoprolol vs. placebo and clopidogrel plus aspirin vs. aspirin alone: 45 852 patients within 24h of suspected AMI onset. Outcomes: the composite of death, reinfarction, cardiac arrest (metoprolol only), and stroke (clopidogrel only); death from any cause.	No difference in death from any cause with the use of metoprolol (OR 0.99; 95%CI 0.92-1.05). Metoprolol use was associated with significantly decreased risk of reinfarction (OR 0.82; 95%CI 0.72-0.92) and ventricular fibrillation (OR 0.83; 95% CI 0.75–0.93), but increased risk of cardiogenic shock (OR 1.30; 95%CI 1.19–1.41). Clopidogrel use resulted in a reduction in death, reinfarction, or stroke (OR 0.91; 95%CI 0.86-0.97), without a significantly increased risk of major bleeding regardless of age.
***Ibanez* et al (2013)[82]** **METOCARD-CNIC clinical trial (Effect of Metoprolol in Cardioprotection During an AMI)**	Metoprolol (Beta-blocker)	STEMI patients + PCI within 6 hours: IV metoprolol (up to three 5-mg boluses of metoprolol tartrate 2 minutes apart) (n=131) or not (control, n=139) before reperfusion.	Early IV metoprolol before reperfusion reduced infarct size by CMR imaging compared with control (25.6±15.3 versus 32.0±22.2 g; adjusted difference, -6.52; 95% confidence interval, -11.39 to −1.78; *P*=0.012) Early IV metoprolol also increased left ventricular ejection fraction group (adjusted difference, 2.67%; 95% confidence interval, 0.09–5.21; *P*=0.045) with no excess of adverse events during the first 24 hours after STEMI.
***Mateos* et al (2015)[81]** **METOCARD-CNIC (subgroup) clinical trial**	Metoprolol (Beta-blocker)	Subgroup analysis of the METOCARD-CNIC study: 74 patients receiving IV metoprolol by Emergency Medical Service team (EMS) and compared them with others(n=73) treated by EMS but not receiving IV metoprolol.	Out-of-hospital administration of IV metoprolol by EMS within 4.5 hours of symptom onset in our subjects reduced infarct size (23.4 [SD 15.0] versus 34.0 [SD 23.7] g; adjusted difference –11.4; 95% confidence interval [CI] –18.6 to –4.3) and improved LVEF (48.1% [SD 8.4%] versus 43.1% [SD 10.2%]; adjusted difference 5.0; 95% CI 1.6 to 8.4).

***Pizarro* et al (2014)[80]** **METOCARD-CNIC: Long term, clinical trial**	Metoprolol (Beta-blocker)	Long-term CMR imaging performed on 202 patients of the METOCARD-CNIC study (101 per group) 6 months after STEMI.	LVEF at the 6 months CMR was higher after IV metoprolol (48.7 ± 9.9% vs. 45.0 ± 11.7%). The occurrence of severely depressed LVEF (≤35%) at 6 months was significantly lower in patients treated with IV metoprolol (11% vs. 27%, p =0.006). The proportion of patients with indications for ICD was significantly lower in the IV metoprolol group (7% vs. 20%, p=0.012). At a median follow-up of 2 years, occurrence of the pre-specified composite of death, heart failure admission, reinfarction, and malignant arrhythmias was 10.8% in the IV metoprolol group vs 18.3% in the control group, adjusted hazard ratio (HR): 0.55; 95% CI: 0.26 to 1.04; p= 0.065. Heart failure admission was significantly lower in the IV metoprolol group (HR: 0.32; 95% CI: 0.015 to 0.95; p=0.046).
***Roolvink* et al (2016)[83]** **EARLY BAMI clinical trial**	Metoprolol (Beta-blocker)	STEMI patients <12 h from symptom onset in Killip class I to II without AV block: 1:1 randomization to IV metoprolol before reperfusion (2x 5-mg bolus) (n=336) or matched placebo (n=346) before PCI. Primary endpoint was myocardial infarct size as assessed by CMR imaging at 30 days. Secondary endpoints were enzymatic infarct size and incidence of ventricular arrhythmias.	Early IV metoprolol before PCI was not associated with a reduction in infarct size (percent of LV by CMR did not differ (15.3 ± 11.0% metoprolol vs 14.9 ± 11.5% placebo; p ¼ 0.616). Peak and area under the creatine kinase curve did not differ between both groups. LVEF by CMR was 51.0 ± 10.9% in the metoprolol group and 51.6±_ 10.8% in the placebo group (p= 0.68). Metoprolol reduced the incidence of malignant arrhythmias in the acute phase and was not associated with an increase in adverse events.
***Bajaj* et al (2010)[88]**	Abciximab or eptifibatide (Glycoprotein IIb/IIIa platelet receptor antagonists with potent effect on platelets and platelet-leukocyte aggregates)	Prospective, nonrandomized analysis of 241 consecutive patients; 162 received abciximab and 79 received eptifibatide.	Patients receiving abciximab had lower peak CK (2484 U/L) compared with eptifibatide (2650 U/L; *P*=001), had less STEMI, and had better corrected TIMI frame count and better myocardial blush grade. Abciximab was superior to eptifibatide for improving angiographic and electrocardiographic assessment of coronary perfusion, as well as for reducing myocardial infarct size.
***Stone* et al (2012)[89]** **INFUSE-AMI clinical trial**	Abciximab (Glycoprotein IIb/IIIa platelet receptor antagonist)	452 patients presenting within 4 hours of STEMI + primary PCI with bivalirudin anticoagulation: 2 x 2 factorial design to bolus intracoronary abciximab (0.25-mg/kg) delivered locally at the infarct lesion site vs no abciximab and to manual aspiration thrombectomy vs no thrombectomy; Primary endpoint: infarct size (percentage of total left ventricular mass) at 30 days assessed by CMR imaging.	Patients with intracoronary abciximab compared with no abciximab had a significant reduction in 30-day infarct size (median, 15.1%; interquartile range [IQR], 6.8%-22.7%; n = 181, vs 17.9% [IQR, 10.3%-25.4%]; n = 172 ;*P=0.03)*. Patients randomized to intracoronary abciximab also had a significant reduction in absolute infarct mass (median, 18.7 g [IQR, 7.4-31.3 g]; n = 184, vs 24.0 g [IQR, 12.1-34.2 g]; n = 175 ;p=0.03. Patients randomized to aspiration thrombectomy vs no aspiration had

			no significant difference in infarct size at 30 days. Conclusion: Infarct size at 30 days was significantly reduced by bolus intracoronary abciximab delivered to the infarct lesion site but not by manual aspiration thrombectomy.
***Lincoff* et al (2014)[90]** **PROTECT-MI**	Delcasertib (PKC inhibitor which prevents apoptotic cell death)	Patients with anterior STEMI: placebo or one of three doses of delcasertib (50, 150, or 450 mg/h) by IV infusion initiated before PCI and continued for ~2.5 h.	1010 patient study with no effect of IV infusion of Delcasertib at 3 different doses on acute MI size (AUC CK-MB).

AMI: acute myocardial infarction; AUC: area under curve; AV: atrioventricular; CK: creatine kinase; CK-MB: creatine kinase isoenzyme-muscle/brain; CMR: cardiac magnetic resonance; ICD: implantable cardioverter-defibrillator; LV: left ventricle; LVEF: left ventricle ejection fraction; PCI: percutaneous coronary intervention; PKC: protein kinase C; STEMI: ST elevation myocardial infarction; TIMI: thrombolysis in myocardial infarction.

Multitarget strategies to reduce myocardial ischemia-reperfusion injury:

The "immediate" future in clinical investigation

As mentioned before, most drugs/approaches described, either have no efficacy or have low efficacy in terms of reduction infarct size, heart failure development and all-cause mortality. Thus, the single-target therapy in cardiomyocytes has failed and, in most studies, no coronary circulation has been considered as a potential target.

Hausenloy et al (2017)[91] questioned why have there been so many recent clinical cardioprotection studies (Table 2). The fact is: the involvement of multiple pathophysiological pathways in the myocardial IRI phenomenon led to investigate many potential cardioprotective drugs.

Since many pathways are involved, at least one of its therapeutic targets should be unproductive and by inhibiting only one of the fronts, we are not able to act on the others. These multiplicity of pathways and mechanisms led *Hausenloy* et al (2017)[91] and other authors to think about multitarget strategies do reduce myocardia IRI.

It is thus mandatory to find innovative cardioprotective strategies required to diminish the deleterious effects of acute myocardial IRI, as well as to avoid adverse LV remodeling, and heart failure in patients with MI. One of the strategies can be a multitarget strategy which acts on different signaling pathways within the cardiomyocyte. *Davidson* et al[43] defined

"multitargeted cardioprotective therapy" as additive or synergistic cardioprotective effects of multiple cardioprotective agents or interventions directed to distinct targets.

There are multiple studies and ways of combine multitarget therapies, despite none is still approved for effective use.

First, one strategy is to ally more than one cardioprotective agent or intervention with distinct targets within the cardiomyocyte. For this strategy it is important that each cardioprotective agent or intervention is at its maximal "dose", and that each agent has a different intracellular target. For example, in a pig AMI model, the combination of limb RIC (which decreases oxidative stress and endothelial NOS (eNOS)) with either GIK or exenatide (a glucagon-like peptide-1 mimetic, that shifts cardiac metabolism toward increased glycolysis) at the time of reperfusion reduced infarct size to a greater extent than either intervention alone.[52]

Second, other strategy uses multiple cardioprotective agents or interventions combined with noncardiomyocytes targets, for example cells that improve tissue perfusion and or cells that participate in the MVO and are "good" targets, like endothelial cells, fibroblasts, neutrophils, platelets and this can be a more efficient way to promote cardioprotection. One example relatively to this strategy is provided by P2Y12 inhibitors (such as ticagrelor and cangrelor), which are known to reduce infarct size[92] although there is no promising results in trying to relieve MVO and improve microcirculatory flow after MI, even experimentally.

Another strategy is combining more than one cardioprotective agent or intervention and choose them because their action in distinct time-points during ischemia and reperfusion: during ischemia, at reperfusion, and late into reperfusion.

In addition, we have cardioprotective agents or interventions targeting the same signaling pathway but with additive effects, for example, co-administration of the NOS substrate L-arginine and cofactor tetrahydrobiopterin (BH4) just before reperfusion

significantly reduced MI size in both rats and pigs, despite neither being protective on their own.[93, 94]

A single agent or intervention capable of cardioprotection it is the one that can have multiple targets. IV metoprolol administered before reperfusion reduces both infarct size and MVO.[82] The dual-target benefits of metoprolol (acts through the RISK pathway and by blocking beta receptors reducing energy consumption/infarction area), appear to be specific to this drug and not a class effect. The endogenous cardioprotective strategies of IPC, IPost, and RIC are also known to protect the heart through a number of different signaling pathways (PKG, RISK and SAFE).[43] A single miRNA or small interfering RNA can also protect the heart against acute IRI through its effects on a variety of different target mRNAs.[91] Hybrid molecules may have 2 or more structural domains acting as 2 distinct pharmacophores to provide additive cardioprotection. For example, a hybrid compound (ZYZ-803) that combines the adenine nucleus with a moiety that slowly releases hydrogen sulfide (H2S)–induced additive cardioprotection (H2S and NO have protective roles in heart failure; This recent molecule allies the benefits of the two compounds in its constitution to a proangiogenic effect).[95]

Future challenges

The challenge for the next decade is to set up larger phase III trials evaluating clinical outcomes to therapies targeting lethal reperfusion injury. It is very likely that the advances in the next decade will come from refining the therapies already available rather that identifying new drugs.

It is crucial to design well-structured clinical studies that are based on cohesive experimental studies that show encouraging, robust and consistent results in both small and large animal models; it is also important that these models are realistic and assume at least one comorbidity and one medication. Comorbidities and polymedication may be confounders of the

infarct model in humans, and this is hard to mimic in animals. One possible way to control these factors is by doing subgroup analyzes. It is also important to keep in mind, in the projection of clinical studies and trials, that the cardioprotective intervention should be made before PCI, so that the maximum dose is present at the time of the procedure, and this dose should be optimized at a previous phase II study. Another challenge is to analyze endpoints that have relevance in terms of cardioprotection, for example acute and chronic MI size, LV ejection fraction and size, as well as to define inclusion criteria for the selected patients. More important than implementing these measures at a single-centered level, it is important to do it on a multi-centered scale, with globally standardized protocols, since this will allow a much more reliable analysis of the overall picture of myocardial IRI. There are, in fact, many barriers today to being able to carry out well-structured studies in this area, and funds and facilities are needed.

Davidson et al (2019)[43] hypothesize that the ideal multitargeted therapy might be one that can target MVO (e.g., intravenous cangrelor or ANGPL4), target cardiomyocytes (e.g., remote ischemic per-conditioning) and target inflammation (e.g., metoprolol). The timing of administration can also be an important variable, and considering it, we have to keep in mind the unpredictability of the infarction and also that we should not delay life-saving procedures like those we use nowadays for AMI therapy.

A number of questions remain to be answered about melatonin: the combined use with other drugs, the role in regenerative medicine, the cardiac mitochondrial clock processes during IR injury and the potential endothelial protection.

Conclusion

It is essential to realize the scale of the problem worldwide: ischemic heart disease, namely infarction, is the leading cause of death worldwide.

Once established and potentiated the infarct-related therapies, it is important to focus our attention on myocyte death that occurs due to ischemia-reperfusion injury “induced” by PCI.

Mechanical therapeutics were extensively studied, obtaining some positive results.

Pharmacological therapies were studied, with some drugs showing better effects on cardioprotection than others. Due to multiple incongruities in the individualized results of each one of the pharmacological agents it was concluded that allying drugs could be a more effective way of approaching the problem.

Thus, the first combinations of agents have recently begun to be studied at the pre-clinical scale. More recently, melatonin has emerged as an agent capable of acting at the level of myocardial reperfusion injury.

What is certain is that currently there is still no combination of agents that would effectively allow us to say that we are able to reduce the reperfusion injury. Studies in this area are lacking, and there is a gap between experimental and clinical models. This is probably the greatest actual obstacle to draw conclusions on new drugs and therapies that prevent or minimize IRI in humans.

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Anexos

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Primeira página

Título completo (menos de 150 caracteres) em português e em inglês.

Nome e apelido dos autores pela ordem seguinte: nome próprio, seguido do apelido (pode conter dois nomes)

Proveniência (Serviço, Instituição, cidade, país) e financiamento caso haja.

Endereço completo do autor a quem deve ser dirigida a correspondência, fax e endereço electrónico.

Faz-se referência ao número total de palavras do manuscrito (excluindo as tabelas).

Resumo estruturado

O resumo, com um máximo de 250 palavras, está dividido em quatro partes: *a)* Introdução e objectivos; *b)* Métodos; *c)* Resultados e *d)* Conclusões.

Deverá ser elucidativo e não inclui referências bibliográficas nem abreviaturas (excepto as referentes a unidades de medida).

Inclui no final três a dez palavras-chave em português e em inglês. Deverão ser preferencialmente seleccionadas a partir da lista publicada na Revista Portuguesa de Cardiologia, oriundas do Medical Subject Headings (MeSH) da National Libray of Medicine, disponível em: www.nlm.nihgov/mesh/meshhome.html.

O resumo e as palavras-chave em inglês devem ser apresentados da mesma forma.

Texto

Deverá conter as seguintes partes devidamente assinaladas: *a)* Introdução; *b)* Métodos; *c)* Resultados; *d)* Discussão e *e)* Conclusões. Poderá utilizar subdivisões adequadamente para organizar cada uma das secções.

As abreviaturas das unidades de medida são as recomendadas pela RPC (ver Anexo II).

Os agradecimentos situam-se no final do texto.

Bibliografia

As referências bibliográficas deverão ser citadas por ordem numérica no formato 'superscript', de acordo com a ordem de entrada no texto.

As referências bibliográficas não incluem comunicações pessoais, manuscritos ou qualquer dado não publicado. Todavia podem estar incluídos, entre parêntesis, ao longo do texto.

São citados abstracts com menos de dois anos de publicação, identificando-os com [abstract] colocado depois do título.

As revistas médicas são referenciadas com as abreviaturas utilizadas pelo Index Medicus: List of Journals Indexed, tal como se publicam no número de Janeiro de cada ano. Disponível em: http://www.ncbi.nlm.nih.gov/entrez/citmatch_help.html#JournalLists.

O estilo e a pontuação das referências deverão seguir o modelo Vancouver 3.

Revista médica: Lista de todos os autores. Se o número de autores for superior a três, incluem-se os três primeiros, seguidos da abreviatura latina et al. Exemplo:

17. Sousa PJ, Gonçalves PA, Marques H et al. Radiação na AngioTC cardíaca; preditores de maior dose utilizada e sua redução ao longo do tempo. Rev Port cardiol, 2010; 29:1655-65

Capítulo em livro: Autores, título do capítulo, editores, título do livro, cidade, editora e páginas. Exemplo:

23. Nabel EG, Nabel GJ. Gene therapy for cardiovascular disease. En: Haber E, editor. Molecular cardiovascular medicine. New York: Scientific American 1995. P79-96.

Livro: Cite as páginas específicas. Exemplo:

30. Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Mansel Dekker; 1993. P. 33.

Material electrónico: Artigo de revista em formato electrónico. *Exemplo:*

Abood S. Quality improvement initiative in nursing homes: the ANA acts it an advisory role. Am J Nurs. [serie na internet.] 2002 Jun citado 12 Ago 2002:102(6): [aprox. 3] p. Disponível em: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

. A Bibliografia será enviada como texto regular, nunca como nota de rodapé. Não se aceitam códigos específicos dos programas de gestão bibliográfica.

1. Figuras

As figuras correspondentes a gráficos e desenhos são enviadas no formato TIFF ou JPEG de preferência, com uma resolução nunca inferior a 300 dpi e utilizando o negro para linhas e texto. São alvo de numeração árabe de acordo com a ordem de entrada no texto.

- A grafia, símbolos, letras, etc, deverão ser enviados num tamanho que, ao ser reduzido, os mantenha claramente legíveis. Os detalhes especiais deverão ser assinalados com setas contrastantes com a figura.
- As legendas das figuras devem ser incluídas numa folha aparte. No final devem ser identificadas as abreviaturas empregues por ordem alfabética.
- As figuras não podem incluir dados que dêem a conhecer a proveniência do trabalho ou a identidade do paciente. As fotografias das pessoas devem ser feitas de maneira que estas não sejam identificadas ou incluir-se-á o consentimento por parte da pessoa fotografada.

Tabelas

São identificadas com numeração árabe de acordo com a ordem de entrada no texto.

Cada tabela será escrita a espaço duplo numa folha aparte.

- Incluem um título na parte superior e na parte inferior são referidas as abreviaturas por ordem alfabética.
- O seu conteúdo é auto-explicativo e os dados que incluem não figuram no texto nem nas figuras.

2. Artigos de Revisão

Nº máximo de palavras do artigo sem contar com o resumo e quadros- 5.000
Nº máximo de palavras do Resumo - 250
Nº máximo de Figuras - 10
Nº máximo de quadros - 10
Nº máximo de ref. bibliográficas - 100

3. Cartas ao Editor

Devem ser enviadas sob esta rubrica e referem-se a artigos publicados na Revista. Serão somente consideradas as cartas recebidas no prazo de oito semanas após a publicação do artigo em questão.

- Com espaço duplo, com margens de 2,5 cm.
- O título (em português e em inglês), os autores (máximo quatro), proveniência, endereço e figuras devem ser especificados de acordo com as normas anteriormente referidas para os artigos originais.
- Não podem exceder as 800 palavras.
- Podem incluir um número máximo de duas figuras. As tabelas estão excluídas.

4. Casos Clínicos

Devem ser enviados sob esta rubrica.

- A espaço duplo com margens de 2,5 cm.
- O título (em português e em inglês) não deve exceder 10 palavras

Os autores (máximo oito) proveniência, endereço e figuras serão especificados de acordo com as normas anteriormente referidas para os artigos originais.

O texto explicativo não pode exceder 3.000 palavras e contem informação de maior relevância. Todos os símbolos que possam constar nas imagens serão adequadamente explicados no texto.

Contêm um número máximo de 4 figuras e pode ser enviado material suplementar, como por exemplo vídeoclips.

5. Imagens em Cardiologia

- A espaço duplo com margens de 2,5 cm.
- O título (em português e em inglês) não deve exceder oito palavras
- Os autores (máximo seis), proveniência, endereço e figuras serão especificados de acordo com as normas anteriormente referidas para os artigos originais.
- O texto explicativo não pode exceder as 250 palavras e contem informação de maior relevância, sem referências bibliográficas. Todos os símbolos que possam constar nas imagens serão adequadamente explicados no texto.
- Contêm um número máximo de quatro figuras.

6. Material adicional na WEB

A Revista Portuguesa de Cardiologia aceita o envio de material electrónico adicional para apoiar e melhorar a apresentação da sua investigação científica. Contudo, unicamente se considerará para publicação o material electrónico adicional directamente relacionado com o conteúdo do artigo e a sua aceitação final dependerá do critério do Editor. O material adicional aceite não será traduzido e publicar-se-á electronicamente no formato da sua recepção.

Para assegurar que o material tenha o formato apropriado recomendamos o seguinte:

	Formato	Extensão	Detalhes
Texto	Word	.doc ou docx	Tamanho máximo 300 Kb
Imagem	TIFF	.tif	Tamanho máximo 10MB
Audio	MP3	.mp3	Tamanho máximo 10MB
Vídeo	WMV	.wmv	Tamanho máximo 30MB

Os autores deverão submeter o material no formato electrónico através do EES como arquivo multimédia juntamente com o artigo e conceber um título conciso e descritivo para cada arquivo.

Do mesmo modo, este tipo de material deverá cumprir também todos os requisitos e responsabilidades éticas gerais descritas nessas normas.

O Corpo Redactorial reserva-se o direito de recusar o material electrónico que não julgue apropriado.

ANEXO I

DECLARAÇÃO

Declaro que autorizo a publicação do manuscrito:

Ref.ª .....

Título .....

.....

.....

do qual sou autor ou c/autor.

Declaro ainda que presente manuscrito é original, não foi objecto de qualquer outro tipo de publicação e cedo a inteira propriedade à Revista Portuguesa de Cardiologia, ficando a sua reprodução, no todo ou em parte, dependente de prévia autorização dos editores.

Nome dos autores:

.....

.....

Assinaturas:

ANEXO II

Símbolos, abreviaturas de medidas ou estatística

Designação	Português	Inglês
Ampere	A	A
Ano	ano	yr
Centímetro quadrado	cm_2	cm_2
Contagens por minuto	cpm	cpm
Contagens por segundo	cps	cps
Curie	Ci	Ci
Electrocardiograma	ECG	ECG
Equivalente	Eq	Eq
Grau Celsius	°C	°C
Grama	g	g
Hemoglobina	Hb	Hb
Hertz	Hz	Hz
Hora	h	h
Joule	J	J
Litro	L ou L	l ou L
Metro	m	m
Minuto	min	min
Molar	M	M
Mole	mol	mol
Normal (concentração)	N	N
Ohm	Ω	Ω
Osmol	osmol	osmol
Peso	peso	WT
Pressão parcial de CO_2	pCO_2	pCO_2
Pressão parcial de O_2	pO_2	pO_2
Quilograma	kg	kg
Segundo	s	sec
Semana	Sem	Wk
Sistema nervoso central	SNC	CNS
Unidade Internacional	UI	IU
Volt	V	V
Milivolt	mV	mV
Volume	Vol	Vol
Watts	W	W
Estatística:		
Coeficiente de correlação	r	r
Desvio padrão (standard)	DP	SD
Erro padrão (standard) da média	EPM	SEM
Graus de liberdade	gl	df
Média	χ	χ
Não significativa	NS	NS
Número de observações	n	n
Probabilidade	p	p
Teste «t» de Student	teste t	t test