Educational review

Pathophysiology and therapy of myocardial ischemia-reperfusion syndrome

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Abstract

There is a need to find interventions able to reduce the extent of injury in reperfused ST-segment elevation myocardial infarction (STEMI) beyond timely reperfusion. In this review, we summarize the clinical impact of STEMI from epidemiological, clinical and biological perspectives. We also revise the pathophysiology underlying the *ischemia/reperfusion syndrome* (I/R) occuring in reperfused STEMI, including the several players involved in this syndrome, such as cardiomyocytes, microcirculation, and circulating cells. Interventions aimed to reduce the resultant infarct size, known as *cardioprotective therapies*, are extensively discussed, putting the focus on both mechanical interventions (i.e. ischemic conditioning) and promising pharmacological therapies, such as early intravenous metoprolol, exenatide and other glucose modulators, N-acetylcysteine as well as on some other classical therapies which have failed to be translated to the clinical arena. Novel targets for evolving therapeutic interventions to ameliorate I/R injury are also discussed. Finally, we highlight the necessity to improve the study design of future randomized clinical trials in the field, as well as to better select patients who can most likely benefit from cardioprotective interventions.

Keywords

Ischemia/reperfusion injury; cardioprotection; acute myocardial infarction

Learning objectives

To understand the implications of ST-segment elevation myocardial infarction (STEMI) from epidemiological, clinical and biological perspectives.
To have a broad overview of the pathophysiology underlying ischemia/reperfusion (I/R) syndrome and the interventions aimed to reduce its impact (cardioprotective therapies)
To be aware of the importance of the ischemic conditioning phenomenon for the understanding of the mechanisms underlying most cardioprotective therapies as well as for the development of further protective interventions.
To know the most promising cardioprotective therapies: remote ischemic conditioning, metoprolol and glucose modulators among others.
To understand the changing scenario, where different populations, patients and molecular targets should be considered for a successful development of new cardioprotective therapies.

Key points

- Although timely reperfusion limits both myocardial infarct size and the subsequent cardiac remodelling, reperfusion *per se* adds an additional irreversible damage to the myocardium, contributing to final infarct size (IS).
- The relative contribution of ischemia- and reperfusion-related injuries to final IS is unknown and probably varies across different conditions (eg ischemia duration, neutrophil activation, individual susceptibility…).
- Ischemia/reperfusion (I/R) syndrome is multifactorial with several players involved. Cardiomyocytes, microcirculation, and circulating cells are the main compartments affected by this syndrome.
- Ischemic conditioning is the paradigm of mechanical cardioprotective therapy. It can be applied before (pre), after (post), or even during (peri) the index ischemia period, and either in the same organ-heart- (local) or in a distant organ (remote).
- Several pharmacological interventions have shown positive results in ameliorating I/R syndrome, being intravenous metoprolol and exenatide the most promising.
- There is a need to improve the study design of future randomized clinical trials in the field, as well as to better select patients who can most likely benefit from cardioprotective interventions. These cardioprotective therapies must have demonstrated solid results in methodologically well-conducted preclinical studies.

In this review, we briefly present the clinical impact of acute myocardial infarction (AMI) from epidemiological, clinical and biological perspectives (**Figure 1**), as well as the pathophysiology underlying the *ischemia/reperfusion syndrome* (I/R) occuring in the reperfused ST-segment elevation myocardial infarction (STEMI). Interventions aimed to reduce the resultant infarct size, known as *cardioprotective therapies* will be presented as well. We put the focus on some classical therapies, which have been already tested in the clinical arena, as well as on new targets for evolving therapeutic interventions. Of note, the term AMI exclusively refers to STEMI in this review.

From the epidemiological to the experimental perspective

The epidemiological perspective

The implementation of reperfusion and adjuvant pharmacological (acute and maintenance) therapy has resulted in an impressive improvement in prognosis of STEMI patients in Western countries[1,2]. Paradoxically, while in-hospital death rates for STEMI have substantially dropped over the last few decades[3], there is an inversely proportional increase of chronic heart failure (CHF) rates as a result. Patients with severly depressed hearts after AMI formerly would die during hospitalization but today many of them survive at a cost of a high number of subequent long term complications. The incidence of AMI is growing disproportionately in some geographic regions due to the increased cardiovascular risk burden, turning the incidence of STEMI into a major health problem in developing countries[4,5]. Either because the increase of mortality rates associated with a growing incidence or because the raise of morbidity and socioeconomic burden associated with the development of CHF in STEMI survivors[6,7], there is a worldwide need to reduce the life-long impact of AMI.

Besides the necessity to reduce and treat cardiovascular risk factors in the long-term, the implementation of timely reperfusion is currently the cornerstone therapy to substantially improve

mortality and morbidity in AMI patients[2]. It is undebatable that the shortening of symptoms-to-reperfusion time results in better clinical outcomes ("time is muscle")[2,8,9] and there is in many ways still room to improve both the implementation and the timings of primary percutaneous coronary intervention (PPCI, the best reperfusion technique)[10]. However, in some regions, this strategy seems to have reached its own ceiling and, despite having an efficient early PPCI program, 1-year mortaly remains excessively high, reaching 15% in some countries[11]. In countries where the time from STEMI diagnosis to PPCI-mediated reperfusion has been shortened to about 90 minutes, further shortening of this time has not demonstrated to improve mortality[12]. While it is true that today sicker patients undergo PPCI and this might contribute to the plateau in mortality despite better response times[13], these figures highlighting the need for novel therapies to be administered as adjuncts to PPCI in order to both improve patient survival and prevent the onset of heart failure.

The hypothesis that a reduction in myocardial infarct size (IS) translates into an improvement in clinical outcomes has been recently demonstrated in a patient-level meta-analysis, where it was found a steep gradient int the combined endpoint of all-cause mortality and hospitalization for heart failure at 1 year across quartiles of IS, with 8.8% events for the top quartile and 1.2% for the bottom quartile[14,15]. Because timely and complete reperfusion is the most well-established way of limiting IS and the subsequent ventricular remodelling, the focus of this review will be in those cardioprotective interventions beyond reperfusion.

The clinical perspective

The beginning of the reperfusion era in AMI patients starts in 1972 with the seminal studies by Ross and co-workers, who demonstrated in a dog model that reperfusion after 3 h coronary occlusion limited the progression of necrosis[16–18]. Soon afterward Braunwald & Kloner popularized the concept that reperfusion itself induces additional injury in the vulnerable ischemic tissue[19]. Since then, it is well accepted that the extension of irreversibly injured myocardium (i.e.

infarct size) is the result of ischemia- and reperfusion-related damage, thus the term I/R injury. After several decades of intense research in the topic, it is still debated what is the relative contribution of ischemia- and reperfusion-related injuries to final infarct size. One reason for this uncertainty is the close interplay between both types of injury: since the degree if ischemic injury at the time of blood flow restoration is a main factor contributing to reperfusion-related damage, it is impossible to separate both types of damage. Another factor contributing to this vagueness is the absence of tools to differentiate ischemia- and reperfusion-related injuries. Cardiac magnetic resonance (CMR) imaging is able to visualize some features of reperfusion-related injury, like edema formation, microvascular obstruction, and others[20–24], but others like cardiomyocyte´s mitochondria damage are not amenable for CMR imaging at present.

GISSI and ISIS-2 trials concurrently demonstrated that reperfusion through intravenous thrombolysis was not just feasible[25], but also able to improve clinical outcomes in STEMI patients[26,27]. Mechanical reperfusion using PPCI was later demonstrated to be superior to thrombolysis and thus the preferred mode of reperfusion, if available in a timely fashion[28]. However, while early reperfusion therapy solves part of the problem (ischemic-related injury), the other part (reperfusion-related injury) still remains to be clinically addressed and has become one of the top ten unmet clinical needs in cardiology[29]. One clinical observation led to the change in the way researchers envisioned damage associated with AMI: patients suffering pre-infarction angina had a better prognosis than patients whose first symptom was at the index AMI[30]. This clinical observation was experimentally mimicked in in dogs by Murry *et al*[31] by inducing brief periods of coronary occlusion and reperfusion before prolonged occlusion (i.e. index AMI insult), something known as ischemic preconditioning. Preconditioning resulted in massive reductions in infarct size. This simple experiment, emulating the clinical scenario, demonstrated that, beyond early reperfusion, an intervention could reduce infarct size. Ischemic preconditioning has been shown to protect when applied in the actual heart, as the seminal experiment by Murry *et al*[31], and also when applied in a distant organ (something known as remote ischemic conditioning). In addition, it has been shown that conditioning stimulus can be applied at the end of the ischemia

duration (post-conditioning) and even at the middle of it in a remote organ (remote per-conditioning).

The experimental perspective

The impact of myocardial reperfusion at the histological level was first postulated in 1960 by Jennings *et al* in a landmark study describing pathology features of the reperfused ischemic canine myocardium, such cell swelling, contracture of myofibrils, disruption of the sarcolemma and the appearance of intra-mitochondrial calcium phosphate particles[32]. Later on, the concept of reperfusion injury was further expanded to other manifestations[33], such as myocardial stunning, reperfusion arrhythmias and the no-reflow phenomenon related to microvascular damage. However, the reperfusion-triggered death of cardiomyocytes that have been reversibly injured during the ischemia has been considered the paradigm target for all developing cardioprotective therapies during the last decades. This phenomenon (known as *lethal reperfusion injury*) is driven by a cytosolic and mitochondrial calcium overload, oxidative stress and rapid restoration of intracellular pH resulting in the opening of the mitochondrial permeability transition pore (mPTP). Researchers have focused on investigating molecular targets in the cardiomyocyte, under the hypothesis that their activation at reperfusion onset ameliorates the impact of myocardial I/R, reducing the resultant IS (and having subsequently an improvement in clinical outcomes).

The pathophysiology of myocardial I/R and cardioprotection

By volumetric determination, cardiomyocytes represent around 75-80% of the total myocardium, whilst other cells appears to contribute little to the volume – endothelium by 3% and fibroblasts by 2%[34]. However, when it comes to cell numbers, the proportions are slightly different and the adult myocardium is composed of ~56% myocytes, 27% fibroblasts, 7% endothelial cells, and 10% vascular smooth muscle cells, with a similar percentages in the left and

right ventricles, as demonstrated by Banerjee *et al*[35]. Given that the experimental gold-standard measure of area at risk and infarct size in the experimental settings, provided by Evans blue and Triphenyl Tetrazolium Chloride (TTC) respectively, is based in volumetric measures, the cardiomyocyte has been assumed for a long-time to be the only target in cardioprotection. Moreover, cardiomyocyte death is the main cause of pump failure, arrhythmias and death in patients with STEMI[9]. Therefore, it has been taken for granted that the effect of cardioprotective interventions should specifically apply to the cardiomyocyte and this cell type has become central to recapitulate reductionist models of protective therapies against I/R (i.e. hypoxia/reoxygenation experiments). However, there is a growing body of evidence showing a role for neutrophils, platelets, endothelial cells and fibroblasts on top of cardiomyocytes[36].

Cardiomyocyte death: the endstage of I/R injury

The heart is a contractile organ, with cardiomyocytes being the contractile units. Cardiomyocytes are thus at the center of the heart´s function. Loss of contractile units lead to failure of cardiac function. As mentioned before, cardiomyocytes represent up to 80% of heart volume and thus loss of these cells has a significant impact on cardiac function. Despite I/R injury is a multi-compartment syndrome, cardiomyocytes are at the end of the chain of events. For this reason, most of the attempts to find therapies able to reduce I/R injury have been focused into cardiomyocytes. Deciphering the intra-cardiomyocyte events occurring during I/R injury have led to the identification of potential targets to protect these cells during I/R Experimental studies over the last three decades have identified a complex signalling map within the cardiomyocyte to explain how most cardioprotective therapies exert their protective effect against I/R injury[37]. These findings have been firstly described to elucidate the mechanism underlying some forms of ischemic conditioning, but they have been subsequently extended to most pharmacological interventions. Briefly, there is a consensus to recognize three hierarchical levels of signal transduction[38]: 1) triggers (usually sarcolemmal membrane receptors, such as G-protein-couple receptors and tyrosine kinase receptors); 2) intracellular mediators (the signalling cascades that

help initiate and propagate the signal); and 3) end-effectors (mechanisms that actually cause the attenuation of cellular injury and death during the lethal ischemic insult). This sequential three-step mechanism can be easily illustrated with a well-known cardioprotective therapy: insulin (the trigger) activates PI3Kα, which in turn recruits Akt and its downstream cascade (mediators) to end up inhibiting the mitochondrial permeability transition pore (mPTP, the end-effector), which in the last link of the chain to avoid cardiomyocyte death[39].

There have been attempts to pharmacologically manipulate these three levels of signal transduction. At trigger-level, insulin or adenosine have been used to activate the cardioprotective response[39]. At mediator level, three pro-survival cascades have received special attention[38,40]: the Reperfusion Injury Salvage Kinase (RISK) pathway (comprising PI3K-Akt and MEK1/2-ERK1/2) [41–43], the Survivor Activator Factor Enhancement (SAFE) pathways (comprising TNFα and JAK-STAT3) [44] and the PKG/eNOS signalling cascade[45]. Importantly, these pro-survival pathways are activated at the onset of reperfusion[46,47], hence getting a translational value if they can be activated either in the ambulance or the cath lab. Finally, at end-effector level, all the three pro-survival cascades seems to converge in the mitochondria[48].

Non-cardiomyocyte compartments involved in I/R injury

While cardiomyocytes are the endstage of I/R injury, there are several processes occurring upstream that contribute to the loss of contractile units. No matter how "strong" is the cardiomyocyte (e.g. from the intracellular signalling pathways perspective) that if there is no efficient tissue perfusion due to microvascular damage, the cell will not survive the episode. Similarly, a cardiomyocyte able to survive the ischemic insult that is surrounded by highly activated neutrophils of other inflammatory cells-mediators is at high risk of dying hours/days after reperfusion. Recent evidences have shown that therapies targeting non-cardiomyocyte compartments can be efficient in reducing I/R injury and ultimately infarct size[36].

Due to the high metabolic demand, the heart has an extensive microvascular blood supply system. Some advocate that the endothelium might have a relevant role in cardioprotection due to both its optimal situation to interact with blood signals and its paracrine ability[49]. As first point of contact between the myocardium and humoral factors, the endothelium constitutes a "blood-heart barrier"[50]. There is some evidence demonstrating that the efluent collected from preconditioned endothelial cells is able to provide some protection against I/R in naïve primary cardiomyocytes[51]. Similarly, Teng *et al*. demonstrated with a transgenic mouse model, which restricted the expression of EPO receptor to hematopoietic and endothelial cells, that the administration of EPO in these mice can protect the heart against I/R, therefore suggesting a major role for the endothelial cell response to EPO to achieve an acute infarct-limiting effect[52]. As a paracrine organ, the endothelium has been demonstrated to trigger protection in cardiomyocytes through receptor/ligand interaction and gaseotransmitter. Endothelin-1 (ET1) receptor and bradykynin B(2) receptor are both present in cardiomyocytes[53,54] and when pharmacologically activated both trigger a preconditioning-like effect[53–55]. In regard to gaseous signals, nitric oxide has been long associated with ischemic conditioning though the role of eNOS (the endothelial isoform of nitric oxide synthase), as demonstrated in eNOS knockout mice[56]; however, therapies with inhalated nitrid oxide have failed in their translation to the clinical arena [57].

Besides being a provider of protective triggers and activated mediators to cardiomyocytes, there is also the possibility for the endothelium to be a target itself for cardioprotection[49]. Most signalling pathways and end-effector mechanisms described in cardioprotection are most likely not specific to cardiomyocytes. Some publications have shown a higher vulnerability to I/R of the endothelium when compared to cardiomyocytes[58,59]. Targeting endothelial receptors through adenosine agonists A1 and A3 or angiotensin II[60] preserve not only the endothelium-dependent vasodilation, but also cardiomyocyte viability. Indeed preserving microvascular function will provide further blood supply to the injured cardiomyocytes. In the clinical setting, pre-infarct angina (a preconditioning clinical manifestation) has been associated with attenuation of no-reflow in STEMI patients undergoing PPCI[61].

Other cells can also be relevant in cardioprotection, such as platelets, where recent experimental data have demonstrated that P2Y12 inhibitors are protective at the onset of reperfusion through RISK activation[62,63], or neutrophils, that can be targeted by metoprolol to inhibit neutrophil-platelet interaction[36].

Cardioprotective interventions beyond reperfusion

Mechanical interventions

The phenomenon whereby the myocardium can endogenously be protected from lethal I/R was firstly reported by Murry *et al* in 1986[31]. As briefly introduced before, in this landmark study, the myocardial IS reduction obtained from the application of several brief cycles of non-injurious ischemia and reperfusion before the subsequent sustained index ischemic insult was coined with the term "ischemic preconditioning" (IPC)[31]. This finding, firstly described in dogs but subsequently replicated in numerous pre-clinical models[64], became the cornerstone of the field for two main reasons: 1) the concept of local IPC evolved into "ischemic conditioning", a broader term that encompasses a number of related endogenous cardioprotective strategies, applied either to the heart (ischemic preconditioning or postconditioning)[65] or to a distant organ (remote ischemic pre-, per- or postconditioning)[65,66]; 2) its underlying signalling architecture has been extrapolated to several cardioprotective therapies and has helped to identify molecular targets amenable to pharmacological modulation. **Table 1** illustrates the landmark studies in I/R and conditioning-related cardioprotective therapies in chronologic order.

The translational potential of local IPC is inevitably limited by the necessity to apply the intervention before the index ischemia, which is unpredictable in many clinical scenarios such as the STEMI. Ischemic postconditioning has been already tested in the clinical setting with mixed results in proof-of-concept studies[22,67,68] but overall disappointing findings when assessing hard clinical outcomes[69]. On the contrary, remote ischemic conditioning (RIC) has emerged as a non-

invasive alternative that can be applied either before (pre-conditioning), during (per-conditioning) or after (post-conditioning) index ischemia[70]. Overall, large clinical trials assessing the impact of RIC on hard endpoints in the context of cardiac surgery have been disappointing[71,72] – most likely because the small amount of myocardium at risk, the short ischemic time, the routine use of cardioplegia and hypothermia and the type of death in this clinical scenario (more related to surgical complications than to the myocardial injury). In the end, cardiac surgery-induced infarct myocardial damage is no longer a relevant problem with current approaches. However, it is acknowledged that the patophysiology underlying STEMI is completely different and there is great expectation for the outcome of two large ongoing clinical trials which have been combined into a single one, namely the CONDI2/ERIC-PPCI study[73] – this trial have already finished recruitment of 5400 STEMI patients undergoing PPCI in Europe (Denmark, UK, Spain, and Serbia,). Patients were randomized to RIC or control, and the primary outcome is the composite of cardiac death and heart failure hospitalization over 12 months follow-up. Results will be reported late in 2019.

The aplication of high mechanical index impulses using a regular clinically-available ultrasound transducer during commercially-available intravenous micobubbles infusion (the so calle sonothrombolysis) has shown to be effective reducing cardiac injury in STEMI patients[74]. The recent and promissing MRUSMI trial concluded that sonothrombolysis has an important role restoring epicardial flow and reducing infarct size[74]. This effect is probably due to the microbubbles growth and collapse produced by the ultrasound stimulation. This mechanical intervention promotes cavitation forces capable of thrombus dissolution [75].

Pharmacological strategies

Several pharmacological strategies have been tested in experimental and pilot clinical trials with promising results[76]. Many of them have failed as they progressed in the clinical arena. We are focusing this chapter into the therapies that are still promising and continue in the race of

finding a robust cardioprotective agent. We also briefly comment therapies that were highly promising but withdraw from this career.

Metoprolol

There is solid preclinical data showing that the administration of metoprolol before reperfusion reduces myocardial infarct size in a pig model of AMI[77–79]. Moreover, there are incipient data supporting the idea that, unlike most cardioprotective therapies, metoprolol targets the neutrophil instead of the cardiomyocyte[36]. Two recent trials have evaluated the cardioprotective effect of metoprolol when administered before reperfusion. The Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) trial randomized 270 anterior STEMI patients to early intravenous metoprolol (started during ambulance transfer if possible) or control. This trial demonstrated a significant reduction in myocardial IS and an improvement in left ventricular systolic function[80,81]. Moreover, smaller infarcts were observed in those patients recruited during transfer (early treated with metoprolol) in comparison with those at the PCI center[82]. In a subsequent attempt to assess the cardioprotective effect afforded by metoprolol, the Early Intravenous Beta-Blockers in Patients With ST-Segment Elevation Myocardial Infarction Before Primary Percutaneous Coronary Intervention (EARLY-BAMI) trial showed neutral results for myocardial IS reduction in 683 STEMI patients. Several features of the trial design might explain these conflicting results, such as the evaluation of the effect in a non-restricted STEMI population with infarcts in any location, the extended time window for recruitment from 6 to 12h, or the application of a lower metoprolol dose in comparison with the METOCARD-CNIC trial. In light of the known impact of timing of administration of metoprolol on its cardioprotective abilities[77], EARLY BAMI probably failed due to the very late administrarion of the drug (i.e very close to reperfusion). However, this is speculative at this moment, and the definite answer to whether metoprolol ameliorates I/R and this is translated into an improvement in clinical outcomes requires an ultimate clinical trial. The

Impact of pre-reperfusion Metoprolol On clinical eVEnts after myocardial infarction (MOVE ON!) trial[9] has been designed taking into consideration all these facettes.

Exenatide and other glucose modulators

The potential therapeutic use of insulin to protect ischemic cardiomyocytes was proposed more than 50 years ago by Sodi Pallares[83]. This protective effect was first attributed to its ability to modulate glucose metabolism. The infusion of glucose-insulin-potassium (GIK), known as metabolic cocktail, was evaluated in acute myocardial infarction experimental models under the hypothesis that GIK reduces free fatty acids metabolism, therefore providing an optimal metabolic milieu to resist both ischemic and reperfusion injury[84]. In this line, the Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care (IMMEDIATE) trial recruited patients with suspected acute coronary syndrome and randomized them to GIK or placebo during the hospital transfer and failed to demonstrate efficacy in its primary endpoint. However, GIK significantly reduced myocardial IS in the subgroup of patients presenting with STEMI who underwent cardiac magnetic resonance[85]. Notably, other glucose modulators have shown promising results in cardioprotection. Findings from Yellon's lab have revealed that the administration of either GLP-1 native peptide or the inhibition of DPP-4 protects the heart against I/R injury in an *ex vivo* rat model of AMI through a mechanism not driven by the stimulation of insulin secretion, but by the activation of intracellular prosurvival kinases cascades[86,87]. In the clinical setting, Lonborg *et al.* showed that the infusion the GLP-1analogue exenatide, prior to PPCI, increases myocardial salvage in STEMI patients [88]. The potential protective effect provided by the new SGLT2 inhibitors remains largely unknown at the moment, but it might become a new potential target to limit myocardial IS in AMI patients[89].

N-acetylcysteine

The cardioprotective effect of N-acetylcisteine (NAC), a sulfhydryl-containing antioxidant agent, has been widely tested in both the experimental and clinical setting, with some controversial results. Despite the preclinical positive findings, the cardioprotective effect of NAC administration in STEMI patients was disappointing in the Prospective, Single-Blind, Placebo-Controlled, Randomized Leipzig Immediate PercutaneouS Coronary Intervention Acute Myocardial Infarction N-ACC (LIPSIA-N-ACC) trial[90] - high-dose NAC reduced oxidative stress but did not provide clinical when compared to placebo. In contrast, the recent -acetylcysteine in Acute Myocardial Infarction (NACIAM) trial[91] has shown in a small cohort of STEMI patients that the intravenous administration of high dose of NAC on top of low dose of intravenous nitroglicerine (NTG) reduces myocardial infarct size mesured by CMR and enzymatic concentration when compared to placebo. It is plausible that the combination of both therapies, linked to the potentiation of vasodilator and antiaggregant effects, have increased the chance for NAC to demonstrate a protective effect. Despite the role of each drug per separate have not been clarified, the promising results should be an stimulus to mechanistics studies and larger clinical trials aimed to evaluate synergystic cardioprotective effects of NAC with NTG, or even in combination with other interventions.

Promising agents not fulfilling expectations

From the long list of failures, some have been specially painful because they were preceded by solid experimental and pilot clinical experiences[9,76]. Some of them are presented for the sake of historical perspective:

- Adenosine

Prior to index ischemia, adenosine has been shown to reduce myocardial infarct size in animal models of acute I/R injury through mechanisms related to nitric oxide and protein kinase G [92]. The infarct size reduction by adenosine at reperfusion has been contentious in animal

models[93], and its translation to the clinical setting has been equally contentious. The AcuteMyocardial Infarction STudy of Adenosine (AMISTAD) trial reported reductions in infarct size with high-dose intravenous administration[94,95], though AMISTAD-II was neutral for clinical outcomes in patients with STEMI undergoing reperfusion therapy[95]. Two recent small placebo-controlled trials tested intracoronary adenosine to reduce infarct size, as evaluated by CMR.[96,97] In both trials, intracoronary adenosine was not associated with smaller infarctions of less microvascular obstruction, being the last nail in the coffin of this intervention. Overall, no consistent benefit has been observed for adenosine on infarct size and clinical outcomes[94–96,98,99],177–183, though a meta-analysis found less microvascular injury and heart failure outcome only with intracoronary adenosine[100].

- Cyclosporine

Cyclosporin has demonstrated to reduce myocardial infarct size in many experimental studies, with few contentious results[101]. After a pilot proof-of-concept study on the use of intravenous cyclosporine A immediately prior to reperfusion in STEMI patients had shown an increased LVEF and reduced infarct size by enzymatic release[102], the larger follow-up Cyclosporine to ImpRove Clinical oUtcome in ST-elevation myocardial infarction patients (CIRCUS) trial[48] failed to improve clinical outcomes at 1 year and reproduce the results in terms of infarct size in anterior STEMI patients. The CYCLosporinE A in Reperfused AcuteMyocardial Infarction (CYCLE) trial[103] in patients with large reperfused myocardial infarctions failed to demonstrate enzymatic infarct size reduction and ST-segment resolution. Overall, there is solid evidence demonstrating that inhibiting the mPTP opening is protective, hence a reasonable explanation for this unsuccessful translation might be that in the clinical setting cyclosporine A reaches its molecular target too late as to confer substantial protection.

Cardioprotection: an unsuccessful translational story to date

Many cardioprotective therapies aimed at reducing I/R have been successfully evaluated in the experimental setting. It is important to remark that not all these experimental experiences were done with rigor according to accepted preclinical guidelines[104]. Despite attenuating I/R at the bench, not all of them have subsequently demonstrated an IS-limiting effect at the bedside, and none have demonstrated to date clear benefits in terms of mortality or heart failure rehospitalization[105]. The reasons for these disappointing translation from experimental and proof-of-concept trials to clinical practice have been widely discussed elsewhere and are a matter of intense debate [93,105–107]. They are briefly summarized in **Table 2**. One common denominator of many of the failures is that the experimental setting where the therapies were proven beneficial are vey different from the clinical scenario in which they were validated. As an illustrative example, therapies targeting the mitochondrial permeability transition pore should reach the cardiomyocyte before reperfusion or at immediate reperfusion the latest. This is achievable at the experimental level but not at the clinical. Recently is has been shown that the administration of a bloodless oxygen carrying solution (enriched with other nutrients) can stop ongoing necrosis without immediate blood flow restoriation [108]. This strategy allows a total control of conditions (temperature, pH, absence of inflammatory cells or mediators etc) at early stages of reperfusion. This platform could serve to test some of these agents that only are beneficial if reach the myocardium before full-blown reperfusion conditions occur.

Current challenges from epidemiological, clinical and experimental perspectives

The epidemiological perspective

The impact of evidence-based medicine in clinical trials in cardioprotection

It is becoming challenging to find a balance between what it is clinically relevant and what it is economically feasible. The progressive decline in cardiovascular events in post-MI patients[1,9] has a huge impact on the statistical power of randomized clinical trials (RCTs) – i.e. the EARLY-BAMI was powered to detect a reduction in IS from 28% to 23.5%, whereas the final estimated IS for the placebo group was actually 14.9%, thus making the conclusions hard to interpret because the lack of statistical power[109]. Adding extra events to mortality and heart failure rehospitalization increases the event rate, but it might dilute the effect size[105].

Selecting the most suitable outcomes in proof-of-concept and phase III RCTs

In the classic sequential approach for performing translational research, once robust data is obtained from animal experiments[104], the subsequent logical step would be to carry out a "proof-of-concept" clinical trial[107,110]. There is a need to evaluate not only myocardial infarct size, but also left ventricular function. It is no clear-cut to what extent one is the surrogate for mortality and the other for morbidity – no matter the true relationship, both surrogate outcomes are clinically relevant and the composite outcome of mortality and heart failure rehospitalization have been already used in many cardiovascular RCTs[111]. However, it is unknown how potent an infarct-limiting (or LVEF-increasing) intervention has to be to provide a clinically meaningful impact – there is no a clear threshold, and it might the case that rather than a relevant decrease in a relative percentage, some other measures (such as number of patients requiring implantable cardioverter defibrillator) might better measure the impact of cardioprotective therapies.

The clinical perspective

Selecting the most suitable patient profile

Patients with larger, preferably anterior infarcts and with an ongoing occluded coronary artery at the time intervention would benefit more than patients with smaller infarcts and partial reperfusion at the time of the protective therapy[112]. There might be other clinical scenarios where cardioprotective interventions might have a relevant role, such as in patients with cardiac arrest, who are under global I/R[113,114].

Selecting the adequate window opportunity

If reperfusion occurs shortly after the onset of symptoms, no intervention or drug would have real impact on IS or subsequent clinical outcomes because the so successful reperfusion leaves little room to cope with I/R. In contrast, there remains little salvageable myocardium in late reperfusion[8]. Many pharmacological agents have proved effective only in those patients presenting shorter periods of ischemia. In a subgroup analysis of the Acute Myocardial Infarction Study of Adenosine-II (AMISTAD-II)[95], patients reperfused within less than 3.2 h of symptom onset (median time to reperfusion) showed that adenosine reduced the composite endpoint of death and congestive heart failure[115]. Similarly, exenatide seems to be more effective in STEMI patients with shorter ischemic times[116], however, metoprolol could be more effective with longer ischemic times[77].

Selecting the optimal time to administer the cardioprotective intervention

It has been largely assumed for a long time that any cardioprotective intervention can be given prior to or at the time of PPCI to reduce myocardial IS and preserve LVEF. This assumption was made based on the acute recruitment of pro-survival kinases at the onset of reperfusion[43,47]. Thus, most cardioprotective interventions has been tested at reperfusion in the experimental and

clinical setting[112], despite being feasible to be administered at any time between first patient contact and time of reperfusion, as demonstrated by several proof-of-concept clinical studies assessing RIC[117], glucose-insulin-potassium therapy[85] and metoprolol[80,81].

Unlike applying the intervention at the time of PPCI, there is a growing body of evidence pointing towards a greater benefit to do it at an earlier time-point – i.e. in the ambulance while in transit to the cath lab. Several publications support the notion that the longer the cardioprotective therapy is on board, the more effective it is reducing myocardial IS. Both RIC[118] and metoprolol[77] have demonstrated that the sooner the intervention is applied in the course of the infarction, the better the surrogate outcome. Similarly, recent findings suggest that therapies effectively reducing myocardial IS exert an important effect not only on reperfusion, but also on ischemic injury – as illustrated by the impact of per-RIC on attenuatting ST-segment elevation during ongoing coronary occlusion in a pig model of AMI[119]. The clinical consequence is that these therapies should be applied as soon as possible on myocardial infarction diagnosis, which is usually the out-of-hospital setting.

The experimental perspective

Using adequate experimental models

Differences in animal physiology should be considered when attempting to translate an intervention[107,120]. Within the framework of the Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR) initiative, Jones *et al.* described a species-related effect-size gradient when local IPC was applied (IS reduction was largest in mice, intermediate in rabbits and lower in pigs). Even within the same species, substantial differences in the response to AMI have been also observed across different mice strains with variations up to 30% of myocardial IS using the same procedure[121]. As a general rule, rodent models are optimal when testing novel therapies and elucidating their underlying mechanisms, whereas large-animal models, that closely resembles human physiology, are more useful before performing proof-of-concept studies in the clinical arena[122]. In any case, it is fundamental to thoroughly understand the physiopathology and

the underlying temporal evolution of the AMI in each specific preclinical models, as well as the effects of a given cardioprotective therapy in such features.

New targets

There are some novel therapeutic targets that are currently under intense investigation, such as theimmune system (monocytes, macrophages, extracellular DNA and RNA, inflammasomes), platelet – inflammatory cell interactions, exosomes and microvesicles, GPCRs, Toll-like receptors (TLRs) and proteases such as matrix metalloproteinases and calpains among others, with especial emphasis on mitochondria [123]. A high number of functional mitochondria are needed for the myocardium to keep producing the necessary ATP for a regular contractile function, in addition to participate in other basic biological processes, such as ion homeostasis and calcium exchange[124]. Among all the events triggered by I/R injury, mitochondrial disturbances play a major role, being probably the final confluence of many simultaneous pathways, as it happens with ischemic conditioning cascades finishing in the mitochondria as end-effector[124]. Some drugs directly targeting the mitochondria have been tested in myocardial I/R injury, such as the mitochondrial permeability transition pore inhibitorcyclosporine-A, although they have failed in their final clinical translation to date [48]. Despite this initial disappointing results, mitochondria remains yet an attractive target in AMI and new approaches using drugs aimed to modify mitochondrial dynamics (fission and fusion)[125] or even transplantating complete organelles have been recently proposed[126].

The complexity of the underlying pathophysiological processes in the myocardium subjected to I/R injury, as well the different cell types involved and the unknown impact of time over both components of the injury suggests that mimicking a single pathway is probably a too simplistic approach.. Therefore, the synergistic application of more than one therapy either acting on different processes involved in the I/R injury, or just adding further effect into the the same biological process is getting popular in some laboratories - i.e.in a pig AMI model, the combination of RIC with either GIK or exenatide at the time of reperfusion have shown to reduce myocardial IS to a greater extent than either intervention alone[127]. The rational for using

combination therapies is expanded elsewhere[128]. These positive results have lead these researchers to conduct a clinical trial combining these two therapeutic interventions (the COMBINATION Therapy in Myocardial Infarction, or COMBAT-MI Trial).

Conclusions

Although timely reperfusion limits both myocardial infarct size and the subsequent myocardial remodelling, reperfusion *per se* adds an additional irreversible myocardial damage, contributing to final infarct size. Ischemia/reperfusion syndrome is multifactorial with several players involved. Cardiomyocytes, microcirculation, and circulating cells are the main compartments affected by this syndrome. There is yet a need to translate cardioprotective therapies into the clinical setting, being intravenous metoprolol, exenatide and N-acetilcysteine the most promising therapies. Several opportunities have been found to improve the translationability of these interventions: selecting the most suitable outcomes in proof-of-concept and phase III randomized clinical trials, the most suitable patient profile, the adequate window opportunity and the optimal time to administer the cardioprotective intervention. In the experimental setting, using adequate experimental models (i.e. rodent models for mechanisms and large-animal models for translational studies) and evaluating new targets (i.e i.e. microcirculation, circulating cells, edema, mitochondria, combined therapies) can help to move this field forward.

Conflict of interest

The authors declare that there is no conflict of interest.

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Figures

Figure 1. Challenges and opportunities to translate therapies ameliorating myocardial ischemia-reperfusion injury from a epidemiological, clinical and biological perspective

Table 1. Landmark studies in I/R and conditioning-related cardioprotective therapies

Year	Author	Achievement
1972	Maroko *et al.* [16]	First evidence that reperfusion limits extent of necrosis
1977	Reimer *et al.* [126]	Description of the wavefront progression of necrosis (from endocardium to epicardium)
1986	Murry *et al.* [31]	First evidence that ischemic preconditioning reduces IS (first window of protection)
1993	Marber *et al.* [127]	Description of the second window of protection
1993	Przyklenk *et al.* [128]	First evidence that remote ischemic preconditioning within the heart reduces IS
1997	Birnbaum et al. [129]	First evidence that the application of non-coronary remote conditioning confers protection to the heart
2002	Schulman *et al.* [41]	Description of the Reperfusion Injury Salvage Kinase (RISK) pathway
2003	Zhao *et al.* [130]	First evidence that ischemic postconditioning reduces IS
2005	Lecour *et al.* [131]	First evidence of a RISK-independent signalling pathway, later on coined as the Survivor Activator Factor Enhancement (SAFE) pathway
2010	Bøtker *et al.* [114]	First evidence that remote ischemic perconditioning increases myocardial salvage in man
2017	García-Prieto et al[36]	First demonstration that the β1 selective blocker metoprolol reduces MVO and infarct size by targeting neutrophils
2019	CONDI2/ERIC-PPCI study [73]	Ongoing randomized clinical trial involving 5400 STEMI patients undergoing PPCI assessing improvement in long-term clinical outcomes following the application RIC

I/R, ischemia/reperfusion injury; IS, infarct size; mPTP, mitochondrial permeability transition pore; PPCI, primary percutaneous coronary intervention; RIC, remote ischemic conditioning; STEMI, ST-segment elevation myocardial infarction

Table 2. Potential reasons explaining the translational failure of cardioprotective therapies

1	Differences between AMI experimental models and patients with STEMI (i.e atherothrombosis vs artery ligation)
2	Differences in animal physiology (i.e. a different IPC effect size gradient was observed across species in the CAESAR initiative)[132]
3	Reductionist approaches for experimental designs to an increasingly complex clinical situation (i.e. single interventions are tested without the background of co-morbidities or other therapies)[104]
4	Performance of proof-of-concept clinical trials without solid experimental evidence or large animal data[9]
5	Lack of reproducibility and poor reporting[133]
6	Lack of "strict" validation of surrogate endpoint in clinical trials[102]
7	Randomized clinical trial design: eligibility criteria for patient selection (i.e. anterior infarct would benefit the most), misuse of some composite endpoints

AMI, acute myocardial infarction; CAESAR,Consortium for preclinicAl assESsment of cARdioprotective therapies; IPC, ischemic preconditioning; STEMI, ST-segment elevation myocardial infarction

Figure 1