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THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Evolving Therapies for Myocardial Ischemia/Reperfusion Injury

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CME Objective for This Article: After reading this article, the reader should be able to: 1) relate the importance of infarct size (amount of myocardium irreversibly injured during an ST-segment elevation acute myocardial infarction [STEMI]), and the need to find novel/better therapies able to reduce infarct size; 2) discuss the difference between ischemic and reperfusion injuries; 3) acknowledge that, on the basis of a timely reperfusion, additional interventions/therapies are needed to reduce the impact of reperfusion injury and, ultimately, infarct size; 4) discuss the global general pathways implicated in reperfusion-mediated injury; and 5) describe the main interventions holding the potential to reduce ischemia/reperfusion injury.

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Evolving Therapies for Myocardial Ischemia/Reperfusion Injury

ABSTRACT

The damage inflicted on the myocardium during acute myocardial infarction is the result of 2 processes: ischemia and subsequent reperfusion (ischemia/reperfusion injury). During the last 3 decades, therapies to reduce ischemic injury (mainly reperfusion strategies) have been widely incorporated into clinical practice. The remarkable reduction in death rates achieved with these therapies has resulted in a shift in emphasis from efforts to reduce mortality to a focus on tackling the downstream consequence of survival: post-infarction heart failure. Infarct size is the main determinant of long-term mortality and chronic heart failure, and thus, the possibility of limiting the extent of necrosis during an ST-segment elevation myocardial infarction is of great individual and socioeconomic value. After the great success of therapies to reduce ischemic injury, the time has come to focus efforts on therapies to reduce reperfusion injury, but in the recent few years, few interventions have successfully passed the proof-of-concept stage. In this review, we examine the past, present, and future therapies to reduce ischemia/reperfusion injury. (J Am Coll Cardiol 2015;65:1454–71)

Acute myocardial infarction presenting as ST-segment elevation (STEMI) is the result of abrupt occlusion of an epicardial coronary artery. As a result, the myocardium distal to the occlusion site becomes ischemic. Unrelieved ischemia causes permanent damage to the myocardium previously supplied by the occluded artery. Myocardium is destroyed and replaced by fibrous scar tissue. Because scar tissue does not contribute to myocardial contractile function, if the scar is large, global left ventricular (LV) contractile function is impaired, resulting in progressive chronic heart failure. After the demonstration that coronary thrombosis was the cause (not the result) of STEMI in the vast majority of cases, timely restoration of blood flow to the ischemic myocardium (reperfusion) became the standard treatment for these patients. Reperfusion was rapidly demonstrated to limit infarct size, improve long-term myocardial function, change the healing pattern of the infarcted zone, and more importantly, reduce mortality. A large body of experimental and clinical evidence supports the notion that reperfusion induces additional damage to the myocardium, known as reperfusion injury. As a result, the damage inflicted on the myocardium during an STEMI is better defined as *ischemia/reperfusion (I/R) injury*, the result of ischemic and reperfusion processes. Myocardial I/R injury is a complex phenomenon involving many players, all contributing to the final damage inflicted on the heart (**Central Illustration**).

In the present review, we describe the evolving therapies for the treatment of myocardial I/R injury. These include therapies targeting both ischemic and reperfusion damage. To explain the rationale for the quest for new and better therapies, we describe the pathophysiology of myocardial I/R injury and the translational path of research, from the pre-clinical discovery phase, through proof-of-concept clinical trials, to large trials aimed at changing clinical practice. In the context of this paper, the term myocardial infarction always refers to STEMI.

IMPACT OF STEMI IN 2015: A PARADIGM SHIFT

The incidence of STEMI in Western countries has declined during the last decades due to the progressive implementation of preventive therapies and better control of risk factors (1). Despite the progressive and gradual decrease in its incidence, STEMI remains a significant health problem, representing a major contributor to mortality/morbidity worldwide (1). As detailed later in this review, the implementation of timely reperfusion has resulted in a very significant reduction in the acute mortality associated with STEMI. Risk-adjusted in-hospital mortality has decreased from ≈20% in the late 1980s to ≈5% among STEMI patients treated in routine practice in 2008 (2), reaching a plateau thereafter (3). However, these impressive reductions in mortality rates, resulting from the widespread use of reperfusion strategies and adjuvant pharmacological therapies, have resulted in an increase in the incidence of chronic heart failure. Although this outcome might at first seem paradoxical, the explanation is simple: patients with a severely depressed cardiac function would not have survived

ABBREVIATIONS AND ACRONYMS

AAR = area at risk

CMR = cardiac magnetic resonance

I/R = ischemia/reperfusion

LVEF = left ventricular ejection fraction

MPTP = mitochondrial permeability transition pore

MVO = microvascular obstruction

PCI = percutaneous coronary intervention

STEMI = ST-segment elevation myocardial infarction

the acute STEMI phase in the past, but with the advent of reperfusion, they now survive the index episode and live with a significantly damaged heart (2). In fact, STEMI is 1 of the major contributors to chronic heart failure. Post-infarction reduced left ventricular ejection fraction (LVEF) is 1 of the principal causes of chronic heart failure worldwide (4). The great success of reperfusion therapies has resulted in a paradigm shift in clinical research in the field of STEMI: attention is no longer solely aimed toward reducing mortality (already very low), but increasingly, is to tackle the downstream consequence of improved survival: post-infarction heart failure.

Successful clinical research has led to interventions for chronic heart failure (drugs and devices) that reduce long-term mortality in STEMI survivors with low LVEF (5). However, these strategies are economically costly, precluding their universal implementation (6). Chronic treatment of heart failure represents a huge socioeconomic burden on individuals and health care systems. As explained later in this paper, infarct size is the main determinant of adverse post-infarction outcomes, including heart failure (7). Therapies able to reduce infarct size are, therefore, urgently sought under the hypothesis that smaller infarctions will result in better long-term heart performance and that this will translate into fewer adverse clinical events (8,9). As we detail throughout this paper, the identification of refined or new therapies better able to reduce infarct size is a major challenge to 21st century society.

PATHOPHYSIOLOGY OF I/R INJURY

GENERAL CONSIDERATIONS. After the occlusion of an epicardial coronary artery, the myocardium previously perfused by the occluded artery is in jeopardy. The hypoperfused myocardial zone during myocardial infarction is known as the area at risk (AAR). If the coronary artery is not rapidly reperfused and no collateral circulation is present, most of the AAR becomes necrotic. Given that many patients receive timely reperfusion therapy, part of the AAR remains free of necrosis: the so-called salvaged myocardium. The typical morphological features of reperfused myocardial infarction are contraction bands, karyolysis, mitochondrial swelling and disruption, and membrane disruption in cardiomyocytes, accompanied by microvascular destruction, interstitial hemorrhage, and inflammation (10,11). Experimental studies identified the determinants of myocardial infarct size as: 1) the size of the AAR (12); 2) the duration of myocardial ischemia (13,14); 3) the amount of residual blood flow through collaterals (12,13); 4) the temperature of the tissue during ischemia; and 5) the hemodynamic situation during ischemia (15). The most notable hemodynamic parameter is heart rate, which determines not only myocardial demand, but also coronary blood flow (16); however, hemodynamics influence infarct size only to a limited degree, and infarct size is, thus, largely determined by lack of blood/energy supply and less by myocardial demand, which is significantly reduced by the regional lack of contraction (17).

The seminal studies by Maroko et al. (18) and Ginks et al. (19) 40 years ago first demonstrated that reperfusion salvages myocardium from infarction, and these studies initiated the ongoing success story of reperfusion therapy (20). The potential of reperfusion to induce additional injury secondary to the ischemic damage emerged soon afterward with the identification of stunning as a reversible form of myocardial reperfusion injury (21). Although the contribution of reperfusion injury to final infarct size has been disputed in the past, today it is accepted that reperfusion can induce additional damage to the myocardium. This view is supported by strong evidence that interventions applied at the end of the ischemic period (i.e., coinciding with reperfusion) can reduce infarct size. It was already recognized in the mid-1980s that gentle reperfusion at low pressure resulted in significantly less edema and a smaller infarct size than standard abrupt reperfusion at normal pressure (22). This idea was later developed by Zhao et al. (23), who demonstrated reduction of infarct size by brief episodes of coronary reocclusion/reflow at the time of reperfusion, a strategy called ischemic post-conditioning (24). Because these interventions are applied at the end of the ischemic period, they cannot reduce infarct size by reducing ischemic damage and, thus, must reduce reperfusion-related damage. From these observations it is clear not only that reperfusion injury contributes to infarct size, but also that all conditioning strategies that protect the myocardium and reduce infarct size act only in conjunction with eventual reperfusion (8,25,26).

ROLE OF MICROCIRCULATION IN INFARCT SIZE AFTER STEMI. The coronary microcirculation is a critical player in the complex phenomenon of myocardial I/R (**Central Illustration**). The microcirculatory network is the interface between the epicardial vessel and the cardiomyocytes. Thus, no matter how

efficiently and rapidly the blood flow is restored to the epicardial artery, if there is a microvascular obstruction (MVO) the myocardial tissue will remain without efficient perfusion. MVO (also termed the no-reflow phenomenon) during I/R is a major contributor to final infarct size and is an independent predictor of morbidity/mortality (27). The no-reflow phenomenon was first characterized by Kloner et al. (11,28) in dogs subjected to 90 min of coronary occlusion and subsequent reperfusion; the coronary microcirculation of these animals showed severe capillary damage, notably swollen and ruptured endothelial cells, and intraluminal thrombosis, and was surrounded by swollen and irreversibly injured cardiomyocytes. In the clinic, the no-reflow phenomenon is seen in 10% to 30% of patients with reperfused STEMI (29,30) despite successful recanalization of the epicardial coronary arteries; MVO in these patients is detected angiographically from slow or no reflow of contrast medium or by cardiac magnetic resonance (CMR). MVO develops within minutes of established reperfusion (31,32) and persists for at least 1 week (33,34). MVO is usually confined to the infarcted myocardium (14,28); however, the existence of no-reflow phenomena within the AAR but outside the infarcted area has not been systematically excluded. Notably, MVO can impair the washout of reduction equivalents and dehydrogenases that is mandatory for valid delineation of infarcted tissue by TTC staining, thus contributing to underestimation of infarct size (35). Reduced coronary blood flow is also observed outside of the AAR, but this does not reflect a no-reflow phenomenon, and is instead the result of reflex-mediated alpha-adrenergic coronary vasoconstriction (36–38).

A number of mechanisms have been proposed as contributors to MVO: 1) embolization of particulate debris from the ruptured culprit atherosclerotic lesion, with physical obstruction of the coronary microcirculation (39); 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 (40); 3) intense vasoconstriction induced by soluble vasoconstrictor substances released from the culprit lesion (41,42); 4) extravascular coronary microvascular compression due to edema in the surrounding myocardium (43); and 5) primary physical destruction of the capillary endothelium (28). These different mechanisms are not mutually exclusive and can act in concert, and their individual contribution to impaired myocardial reperfusion may vary temporally and spatially. Irreversible injury to cardiomyocytes and the coronary microcirculation are intimately related (44). High intramyocardial pressure, with a predominant contribution from edema, might be the principal cause of MVO in the endocardial layer, whereas microembolization might underlie infarct expansion in the border zone (45). However, there is currently no evidence to support a causal role for microvascular coronary obstruction in myocardial infarction, although it is intuitive to argue that the absence of efficient tissue perfusion in areas of MVO will maintain the muscle ischemia and thus contribute to infarct expansion.

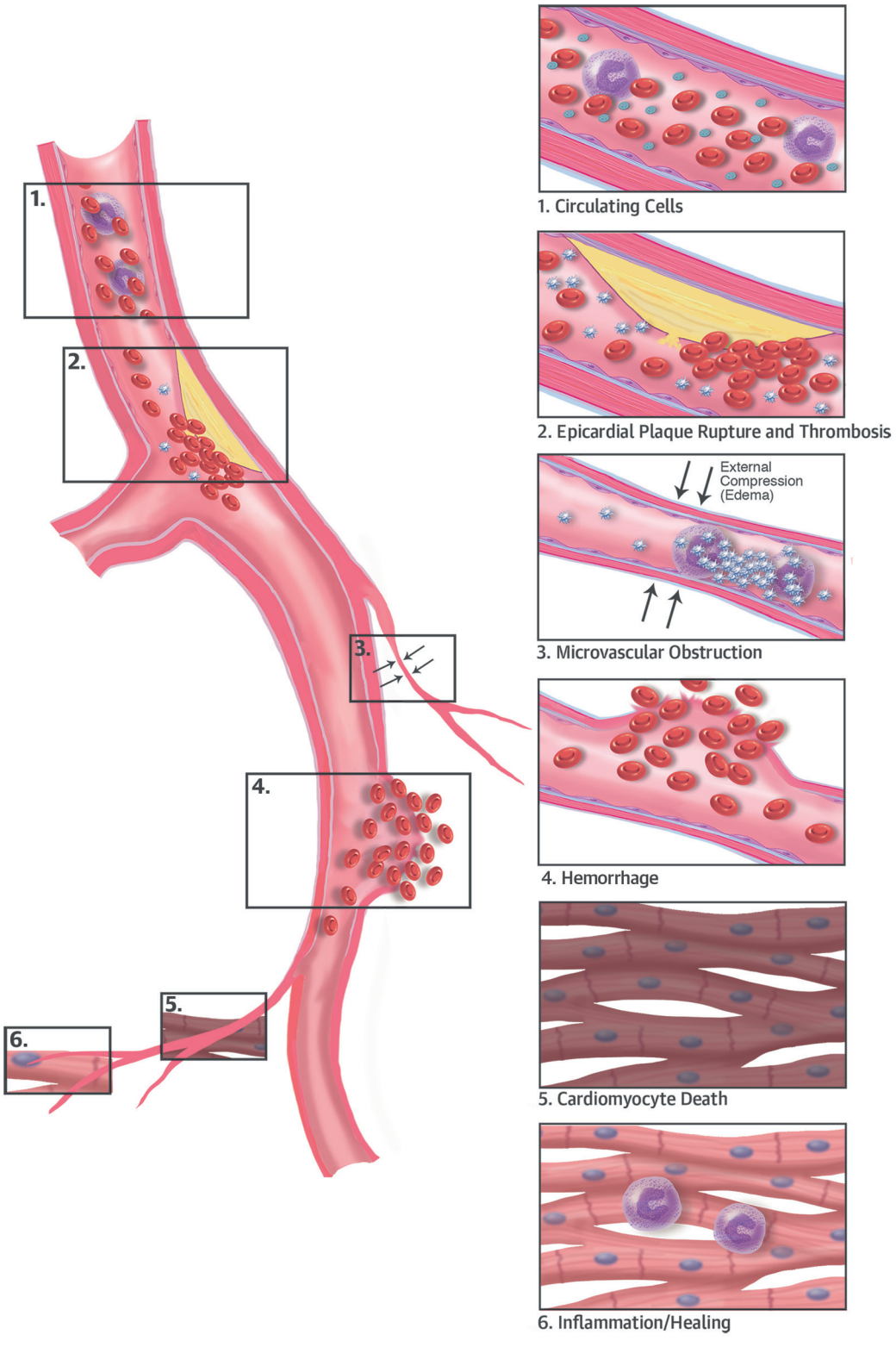
The role of leukocytes in myocardial infarct development is contentious, and leukocyte infiltration may be more important for infarct healing and remodeling rather than the determination of infarct size (46). However, the potential contribution of intravascular leukocytes to MVO and infarct size deserves more attention. Myocardial infarction and MVO currently appear to be parallel phenomena that result from similar pathomechanisms: a primary energetic deficit and subsequent excessive formation of reactive oxygen species upon reperfusion. It is, therefore, not surprising that post-conditioning reduces not only myocardial infarct size, but also MVO (23,34).

CARDIOMYOCYTE NECROSIS, APOPTOSIS, AUTOPHAGY, AND NECROPTOSIS: DOES MODE OF DEATH MATTER?

Myocardial infarction has traditionally been viewed as a manifestation of necrotic cell death, but recently, different forms of cardiomyocyte death have been identified during I/R and are proposed to contribute to final infarct size.

Necrosis is morphologically characterized by myofibrillar contraction bands, swollen and ruptured mitochondria, destruction of cardiomyocyte membranes, microvascular destruction, hemorrhage, and inflammation. Most of these morphological features are aggravated and are made more manifest by reperfusion (11,13,14,47,48). Necrosis is thought to result from unregulated and uncoordinated pathophysiological mechanisms. During ischemia, the developing acidosis from anaerobic glycolysis increases the influx of Na^+ through the Na^+/H^+-exchanger, and intracellular Na^+ accumulation is increased by the inhibition of Na^+/K^+-ATPase due to the lack of available ATP (49,50). The subsequent exchange of Na^+ for Ca^{++} by reverse mode operation of the sarcolemmal Na^+/Ca^{++}-exchanger induces intracellular Ca^{++} overload. Upon reperfusion, the rapid normalization of pH and re-energization in the context of elevated cytosolic Ca^{++} induces oscillatory release and reuptake of Ca^{++} into the sarcoplasmic reticulum, causing uncontrolled excess myofibrillar hypercontraction (50–52). The

CENTRAL ILLUSTRATION **Players Involved in Ischemia/Reperfusion Injury**



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normalization of the acidic pH also activates calpain, which digests the cytoskeleton and the sarcolemma (53). The high cytosolic concentrations of Na^{+} and Ca^{++} result in intracellular edema when extracellular osmolarity is rapidly normalized by reperfusion. Finally, excess formation of reactive oxygen species contributes to sarcolemmal disruption (54). Necrosis is typically followed by an inflammatory response.

Unlike necrosis, apoptosis, autophagy, and necroptosis are regulated processes with specific underlying signal transduction mechanisms (55,56). *Apoptosis* is an energy-consuming form of cell death characterized by characteristic deoxyribonucleic acid strand breaks that are identified by deoxyribonucleic acid laddering and/or terminal deoxynucleotidyl transferase dUTP nick-end labeling staining (57). Apoptosis can be initiated extrinsically by activation of sarcolemmal receptors, notably FAS and tumor necrosis factor α receptors (58), or intrinsically by mitochondrial release of cytochrome c, which initiates a cascade of caspase activation leading to intracellular proteolysis, typically without an inflammatory response (56). A pivotal event in the initiation of apoptotic cell death is the opening of the mitochondrial permeability transition pore (MPTP) (59). The MPTP is a large-conductance megachannel, which is closed under physiological conditions but opens in response to increased concentrations of calcium, inorganic phosphate, or reactive oxygen species and to a decreased inner mitochondrial membrane potential, all of which are present in myocardial I/R (60,61). Formation and opening of the MPTP results in mitochondrial matrix swelling, ultimately leading to rupture of the outer membrane and release of cytochrome c to the cytosol, where it activates the caspase cascade. Proapoptotic and antiapoptotic proteins of the Bcl-family interact with the MPTP (62). Recently, the traditional view of the MPTP has been questioned, because all of its purported constituents are dispensable under some conditions, and it is possible that the MPTP originates from F-ATP synthase (63).

Autophagy is a regulated process of lysosomal degradation and recycling of proteins, including mitochondrial proteins (mitophagy) (64). Autophagy is characterized by the presence of double-membrane vesicles (autophagosomes) and increased expression of beclin-1, light chain 3, the autophagy-related gene 5-12 complex, p62, and parkin, the last 2 of which are essential for mitophagy (65). Somewhat paradoxically, cell death by autophagy is considered protective rather than detrimental (66). For example, in pigs subjected to 45 min of coronary occlusion and reperfusion, the purported autophagy inducer chloramphenicol reduced infarct size (67). However, the role of autophagy in myocardial I/R injury in humans remains contentious (68,69).

Necroptosis shares features with necrosis and apoptosis, but is distinctly regulated by activation of receptor-interacting protein kinases 1 and 3 (70) and can be inhibited by substances such as necrostatin (71).

It is currently unclear to what extent necrosis, apoptosis, autophagy, and necroptosis are mutually exclusive processes and to what extent each contributes to infarct size. Typical features of apoptosis (terminal deoxynucleotidyl transferase dUTP nick-end labeling staining) and autophagy (characteristic protein expression) are both found in the TTC staining-defined infarct zone, which has traditionally been considered necrotic. The opening of the MPTP appears to be decisive for necrosis, apoptosis, and

CENTRAL ILLUSTRATION

Myocardial ischemia/reperfusion injury is a complex phenomenon in which many players contribute to the final damage inflicted to the myocardium. **1.** The first critical player is the epicardial artery. Atherosclerotic plaque rupture with superimposed thrombus results in an abrupt stop of oxygen and nutrient supply distal to the occlusion site. The opening of the epicardial vessel by mechanical or pharmacological means, as well as the thrombus burden reduction by adjuvant antiplatelet/anticoagulant therapies is only the first step toward the salvage of myocardium. During the reperfusion process (either if it is mechanical by primary angioplasty or pharmacological by thrombolytics), thrombus material and other plaque debris can be distally embolized contributing to microvascular obstruction. **2.** Circulating cells contribute to the damage inflicted to the myocardium: activated platelet and leukocytes in the bloodstream not only contribute to the thrombus generation, but also can form plugins that can embolize distally into the microcirculation upon resting blood flow across the culprit lesion (a process independent from plaque debris microembolization). **3.** The microcirculation (net of capillaries) is a critical player in the fate of the myocardium during ischemia/reperfusion. Once the epicardial vessel flow is restored, efficient tissue perfusion is dictated mainly by the microcirculation. Plaque debris and platelet/neutrophil aggregates can induce a mechanical obstruction of the microcirculation precluding efficient tissue perfusion. The generation of tissue edema following reperfusion can result in external compression of the microcirculation, reducing the perfusion capacity of the capillary network **(double arrows)**. Finally, the microcirculation can disintegrate due to the previous damage and allow the leakiness of circulating cells into the interstitial space. **4.** Red blood cell deposits (hemorrhage) are especially harmful due to the release of iron, contributing to the subsequent inflammatory reaction. **5.** Cardiomyocytes that have survived the ischemic phase suffer during the reperfusion period due to several intracellular pathways triggered at reperfusion (see text for detailed information about these processes). **6.** After the entire ischemia/reperfusion insult has passed, the significant infiltration of myocardial tissue by inflammatory cells can induce an additional damage to the myocardium.

necroptosis, and mitochondria are also decisive in mitophagy/autophagy. The importance of regulated forms of cardiomyocyte cell death in I/R injury is probably related more to their specific signal transduction mechanisms. Recognition of the different modes of cardiomyocyte death during infarction suggests the possibility of identifying therapeutic targets that can modulate these processes. From the clinical perspective, cardiomyocyte death is equally relevant whatever the mechanism.

PRE-CLINICAL MODELS OF MYOCARDIAL I/R INJURY

In a typical clinical scenario, a patient of middle-advanced age has various risk factors, comorbidities, and comedications (72) and has a coronary circulation that has already undergone functional and structural remodeling before STEMI (73). The affected individual may or may not have a developed collateral circulation (74), a history of episodes of prodromal angina that may induce protection by ischemic preconditioning (75,76), or prior coronary microembolization that has induced patchy microinfarcts (39). STEMI is usually initiated by the sudden rupture of an atherosclerotic plaque and more-or-less complete occlusion of an epicardial coronary artery, most often followed by spontaneous or interventional reperfusion, but sometimes occurring without reperfusion (17). Because all of these factors contribute to the final infarct size, it is clear that no animal model can recapitulate a clinical scenario exactly. However, most of our knowledge about myocardial infarction is derived from studies in anesthetized, young, healthy animals subjected to sudden coronary occlusion and reperfusion. Animal models are critical to our understanding of the pathophysiology of human conditions, but the information obtained with any particular model can only solve a part of the puzzle. There is thus no superior animal model, and each has its uses. Rodent models help to identify potential mechanisms, but their huge anatomic/physiological differences from humans make it imprudent to extrapolate results to the clinical setting. Pilot clinical trials are justified only when solid and incontestable benefits are found in large-animal models with a much closer match to human anatomy and physiology. The history of cardiovascular medicine is littered with failed clinical experiences caused by taking shortcuts from this translational path.

VALUE OF SMALL ANIMAL MODELS OF MYOCARDIAL INFARCTION. Mice are increasingly used in experimental infarct studies for their ease of breeding, their low cost, and the availability of transgenic models. But, translation of results is highly limited because of their high heart rate (an order of magnitude higher than that of humans) and the small size of their hearts, which ensures that, during permanent coronary occlusion, the inner myocardial layers continue to be served with oxygen and nutrients by diffusion. Thus, infarction in mice develops within 45 to 90 min (77,78), but no more than 70% of the AAR is infarcted even with permanent coronary occlusion (79). Rodents generally have a higher heart rate than larger mammals, but whereas rats and rabbits have little collateral blood flow and fast infarct progression (80), guinea pigs have substantial collateral blood flow and very little infarct progression (81). Significant differences in the response to myocardial infarction are also found across different mice strains. Depending on the genetic background, infarct size after the same procedure can vary by 30% (82).

LARGE ANIMAL MODELS OF MYOCARDIAL INFARCTION. Large animals are the obligatory step before initiating human trials. Among the larger mammals, pigs and primates have little collateral blood flow, whereas cats and especially dogs have a sizeable innate collateral circulation (81). In pigs, infarction starts after 15 to 35 min of coronary occlusion and spreads such that, after 60 to 180 min, infarction is complete and affects more than 80% of the AAR (83,84). Tolerance to I/R varies notably across different pig strains. Primates show a surprising resistance to infarction, with little or no infarction evident after 40 to 60 min of coronary occlusion, and even after 90 min of occlusion, the infarct size is smaller than that in pigs (85). In dogs, infarction is largely subendocardial after 40 min of coronary occlusion and progresses to affect about 70% of the AAR after 6 h, but even permanent occlusion leaves a small zone of viable myocardium in the subepicardium (13,14). Given the variable but significant collateral blood flow in dogs, infarct size is best quantified as a fraction of the AAR and by its inverse relation to the residual blood flow (17). Notwithstanding the confounders detailed previously (age, comorbidities, comedication), infarct development in humans appears to be slower than in these large mammals. From contrast-enhanced CMR analysis (86–88) and the amount of salvageable ischemic myocardium at the time of reperfusion (89,90), one can grossly estimate that about 30% to 50% of the AAR remains viable after 4 to 6 h from the onset of anginal symptoms. Even after 12 h of coronary occlusion, interventional reperfusion can significantly limit infarct size (91). It is currently unclear whether the slower infarct progression in humans than in larger mammals is

related to a developed collateral circulation close to that in dogs (74), a species-specific greater resistance to infarction as in primates (85), pre-infarction anginal episodes that protect by ischemic pre-conditioning (76), some degree of short-term hibernation with contractile and metabolic adaptation to the reduced blood flow (92,93), or background medication, notably with platelet inhibitors (94).

Aside from the differences between animal models, there are other important factors that must be considered when performing pre-clinical studies and, more importantly, when comparing results from different models or even different laboratories. For example, the time of the day at which I/R occurs has a significant influence in the tolerance of the heart to I/R. After the initial demonstration in mice, this phenomenon has also been observed in patients (95). Similarly, the season and even the day of the week might have an effect on the results observed in animal models and, eventually, in the response to protective therapies.

THERAPIES TO REDUCE ISCHEMIC INJURY

Four landmark studies published more than 30 years ago, 2 experimental and 2 clinical, changed the course of STEMI treatment in less than a decade, leading to the huge development of strategies to reduce ischemic injury.

1. The demonstration of a spatial progression of necrosis during an infarction. Reimer et al. (13,14) subjected anesthetized dogs to coronary occlusion of various duration and reported the progression of a wave front of myocardial necrosis from the central subendocardial layers, where ischemia is most severe, to the less ischemic lateral boundaries of the AAR and the subepicardium.
2. The demonstration of the reduced infarct size with reperfusion. Maroko et al. (18) and Ginks et al. (19) first demonstrated the existence of myocardial salvage by reperfusion at 3 h after coronary occlusion (“time is muscle”).
3. The unequivocal demonstration that coronary thrombosis is present in most cases of ongoing STEMI. DeWood et al. (96) reported detailed angiographic findings in patients studied shortly after the onset of STEMI.
4. The first experience with the administration of intracoronary thrombolytics (streptokinase), as reported by Chazov et al. (97) and later, in a larger group of patients, by Rentrop et al. (98).

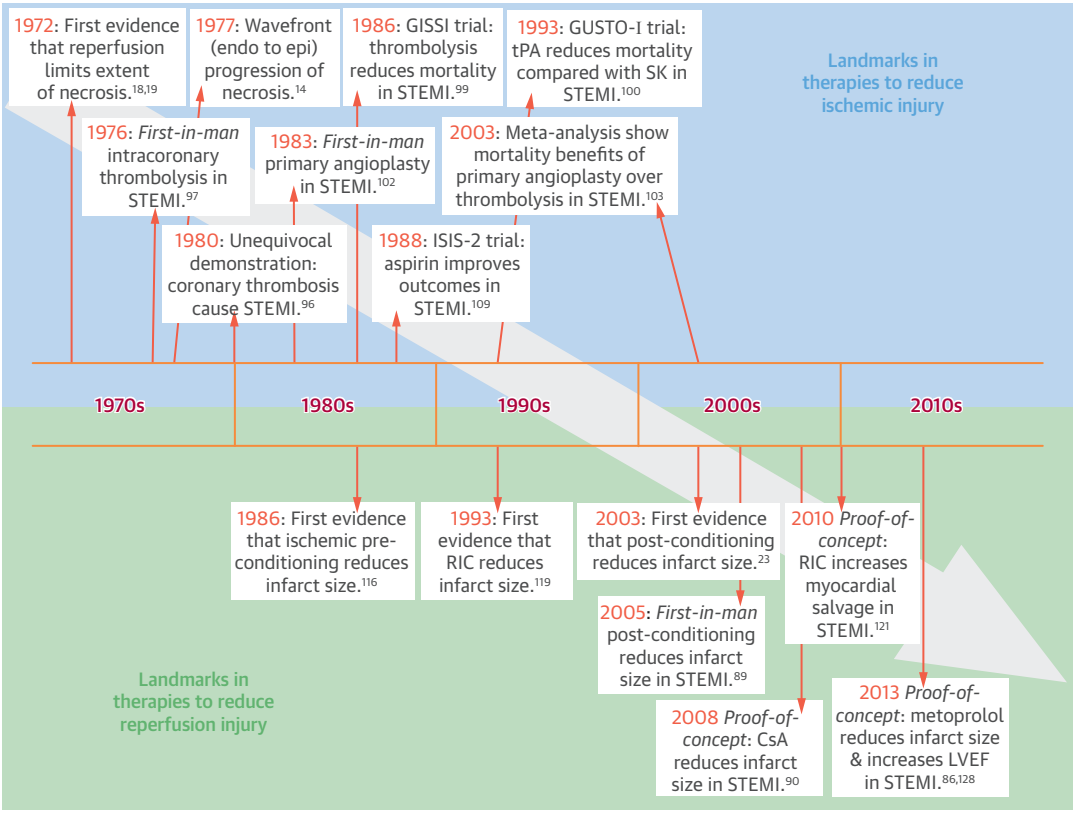
The conclusions reached, although obvious today, were revolutionary when these studies were published. We now take for granted that STEMI is generally caused by an acute thrombotic occlusion of an epicardial coronary artery and that timely recanalization of the occluded artery salvages jeopardized ischemic but still viable myocardium. Thirty years ago, such notions were thought by many to be heretical. **Figure 1** summarizes the most relevant landmarks in the history of STEMI therapy (20).

The development of reperfusion as a therapy for limiting ischemic damage during STEMI is 1 of the greatest success stories in the treatment of human disease. Before this paradigm was established, early mortality was ≈20%; for example, the mortality rate in the control group of the GISSI-1 (Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardio) trial was 18% (99). This figure has since declined to reach ≈5% in recent randomized clinical trials focusing on either pharmacological revascularization, mechanical revascularization, or both. Aside from reperfusion itself, the progressive reduction in the time between STEMI diagnosis and reperfusion has made an important contribution to this reduced mortality. Following the “time is muscle” principle, huge efforts have been made to ensure early reperfusion. It is now widely accepted that the shortening of door-to-balloon time (the time between first medical contact and mechanical reperfusion) results in greater myocardial salvage and better outcomes. A major multidisciplinary effort has resulted in a significant decline in door-to-balloon times over the last 10 years in all registries. However, despite these improvements, a large U.S. study of almost 100,000 STEMI patients found that in-hospital mortality has remained unchanged, indicating the need to target other components of the total ischemic time (3) and other factors that contribute to infarct size.

REPERFUSION BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION, THROMBOLYSIS, OR BOTH. Reperfusion is the most effective therapy ever developed against ischemic damage during STEMI. Pharmacological, mechanical, and combined reperfusion techniques have been refined over the years, and their efficacy has been greatly advanced by the development of adjunctive therapies. The use of lytic agents in placebo-controlled trials was pivotal in the elucidation of the benefits of sustained reperfusion. The first large-scale trial to definitively show a significant reduction in mortality by reperfusion with intravenous administration of thrombolytic agents was the landmark GISSI-1 trial (99). This study was followed by other landmark trials in the field (100,101).

Another revolution in the search for improved therapies to reduce the ischemic damage associated

FIGURE 1 Landmarks in STEMI Therapy



Some of the most relevant landmarks in the quest for therapies to reduce ischemic- **(top, blue)** and reperfusion-related **(bottom, green)** injuries are represented. Landmarks chosen represent those with a greater effect on clinical practice (there are many other mechanistic landmarks not represented in the figure because they have not yet reached the clinical arena). As suggested by the **gray arrow**, during the early years of this quest against ischemia/reperfusion injury, most of the efforts and discoveries were in the ischemic-injury side. In the last decades, and after the ischemic damage was very well approached by clinically available therapies, reperfusion injury therapies have started to arise. The proof-of-concept era for therapies targeting ischemic injury was in the 1970s, and the large trials arose in the 1980s. For reperfusion injury-targeting therapies, the proof-of-concept era was in the late 1990s and 2000s, and it is expected that large trials testing the effect of therapies to reduce reperfusion injury will be undertaken in the 2010s. CsA = Cyclosporin-A; endo = endocardial; epi = epicardial; LVEF = left ventricular ejection fraction; RIC = remote ischemic conditioning; SK = streptokinase (thrombolytic); STEMI = ST-segment elevation myocardial infarction; tPA = recombinant tissue-type plasminogen activator (thrombolytic).

with STEMI was the use of mechanical reperfusion by percutaneous coronary intervention (PCI). PCI for STEMI (primary angioplasty) was first described as a rescue intervention in cases in which thrombolysis was unsuccessful. It was also implemented widely as adjunctive therapy to thrombolysis, and it was performed systematically to evaluate the coronary anatomy and residual stenosis or electively in cases of spontaneous or inducible angina days after successful thrombolysis. The use of primary angioplasty as an alternative to thrombolysis was first described in 1983 (102).

The many studies comparing these strategies leave no doubt that timely PCI by an experienced team is superior to in-hospital thrombolytic therapy (103). Furthermore, progress in stent therapy has markedly reduced the incidence of acute and late stent thrombosis (104) and refined the primary PCI strategy. Despite the clear advantages of PCI over thrombolysis in head-to-head comparisons, the clinical scenario is sometimes more complicated. Although no prospective studies have been performed to prove it, primary PCI may not exhibit a mortality advantage over immediate thrombolysis when performed after a delay of 120 min. In some patients (those who present early with a large AAR and a low bleeding risk), this maximum acceptable delay may be significantly shorter (105).

A slightly different approach that was recently developed for patients who cannot get timely PCI is to follow the classical thrombolytic therapy and delay the planned PCI until 3 to 24 h after lytic administration (this differs from the facilitated approach, in which PCI is performed as soon as the patient arrives at the PCI center). In the STREAM (Strategic Reperfusion Early After Myocardial Infarction) trial, pre-hospital administration of the thrombolytic tenecteplase (half-dose in the elderly) to ≈2,000 STEMI patients who could not get PCI within 1 h resulted in rates similar to standard primary PCI for the composite of death, shock, congestive heart failure, or reinfarction at 30 days (106). One-year mortality rates in the 2 groups were almost identical (107). This approach needs to be further explored in patients with long transport times to the PCI hospital.

The use of adjunctive antithrombotic agents with thrombolytics is predicated on the fact that the intensity and net extent of thrombolysis reflects a competition between lysis and ongoing thrombosis (108). Convincing evidence of the effectiveness of aspirin as an adjunctive agent was first acquired in the ISIS-2 (Second International Study of Infarct Survival) trial (109), in which the benefits of aspirin and streptokinase were additive. Aspirin has also been used as an adjunctive therapy in STEMI in patients undergoing reperfusion by primary PCI. Further clinical benefits are obtained by supplementing aspirin with new antiplatelet agents such as clopidogrel, ticagrelor, or prasugrel. Given the clear beneficial effect of optimal antiplatelet therapy in STEMI, it should be implemented in the testing of any cardioprotective strategy. Further information on reperfusion strategies and adjunctive pharmacological therapy can be found in dedicated review papers (20).

OTHER THERAPIES TO REDUCE ISCHEMIC INJURY. The possibility of slowing the progression of ischemic damage during ongoing ischemia has been investigated for several decades. Drugs able to reduce oxygen consumption have been tested in randomized clinical trials. Among them, β-blockers were widely evaluated in many STEMI trials, but no clear reduction in infarct size was detected, bringing the cardioprotective potential of β-blockers in STEMI into question. However, all of these trials addressing the effect of β-blockers on infarct size, with the exception of 1 (110), were performed in patients not undergoing reperfusion. More recently, interest in the β1-selective blocker metoprolol has been revitalized due to its demonstrated ability to minimize reperfusion injury (discussed in the following text).

Hypothermia has been unequivocally shown to reduce the rate of progression of ischemic damage in animal models of STEMI (111), but clinical application of hypothermia has been extremely challenging due to the lack of a safe cooling procedure able to reduce temperature fast enough to affect ischemic damage (well before reperfusion). After several small studies, the CHILL-MI (Rapid Endovascular Catheter Core Cooling combined with cold saline as an Adjunct to Percutaneous Coronary Intervention For the Treatment of Acute Myocardial Infarction) trial recruited 120 STEMI patients scheduled for PCI and randomized them to standard care or a rapid cooling protocol (infusion of cold saline plus endovascular cooling device). Hypothermia did not reduce infarct size (normalized to AAR) as measured by CMR (112), despite achievement of the target temperature (<35°C) in >75% of patients at the time of reperfusion. Attaining significant reductions of ischemic damage (and infarct size) with hypothermia would require the target temperature to be reached long before reperfusion. With the significant reductions seen in door-to-balloon times, this seems very unlikely to be achieved with the available techniques.

THERAPIES TO REDUCE REPERFUSION INJURY

Although the history of therapies to reduce ischemic damage, mostly involving reperfusion therapy, is full of rapid successes, the development of therapies to reduce reperfusion injury has been disappointing. This disparity reflects the contrast between the straightforward problem presented by reducing ischemic injury (restoration of blood flow) and the more complex processes associated with reperfusion injury.

THE LONG DEBATE IS COMING TO AN END: LETHAL REPERFUSION INJURY IS A REALITY. The term "reperfusion injury" has been used for many decades to describe several events associated with reperfusion, some transitory (e.g., ventricular arrhythmias, myocardial stunning, and so on) and others permanent (e.g., death induced by reperfusion, known as lethal reperfusion injury). The existence of lethal reperfusion injury in STEMI has long been a matter of debate (24,113). Despite the convincing experimental evidence already outlined in this review, definitive clinical demonstration has been lacking. This is partly due to the gap between well-defined and controlled experimental models on the one hand and unclear human proofs-of-concept (i.e., "clinical models") and trial designs on the other (114). Negative findings in infarct size reduction trials accumulated, giving currency to the idea, common among cardiologists and in

the pharmaceutical industry, that reperfusion injury was either a fantasy or at best a laboratory artifact. Studies in the early 1990s showed that reperfusion did not increase the transmural extent of infarction in canine hearts, suggesting an absence of reperfusion injury (115). The dog is a particular case, however, in which injury progresses slowly, and 90 to 180 min of coronary occlusion might not provoke significant reperfusion injury; it is known that ischemic and reperfusion injuries are linked, the degree of the former determining the extent of the latter. The idea of lethal reperfusion injury has since won progressive acceptance on the basis of evidence coming from clinical and basic science studies. Lethal reperfusion injury can be defined as a potentially preventable death of myocardium that was viable at the time of reperfusion and that is the consequence of events triggered or magnified by reperfusion. The fact that preventive maneuvers like post-conditioning limit infarct size without affecting ischemic injury is the best demonstration of the reality of lethal reperfusion injury.

NONPHARMACOLOGICAL INTERVENTIONS TO REDUCE REPERFUSION INJURY. The first major breakthrough in nonpharmacological interventions was the definition of "*ischemic pre-conditioning*" by Murry et al. (116), who, in 1986, reported that brief cycles of ischemia and reperfusion performed *before* a prolonged coronary artery occlusion with reperfusion could dramatically reduce final infarct size in dogs. Given that total ischemic time was unaltered (even increased) by this intervention, this study suggested that there was more to limiting infarct size than a shorter duration of ischemia. This finding ushered in a paradigm shift, which stimulated a wave of research that has increased our understanding of the pathophysiology of I/R injury at the molecular level, thereby preparing for the identification of new targets for future innovative therapies. Many years later, the infarct-limiting effects of ischemic pre-conditioning were shown to be due to a large extent to a reduction in reperfusion injury (117), although it is also plausible that pre-conditioning can reduce ischemic damage. The unpredictability of coronary artery occlusion in STEMI patients means that ischemic preconditioning cannot be applied in this clinical setting, but it could have an important role in planned procedures like cardiac surgery (8,75). The second breakthrough was the description of "*ischemic post-conditioning*" by Zhao et al. (23) in 2003. They showed that brief episodes of ischemia and reperfusion performed immediately *after* reflow following prolonged ischemia could reduce final infarct size in dogs by 30% to 40% (23). This effect was even more surprising than pre-conditioning, because the intervention was applied after reflow; thus, it has no connection to the duration of ischemia or any associated event and must be related to the prevention of events occurring after reperfusion. With this simple experiment, this group demonstrated that lethal reperfusion injury is a reality (24), is quite significant in an experimental setting (30% to 40% of final infarct size), and that it is amenable to timely intervention.

With these breakthroughs, the time had come to test whether lethal reperfusion injury could be attenuated in STEMI patients. The first to do so were Staat et al. (89), who demonstrated in 2005 that ischemic post-conditioning can reduce infarct size in STEMI. In this proof-of-concept trial, ischemic post-conditioning was applied within 1 min after reflow by inflating/deflating the angioplasty balloon (low-pressure, upstream of the stent) in 4 1-min cycles. This resulted in a 36% reduction of the area under the curve for creatine kinase release, a surrogate marker of infarct size. Most, not all, of the small trials performed have shown infarct size reduction in patients undergoing post-conditioning (8). However, the largest randomized clinical trial of post-conditioning in STEMI was neutral (118). The protocol for the POST (Effects of Postconditioning on Myocardial Reperfusion in Patients With ST-Segment Elevation Myocardial Infarction) trial allowed the discretional use of thrombectomy, pre-dilation, and other maneuvers. The repeated balloon inflation-deflation at the site of the culprit lesion might have been responsible for excessive inadvertent thrombus microembolization, as suggested by the low rate of ST-segment resolution. And, although the protocol stipulated post-conditioning within 1 min of STEMI, the high frequency of thrombectomy (50%) likely delayed post-conditioning beyond the protective 1-min time-frame, and this might have diluted the benefits of this protective strategy. The fact that the largest trial was neutral calls for caution in the interpretation of the infarct-limiting effects of post-conditioning until new trials are completed. In addition, the details of the POST trial highlight that application of this strategy in a real-life scenario is more challenging than in the proof-of-concept trials, in which patient selection and protocol application is more controlled. In the DANAMI-3 (Danish Study of Optimal Acute Treatment of Patients With ST-elevation Myocardial Infarction-3) (NCT01435408), 2,000 STEMI patients will be allocated to 1 of 3 study interventions: conventional PCI (immediate stent implantation), post-conditioning (with stent implantation after the end of 4 30-s post-conditioning cycles), or deferred stenting (opening the culprit

artery with the wire only, followed by thrombectomy and/or low pressure balloon inflation and stenting after 48 h). The combined endpoint will be all-cause mortality or heart failure at 2 years.

Another form of myocardial conditioning well described in animal models is remote ischemic conditioning: conditioning performed in a distant organ (119). Remote ischemic conditioning is reviewed in detail elsewhere (120). For the purpose of this review, it is important to mention that remote ischemic preconditioning (4 5-min brachial cuff inflations applied during ongoing STEMI: i.e., during ambulance transfer to the PCI center and before PCI reperfusion) resulted in increased myocardial salvage compared with regular PCI (121), and this might translate into fewer long-term clinical events (122). The possibility should be confirmed in the ongoing CONDI-2 (Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing pPCI) trial (NCT01857414).

PHARMACOLOGICAL INTERVENTIONS TO REDUCE REPERFUSION INJURY. During the past 10 years, many phase II clinical trials have been performed to find coadjuvant pharmacological interventions to ameliorate the myocardial damage associated with STEMI. We will describe here the most promising pharmacological strategies as well as examples from the long list of failures.

DRUGS WITH PROMISING RESULTS IN PILOT/PHASE II TRIALS

CYCLOSPORINE-A. Because local conditions (coronary anatomy, thrombus burden) can make it difficult to apply ischemic post-conditioning during PCI, pharmacological agents that trigger similar pathways to ischemic conditioning have been extensively investigated at the pre-clinical level and then translated into pilot clinical trials (123). Cyclosporine-A is the paradigm pharmacological post-conditioning agent. Cyclosporine-A acts by inhibiting the opening of the MPTP, an event also seen with post-conditioning. Piot et al. (90) randomized 58 patients to receive a single bolus of cyclosporine A or placebo immediately before PCI. Infarct size, measured by the area under the curve of creatine kinase, was significantly smaller in the cyclosporine-A group. The ongoing multicenter, randomized, placebo-controlled CIRCUS (Cyclosporine and Prognosis in Acute Myocardial Infarction [MI] Patients) trial (NCT01502774) recruited 975 anterior STEMI patients (Thrombolysis In Myocardial Infarction flow grade 0 to 1 left anterior descending occlusion) and randomized them to cyclosporine A or placebo. The combined primary endpoint (total mortality; hospitalization for heart failure; and LV remodeling [increase of LV end-diastolic volume >15%]) will be assessed at 1-year follow-up.

METOPROLOL. The effect of β-blockers on infarct size in STEMI patients has been intensely debated. Different β-blocker agents were tested in many STEMI trials in the 1970s to 1980s with no definite conclusion on their cardioprotective effect. However, these trials were performed before reperfusion became established practice, and it is not surprising that β-blockers showed no consistent infarct limiting effect because, with no reperfusion, the chances of myocardium salvage are negligible. In the era of thrombolysis as the standard treatment for STEMI, the 1 randomized clinical trial performed showed neutral effects of intravenous (IV) atenolol on infarct size (110). Pre-clinical data from the pig model of infarction demonstrated that IV metoprolol very significantly reduces infarct size when administered before reperfusion (124,125). Contrary to the classical theory of reduced myocardial oxygen consumption, the mechanism responsible for this infarct-limiting effect is proposed to be related to a reduction in reperfusion injury due to the effect of metoprolol on circulating cells (neutrophils/platelets) rather than cardiomyocytes (126). This pre-clinical evidence led to the METOCARD-CNIC (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction) trial, in which 270 anterior STEMI patients undergoing PCI were randomized to early IV metoprolol or control before reperfusion. Infarcts, measured by CMR, were significantly smaller in the IV metoprolol group (86), and the effect was more pronounced in patients recruited during ambulance transfer to the PCI center (127). Six-month CMR follow-up of more than 200 patients showed that the IV metoprolol group had a significantly higher mean LVEF and had significantly less cases of severe LVEF depression (128).

The encouraging results from the METOCARD-CNIC trial appear to contradict findings from the much larger COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial). In this mega trial, STEMI patients undergoing thrombolysis were randomized to early IV metoprolol followed by oral metoprolol or matching placebo. The COMMIT trial did not report data on infarct size; yet, it showed significantly reduced rates of reinfarction and ventricular fibrillation in response to early IV metoprolol, but this was counterbalanced by an excess cardiogenic shock, resulting in a net neutral effect on mortality (129). However, treatment of patients in the COMMIT trial was not optimal according to current guidelines. First, STEMI patients received thrombolytic therapy

at a mean of >10 h after symptom onset (129). The amount of myocardial salvage that can be achieved after 10 h of coronary occlusion is residual at best, especially when thrombolysis is used as the reperfusion strategy (130). Second, the COMMIT trial included STEMI patients in Killip class III, who received the full regime of metoprolol administration; metoprolol resulted in relative mortality reductions of 5% and 2.5% in Killip class I and II patients, respectively, but increased mortality by 19% in Killip class III patients. Finally, metoprolol also increased mortality in patients with systolic blood pressure <120 mm Hg. These results reinforce the contraindications for IV β-blocker therapy in patients with overt heart failure or who are hemodynamically compromised, who have been systematically excluded from other β-blocker studies. In contrast with COMMIT, the METOCARD-CNIC trial recruited early presenters (<6 h from STEMI onset), and patients with Killip class ≥III were excluded. The patient population in the METOCARD-CNIC trial is thus more representative of the current standard of care for STEMI patients. The ongoing EARLY BAMI (Beta-blocker Administration before primary PCI in patients with ST-elevation Myocardial Infarction) trial (131) is testing the infarct-limiting effects of IV metoprolol in STEMI patients recruited during ambulance transfer to the PCI center, with a similar design to METOCARD-CNIC, except that it includes patients with infarcts in any location (METOCARD-CNIC recruited only patients with anterior STEMI) and extends the time window for recruitment to 12 h (compared with 6 h in METOCARD-CNIC). Finally, the hard endpoint-powered MOVE ON! (Impact of pre-reperfusion Metoprolol On clinical eVEnts after myocardial infarctiON) trial will definitively answer whether the amelioration of I/R injury exerted by early IV metoprolol (132) translates into a real clinical benefit. Given that not all β-blockers have the same intracellular effects (due to their disparate lipophilicity among other differences) and do not even share the same mechanistic effect (despite being considered β1 selective), it should not be assumed that all will have similar infarct-limiting effects.

GLUCOSE MODULATORS. The possible therapeutic use of glucose to protect cardiomyocytes from energy depletion during myocardial infarction was proposed several decades ago by Sodi Pallares et al. (133). Combined administration of glucose/insulin/potassium (GIK) during ongoing myocardial infarction has been tested in several trials with some encouraging results. The IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care) trial recruited patients with suspected acute coronary syndrome and randomized them to GIK or placebo during transfer to the hospital. In the subgroup of patients presenting with STEMI, GIK significantly reduced CMR-evaluated infarct size (134). However, these promising results need to be confirmed in a prospective trial powered to detect differences in infarct size. Another approach has been the use of glucagon-like peptide-1 (GLP1) analogs. After promising preclinical results, Lonborg et al. (135) randomized 172 STEMI patients to receive IV injection of the GLP1 analog exenatide or placebo. Myocardial salvage on CMR was significantly higher in the exenatide group (135).

ABCIXIMAB. Glycoprotein IIb/IIIa inhibitors were developed for the reduction of thrombotic events due to their potent effect on platelets and platelet-leukocyte aggregates implicated in I/R injury. The INFUSE-AMI trial recruited 452 anterior STEMI patients undergoing PCI and performed an open-label, 2×2 factorial randomization to test the effect of abciximab and/or thrombectomy on infarct size, as evaluated by CMR. Thrombus aspiration had no effect on infarct size, but intracoronary administration of abciximab significantly reduced infarct size (136). Given that the current standard of care for STEMI patients includes the use of potent oral antiplatelet agents, glycoprotein IIb/IIIa inhibitors are left for a selected STEMI population.

EXAMPLES FROM THE LONG LIST OF FAILED CLINICAL TRIALS FOR REDUCING REPERFUSION INJURY

Many clinical trials have attempted and yet failed to demonstrate the ability of a given therapy to limit infarct size by reducing reperfusion injury. A common denominator in many of these failures is the weak or unclear benefit in the pre-clinical phase (137,138). A description of all of the negative trials in the field is beyond the scope of this review; here, we provide an update on the more recent negative trials.

Adenosine has been evaluated in several trials and did not show a clear infarct-limiting effect. In the most recent trials, adenosine was administered by intracoronary injection at high doses, and myocardial salvage was evaluated by CMR. Adenosine had no effect on myocardial salvage or MVO (87,139). The effect of inhaled nitric oxide on infarct size in 250 STEMI patients undergoing PCI was very recently tested in the NOMI (Nitric Oxide for inhalation to reduce reperfusion injury in acute STEMI) trial (140). Nitric

oxide inhalation did not reduce infarct size as evaluated by CMR. The effect of IV sodium nitrite on infarct size was evaluated in 229 STEMI patients undergoing PCI in the NIAMI (Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial) trial (141). The authors did not find any difference in infarct size as evaluated by CMR, the primary endpoint of the study. The effect of intravenous TRO40303, a drug binding to an unclear molecular target in the outer mitochondrial membrane, was tested in the MITOCARE (Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction) trial as an adjunct to primary percutaneous coronary intervention for acute STEMI. This study of 163 STEMI patients randomized to TRO40303 or placebo found no differences in infarct size either by biomarker release (primary endpoint) or CMR. Salvage index by CMR was also not different between groups (142). Several other trials have shown negative results in the past (reviewed in more detail by Kloner [9]).

CLINICAL TRIALS TARGETING ISCHEMIA/REPERFUSION INJURY: IMPORTANCE OF ENDPOINTS

Incorporation of new clinical evidence into clinical practice guidelines requires the demonstration of a clear clinical benefit (an effect on hard endpoints). This demonstration usually requires large phase III trials. Given the high costs associated with large trials, the natural next step after obtaining strong preclinical data is to perform a pilot (phase II) clinical trial. These small trials usually choose a primary endpoint accepted as a surrogate for hard clinical endpoints. Infarct size is the most intuitive parameter evidencing the cardioprotective effect of a given intervention and also correlating with clinical events. More importantly, it is well-defined in animal models, where it is recognized as the hallmark of cardioprotection. Although single photon emission computed tomography (121), electrocardiogram parameters (118), and biomarker release (90) have been widely used, CMR-measured infarct size is currently the most widely recommended technique for assessing infarct size in STEMI trials. LVEF is the classical surrogate functional parameter, because it has been clearly associated with long-term mortality and morbidity after STEMI (143). In addition to infarct size, other readouts of reperfusion injury or determinants of infarct size can be measured to more accurately evaluate protective interventions. For example, MVO is associated with poor clinical outcomes when evaluated by various techniques, mainly CMR (27,144). AAR has been used in recent trials to normalize infarct size or to depict myocardial salvage (% of AAR with no infarction). AAR can be estimated by angiographic means (BARI [Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index]/APPROACH [Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease] coronary angiographic scores or LV angiographic scores) or by T2-CMR. Although T2-CMR is increasingly used, its capacity to depict true AAR is debated (the pros and cons of the use of CMR to depict AAR are reviewed elsewhere [145,146]). Some studies have reported a prognostic value of myocardial salvage (147); however, this parameter has not been extensively validated. Myocardial salvage is a surrogate of infarct size used to control for variations in AAR between study groups. In addition, very recently, therapies that reduce infarct size, like postconditioning (148) and remote conditioning (149), were shown to reduce CMR-evaluated AAR. Studies of remote ischemic conditioning and the GLP1 analog exenatide have shown increases in myocardial salvage, but no significant effect on infarct size or LVEF (121,135). These results should, moreover, be interpreted with caution, because the real surrogate markers of hard clinical endpoints were neutral. Trials using infarct size (a known surrogate of clinical events) as an endpoint are easier to interpret than trials using myocardial salvage (a surrogate of a surrogate of clinical events). The use of CMR to visualize post-infarction edema has recently been made more complex by the demonstration that the edematous reaction of the myocardium to ischemia/reperfusion is bimodal (150). Comprehensive serial CMR evaluation in pigs showed that there are 2 independent waves of edema after ischemia/reperfusion: an initial wave appearing abruptly upon reperfusion, and a deferred wave occurring a few days later (150). This bimodal post-ischemia/reperfusion edematous reaction highlights the need for caution in the use of CMR to visualize edema as a marker of ischemic memory.

Sample size calculation for clinical trials is based on the anticipated treatment effect on a principal outcome (a reduction or increase in the experimental arm vs. control). Normally, several additional secondary endpoints are prospectively evaluated. It is important to stress that when the primary endpoint of a trial is negative, all other findings are exploratory at best, and the secondary outcome, if relevant, should be tested in a dedicated trial. It is also important to highlight the risk of overinterpreting subgroup analyses. Some of the phase II trials described here as negative have "exploratory" analyses in which

subgroups (e.g., anterior STEMI or patients presenting early) show a positive result. These analyses are biased because the subgroup was not randomized, and thus, the results could be affected by potential unknown confounders. This is especially true when the overall population does not show a significant treatment effect. The interpretation of endpoints in clinical trials was recently reviewed by Pocock and Gersh (151).

Other relevant questions regarding the selection of populations for clinical trials are related to the better identification of potential responders according to the expected mechanism of the study intervention. The response to protective agents administered at reflow appears to vary according to the duration of ischemia, and some trials using pharmacological agents suggest that only patients with short duration of ischemia can benefit (112). This suggests that the dynamics of lethal reperfusion are influenced by the duration of the preceding ischemia and are probably not linear. In addition, indirect evidence suggests that age, sex, comorbidities (e.g., diabetes, hypertension, smoking), and cotreatments (statins, antiplatelet agents) may have an effect on infarct size and on protection by conditioning interventions (94,152).

THE FUTURE

Over the past decades, important progress has been made in the quality of phase II trials evaluating protective interventions against lethal reperfusion injury. Although these trials will always be a mandatory preliminary step, the challenge of the next decade is to set up larger phase III trials evaluating clinical outcomes to therapies targeting lethal reperfusion injury. As in other disciplines, we envision that the advances in the next decade will come from refining the therapies already available rather that identifying new drugs. The commitment of funding agencies, scientific societies, and industrial partners is needed to achieve this challenging goal.

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