Reperfusion Injury, Microvascular Dysfunction, and Cardioprotection

The "Dark Side" of Reperfusion

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Early restoration of coronary perfusion is the single most important objective in the management of ST-segment elevation myocardial infarction (STEMI). Failure to do so is associated with larger myocardial infarcts and a greater likelihood of ventricular arrhythmias, cardiac failure, cardiogenic shock, and death.[1] Thus, the immediate goal of reperfusion therapy is to restore normal epicardial blood flow promptly, which is achieved most effectively with primary percutaneous coronary intervention (PCI).[2,3] Advances in adjunctive interventional techniques and pharmacological therapy have made it possible to achieve normal (Thrombolysis in Myocardial Infarction grade 3 flow) epicardial flow in as many as 95% of patients undergoing primary PCI.[2] Nevertheless, despite timely reperfusion of the infarct-related artery and the establishment of a patent epicardial coronary vessel, restoring optimal myocardial (microvascular) perfusion and salvage in all patients has remained elusive, representing perhaps the last frontier of reperfusion therapy.[3]

Potential mechanisms that may limit myocardial salvage include microvascular dysfunction and lethal reperfusion injury (RI). The entity of microvascular dysfunction is well documented clinically and thought to be the consequence of $\geq$1 of the following: distal coronary emboli resulting from thrombus, platelets and atheroma, in situ thrombosis, vasospasm, and/or inflammation.[4] RI, as it relates to the heart, may be defined as myocardial and vascular injury occurring as a direct result of blood flow restoration to the ischemic tissue.[5] It is proposed that the resulting cellular dysfunction, apoptosis, and necrosis mediate myocardial stunning, no reflow, reperfusion arrhythmias, and additional loss of myocardium (lethal RI).[6] The precise pathophysiology of RI remains to be established, although several mechanisms have been proposed (Figure 1).[6] The current understanding of the etiology of RI is derived predominantly from animal studies in which interventions that inhibit the putative mediators of RI have been shown to reduce infarct size. Importantly, many of these mechanisms are activated within the first few minutes after reperfusion, although the processes may continue over hours, leading to progressive necrosis by a wave front of injury.

Animal models of RI indicate that as much as half the infarct size may be related to the restoration of flow.[7] The reproducibility of the phenomenon in numerous species through the use of a variety of pharmacological and nonpharmacological strategies led to considerable initial enthusiasm for the therapeutic potential to limit RI. However, the vast majority of human studies have failed to demonstrate any cardioprotective benefits of adjunctive therapies aimed at modifying RI.[8] This has led some to doubt the clinical relevance of the concept,[9] although there is now renewed interest after recent randomized trials have demonstrated efficacy using interventions such as ischemic postconditioning (IP),[10] administration of adenosine,[11] inhibition of the mitochondrial permeability transition pore (MPTP),[12] and intracoronary delivery of supersaturated oxygen.[13] Moreover, an increased understanding of cardioprotective pathways such as RI salvage kinase (RISK) pathway and their interaction with promoters of apoptosis has helped to provide a mechanistic understanding of how a new generation of agents may potentially reduce RI. Thus, the aim of this review is to reassess the potential for cardioprotection around the time of reperfusion. Our focus is on primary PCI because of its marked superiority over thrombolysis in achieving normal epicardial flow and thereby providing a more standardized platform from which to study adjunctive therapies.

Failure of Translation to Clinical Practice

It has been estimated that hundreds of interventions have been investigated in experimental studies for their potential to ameliorate RI.[14] However, despite the large body of basic science, which has resulted in an extensive clinical trial agenda, translation into clinical practice has been disappointing. Virtually all trials have failed to demonstrate efficacy with respect to their primary clinical end point.[8] The strategies tested in these trials include the inhibition of calcium overload, generation of reactive

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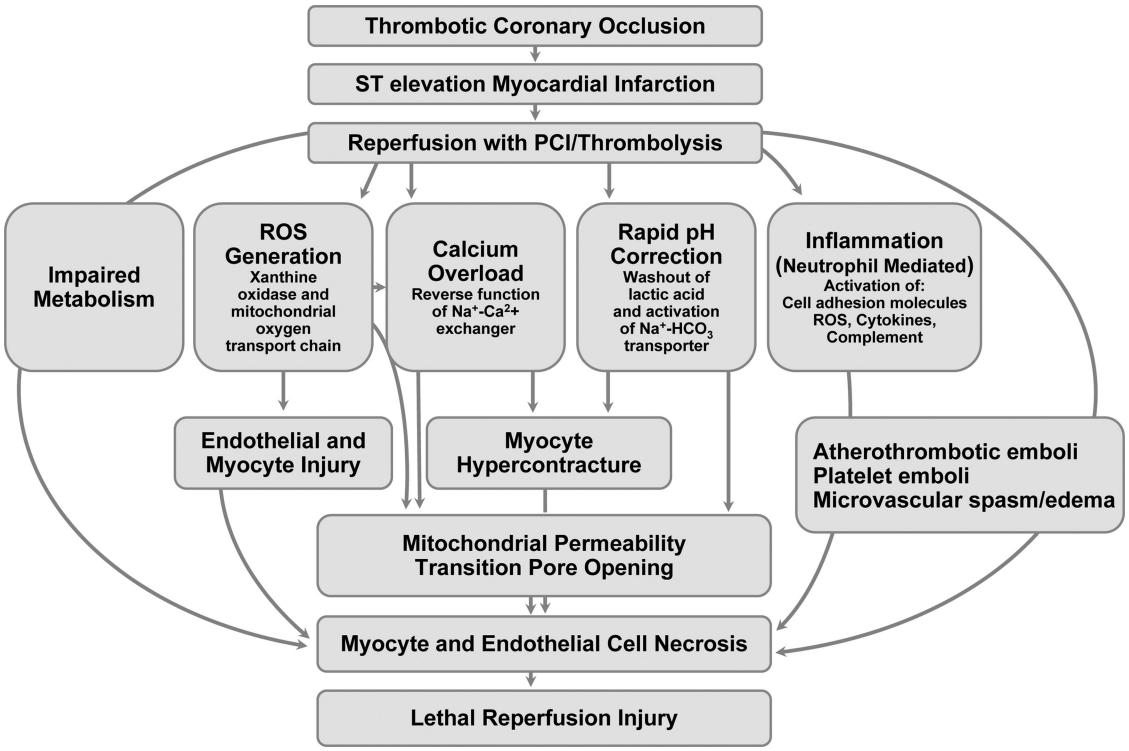

Figure 1. Pathophysiology of lethal RI. ROS indicates reactive oxygen species.

oxygen species, inflammation, and modulation of the myocardial metabolism.

One explanation for the failure to translate cardioprotection into clinical practice is that RI does not occur in humans. However, it would be unusual for a biological phenomenon to be widely prevalent in animals and yet be absent in humans. Alternatively, if we accept that RI does occur in humans, the inability to show benefit in most clinical trials may result because it cannot be prevented in humans or because the strategies that have been applied are ineffective or suboptimal. The possibility that RI is not preventable in some, if not all, patients is real because, unlike young healthy animals studied in controlled settings and subjected to a relatively brief period of ischemia (30 to 90 minutes), patients with STEMI are typically elderly with an atherothrombotic vasculature, present with variable duration of ischemia, have multiple comorbidities, and are concomitantly treated with drugs, all factors that may interfere with cardioprotection.[15]

If we assume that RI is potentially preventable in humans, then there are several reasons why clinical studies conducted to date may be considered to have been suboptimally designed. First, some trials have tested agents that have not been rigorously investigated in the preclinical stage.[6,8] Second, most studies have applied cardioprotective strategies without a strict inclusion criterion for the duration of ischemia (symptom onset to reperfusion time). We propose that there is a relatively narrow therapeutic window during which RI may be preventable (Figure 2). Thus, patients who present very early and receive effective reperfusion therapy have small infarcts and gain little additional benefit from an adjunctive strategy.[16,17] On the other hand, patients who present late sustain large infarcts and may have little potential for myocardial salvage. The validity of this hypothesis must be considered in context of the fact that the precise timing of the onset of lethal ischemia is often difficult to establish in patients and may be influenced by intermittent spontaneous reperfusion and/or the presence of a collateral circulation. Third, although measurement of infarct size in the explanted heart from animals is relatively accurate, infarct size determination in humans is more challenging despite major improvements in imaging techniques. Moreover, the magnitude of the infarct in patients with STEMI is smaller than that generally induced in animal models. Hence, a small effect

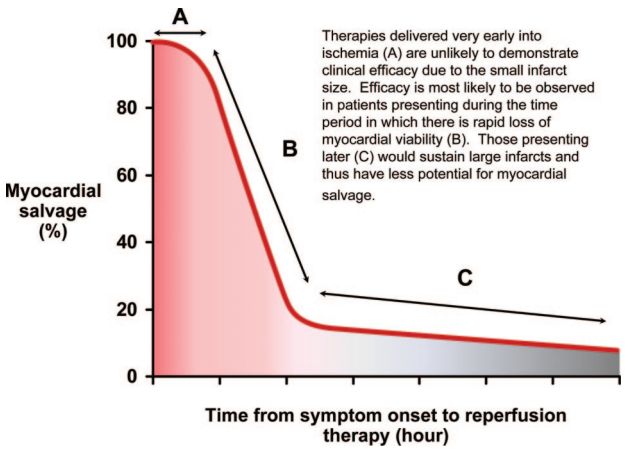

Figure 2. Hypothetical construct of the therapeutic window for cardioprotection. Adapted from Gersh BJ et al. Pharmacological facilitation of primary percutaneous coronary intervention for acute myocardial infarction. Reproduced from Gersh et al[17] with permission of the publisher.

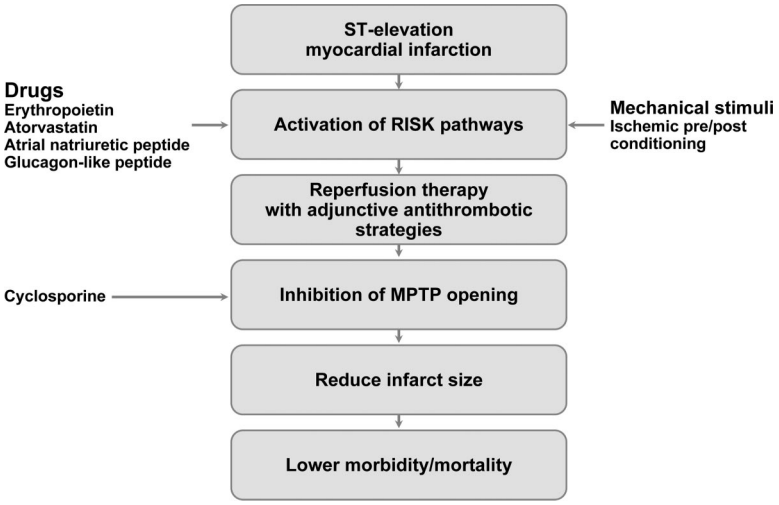

Figure 3. Potential strategies for cardioprotection.

from an adjunctive therapy may be difficult to demonstrate, especially in patients with small infarcts.[18,19] In addition, infarct size in STEMI may be modified by the variable location of the arterial occlusion and hence the area at risk, as well as the presence of a collateral circulation and spontaneous fibrinolysis, factors that are not accounted for in the carefully controlled animal experiments.[20] Fourth, improvements in treatment for STEMI have resulted in low mortality rates in contemporary practice. In the recent large-scale Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial (5745 patients with anterior and large nonanterior infarction) and The Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction (HORIZONS-AMI) trial (3602 patients with anterior and nonanterior infarction, including cardiogenic shock) of primary PCI, 30-day mortality was 4% and 2.6%, respectively.[16,21,22] Clinical trials designed to further reduce such low mortality rates by 20% would require enrollment of >15 000 patients, which is not a feasible undertaking. Finally, in almost every study of acute myocardial infarction, the cardioprotective intervention had been initiated after reperfusion was established. In most animal models, the treatment is administered before either the induction of ischemia or restoration of coronary blood flow. Because RI starts almost immediately on restoration of blood flow, it would seem necessary that cardioprotective strategies be initiated before reperfusion therapy.

Emerging Novel Approaches for Limiting RI

RISK Pathway

The RISK pathway is a family of protein kinases (eg, protein kinase Cϵ) that appear to be activated during reperfusion and to confer cardioprotection by preventing myocyte calcium overload, inhibiting MPTP opening, and recruiting antiapoptotic pathways (Figure 3).[23] It has been proposed that pharmacological targeting of the RISK pathway may be a potential way to diminish RI. Drugs such as atorvastatin, erythropoietin, and atrial natriuretic peptide (ANP) have been shown to reduce infarct size in animal studies, in part, by activating the RISK pathway.[24] Indeed, preliminary evidence for the clinical efficacy of ANP has been reported in the Japan Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage by ANP (J-WIND-ANP) trial, conducted among 569 patients undergoing primary PCI. Randomization to a 3-day infusion of ANP was associated with a 15% reduction in infarct size, improved left ventricular ejection fraction, and lower combined end point of death or cardiac failure.[25] Additional randomized clinical trials investigating the efficacy of erythropoietin and atorvastatin are currently being conducted in patients undergoing primary PCI.[26]

Inhibition of the MPTP

Inhibition of MPTP opening appears to be an attractive strategy because it is a putative downstream mediator of lethal RI (Figures 1 and 3).[23,27] The most compelling evidence that this may have the potential to translate to clinical benefit has been reported by Piot and colleagues[12] using cyclosporine, a potent inhibitor of MPTP. They demonstrated in a randomized trial of 58 patients that the administration of a single 2.5-mg/kg bolus of the drug immediately before primary PCI reduced infarct size (measured with biomarkers and cardiac magnetic resonance imaging) by as much as 40%. These data provide proof of concept and suggest that lethal RI does occur in humans. However, the findings must be regarded as preliminary, with efficacy and safety requiring confirmation in a large clinical trial.

Ischemic Preconditioning and IP

Activation of the endogenous protective mechanisms against RI using a mechanical stimulus has also emerged as an attractive approach. The feasibility of this approach was demonstrated almost 2 decades ago by Murry and colleagues.[7] They found that a few brief cycles of sublethal ischemia followed by reperfusion (ischemic preconditioning), before the onset of lethal ischemia, was cardioprotective. In animal studies, preconditioning reduces infarct size, reperfusion arrhythmias, myocyte energy requirements, and endothelial injury and accelerates myocardial functional recovery.[28] However, the application of ischemic preconditioning in patients with STEMI has not been feasible because of the unpredictable nature of the event.

Interest in this field was recently brought back to the forefront by Zhao and Vinten-Johansen,[29] who demonstrated in dogs that three 30-second cycles of reperfusion interrupted by 30 seconds of ischemia after sustained lethal ischemia reduced infarct size. They called this form of modified reperfusion IP. The precise mechanisms involved in IP are unknown, but they appear to resemble those observed in preconditioning with multiple triggers, mediators, and end effectors.[29] Receptor-dependent triggers such as endogenous adenosine and non–receptor-dependent triggers such as nitric oxide have been proposed. The current paradigm is that these triggers activate mediators such as mitochondrial ATP-sensitive potassium (K-ATP) channels and the RISK pathways, which ultimately inhibit the opening of MPTP during reperfusion (Figure 3).

The potential clinical application of IP in STEMI was recognized early, and 3 recently published proof-of-concept studies have demonstrated efficacy. The first, by Staat and colleagues,[30] enrolled 30 patients who were randomized to standard primary PCI with direct stenting or direct stenting after IP. IP was performed within 1 minute of restoring flow by 4 cycles of balloon inflation in the culprit coronary artery at the site of the lesion for 1 minute interspersed with 1 minute of deflation. Infarct size, measured as the magnitude of creatine kinase release over 72 hours, was reduced by 36% in the IP-treated patients. Microvascular perfusion, measured by angiographic blush grade, was also significantly improved. In another study of 38 patients, the same group of investigators reported a sustained benefit of IP, with smaller infarct size at 6 months and a 7% higher left ventricular ejection fraction.[10] Similar findings were reported in 2 further small studies.[31,32] The largest and most recent study to date randomized 118 patients with STEMI with an occluded culprit artery to either four 30-second cycles of ischemia-reperfusion immediately before primary PCI or primacy PCI alone. IP was associated with an ≈18% ($P=0.037$) reduction in the primary end point of infarct size, measured by cardiac magnetic resonance imaging at 3 months.[33]

Despite the promise of IP, it is limited by the fact that it can be applied only after the patient reaches a cardiac catheterization laboratory. A potential solution to this problem has been suggested from animal studies by investigators who have reported that ischemia/reperfusion of distant tissue or organ may also offer cardioprotection, a phenomenon known as remote IP.[34] The translation of this concept to the clinical arena would open the possibility of administering the benefits of postconditioning earlier and to a greater number of patients. Indeed, the findings of 1 study exploring the forearm circulation in healthy volunteers and another in patients undergoing elective PCI suggest that remote IP may indeed occur in humans.[35,36] A recent randomized trial among 333 STEMI patients requiring primary PCI provides the most compelling evidence for clinical applicability. Patients were randomized to 4 cycles of 5 minutes of forearm ischemia followed by reperfusion or no adjunctive therapy while being transported in the ambulance to the catheterization laboratory. The primary end point of myocardial salvage index at 1 month, measured by single-photon emission computed tomographic imaging, was increased in the remote IP group (0.56 versus 0.76; $P=0.02$). However, there was no improvement in secondary end points such as ST-segment resolution, peak troponin levels, and left ventricular ejection fraction.[37]

Thus, the preliminary data for cardiac and remote IP are very encouraging. However, confirmation in larger randomized multicenter studies that are adequately powered to assess clinical end points is required.

Other Pharmacological Targets

Opening of sarcolemmal and mitochondrial K-ATP channels has been implicated as another endogenous signal for cardioprotection. Nicorandil is a K-ATP channel opener; its efficacy was tested in the Japan Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage by KATP (J-WIND-KATP) randomized trial, which enrolled 545 patients. Treatment with a bolus at the time of PCI followed by a 24-hour intravenous infusion of nicorandil did not reduce infarct size as an adjunct to primary PCI.[25] This was is contrast to the study by Ishii and colleagues,[38] in which a single bolus of nicorandil at the time of PCI reduced the composite end point of cardiovascular death or hospitalization for worsening congestive heart failure. One explanation offered for the different outcomes is that the bolus dose in the latter study was 3-fold higher than in the J-WIND-KATP trial. Thus, the cardioprotective role of nicorandil remains to be established and, if present, may be dose dependent.

Adenosine, acting by stimulating the A_1 and A_3 receptors, has been shown in animal models to mimic ischemic preconditioning responses and to potentially interfere with RI on numerous levels.[39] However, large-scale clinical trials of adenosine and its analog have failed to demonstrate clinical efficacy.[13,39–41] In the pilot Acute Myocardial Infarction Study of Adenosine (AMISTAD)-I trial, intravenous adenosine reduced infarct size in patients treated with fibrinolysis, a benefit that was limited to the anterior wall.[42] The subsequent AMISTAD-II trial was conducted in patients with anterior STEMI receiving either fibrinolysis or primary PCI. A 3-hour infusion of adenosine did not reduce the primary clinical end point, although in a posthoc subgroup analysis, a benefit was seen in those reperfused within 3 hours.[43] In addition, a significant reduction in infarct size was reported in the high-dose adenosine subgroup (median infarct size, 27% of the left ventricle with placebo versus 11% with high-dose adenosine; $P=0.02$).[11] Thus, further studies are warranted to investigate the efficacy of an optimal dose ($70\ \mu g \cdot kg^{-1} \cdot min^{-1}$) of adenosine in patients presenting with large STEMI with a short ischemic time. Additionally, novel selective adenosine receptor agonists are being developed that may merit clinical evaluation.

Protein kinase Cδ is an isoenzyme of the protein kinase C family that has been identified as a mediator of lethal RI through its binding to mitochondria.[44] KAI-9803 is a novel peptide that inhibits protein kinase Cδ activity. The safety of 2 intracoronary boluses, 1 before and 1 after primary PCI, of KAI-9803 has been recently established in the Protein Kinase C Enzyme to Limit Total Infarct Size in Acute Myocardial

Infarction (DELTA-MI) trial conducted in patients with anterior STEMI.[45] A larger trial is being conducted to test the efficacy of an intravenous infusion of KAI-9803 in patients presenting with 6 hours of an STEMI.

Finally, in the Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury (FIRE) trial, FX06, a naturally occurring peptide derived from human fibrin, was evaluated for its efficacy in reducing RI.[46] In this randomized, placebo-controlled, multicenter study of 234 patients with STEMI, FX06 did not reduce troponin levels or infarct size at 5 days. There was, however, a significant reduction in the secondary end point of necrotic core size (measured by cardiac magnetic resonance imaging), although the significance of this finding is uncertain given that there was no difference in the size of the scar at 4 months.

Metabolic Modulation Through Insulin

The most widely explored strategy for metabolic modulation has been the combined administration of glucose-insulin-potassium[43] with the goal of shifting substrate use in the ischemic myocardium from free fatty acid to glucose. Although the preclinical data have been encouraging with respect to reducing infarct size, clinical trials have repeatedly failed to demonstrate efficacy.[47–52]

Novel Antithrombotic Mechanical and Pharmacological Concepts

Encouraging data are emerging for mechanical thrombectomy to prevent distal embolization of atherothrombotic debris during PCI.[4] The embolized material not only occludes the microcirculation but also contains vasoactive and proinflammatory mediators that may contribute to RI. Several devices have been investigated that can be categorized broadly as distal protection devices, passive aspiration catheters, and active mechanical thrombectomy systems such as the AngioJet Rheolytic catheter (Possis Medical Inc, Minneapolis, Minn). The most convincing evidence for adjunctive aspiration thrombectomy was reported from the single-center Thrombus Aspiration During Percutaneous Coronary Intervention in Acute Myocardial Infarction Study (TAPAS) trial, in which removal of thrombus during primary PCI with a simple aspiration device resulted in superior microvascular perfusion and lower mortality compared with PCI alone.[53–55] In addition, the Thrombectomy With Export Catheter in Infarct-Related Artery During Primary Percutaneous Coronary Intervention (EXPIRA) trial has recently demonstrated that aspiration thrombectomy leads to a reduction in infarct size.[56] Further large, multicenter, randomized trials to confirm the findings of the TAPAS trial would be helpful in establishing adjunctive thrombectomy as the standard of care.

Intracoronary delivery of antithrombotic drugs is also regaining focus as a concept for adjunctive therapy on the basis of data from randomized trials. In the Leipzig trial, 154 patients were randomized to receive either an intracoronary or an intravenous bolus of abciximab followed by a 12-hour infusion.[57] The primary end points of infarct size and microvascular obstruction at 48 hours were both significantly lower in the intracoronary drug delivery group and associated with a trend toward better clinical outcomes. Similarly, Sezer and colleagues[58] randomized 41 patients requiring primary PCI for STEMI to receive either streptokinase 250 kU over 3 minutes immediately after PCI or no additional therapy. They reported that microvascular function was significantly better in the intracoronary streptokinase group compared with the control group. There was a trend toward a smaller infarct size and better ejection fraction at 6 months in the streptokinase-treated group, although this was not statistically significant. Larger multicenter trials of intracoronary drug delivery are underway to confirm these results.

Mechanical Adjunctive Strategies

Paradoxically, although normoxemic reperfusion leads to the generation of reactive oxygen species, experimental data suggest that RI can be diminished by the use of high oxygen tensions.[59] In canine and porcine models, the site-specific infusion of supersaturated oxygen (Po_2, 760 to 1000 mm Hg) for 90 minutes after successful reperfusion has been shown to reduce infarct size, to improve microcirculatory flow by decreasing capillary endothelial cell swelling, and to reduce myeloperoxidase levels and RI. This concept was initially evaluated in the Acute Myocardial Infarction With Hyperoxemic Therapy (AMIHOT) trial among 269 patients undergoing primary PCI within 24 hours of symptom onset.[60] Patients were randomized after a successful procedure to either supersaturated oxygen (TherOx Inc, Irvine, Calif) for 90 minutes delivered into the infarct-related artery or control. Hyperoxemia neither reduced infarct size nor improved regional wall motion at 14 days in the entire study population. However, a benefit of supersaturated oxygen was noted in patients with large anterior infarction reperfused within 6 hours of symptom onset. The AMIHOT II trial, subsequently conducted exclusively among this patient population, was designed to allow partial hierarchical pooling of data from the AMIHOT I trial if the results were similar. In the bayesian pooled analysis from the 2 trials, supersaturated oxygen therapy was associated with a statistically significant 6.5% reduction in infarct size.[61] As a result, this device is currently undergoing Food and Drug Administration review for approval to reduce infarct size in patients with anterior STEMI undergoing successful primary PCI within 6 hours of symptom onset.

Therapeutic hypothermia is another mechanical adjunctive strategy that has been tested in STEMI. The rationale is that myocardial temperature is an important determinant of the magnitude of tissue necrosis during myocardial infarction. Animal studies suggest that lowering myocardial temperature by a few degrees reduces metabolic demand and infarct size.[62] The feasibility and safety of this concept were established with endovascular cooling (Radiant Medical Inc, Redwood City, Calif) in a randomized trial of 42 patients.[19] However, 2 completed randomized trials of this approach have been negative, possibly because of an inability to achieve myocardial cooling before reperfusion.[63,64]

Conclusions and Future Perspectives

Important lessons have been learned about how best to conduct future research into cardioprotection. First, basic

studies should be conducted in large-animal models that mimic human disease. This may be achieved, for example, by the use of hypercholesterolemic animals with atherosclerosis and/or comorbid conditions such as diabetes mellitus. To minimize bias, the preclinical studies should ideally be conducted with a randomized, blinded, multicenter design. Basic scientists and clinical investigators working in this field should form collaborative networks to optimize the likelihood of selecting and testing promising agents.[65] Second, it is essential that future clinical trials enroll patients presenting within 2 to 6 hours of symptom onset at risk for large myocardial infarction to maximize the chances of showing a positive treatment effect. This implies that estimation of the area at risk must be an inclusion criterion, and the presence of good collateral flow or normal antegrade preprocedural flow (Thrombolysis in Myocardial Infarction grade 3 flow) in the infarct territory should be an exclusion criteria. Although technetium-99m-sestamibi single-photon emission computed tomographic imaging or cardiac magnetic resonance imaging can reliably measure infarct size, large trials demonstrating a clinical benefit would be optimal. Unfortunately, the size of trials required to demonstrate a further reduction in mortality beyond that achieved with contemporary reperfusion therapy makes mortality trials unlikely, necessitating reliance on composite measures with softer end points such as new-onset heart failure. Although the challenges of conducting such clinical studies are formidable, recent developments suggest that cardioprotection in patients undergoing reperfusion therapy is an achievable goal, promising to further reduce morbidity and mortality for patients with STEMI.

Disclosures

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