Journal of the American College of Cardiology Vol. 59, No. 11, 2012

Published by Elsevier Inc.
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.07.054

STATE-OF-THE-ART PAPER

An Update on Cardioprotection

A Review of the Latest Adjunctive Therapies to Limit Myocardial Infarction Size in Clinical Trials

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Acute myocardial infarction (AMI) with subsequent left ventricular dysfunction and heart failure continues to be a major cause of morbidity and mortality in the Western world. Rapid advances in the treatment of AMI, mainly through timely reperfusion, have substantially improved outcomes in patients presenting with acute coronary syndrome and particularly ST-segment elevation myocardial infarction. A vast amount of research, both translational and clinical, has been published on various pharmacological and interventional techniques to prevent myocardial cell death during the time of ischemia and subsequent reperfusion. Several methods of cardioprotection have shown the ability to limit myocardial infarction size in clinical trials. Examples of interventional techniques that have proven beneficial are ischemic post-conditioning and remote ischemic per-conditioning, both of which can reduce infarction size. Lowering core body temperature with cold saline infusion and cooling catheters have also been shown to be effective in certain circumstances. The most promising pharmaceutical cardioprotective agents at this time appear to be adenosine, atrial natriuretic peptide, and cyclosporine, with other potentially effective medications in the pipeline. Additional pre-clinical and clinical research is needed to further investigate newer cardioprotective strategies to continue the current trend of improving outcomes following AMI.
(J Am Coll Cardiol 2012;59:969–78) © 2012 by the American College of Cardiology Foundation

Acute myocardial infarction (AMI) remains the leading cause of death in the United States, with nearly 1 million AMIs occurring annually, approximately 29% of which are ST-segment elevation myocardial infarctions (STEMIs) (1). Advances in our understanding of the pathogenesis of coronary artery occlusion and myocardial necrosis have led to dramatic improvements in morbidity and mortality following AMI. By 1986 (2), re-establishing flow in the occluded coronary artery by intravenous thrombolytic therapy was the standard of care for both reducing the incidence of subsequent heart failure and decreasing mortality (3,4). The theory of salvaging myocardium that would otherwise be condemned to ischemic death by restoring blood flow proved to be viable, and better techniques of opening the occluded coronary artery were quickly developed. Percutaneous transluminal coronary angioplasty (5–7), and coronary stenting (8–11) were extensively studied during the 1990s, and by 2000, they were considered, when available, the preferred strategy over thrombolytics (12,13). Over the last decade, many adjuvant medications (primarily antiplatelet drugs to keep the artery open) have been shown to be beneficial in the setting of AMI with mechanical reperfusion (14–16).

Although decreases in delays to establish coronary reperfusion and medications to maintain coronary patency have led to better outcomes following AMI, further improvements in morbidity and mortality from refinement of these techniques are likely to be small. Since the 1980s, an extraordinary amount of research has been conducted to find an agent that would render myocardial cells more resistant to the deleterious effects of ischemia followed by reperfusion. This idea of *cardioprotection* encompasses manipulation of the cellular events by pharmacotherapy or other therapies during ischemia and reperfusion to reduce the amount of myocardial cell death. This differs from the current (and well-proven) strategy of minimizing the time that the myocardium is ischemic by re-establishing blood flow as soon as possible. Although many potential cardioprotective agents have floundered in clinical trials over the years (Table 1) (17), a better understanding of the cellular mechanisms behind ischemia-reperfusion injury has led to improved results in recent clinical trials of several new cardioprotective drugs and interventions. The following review attempts to bring to light some promising new adjunctive therapies for reperfusion-treated AMI.

Ischemic Conditioning and Drugs That Mimic Its Effect

Pre-conditioning. Activating the body's endogenous protective mechanisms through repeated nonlethal bouts of

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Manuscript received May 10, 2011; revised manuscript received June 13, 2011, accepted July 19, 2011.

Abbreviations and Acronyms

AMI = acute myocardial infarction
ANP = atrial natriuretic peptide
LV = left ventricular
MI = myocardial infarction
MPTP = mitochondrial permeability transition pore
PCI = percutaneous coronary intervention
PDE5 = phosphodiesterase-5
STEMI = ST-segment elevation myocardial infarction

ischemia-reperfusion before a prolonged episode of ischemia is a powerful way to limit infarct size. This technique, known as ischemic pre-conditioning, was described 25 years ago by Murry et al. (18), and it continues to be the gold standard against which all experimental cardioprotective agents are judged. By definition, ischemic preconditioning must be applied *before* the ischemic event, making it difficult to use in the setting of AMI. It is, however, effective in cardiac surgery (19) and elective percutaneous coronary intervention (PCI) (situations in which myocardial ischemia is planned) (20).

Perhaps even more valuable, the study of preconditioning has led to improved understanding of the mechanisms of ischemia-reperfusion injury, which has opened the door for pharmacological agents that can mimic the effect of ischemic conditioning. The physiological, cellular, and molecular mechanisms of ischemic preconditioning have been studied for many years, and a detailed discussion is beyond the scope of this review. Briefly, the short episodes of ischemia-reperfusion recruit cell surface receptors to various autocoids (e.g., adenosine, bradykinin, opioids) that are released during ischemia. These receptors initiate a complex signaling cascade involving many protective kinases, including the reperfusion injury salvage kinase group, reactive oxygen species, and mitochondrial components (all targets for pharmacotherapy). The end result is a cardioprotective state that is mediated by several effector molecules, such as the mitochondrial permeability transition pore (MPTP) (21) and others. The reader is referred to several fine reviews on the mechanisms of cardioprotection and conditioning (21–23).

Additionally, other methods of ischemic conditioning that are more clinically applicable have been developed and have proven to reduce myocardial infarct size both in animal models and in clinical studies.

Post-conditioning and cyclosporine. Although ischemic pre-conditioning has consistently proven to be cardioprotective, its clinical application is clearly limited. In hopes of making ischemic conditioning clinically relevant, Zhao et al. (24) used the principles of ischemic pre-conditioning but applied them *after* the ischemic event took place (closely mimicking the setting of an AMI) in a canine model of myocardial ischemia-reperfusion. Indeed, these brief episodes of coronary artery occlusion applied immediately after the ischemic insult limited the MI size in the same manner as ischemic pre-conditioning.

Although not all animal models have consistently shown a benefit of post-conditioning on infarct size, there are compelling data in humans. Within 2 years of the initial

Table 1 Recent Cardioprotection Trials That Failed to Show a Reduction in Infarct Size

Therapeutic Agent	Trial Design (Ref. #)	Year	Outcome
LeukoArrest CD11/CD18 leukocyte integrin receptor inhibitor	HALT-MI (96) 420 patients randomized to LeukoArrest 0.3 mg/kg, LeukoArrest 1.0 mg/kg, or placebo before reperfusion	2002	No difference in infarct size measured by SPECT No improvement in clinical events
Eniporide Na^+/H^+ exchange inhibitor	ESCAMI (97) 959 patients randomized to 10-min infusion of eniporide 100 mg, eniporide 150 mg, or placebo before reperfusion	2001	No difference in infarct size measured by mean release of α-hydroxybutyrate dehydrogenase No improvement in clinical outcomes
Caldaret Intracellular Ca^{2+}-handling modulator	CASTEMI (98) 387 patients randomized to a 48-h infusion of caldaret 57.5 mg, caldaret 172.5 mg, or placebo initiated before reperfusion	2006	No difference in infarct size measured by SPECT No difference in LVEF on day 7 or 30
Nicorandil K^+ channel opener/vasodilator	J-WIND-KATP (58) 545 patients randomized to 0.067-mg/kg bolus followed by 24-h infusion of 1.67 μg/kg/min of nicorandil or placebo	2007	No difference in infarct size measured by CK-MB release
GIK glucose-insulin-potassium	CREATE-ECLA (99) 20,201 patients randomized to usual care plus 24-h infusion of GIK or usual care alone	2005	No difference in mortality, cardiac arrest, cardiogenic shock, reinfarction, or heart failure
Delcasertib δ-protein kinase C inhibitor	PROTECTION-AMI (100) Anterior MI cohort: 1,010 patients randomized to 2.5-h infusion of delcasertib 50 mg/h, 150 mg/h, 450 mg/h, or placebo initiated before PCI Inferior MI cohort: 166 patients randomized to 2.5-h infusion of delcasertib 450 mg/h or placebo initiated before PCI	2011	No difference in infarct size measured by CK-MB release or ST-segment resolution in either cohort No difference in clinical outcomes
Erythropoietin	REVEAL (101) 222 patients randomized to intravenous epoetin-alfa or placebo within 4 h of reperfusion	2011	No difference in infarct size as measured by cardiac MRI at 2 to 6 days or 12 ± 2 weeks Increased infarct size in patients ≥70 yrs who received epoetin-alfa Increased incidence of composite outcome of death, MI, stroke, or stent thrombosis in epoetin-alfa group

CK-MB = creatine kinase-MB; GIK = glucose-insulin-potassium; LVEF = left ventricular ejection fraction; MI = myocardial infarction; MRI = magnetic resonance imaging; PCI = percutaneous coronary intervention; SPECT = single-photon emission computed tomography.

animal studies of Zhao et al. (24), ischemic post-conditioning was already being investigated clinically (Table 2). In 2005, Staat et al. (25) published a landmark study of post-conditioning. This was a multicenter randomized controlled trial of 30 patients with STEMI involving the left anterior descending coronary artery or the right coronary artery that was undergoing PCI. Their protocol of post-conditioning consisted of 4 cycles of 1-min balloon inflation followed by 1 min of balloon deflation within 1 min of reflow after deployment of a coronary stent. Patients in the post-conditioning group were found to have 36% smaller infarctions as determined by serum creatine kinase (CK) release during the first 72 h of reperfusion and lower peak CK levels. The blush grade was also significantly higher in the post-conditioning group than controls, likely representing improved myocardial perfusion in the first minutes following reperfusion.

In 2008, Laskey et al. (26) used a protocol of 2 cycles of 90 s of balloon inflation followed by 3 min of reperfusion in patients presenting within 6 h of a transmural anterior wall AMI. They found that post-conditioned patients had significantly lower peak CK levels, greater and more rapid ST-segment resolution, and improved distal coronary flow velocity parameters.

Despite subtle differences in post-conditioning protocols including measurement of risk zone by Staat et al. (25–27), both Laskey and Staat demonstrated cardioprotective effects of post-conditioning.

These small clinical trials of ischemic post-conditioning are promising and show the potential of post-conditioning to improve both early markers of infarct size and myocardial function up to 1 year after infarction (Table 2). A major limitation of ischemic post-conditioning, however, is that it can only be performed in the catheterization laboratory during PCI. Although the benefits of primary PCI are well established, many patients still receive thrombolytic therapy as a method of reperfusion. A pharmacological agent that mimics the effects of post-conditioning could be administered to patients at the time of reperfusion, regardless of the modality being used.

To date, cyclosporine is the most promising pharmacological post-conditioning mimetic. Cyclosporine is thought to derive its cardioprotective effects from inhibiting formation of the MPTP, a key component of lethal reperfusion injury (28). The MPTP appears to form in the early stages of reperfusion in response to the calcium overload and reactive oxygen species generation that develops with reperfusion (29). This large-diameter pore forms in the

Table 2 Clinical Trials Examining Post-Conditioning in Acute STEMI

First Author, Year (Ref. #)	Study Design	Post-Conditioning Protocol	Primary Endpoints	Results
Staat et al., 2005 (25)	Prospective, open-label multicenter randomized controlled study (N = 30)	4 cycles of 1-min occlusion/1-min reperfusion within 1 min of index reperfusion	Serum CK release during first 72 h of reperfusion Peak serum CK level Blush grade during reflow ST-segment shifts at 48 h	36% decrease in area under the curve of CK release Increased blush grade
Laskey 2005 (102)	Prospective, open-label single-center randomized controlled study (N = 17)	2 cycles of 90-s occlusion/ 3–5-min reperfusion	Doppler-derived distal coronary flow velocity parameters ST-segment shift	Decreased magnitude of final ST-segment elevation Increased rate of ST-segment resolution Improved coronary flow velocity reserve
Laskey et al., 2008 (26)	Prospective, open-label single-center randomized controlled study (N = 24 patients with anterior STEMI)	2 cycles of 90-s occlusion/ 3-min reperfusion	Doppler-derived distal coronary flow velocity parameters ST-segment shift Peak CK release	Greater and more rapid ST-segment resolution Improved coronary flow velocity reserve Lower peak serum CK level
Ma et al., 2006 (103)	Prospective, open-label single-center, randomized controlled study (N = 94 patients with first STEMI)	3 cycles of 30-s occlusion/ 30-s reperfusion within 1 min of index reperfusion	Serum CK/CK-MB release during first 72 h Myocardial contractile function 8 weeks after infarction Corrected TIMI frame count Level of oxidative stress (MDA levels)	Faster CTFC Greater change in WMSI Lower peak CK-MB level Lower MDA-reactive products
Thibault et al., 2008 (104)	Prospective, open-label single-center randomized controlled study (N = 38 patients with first STEMI)	4 cycles of 1-min occlusion/ 1-min reperfusion within 1 min of index reperfusion	Infarct size (by CK and troponin) SPECT rest-redistribution index at 6 months Echocardiography at 1 yr	39% decrease in SPECT rest-redistribution index 40% reduction in infarct size Improved EF at 1 yr Improved WMSI at 1 yr
Lonborg et al., 2010 (105)	Prospective, open-label single-center randomized controlled study (N = 118)	4 cycles of 30-s occlusion/ 30-s reperfusion within 1 min of index reperfusion	Myocardial salvage at 3 months as assessed by delayed enhancement cardiac MRI	19% reduction in infarct size 31% increase in salvage ratio

CK = creatine kinase; CK-MB = creatine kinase-MB; CTFC = corrected thrombolysis in myocardial infarction frame count; EF = ejection fraction; MDA = malondialdehyde; SPECT = single-photon emission computed tomography; MRI = magnetic resonance imaging; STEMI = ST-elevation myocardial infarction; WMSI = wall motion severity score index.

mitochondrial inner membrane, allowing free passage of all molecules <1.5 kDa into the mitochondrial matrix, causing osmotic swelling, damage, and/or destruction of the mitochondria. Additionally, the inner membrane becomes freely permeable to protons, effectively uncoupling oxidative phosphorylation and disrupting ATP production (30). There is a large body of evidence in multiple animal models suggesting that, through inhibition of MPTP formation, cyclosporine can significantly reduce infarct size when given at the time of reperfusion (28,29). Interestingly, some of the cardioprotective effects seen in both pre- and post-conditioning are due to inhibition of MPTP formation, suggesting a common pathway linking these forms of protection (31).

In a small proof-of-concept clinical trial, Piot et al. (32) demonstrated that cyclosporine administered at the time of PCI could reduce the size of MI compared with placebo. Fifty-eight patients presenting with acute STEMI were randomized to receive either an intravenous bolus of cyclosporine 2.5 mg/kg of body weight or normal saline. Infarct size, as measured by CK release after reperfusion, was reduced by 40% in the cyclosporine group ($p = 0.04$) compared with the control group (138,053 arbitrary units [interquartile range (IQR): 114,008 to 283,461] for the cyclosporine group vs. 247,930 arbitrary units [IQR: 145,639 to 404,349] for the control group). The median area under the curve for troponin I release showed a trend toward being lower in the cyclosporine group but was not statistically significant. In a subgroup of 27 patients, cardiac magnetic resonance imaging (MRI) was performed at 5 days and 6 months after infarction. Patients in the cyclosporine group had a significantly smaller area of hyperenhancement than patients in the control group at 5 days (37 g [IQR: 21 to 51 g] for the treated patients vs. 46 g [IQR: 20 g to 65 g] for the controls; $p = 0.04$). In a subsequent published report, the same group related that the significant difference in infarct size as measured by cardiac MRI persisted 6 months later (29 ± 15 g in the cyclosporine group vs. 38 ± 14 g in the control group; $p = 0.04$) (33). Currently, a large multicenter trial involving approximately 1,000 patients is underway to further investigate cyclosporine as a cardioprotective agent.

Remote ischemic conditioning. There is accumulating evidence that ischemic conditioning can limit myocardial infarct size even when applied to a distant organ's vascular bed. This technique, known as "remote ischemic conditioning," has been shown to be cardioprotective in various animal models when administered before the ischemic event (remote pre-conditioning) (34,35), after the ischemic event (remote post-conditioning) (36,37), and *during* the ischemic event (remote per-conditioning) (38). Practically, remote conditioning is very attractive because even limb ischemia induced by inflation of a blood pressure cuff can reduce myocardial ischemic injury (38). Mechanistically, it appears that remote conditioning activates similar signaling pathways as pre- and post-conditioning; however, it is unknown how the conditioning stimulus applied to a remote organ is able to protect the heart. A current theory involves an unknown humoral substance that is released from the conditioned organ and exerts its effect on the heart (39,40). Alternatively, a signal may be carried from afferent neurons in the conditioned organ to efferent neurons terminating in the heart (41). Further research is underway to clarify the underlying mechanism.

In a recent study by Botker et al. (42), 333 patients with acute STEMI were randomized to receive either PCI alone or remote ischemic conditioning before PCI. The remote per-conditioning began in an ambulance en route to the hospital and consisted of intermittent arm ischemia through 4 cycles of alternating 5-min inflation to 200 mm Hg and 5-min deflation of a standard upper-arm blood pressure cuff. The primary endpoint of this study was myocardial salvage index 30 days after PCI as measured by gated single-photon emission computed tomography. The researchers found a significant improvement in myocardial salvage index in the per-conditioning group as compared with controls (0.69 ± 0.27 for treated patients vs. 0.57 ± 0.26 for controls; $p = 0.03$). There was also a trend, although not statistically significant, toward smaller final infarct size in the per-conditioning group ($8 \pm 10\%$ vs. $12 \pm 13\%$; $p = 0.10$). This study adds to the growing body of evidence that suggests that ischemic conditioning is both feasible and effective in real-world clinical situations. In addition, this specific technique of using upper-extremity limb ischemia en route to the hospital is particularly exciting because of its convenience and effectiveness. Larger clinical trials are underway to refine this novel technique of cardioprotection.

Adenosine: AMISTAD I and II and Beyond

As the understanding of ischemic conditioning grew, it became apparent that adenosine plays a central role in triggering this pre-conditioned cardioprotective state (43,44). Many different adenosine receptors and receptor subtypes are expressed in cardiomyocytes, and their exact role in the different methods of cardioprotection is not completely understood. However, it is known that A1 and A3 receptors, when engaged by adenosine or adenosine agonists before lethal ischemia, trigger the pre-conditioned state (43,44). The molecular signaling cascade by which this occurs is complex and beyond the scope of this review (45); however, the common final pathway again appears to be inhibition of MPTP formation.

Although adenosine was clearly shown to be cardioprotective when administered before lethal ischemia, its ability to reduce infarct size in experimental models when administered during ischemia before reperfusion is somewhat more controversial (46–50). Despite mixed results in various animal models of ischemia-reperfusion, large-scale clinical trials were undertaken to examine the role of adenosine administered during evolving acute STEMI.

AMISTAD I (Acute Myocardial Infarction Study of Adenosine) was the first large-scale clinical trial testing

adenosine as an adjunct to reperfusion therapy (51). Published in 1999, this multicenter randomized placebo-controlled trial of 236 patients with STEMI treated with thrombolytic therapy showed that adenosine infusion caused a 33% relative reduction in infarct size. However, this benefit was only observed in patients who suffered an anterior MI, and in fact, patients with nonanterior infarctions who received adenosine showed a trend toward increased adverse clinical events.

AMISTAD II was designed to examine the effect of adenosine as an adjunct therapy for patients with acute anterior STEMI undergoing either thrombolysis or PCI (52). In this trial, 2,118 patients were randomized to receive a 3-h intravenous infusion of low-dose adenosine (50 μg/kg/min), high-dose adenosine (70 μg/kg/min), or placebo before PCI or within 15 min of the initiation of fibrinolysis. No difference in the primary clinical composite endpoint of death, new-onset congestive heart failure, or rehospitalization for congestive heart failure within 6 months was observed between the high-dose adenosine group, low-dose adenosine group, or pooled adenosine dose group and placebo. The high-dose adenosine group, however, did show a significant reduction in infarct size compared with the control group (11% in the treated group vs. 27% in the controls; $p = 0.023$). These findings confirmed the infarct size–reducing effects of high-dose adenosine observed in AMISTAD I. A subsequent post hoc analysis of AMISTAD II by Kloner et al. (53) showed significant improvement in mortality and event-free survival in a subset of patients who received adenosine and reperfusion therapy within 3.17 h of symptom onset, and this population of patients should be studied further in a randomized clinical trial (54). Patients reperfused late (i.e., beyond 3.17 h of symptom onset) did not demonstrate any clinical benefit of adenosine.

Intracoronary administration of adenosine has been studied in several clinical trials, with mixed results (55,56). Recently, a randomized controlled study of 112 patients presenting within 12 h of STEMI demonstrated no beneficial effect of a 4-mg intracoronary bolus of adenosine immediately before reperfusion (57). There was no significant difference in the primary endpoint of myocardial salvage index as measured by MRI between the 2 groups (41.3% [IQR: 20.8% to 66.7%] for the treated group vs. 47.8% [IQR: 39.8% to 60.9%] for controls; $p = 0.52$). A major limitation of this study, however, was that adenosine was administered as an intracoronary bolus. The half-life of adenosine is only several seconds, making a bolus injection unlikely to be effective. Intravenous/intracoronary infusion of the drug allows it to remain in circulation longer, perhaps allowing adequate time to be beneficial.

J-WIND Atrial Natriuretic Peptide

The J-WIND (Japan-Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage) trial was 2 independent clinical trials that examined the effects of atrial natriuretic peptide (ANP) and nicorandil on myocardial infarct size, cardiac function, and clinical outcomes (58).

In J-WIND-KATP, 545 patients were randomized to receive either nicorandil by bolus injection of 0.067 mg/kg followed by an intravenous infusion of 1.67 μg/kg/min for 24 h or saline infusion by the same method. No differences in infarct size, chronic LV function, or cardiac events were seen.

J-WIND-ANP was a multicenter randomized placebo-controlled trial involving 27 hospitals. A total of 277 patients were randomized to receive a continuous infusion of ANP for 3 days following reperfusion by either PCI or thrombolytic therapy, and 292 patients were randomized to receive placebo infusion (5% glucose) for the same time period. The researchers showed that ANP, given acutely, reduced total CK release (66,459.9 IU/ml per h in the treated group vs. 77,878.9 IU/ml per h in controls), which corresponded to a 14.7% reduction in infarct size. Patients in the ANP group also had significantly less reperfusion injury and a small but significant increase in ejection fraction at 6 to 12 months compared with controls (44.7% vs. 42.5%). Finally, ANP significantly decreased cardiac death and hospitalization due to heart failure over a median follow-up time of 2.7 years.

Beta-Blockade

Cardioselective beta-blockers have an important and well-established role in the treatment of AMI. The beneficial effects of beta-blockers on LV remodeling, reinfarction, life-threatening arrhythmias, and most importantly, mortality are well documented in published reports (59,60). However, their effect on infarct size per se is somewhat more controversial. Pre-clinical data on the infarct-sparing effects of beta-blockers are mixed, and the majority of large clinical trials that looked at the usefulness of beta-blockade in AMI was carried out in the "pre-thrombolytic era." The consensus from these early trials was that beta-blockade might reduce infarct size when given very early after the onset of symptoms (4 to 7 h) (60–63).

Contemporary data on the cardioprotective effects of beta-blockers are certainly sparse; however, the lack of data provides an opportunity to discuss the process by which potentially cardioprotective therapies should be investigated. Over the last 25 years, there has been an obvious disconnect between the promising results seen in pre-clinical cardioprotection studies and clinical success (17,64) (Table 1). There are many reasons for this; however, the undertaking of premature clinical trials before adequate pre-clinical research has been completed appears to be a significant problem. Recently, the multicenter CAESAR (Consortium for Preclinical Assessment of Cardioprotective Therapies) initiative was supported by the National Heart Lung and Blood Institute (54). This initiative was developed from a 2003 National Heart Lung and Blood Institute working group that was convened to discuss methods to

better translate cardioprotective therapies to clinical practice. CAESAR was created to more systematically examine potential therapies in multiple laboratories and in different animal models using a centralized core laboratory for assessment of infarct size. This standardized approach, more closely resembling a multicenter clinical trial, will hopefully yield more accurate, reliable results before a therapy is tested in clinical trials.

Although there is excitement about the cardioprotective potential of new beta-blockers such as nebivolol (65,66), sufficient pre-clinical investigation, including reproducible results in multiple animal models, should be completed before large-scale clinical testing.

Therapies That Show Promise Under the Right Circumstances

Mild hypothermia. Therapeutic hypothermia is a well-studied method of protecting the ischemic heart from infarction. Multiple animal models have demonstrated the cardioprotective effect of hypothermia, from mild hypothermia (32°C to 35°C) to moderate (28°C to 32°C), severe (20°C to 28°C), and profound (<20°C) hypothermia (67–71). Although the mechanism of protection in moderate and severe hypothermia appears to involve metabolic slowing, mild hypothermia likely involves alterations in signaling pathways (67,72). Interestingly, Yang et al. (73) recently published a study that suggested that preservation of extracellular signal–regulated kinase activity by mild hypothermia contributed to a reduction in infarct size in rabbits (73). Extracellular signal–regulated kinase is a member of the reperfusion injury salvage kinases group of protective kinases, which are involved in protection from ischemia-reperfusion injury and have been shown to be activated in other methods of cardioprotection such as ischemic conditioning.

Several early clinical trials attempted to translate this benefit to humans, with limited success (74); however, hypothermia can indeed limit infarct size under certain conditions. COOL-MI (Cooling as an Adjunctive Therapy to Percutaneous Intervention in Patients With Acute Myocardial Infarction) was a multicenter randomized controlled clinical trial that evaluated the effectiveness of hypothermia in reducing myocardial infarct size (74). In this trial of 357 patients, 177 patients were randomized to receive an endovascular cooling catheter (Reprieve, Radiant Medical, Redwood City, California) positioned in the inferior vena cava through the femoral vein. When the patients arrived in the catheterization laboratory, hypothermia was induced by circulating cooled saline through the device, thereby cooling the blood as it passed along the catheter. Although this study failed to show an overall difference in infarct size, the investigators did notice a trend toward smaller infarctions in patients receiving hypothermia who had anterior MIs versus those with inferior MIs. Further investigation showed that patients with anterior MIs who were cooled to temperatures <35°C before reperfusion had significantly smaller infarctions than controls (9.3% in treated patients vs. 18.2% in controls; $p = 0.05$), albeit in a small number of patients ($n = 16$).

A major limitation to COOL-MI was that only a minority of patients was able to reach the target temperature of <35°C before reperfusion—likely due to the rapid door-to-balloon time and a slow cooling process. The RAPID-MI-ICE (Rapid Intravascular Cooling in Myocardial Infarction as Adjunctive to Percutaneous Coronary Intervention) trial was designed to evaluate the safety and feasibility of rapidly induced hypothermia before reperfusion (75). Twenty patients were randomly assigned to hypothermia or control. In addition to an endovascular cooling catheter, patients in the hypothermia group received an intravascular infusion of 1 to 2 liters of cooled saline. All patients in the hypothermia group attained a core body temperature of <35°C before reperfusion without a significant delay in door-to-balloon time. Rapidly induced hypothermia was found to reduce infarct size normalized to area at risk by 38% compared with controls (29.8 ± 12.6% in treated patients vs. 48.0 ± 21.6% in controls; $p = 0.04$), as assessed by cardiac MRI performed 4 ± 2 days following MI. Additionally, no increase in adverse events was seen. This pilot study demonstrated that attaining a core body temperature of <35°C may be effective in reducing myocardial infarct size and can be quickly and safely induced without a significant delay in reperfusion or increase in adverse events.

Supersaturated oxygen therapy: AMIHOT I and II. Although still somewhat controversial, lethal reperfusion injury may play an important role in myocardial ischemia-reperfusion injury (76,77). Although complex and damaging, reperfusion injury provides an attractive target for adjunctive cardioprotection therapy because, unlike the initial ischemic event, the moment of reperfusion can usually be controlled.

An adjunctive therapy that may attenuate reperfusion injury is hyperoxemic therapy, and a recently published pivotal trial demonstrated this approach to be effective. AMIHOT II (Acute Myocardial Infarction With Hyperoxemic Therapy II) was a prospective multicenter study of 301 patients with anterior STEMI who presented within 6 h of symptom onset (78). Patients were randomized to receive a 90-min intracoronary infusion of supersaturated oxygen immediately following PCI or standard of care therapy. This trial was designed following the disappointing results of AMIHOT I, which evaluated hyperoxemic therapy in patients with anterior or large inferior MIs presenting within 24 h of symptom onset (79). AMIHOT I failed to meet its primary efficacy endpoints of change in wall motion severity index at 3 months, ST-segment curve area 3 h after PCI, and final infarct size at 14 to 21 days. However, post hoc analysis indicated a possible benefit in patients with anterior MIs presenting within 6 h of symptom onset.

AMIHOT II indeed demonstrated positive results (78). Using Bayesian hierarchical modeling, the research-

ers were able to borrow evidence from patients enrolled in AMIHOT I that met inclusion criteria for AMIHOT II. This underused yet well-established technique allows for a smaller sample size and therefore more rapid completion of pivotal clinical trials (80). When data from AMIHOT I and II were pooled, infarct size was found to be significantly reduced (25% [IQR: 7% to 42%] in the control group vs. 18.5% [IQR: 3.5% to 34.5%] in the treated group; $P_{Wilcoxon} = 0.02$; Bayesian posterior probability of superiority 96.9%). Additionally, in patients with a baseline LV ejection fraction of <40%, infarct size was reduced from 33.5% in controls to 23.5% in the treatment group. This incremental myocardial salvage of 10% is even better than the 6.5% salvage seen in the entire study population, indicating that this therapy may be most beneficial in patients with more devastating infarctions.

Promising Therapies for the Future

Nitrates. There is a renewed interest in nitrates as a cardioprotectant in recent years because new clinical and animal model studies have shown promising results. Early clinical trials in the pre-thrombolytic era of the 1970s and 1980s suggested that nitrates could reduce mortality in the setting of AMI, and a large meta-analysis published in the *Lancet* in 1988 confirmed this mortality benefit (81). However, 2 large clinical trials, GISSI-3 (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico 3) (82) and ISIS-4 (Fourth International Study of Infarct Survival) (83), were designed to address the possible mortality benefit of nitrates in the setting of AMI with thrombolytic therapy, and both trials failed to show a benefit.

More recently, several clinical studies have shown the potential of nitrates to limit myocardial infarct size. Ambrosio et al. (84) retrospectively analyzed the GRACE (Global Registry of Acute Coronary Syndromes) data set to determine if antecedent nitrate therapy led to less myocardial necrosis during the ischemic event. The researchers found that 18% of chronic nitrate users were diagnosed with STEMI compared with 41% of nitrate-naive patients. Likewise, 82% of nitrate users presented with non–STEMI compared with 59% of patients who were nitrate naive. They also found that antecedent nitrate use was associated with lower CK-MB and troponin levels, regardless of acute coronary syndrome type.

Nitroglycerin has also been shown to have preconditioning mimetic action in several clinical circumstances. In patients with stable angina, pre-treatment with intravenous nitroglycerin 24 h before an exercise tolerance test improved functional capacity and electrocardiographic manifestations of ischemia exhibited by a decrease in maximal ST-segment depression, total sum of ST-segment depression, and time to resolution of ST-segment depression (85).

The same investigators also showed that nitroglycerin can have cardioprotective effects when given 24 h before coronary angioplasty. Patients who received nitroglycerin had improvement in ST-segment shifts, regional wall motion abnormalities, and chest pain scores after balloon inflation compared with patients who received saline (86).

Phosphodiesterase-5 inhibitors. Currently, there are 3 phosphodiesterase isoform 5 (PDE5) inhibitors available for the treatment of erectile dysfunction: sildenafil, vardenafil, and tadalafil. In the past several years, there has been considerable attention paid to their potential cardioprotective properties, and indeed, there is a growing body of preclinical evidence to support it. The mechanism of PDE5 cardioprotection is complex and beyond the scope of this review, but PDE5 inhibitors appear to be ischemic preconditioning mimetics (87–89) as well as inhibitors of MPTP formation (90). Other mechanisms may involve reduction in arterial blood pressure (91), attenuation of inotropic responses to catecholamines (92,93), and nitric oxide signaling (94,95).

Conclusions and Future Perspectives

The last 3 decades have seen hundreds of potentially cardioprotective adjunctive therapies come and go with none becoming commonplace in clinical practice. The reasons for these translational failures have been discussed at length and well documented in published reports (17). Several therapies, however, have now proven beneficial in clinical trials, and although more studies are needed, we are indeed getting closer to a safe and effective adjunct to reperfusion. Interestingly, candidate treatments with the most potential involve many different treatment modalities, and pre-clinical investigators must use multiple laboratories and animal models to increase the likelihood of bringing a successful therapy to clinical trial.

Although rapid reperfusion of ischemic myocardium remains the standard of care for AMI, we must strive to reduce the amount of cell death even further. With the help of initiatives such as CAESAR and other multilaboratory efforts (106), future large-scale clinical trials will determine which therapy or combination of therapies will be most efficacious in reaching this ambitious yet attainable goal.

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Key Words: cardioprotection ■ hypothermia ■ limit infarct size ■ myocardial infarction ■ necrosis ■ post-conditioning ■ pre-conditioning ■ remote conditioning.