REVIEW ARTICLE

MECHANISMS OF DISEASE

Myocardial Reperfusion Injury

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Coronary heart disease is the leading cause of death worldwide, and 3.8 million men and 3.4 million women die of the disease each year. After an acute myocardial infarction, early and successful myocardial reperfusion with the use of thrombolytic therapy or primary percutaneous coronary intervention (PCI) is the most effective strategy for reducing the size of a myocardial infarct and improving the clinical outcome. The process of restoring blood flow to the ischemic myocardium, however, can induce injury. This phenomenon, termed myocardial reperfusion injury, can paradoxically reduce the beneficial effects of myocardial reperfusion.

The potentially detrimental aspect of myocardial reperfusion injury, termed lethal reperfusion injury, is defined as myocardial injury caused by the restoration of coronary blood flow after an ischemic episode. The injury culminates in the death of cardiac myocytes that were viable immediately before myocardial reperfusion.[1] This form of myocardial injury, which by itself can induce cardiomyocyte death and increase infarct size (Fig. 1), may in part explain why, despite optimal myocardial reperfusion, the rate of death after an acute myocardial infarction approaches 10%,[2] and the incidence of cardiac failure after an acute myocardial infarction is almost 25%.

Studies in animal models of acute myocardial infarction suggest that lethal reperfusion injury accounts for up to 50% of the final size of a myocardial infarct, and in these models a number of strategies have been shown to ameliorate lethal reperfusion injury. Yet, the translation of these beneficial effects into the clinical setting has been disappointing.[3] Nevertheless, recent demonstrations of the benefit of ischemic postconditioning,[4] in which myocardial reperfusion in patients with acute myocardial infarction who are undergoing PCI is interrupted with short-lived episodes of myocardial ischemia,[5-7] have regenerated interest in the reperfusion phase as a target for cardioprotection. The identification of the reperfusion injury salvage kinase (RISK) pathway[8] and the mitochondrial permeability transition pore (PTP)[9,10] as new targets for cardioprotection has also intensified research in this area. These new developments should lead to strategies that improve clinical outcomes in acute myocardial infarction and reduce the risk of heart failure after myocardial infarction.[11]

MYOCARDIAL REPERFUSION INJURY AND CELL DEATH

Myocardial reperfusion injury was first postulated in 1960 by Jennings et al.[12] in their description of the histologic features of reperfused ischemic canine myocardium. They reported cell swelling, contracture of myofibrils, disruption of the sarcolemma, and the appearance of intramitochondrial calcium phosphate particles. The injury to the heart during myocardial reperfusion causes four types of cardiac dysfunction. The first type is myocardial stunning, a term denoting the "mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or near-normal coronary flow."[13] The myocar-

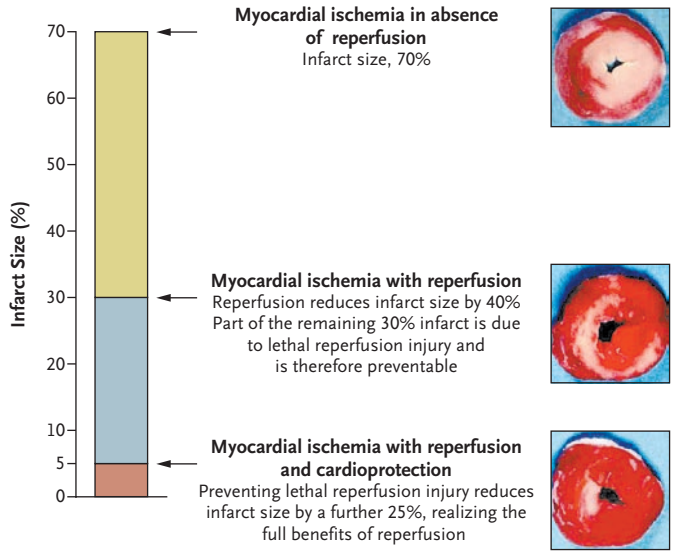


Figure 1. Contribution of Lethal Reperfusion Injury to Final Myocardial Infarct Size.

This hypothetical scheme shows the large reduction in myocardial infarct size obtained by early and successful myocardial reperfusion after a sustained episode of acute myocardial ischemia. The full benefits of myocardial reperfusion are not realized because of the presence of lethal reperfusion injury, which diminishes the magnitude of the reduction in infarct size elicited by myocardial reperfusion. This concept is revealed by the further reduction in myocardial infarct size obtained by preventing lethal reperfusion injury with the administration of a cardioprotective intervention at the beginning of myocardial reperfusion. Infarcted myocardium is depicted in pink, and the viable, at-risk myocardium is stained red. Infarct size is expressed as a percentage of the volume of myocardium at risk for infarction.

dium usually recovers from this reversible form of injury after several days or weeks. The second type of cardiac dysfunction, the no-reflow phenomenon, was originally defined as the "inability to reperfuse a previously ischemic region."[14] It refers to the impedance of microvascular blood flow encountered during opening of the infarct-related coronary artery.[15] The third type of cardiac dysfunction, reperfusion arrhythmias, is potentially harmful, but effective treatments are available.[16] The last type is lethal reperfusion injury. There are several comprehensive reviews of myocardial stunning,[17] the no-reflow phenomenon,[15] and reperfusion arrhythmias.[16]

The concept of lethal reperfusion injury as an independent mediator of cardiomyocyte death, distinct from ischemic injury, has been hotly debated; some researchers have suggested that reperfusion only exacerbates the cellular injury that was sustained during the ischemic period.[18] The uncertainty relates to the inability to accurately assess in situ the progress of necrosis during the transition from myocardial ischemia to reperfusion.[1] As a result, the most convincing means of showing the existence of lethal reperfusion injury as a distinct mediator of cardiomyocyte death is to show that the size of a myocardial infarct can be reduced by an intervention used at the beginning of myocardial reperfusion.[1,19]

POTENTIAL MEDIATORS OF LETHAL REPERFUSION INJURY

OXYGEN PARADOX

Experimental studies have established that the reperfusion of ischemic myocardium generates oxidative stress, which itself can mediate myocardial injury[20] (Fig. 2). Oxidative stress is part of the oxygen paradox,[21] in which the reoxygenation of ischemic myocardium generates a degree of myocardial injury that greatly exceeds the injury induced by ischemia alone.[21] The role of oxidative stress in lethal reperfusion injury is clouded by the inconclusive results of animal and clinical studies[22-52] of cardioprotection by antioxidant reperfusion therapy (Table 1).

Oxidative stress during myocardial reperfusion also reduces the bioavailability of the intracellular signaling molecule, nitric oxide, thereby removing its cardioprotective effects. These effects include the inhibition of neutrophil accumulation, inactivation of superoxide radicals, and improvement of coronary blood flow.[53] Nitric oxide reperfusion therapy to increase nitric oxide levels can reduce the size of a myocardial infarct in animals,[54] but clinical studies of the antianginal nitric oxide donor nicorandil have reported benefit only in terms of improved myocardial reperfusion; results in terms of clinical outcomes after an acute myocardial infarction are mixed (Table 1).[47-49]

CALCIUM PARADOX

At the time of myocardial reperfusion, there is an abrupt increase in intracellular Ca^{2+}, which is secondary to sarcolemmal-membrane damage and oxidative stress–induced dysfunction of the sarcoplasmic reticulum. These two forms of injury overwhelm the normal mechanisms that regulate Ca^{2+} in the cardiomyocyte; this phenomenon is termed the calcium paradox[1] (Fig. 2). The result is intra-

cellular and mitochondrial Ca^{2+} overload, and this excess of Ca^{2+} induces cardiomyocyte death by causing hypercontracture of the heart cells and mitochondrial PTP opening[1] (Fig. 2). Attenuating intracellular Ca^{2+} overload with pharmacologic antagonists of the sarcolemmal Ca^{2+} ion channel, the mitochondrial Ca^{2+} uniporter, or the sodium–hydrogen exchanger decreases myocardial infarct size by up to 50% in experimental studies.[55-57] However, the results of the corresponding clinical studies have been negative.[27,29] That inhibition of sodium–hydrogen exchange at the time of PCI does not protect the myocardium during an acute myocardial infarction is consistent with the results of experimental studies in which the beneficial effects of inhibiting sodium–hydrogen exchange were shown to occur during myocardial ischemia and not reperfusion (Table 1).[29,58] MCC-135, the first in a new class of agents that reduce intracellular Ca^{2+} loading by inhibiting the sodium–hydrogen exchanger and promoting Ca^{2+} uptake by the sarcoplasmic reticulum, has also not influenced infarct size when given during reperfusion (Table 1).[30]

pH PARADOX

The rapid restoration of physiologic pH during myocardial reperfusion, which follows the washout of lactic acid and the activation of the sodium–hydrogen exchanger and the sodium–bicarbonate symporter, contributes to lethal reperfusion injury (Fig. 2). This phenomenon is termed the pH paradox.[59] In neonatal rat cardiomyocytes, experimental studies have shown that reoxygenation with acidic buffer is cardioprotective[60]; this effect may be mediated by the inhibition of mitochondrial PTP opening.[61] However, in clinical studies, delaying the restoration of physiologic pH during myocardial reperfusion using sodium–hydrogen exchange inhibition did not protect the heart (Table 1).[29,58]

INFLAMMATION

After an acute myocardial infarction, the release of chemoattractants draws neutrophils into the infarct zone during the first 6 hours of myocardial reperfusion, and during the next 24 hours they migrate into the myocardial tissue. This process is facilitated by cell-adhesion molecules. These neutrophils cause vascular plugging and release degradative enzymes and reactive oxygen species (Fig. 2).[62]

Experimental studies have shown reductions in infarct size of up to 50% with several interventions aimed at neutrophils during myocardial reperfusion. These interventions include leukocyte-depleted blood[63]; antibodies against the cell-adhesion molecules P-selectin,[64] CD11 and CD18,[65] and the intercellular adhesion molecule 1[66]; and pharmacologic inhibitors of complement activation.[67] However, the corresponding clinical studies have not shown any meaningful cardioprotective effect of such interventions (Table 1).[32-38]

After inconclusive experimental studies,[68,69] clinical studies of the antiinflammatory agent adenosine as an adjunct to PCI have shown an 11% reduction in the size of myocardial infarcts, but benefits in terms of clinical outcomes were limited to patients presenting within 3 hours after the onset of symptoms (Table 1).[39,40]

METABOLIC MODULATION

Several experimental and numerous clinical studies have examined the cardioprotective potential of therapy with glucose, insulin, and potassium administered as an adjunct to myocardial reperfusion.[70,71] These studies have been conducted on the premise that ischemic myocardium benefits more from metabolizing glucose than from fatty acids.[72] A recent very large, randomized, controlled study from several centers reported no cardioprotective benefit from therapy with glucose, insulin, and potassium as an adjunct to myocardial reperfusion in patients with acute myocardial infarction (Table 1).[41] The delay in initiating this therapy, the prolonged period of myocardial ischemia, and high and potentially damaging glucose levels have all been cited as reasons for the lack of cardioprotection.[71] The effect of therapy with glucose, insulin, and potassium administered in the ambulance to patients with acute myocardial infarction before myocardial reperfusion has occurred is being investigated in the Immediate Metabolic Myocardial Enhancement During Initial Assessment and Treatment in Emergency Care (IMMEDIATE) trial.[42]

MAGNESIUM THERAPY

Experimental studies have reported that intravenous magnesium administered during myocardial reperfusion can reduce myocardial infarct size, but the mechanism of this effect is unclear.[73] Initial clinical studies of adjunctive reperfusion therapy with magnesium in patients with acute myocardial

infarction were inconclusive; the timing of its administration was not sufficiently controlled.[43,44] However, subsequent trials with magnesium administered immediately before PCI have also not shown cardioprotection (Table 1).[45,46]

THERAPEUTIC HYPOTHERMIA

Mild hypothermia (33 to 35°C) has been reported to benefit patients surviving a cardiac arrest.[74] Experimental studies have shown a 10% reduction in myocardial infarct size for every 1°C decrease in body temperature[75]; mild hypothermia reduces myocardial infarct size in human-sized pigs.[76] However, initial clinical studies of therapeutic hypothermia in patients with acute myocardial infarction who are undergoing primary PCI have not shown any beneficial effects (Table 1).[50-52]

MITOCHONDRIAL PTP

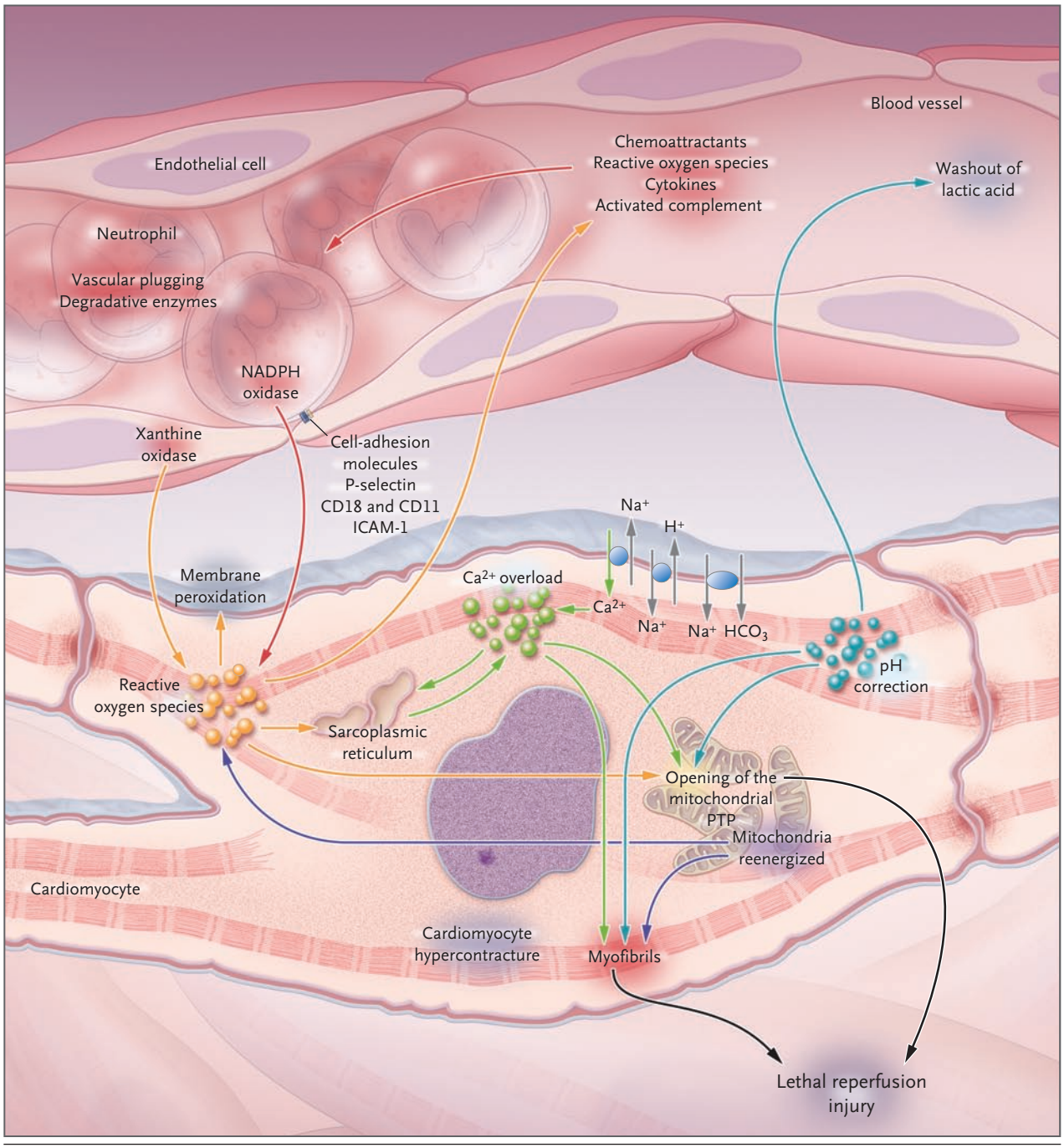
The mitochondrial PTP is a nonselective channel of the inner mitochondrial membrane. Opening the channel collapses the mitochondrial membrane potential and uncouples oxidative phosphorylation, resulting in ATP depletion and cell death.[9] During myocardial ischemia, the mitochondrial PTP remains closed, only to open within the first few minutes after myocardial reperfusion in response to mitochondrial Ca^{2+} overload, oxidative stress, restoration of a physiologic pH, and ATP depletion.[61,77] Therefore, the mitochondrial PTP is a critical determinant of lethal reperfusion injury, and as such it is an important new target for cardioprotection.

TARGETING LETHAL REPERFUSION INJURY

Experimental studies have shown that interventions during myocardial reperfusion can reduce myocardial infarct size by up to 50%, suggesting that lethal reperfusion injury contributes to up to half of the final myocardial infarct size. However, the disappointing attempts to translate the beneficial effects that were shown in animal models into clinical practice have raised the question of whether such infarct models are relevant to myocardial infarction in people.[3] Several reasons have been proposed for the disparity between findings in animals and in patients (Table 2).[3,79] In the clinical setting, the varying degrees of ischemia in an

Figure 2. Major Mediators of Lethal Reperfusion Injury.

During myocardial reperfusion, the acute ischemic myocardium is subjected to several abrupt biochemical and metabolic changes, which compound the changes generated during the period of myocardial ischemia. These changes include mitochondrial reenergization (purple), the generation of reactive oxygen species (ROS) (orange), intracellular Ca^{2+} overload (green), the rapid restoration of physiologic pH (blue), and inflammation (red), all of which interact with each other to mediate cardiomyocyte death through the opening of the mitochondrial permeability transition pore (PTP) and the induction of cardiomyocyte hypercontracture. During myocardial reperfusion, ROS are generated by xanthine oxidase (mainly from endothelial cells) and the re-energized electron transport chain in the cardiomyocyte mitochondria. Several hours later, a further source of ROS is NADPH oxidase (mainly from neutrophils). ROS mediate myocardial injury by inducing mitochondrial PTP opening, acting as neutrophil chemoattractants, mediating dysfunction of the sarcoplasmic reticulum and contributing to intracellular Ca^{2+} overload, damaging the cell membrane by lipid peroxidation, inducing enzyme denaturation, and causing direct oxidative damage to DNA. During myocardial reperfusion, the already Ca^{2+}-overloaded cardiomyocyte is subjected to a further influx of Ca^{2+} through a damaged sarcolemmal membrane, ROS-mediated dysfunction of the sarcoplasmic reticulum, and reverse function of the Na^+–Ca^{2+} exchanger. The generation of ATP by the reenergized electron transport chain in the setting of intracellular Ca^{2+} overload induces cardiomyocyte death by hypercontracture, a process that is facilitated by the rapid restoration of physiologic pH during myocardial reperfusion. Furthermore, the restoration of the mitochondrial membrane potential drives the entry of Ca^{2+} into mitochondria that, in conjunction with the loss of the inhibitory effect of the acidic pH on the mitochondrial PTP and the generation of ROS, act in concert to mediate the opening of the mitochondrial PTP. This opening induces cardiomyocyte death by uncoupling oxidative phosphorylation and inducing mitochondrial swelling. During myocardial reperfusion, the rapid washout of lactic acid together with the function of the Na^+–H^+ and Na^+–HCO_3 transporters mediate the rapid restoration of physiologic pH, facilitating mitochondrial PTP opening and cardiomyocyte hypercontracture. Several hours after the onset of myocardial reperfusion, neutrophils accumulate in the infarcted myocardial tissue in response to the release of the chemoattractants (ROS, cytokines, and the activated complement). The up-regulated cell-adhesion molecules P-selectin, CD18 and CD11, and intracellular adhesion molecule 1 (ICAM-1) then facilitate the migration of neutrophils into the myocardial tissue, where they mediate cardiomyocyte death by causing vascular plugging, releasing degradative enzymes, and generating ROS.



acute myocardial infarction can cause the loss of innate cardioprotective adaptations such as ischemic preconditioning and postconditioning within different regions of the ischemic myocardium. This heterogeneity could contribute to the inconclusive results of clinical studies. Furthermore, of the interventions examined so far, many may have been of questionable benefit in preclinical studies or were given to patients at a dose and schedule that had not been validated in studies in animals.

As a way forward, and in agreement with the working group of the National Heart, Lung, and

Table 1. Previous Attempts to Reduce Lethal Reperfusion Injury in Patients with Acute Myocardial Infarction.*

Cardioprotective Strategy and Trial	No. of Patients	Period of Ischemia	Timing of Intervention	Details of Study	Results
		hr			
Antioxidants					
Flaherty et al.[22]	120	≤4 (92% of patients)	Before PCI	Intravenous bolus of superoxide dismutase (10 mg/kg of body weight) followed by a 60-min infusion of 0.2 mg/kg/min	No difference in recovery of LVEF 4–6 wk after PCI
Downey[23] (EMIP-FR)	19,725	≤6 (83% of patients)	≤15 min after thrombolysis	Intravenous infusion of trimetazidine	No difference in 35-day mortality
Guan et al.[25]	38	4.5	Before PCI	Oral allopurinol	Improved LVEF and less oxidative stress
Tsujita et al.[26]	101	3.5	Before PCI	Intravenous edaravone	Reduced infarct size, less oxidative stress and reperfusion arrhythmias, improved short-term clinical outcomes
Reduction of intracellular Ca^{2+} overload and Na^+–H^+ exchange inhibitors					
Boden et al.[27]	874	≤6 (85% of patients)	After thrombolysis	Oral diltiazem 36–96 hr after onset of infarct symptoms	No effect on death, nonfatal myocardial infarction, or recurrent ischemia but reduction in nonfatal cardiac events, including myocardial revascularization
Théroux et al.[28]	3,439	3	Before PCI	Na^+–H^+ exchange inhibitor cariporide	No effect on infarct size or clinical outcomes
Zeymer et al.[29]	1,389	3	During thrombolysis, before PCI	Na^+–H^+ exchange inhibitor eniporide	No effect on infarct size or clinical outcomes
Bär et al.[30]	387	3.5	Before PCI	Intravenous MCC-135	No effect on infarct size of LVEF measured on SPECT at either 7 days or 30 days
Jang et al.[31]			Before PCI	Intravenous MCC-135	Still recruiting
Antiinflammatory agent					
Baran et al.[32]	394	3.5	Before or during thrombolysis	Anti-CD18 antibody	No effect on infarct size, coronary blood flow, or ST-segment resolution
Faxon et al.[33]	420	3.8	Before PCI	Anti-CD11 and anti-CD18 antibody	No effect on infarct size measured on SPECT at 5–9 days and no effect on TIMI flow or clinical events
Tanguay et al.[34]			During thrombolysis	P-selectin antagonist	No effect on infarct size measured on SPECT or LVEF at 30 days or on ST-segment resolution or clinical outcomes
Mertens et al.[35]	88	≤6	During thrombolysis	P-selectin antagonist	Prematurely discontinued but no effect on myocardial blood flow, LVEF, or ST-segment resolution
Mahaffey et al.[36]	943	2.7	During thrombolysis	Pexelizumab (Alexion) (an anti-C5 complement antibody)	No difference in CK-MB–measured infarct size or 90-day composite end point of death, cardiac failure, or stroke
Granger et al.[37]	960	3.2	Before PCI	Pexelizumab	No difference in CK-MB–measured infarct size or 90-day composite end point of death, cardiac failure, or stroke
Armstrong et al.[38]	5,745	3.2	Before PCI	Pexelizumab	No difference in 30-day mortality or 90-day composite end point of death or cardiac failure

Adenosine					
Ross et al.[39] and Kloner et al.[40]	2,118	3.3	15 min after PCI	Intravenous adenosine	An 11% reduction in infarct size but no effect on clinical outcomes; however, subgroup analysis revealed improved clinical outcomes in patients receiving adenosine ≤3 hr after onset of chest pain
Metabolic modulation (glucose, insulin, and potassium)					
Mehta et al.[41]	20,201	3.9	Both before and after reperfusion	Intravenous glucose, insulin, and potassium given during thrombolysis or PCI	No effect on mortality, cardiac arrest, cardiogenic shock, or reinfarction at 30 days
Beshansky and Selker[42]	15,450		Before reperfusion	Intravenous glucose, insulin, and potassium given in ambulance	Currently recruiting, expected completion in August 2009
Magnesium					
Woods et al.[43]	2,316		During thrombolysis	Intravenous magnesium	Reduced mortality and cardiac failure with magnesium treatment
ISIS-4[44]	4,319		During thrombolysis	Intravenous magnesium	No effect on mortality
Santoro et al.[45]	150	3.3	Before PCI	Intravenous magnesium	No effect on infarct zone wall-motion score or LVEF
Antman et al.[46]	6,213	3.8	Before PCI or before or during thrombolysis	Intravenous magnesium	No effect on 30-day mortality
Nicorandil					
Ono et al.[47]	58	5.6	Before PCI	Intravenous nicorandil	Improved microcirculation and clinical outcomes in short term
Ishii et al.[48]	360	4.8	Before PCI	Intravenous nicorandil	Improved myocardial reperfusion and fewer deaths and less cardiac failure after 2.4-yr follow-up
Kitakaze et al.[49]	545		Before PCI	Intravenous nicorandil	No effect on mortality, infarct size, LVEF, or myocardial reperfusion
Therapeutic hypothermia					
Dixon et al.[50]	42	3.5	Before PCI	Endovascular cooling to 34.7°C for first 3 hr of reperfusion	Nonsignificant reduction in adverse cardiac events and infarct size
O'Neill[51]	400	<6	Before PCI	Endovascular cooling to 34.7°C for first 3 hr of reperfusion	No difference in adverse cardiac events and infarct size, although patients with anterior acute myocardial infarction who are sufficiently cooled before PCI may benefit
Ly et al.[52]	12	3	During PCI	Noninvasive surface cooling to 34.5°C	Safe and feasible

* PCI denotes percutaneous coronary intervention, LVEF left ventricular ejection fraction, SPECT single-photon-emission computed tomography, TIMI Thrombolysis in Myocardial Infarction, and CK-MB creatine kinase MB isoform.

Blood Institute (NHLBI) charged with investigating the recurring issue of the lack of clinical translation of preclinical findings,[3] only agents that have been conclusively shown to be cardioprotective in experiments in animals by multiple investigators should be investigated in the clinical setting.[78]

NEW STRATEGIES FOR PREVENTING LETHAL REPERFUSION INJURY

Targeting individual mediators of lethal reperfusion injury has produced discrepant findings in studies in animals, and clinical studies that use this strategy have not been successful. A more effective approach may be to target more than one mediator at a time (Fig. 3). The recently described interventional strategy of ischemic postconditioning, which by its nature targets several mediators of lethal reperfusion injury, has been shown to reduce myocardial injury in patients with acute myocardial infarction who are undergoing PCI.[5] These findings, along with a number of preclinical studies,[8,19,80] have not only re-ignited interest in the myocardial reperfusion phase as a target for cardioprotection, but they also have provided confirmatory evidence of the existence of lethal reperfusion injury in humans (Table 3).[85,86] Furthermore, the RISK pathway[8] and the mitochondrial PTP[9] are emerging as new targets for preventing lethal reperfusion injury (Fig. 3).

ISCHEMIC POSTCONDITIONING

In 2003, Zhao et al.[4] showed that after a 45-minute episode of sustained myocardial ischemia, the interruption of myocardial reperfusion with three 30-second cycles of myocardial ischemia and reperfusion could reduce the myocardial infarct size in dogs from 47% to 11%.[4] They named this form of cardioprotection ischemic postconditioning, a term that highlights the myocardial reperfusion phase as a target of cardioprotection, although in reality it constitutes another variant of modified myocardial reperfusion.[87]

The mechanism of ischemic postconditioning–induced protection is not fully understood, but the procedure has been shown to target the important mediators of lethal reperfusion injury by reducing oxidative stress, decreasing intracellular Ca^{2+} overload, improving endothelial function, attenuating apoptotic cardiomyocyte death, reducing neutrophil accumulation,[88] and delaying the restoration of neutral pH.[89] Furthermore, ischemic postconditioning activates the RISK pathway[8] and inhibits the opening of the mitochondrial PTP[9] — both important ways of protecting against lethal reperfusion injury. Ischemic preconditioning, a phenomenon in which the size of a myocardial infarct is reduced by initiating episodes of transient myocardial ischemia and reperfusion before the sustained ischemic episode, appears to inhibit lethal reperfusion injury through the same mechanisms as ischemic postconditioning.[90]

Several small studies have used ischemic postconditioning in patients with acute myocardial infarction who are undergoing PCI with a protocol that has reduced myocardial infarct size by 36% and improved myocardial reperfusion (Table 3).[5-7] The effect of ischemic postconditioning on clinical outcomes remains to be determined, however.[85,86]

A closely related strategy for preventing lethal reperfusion injury is to initiate, at the time of myocardial reperfusion, transient episodes of ischemia and reperfusion in a tissue or an organ remote from the heart. This phenomenon is termed remote ischemic postconditioning.[91] Preliminary clinical trials are under way to determine whether transient upper-limb ischemia can reduce myocardial injury in patients with acute myocardial infarction who are undergoing PCI. Given the invasive nature of the ischemic postconditioning protocol and its restriction to patients with acute myocardial infarction who are undergoing PCI, the use of pharmacologic agents that recruit the signal-transduction pathway activated by ischemic postconditioning may be a more effective strategy.

TARGETING THE RISK PATHWAY

The RISK pathway[92] refers to a group of protein kinases that, when specifically activated during myocardial reperfusion, confer cardioprotection by preventing lethal reperfusion injury[8,19]; in a sense, the RISK pathway mediates a form of programmed cell survival. There is extensive preclinical evidence that activation of the RISK pathway by pharmacologic agents[8,80] or by mechanical interventions such as ischemic preconditioning or postconditioning[90] reduces myocardial infarct size by up to 50%. The cardioprotection has been

Table 2. Major Differences between Animal Models and Clinical Studies of Patients with Acute Myocardial Infarction.*

Characteristic	Animal Models	Clinical Studies	Comments
Subjects	Most studies use a homogeneous group of healthy, relatively young animals free of coexisting illnesses.	Studies use heterogeneous, middle-aged patient populations with coexisting illnesses such as diabetes, hypertension, and dyslipidemia, all of which may influence cardioprotection.	Encourage the use of older animals with coexisting illnesses such as diabetes, hyperlipidemia, atherosclerosis, and hypertension to ensure cardioprotection is possible in these settings.
Medication	In most studies, the animals are receiving no other medication.	Patients may be taking different medications that may influence cardioprotection.	Ensure that patients are not receiving medication that could interfere with cardioprotection.
Period of acute myocardial ischemia	Beneficial effects with cardioprotection are observed after relatively short periods of ischemia, ranging from 30 to 60 min. The animals are subjected to the same duration and severity of ischemia.	Most patients present with longer periods of ischemia, ranging from 3 to 12 hr. Both the duration and severity of ischemia vary between patients within the same study; these factors may influence cardioprotection.	Consider selecting certain patient groups such as those presenting early (<3 hr) after symptom onset or those with an anterior myocardial infarction. Alternatively, use more clinically relevant animal models such as a human-sized pig subjected to a long period of ischemia.
Reperfusion time	Most studies assess cardioprotection after relatively short periods of reperfusion, ranging from 120 min to 3 days.	Much longer periods of reperfusion occur in patients, permitting time for the effects of infarct healing and left ventricular remodeling to take place.	Encourage the use of a longer period of reperfusion in studies in animals.
Infarction model	In most studies, acute coronary occlusion is mechanically induced in healthy coronary arteries.	An acute myocardial infarction is an acute inflammatory condition. In most patients with this condition, acute coronary occlusion is due to thrombus formation at a site of a ruptured coronary atherosclerotic plaque.	Consider using more clinically relevant animal models such as animals with atherosclerotic hearts.
Intervention	Many of the interventions administered at the time of myocardial reperfusion have not shown conclusive cardioprotection.	If interventions have not shown conclusive cardioprotection in experimental studies, they are also unlikely to be cardioprotective in the clinical setting.	In the clinical setting, use only interventions rigorously shown in experimental studies to be conclusively cardioprotective. A potential approach would be the use of the intervention in a multicenter, randomized, controlled study in the animal model.†
Timing of intervention	The timing of the intervention relative to the period of ischemia and the onset of myocardial reperfusion is similar in all animals.	The timing of the intervention relative to the period of ischemia and the onset of myocardial reperfusion varies between patients. The timing of the intervention should be guided by the studies in animals.	Consider selecting certain patient groups, such as those presenting after a specific time. In clinical studies, ensure that the intervention is administered before myocardial reperfusion.
Infarct size	Varies from 30% to 60% of the total volume of myocardium at risk, providing a greater scope for cardioprotection.	Infarct sizes of 13% to 16% expressed as a percentage of left ventricular volume (using SPECT) appear to be the normal range, which may limit the scope for cardioprotection.	Encourage the use of more accurate measurement of infarct size using delayed-enhancement cardiac magnetic resonance imaging, which can express infarct size as a percentage of the ischemic risk area.
End points for cardioprotection	Most studies use recovery of left ventricular function or myocardial infarct size as the measured end points.	The clinically relevant end points are outcomes such as short-term and long-term effects on illness and death.	Consider more robust end points in studies in animals, such as long-term effects on left ventricular function and death.

* SPECT denotes single-photon-emission computed tomography.
† Data are from Bolli et al.[3] and Baxter et al.[78]

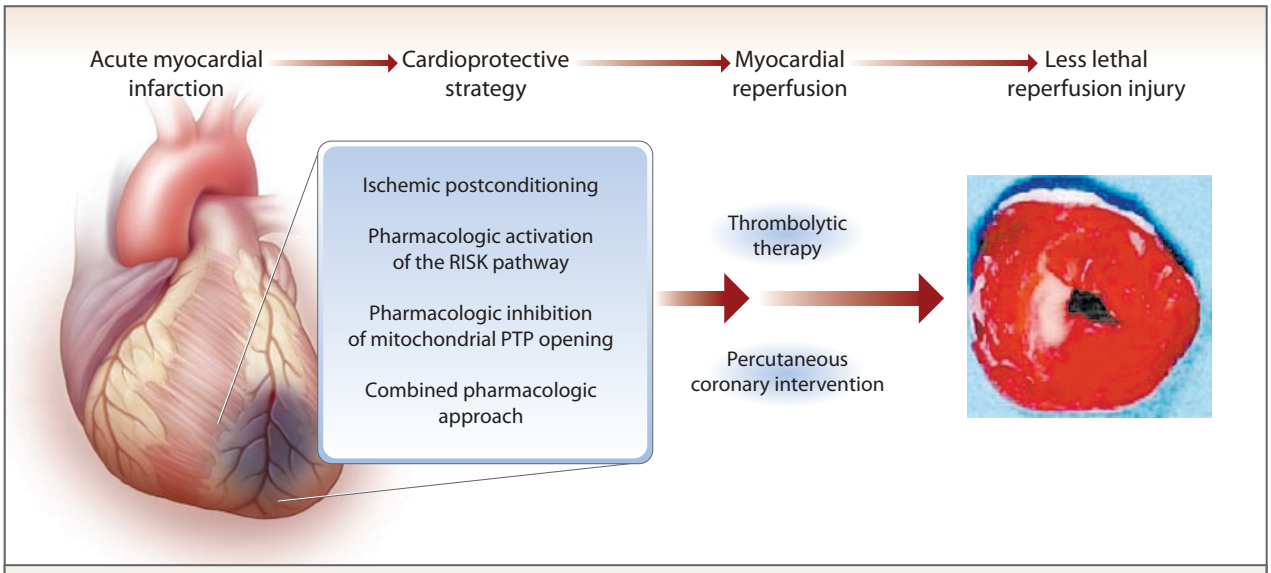


Figure 3. New Cardioprotective Strategies for Reducing Lethal Reperfusion Injury.

For patients with an acute myocardial infarction, ischemic postconditioning or pharmacologic agents that activate the reperfusion injury salvage kinase (RISK) pathway or inhibit the opening of the mitochondrial permeability transition pore (PTP), or a multitargeted pharmacologic approach before or during the immediate onset of myocardial reperfusion, may attenuate lethal reperfusion injury and reduce the final myocardial infarct size.

attributed to inhibition of mitochondrial PTP opening,[93] improved uptake of Ca^{2+} in the sarcoplasmic reticulum,[94] and the recruitment of antiapoptotic pathways.[19]

Pharmacologic agents such as glucagon-like peptide 1,[95] erythropoietin,[96] atorvastatin,[97] and atrial natriuretic peptide,[98] all of which reduce infarct size by activating the RISK pathway, are being examined in proof-of-concept studies in patients with acute myocardial infarction who are undergoing PCI (Table 3).[49,82,83] A recent clinical study has shown that high-dose atorvastatin given to patients with a non–ST-elevation myocardial infarction at the time of urgent PCI reduces myocardial injury during PCI.[84]

Protein kinase C is another potential prosurvival protein kinase implicated in cardioprotection; in animal infarct models, activation of the cardioprotective protein kinase C epsilon isoform or inhibition of the pro-injurious protein kinase C delta isoform reduces myocardial infarct size when administered during myocardial reperfusion.[99] A preliminary clinical study has reported reduced myocardial infarct size in patients undergoing primary PCI who were given intracoronary KAI-9803, an inhibitor of protein kinase delta, during myocardial reperfusion (Table 3).[81]

TARGETING THE MITOCHONDRIAL PTP

Proof-of-concept clinical studies are in progress to determine whether pharmacologic suppression of mitochondrial PTP opening by an intravenous bolus of cyclosporine, administered immediately before PCI, confers cardioprotection during an acute myocardial infarction (Table 3). Pharmacologic inhibition of mitochondrial PTP opening during myocardial reperfusion with the use of cyclosporine or sanglifehrin A reduces myocardial infarct size in studies in animals by up to 50%, suggesting that mitochondrial PTP opening may contribute to half of the final infarct size.[10,100] Furthermore, mice lacking cyclophilin D (a key component of the mitochondrial PTP) have been reported to sustain smaller myocardial infarcts than control animals.[101] Pharmacologic inhibition of mitochondrial PTP in human atrial trabeculae subjected to simulated ischemia–reperfusion injury is also cardioprotective.[102] Studies are under way to investigate the mitochondrial PTP as a target for cardioprotection in the clinical setting. However, more

Table 3. New Cardioprotective Strategies for Reducing Lethal Reperfusion Injury in Patients with Acute Myocardial Infarction.*

Cardioprotective Strategy and Source	No. of Patients	Period of Ischemia	Timing of Intervention	Details of Study	Clinical End Points
		hr			
Ischemic postconditioning					
Staat et al.[5]	30	5.5	During PCI	Four 60-sec low-pressure inflations and deflations of coronary-angioplasty balloon immediately after stent deployment	Reduced infarct size by 36% and improved myocardial reperfusion
Laskey[6]	17	5.7	During PCI	One 90-sec inflation and deflation of coronary-angioplasty balloon immediately after stent deployment	Improved ST-segment resolution and coronary blood flow
Ma et al.[7]	94	7	During PCI	Three 30-sec low-pressure inflations and deflations of coronary-angioplasty balloon immediately after stent deployment	Reduced infarct size, improved wall-motion score index, increased myocardial reperfusion, and improved endothelial function
Atrial natriuretic peptide					
Kitakaze et al.[49]	569		Before PCI	Intravenous infusion	Reduced infarct size by 15%, improved LVEF by 5%, and improved myocardial reperfusion, but no effect on mortality; reduced composite end point of cardiac death and cardiac failure
Protein kinase C–delta inhibitor (KAI-9803)					
Roe[81]	150		Before PCI	Intracoronary bolus of KAI-9803	Reduced infarct size and improved ST-segment resolution
Glucagon-like peptide 1					
Nikolaidis et al.[82]	21	6.3	3 hr after PCI	Intravenous glucagon-like peptide 1 given to patients with poor LVEF	Improved LVEF from 29% to 39%
Darbepoetin alfa (a long-acting erythropoietin analogue)					
Lipsic et al.[83]	22	3.3	Before PCI	Intravenous bolus of darbepoetin alfa	Mobilized endothelial progenitor cells but no effect on left ventricular function
Remote ischemic postconditioning			Before PCI	Remote ischemic postconditioning using transient upper-limb ischemia	In progress
Atorvastatin[84]	171		Before PCI	High-dose atorvastatin administered 12 hr before PCI	Reduced myocardial injury during PCI
Mitochondrial PTP inhibition			Before PCI	Intravenous bolus of cyclosporine	In progress

* LVEF denotes left ventricular ejection fraction, PCI percutaneous coronary intervention, and PTP permeability transition pore.

specific and safer inhibitors of mitochondrial PTP opening need to be developed in order to take advantage of this strategy.

WAVEFRONT OF REPERFUSION INJURY

There is emerging experimental evidence that myocardial infarct size can increase with the duration of myocardial reperfusion, suggesting a potential wavefront of myocardial reperfusion injury mediated by apoptosis and the inflammation-induced death of cardiomyocytes.[103] These findings raise the possibility of reducing myocardial infarct size by intervening late in myocardial reperfusion with the use of antiapoptotic and antiinflammatory agents.[104]

This new strategy and the strategies described above for preventing lethal reperfusion injury are important because they protect cardiomyocytes by means of a mechanism that is effective in all experimental studies; this mechanism also underlies the cardioprotective phenomena of ischemic preconditioning and ischemic postconditioning. Large clinical trials will be required to ensure that these new cardioprotective strategies improve clinical outcomes in patients with acute myocardial infarction. Such new cardioprotective strategies may also benefit patients who sustain acute myocardial ischemia–reperfusion injury during coronary-artery bypass grafting or cardiac-transplant surgery and patients surviving a cardiac arrest.

CONCLUSIONS

For patients presenting with an acute myocardial infarction, early and successful myocardial reperfusion by means of thrombolytic therapy or primary PCI is the most effective interventional strategy for reducing infarct size and improving clinical outcomes. The process of myocardial reperfusion itself, however, can induce injury to the myocardium, thereby reducing the beneficial effects of myocardial reperfusion. The cardiomyocyte death associated with the irreversible, lethal form of myocardial reperfusion injury diminishes the infarct-reducing effects of myocardial reperfusion by independently inducing cardiomyocyte death. For this reason, lethal reperfusion injury would be expected to adversely affect clinical outcomes after an acute myocardial infarction, and it may contribute to the mortality despite early and successful reperfusion.

Until recently, the efficacy that has been shown for most cardioprotective agents in animal models has been difficult to confirm in clinical trials. There is, however, general agreement that ischemic preconditioning and postconditioning are cardioprotective not only in animal hearts but also in human hearts. The increasing understanding of the mechanism of the protection, particularly with regard to the RISK pathway and the inhibition of mitochondrial PTP opening, has led to the development of new pharmacologic interventions to invoke the mechanism at the time of reperfusion (Fig. 3). These pathways, which can target all of the known mediators of lethal reperfusion injury, have already been shown to reduce lethal reperfusion injury in small-scale trials of patients with acute myocardial infarction who are undergoing PCI.[5,7] These clinical results have regenerated interest in the reperfusion phase as a target for cardioprotection. Preliminary clinical data indicate that these new cardioprotective strategies confer a benefit to patients with acute myocardial infarction over and above that provided by myocardial reperfusion alone, but they remain to be confirmed in large-scale clinical studies.

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