C linician Update

Fundamentals of Reperfusion Injury for the Clinical Cardiologist

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Case presentation: S.B. is a 48-year-old man who suffered an acute anterior myocardial infarction and received fibrinolytic therapy. The patient died 12 hours after reperfusion. K.R. is a 68-year-old diabetic woman who underwent conventional coronary artery bypass graft surgery and developed low output syndrome after reperfusion postoperatively. V.A. is a 55-year-old man who developed a stunned myocardium after percutaneous coronary reperfusion. What is reperfusion injury, and why is it important?

Reperfusion of coronary flow is necessary to resuscitate the ischemic or hypoxic myocardium. Timely reperfusion facilitates cardiomyocyte salvage and decreases cardiac morbidity and mortality. Reperfusion of an ischemic area may result, however, in paradoxical cardiomyocyte dysfunction, a phenomenon termed “reperfusion injury.” Modalities for reperfusion include not only thrombolysis, but also percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), and cardiac transplantation. Reperfusion injury has been observed in each of these situations. We discuss here the fundamental principles of reperfusion injury from a mechanistic and pharmacological standpoint.

What is reperfusion injury, and why is it important?
The myocardium can tolerate brief periods (up to 15 minutes) of severe and even total myocardial ischemia without resultant cardiomyocyte death. Although the cardiomyocytes suffer ischemic injury, the damage is reversible with prompt arterial reperfusion. Indeed, such transient periods of ischemia are encountered in the clinical situations of angina, coronary vasospasm, and balloon angioplasty, and are not associated with concomitant myocyte cell death.1,2 With increasing duration and severity of ischemia, however, greater cardiomyocyte damage can develop, with a predisposition to a spectrum of reperfusion-associated pathologies, collectively called reperfusion injury.3 Reperfusion injury results in myocyte damage through myocardial stunning, microvascular and endothelial injury, and irreversible cell damage or necrosis (termed lethal reperfusion injury; Figure 1).3,4

Myocardial stunning is the best-established manifestation of reperfusion injury.5,6 It is defined as “prolonged postsischemic dysfunction of viable tissue salvaged by reperfusion,”1,2,7 and was initially described by Heyndrickx et al in 1975.8 In this scenario, reperfusion of either a globally or regionally ischemic myocardial tissue results in a period of prolonged, yet reversible, contractile dysfunction. The myocardium is essentially “stunned” and requires a prolonged period of time before complete functional recovery. The clinical correlate of a stunned myocardium can be found after reperfusion of a globally ischemic myocardium (cardiac arrest during cardiac surgery), or in the setting of regional ischemia and reperfusion (PCI, thrombolysis, unstable angina, and stress- or exercise-induced angina).1,2,5

Microvascular dysfunction is another manifestation of reperfusion injury.9–11 Reperfusion causes marked endothelial cell dysfunction, which results in vasoconstriction, platelet and leukocyte activation, increased oxidant production, and increased fluid and protein extravasation (discussed below). Although rare, severe microvascular dysfunction may limit adequate perfusion after reperfusion, a phenomenon termed “no-reflow.”

Reperfusion of a severely ischemic myocardium may also result in myocyte death and necrosis (lethal reperfusion injury). This usually occurs in cardiomyocytes that have been severely injured by ischemia but also may develop in revers-
Microvascular injury, and, rarely, lethal myocyte necrosis. Reperfusion injury is manifested as stunned myocardium, reversible and activates neutrophils, platelets, and complement. Reperfusion injury results from several complex and interdependent mechanisms that involve the production of reactive oxygen species, alterations in intracellular calcium handling, microvascular dysfunction, and altered myocardial metabolism.9 Several mechanisms and mediators of reperfusion injury have been described. The most frequently cited include oxygen free radicals, intracellular calcium overload, endothelial and microvascular dysfunction, and altered myocardial metabolism.9–11,16–18

**Oxygen Free Radicals**

The production of excessive quantities of reactive oxygen species is an important mechanism of reperfusion injury. Molecular oxygen, when reintroduced into a previously ischemic myocardium, undergoes a sequential reduction leading to the formation of oxygen free radicals. A landmark study by Bolli and colleagues19 showed that potent oxidant radicals, such as superoxide anion, hydroxyl radical, and peroxynitrite, are produced within the first few minutes of reflow and play a crucial role in the development of reperfusion injury. Oxygen free radicals also can be generated from sources other than reduction of molecular oxygen. These sources include enzymes, such as xanthine oxidase, cytochrome oxidase, and cyclooxygenase, and the oxidation of catecholamines.

Reperfusion is also a potent stimulus for neutrophil activation and accumulation,17 which in turn serve as potent stimuli for reactive oxygen species production. Oxygen-derived free radicals produce damage by reacting with polyunsaturated fatty acids, resulting in the formation of lipid peroxides and hydroperoxides that damage the sarcolemma and impair the function of membrane-bound enzyme systems. Free radicals stimulate the endothelial release of platelet activating factor, which attracts more neutrophils and amplifies the production of oxidant radicals and the degree of reperfusion injury. Reactive oxygen species also quench nitric oxide, exaggerating endothelial injury and microvascular dysfunction. In addition to an increased production, there is also a relative deficiency in endogenous oxidant scavenging enzymes, which further exaggerates free radical–mediated cardiac dysfunction.

**Endothelial Dysfunction and Microvascular Injury**

Reperfusion results in marked endothelial cell dysfunction.9–18 Endothelium-dependent vasodilatation is impaired, whereas the responses to endothelium-dependent vasoconstrictors are exaggerated. Increased production of potent vasoconstrictors, such as endothelin-1 and oxygen free radicals, increases coronary vasoconstriction and reduces blood flow. Furthermore, endothelial dysfunction facilitates the expression of a prothrombotic phenotype characterized by platelet and neutrophil activation, important mediators of reperfusion injury. Once neutrophils make contact with the dysfunctional endothelium, they are activated, and in a series of well-defined steps (rolling, firm adherence, and transmigration) they migrate into areas of tissue injury through endothelial cell junctions9,17,18 (Figure 2).

**Alterations in Calcium Handling**

Changes in intracellular calcium homeostasis play an important role in the development of reperfusion injury.16 Ischemia and reperfusion are associated with an increase in intracellular calcium; this effect may be related to increased sarcolemmal calcium entry through L-type calcium channels or may be secondary to alterations in sarcoplasmic reticulum calcium cycling. In addition to intracellular calcium overload, alterations in myofilament sensitivity to calcium have been implicated in reperfusion injury. Activation of calcium-dependent proteases (calpain I) with resultant myofibril

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**What are the mediators of reperfusion injury?**

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**Figure 1.** Mechanisms and mediators of reperfusion injury. Reperfusion strategies are required to resuscitate the ischemic myocardium. In the clinical realm, these include reperfusion for acute myocardial infarction (AMI) and angioplasty and after CABG surgery and exercise- or stress-induced ischemia. Reperfusion injury results from several complex and interdependent mechanisms that involve the production of reactive oxygen species, alterations in intracellular calcium handling, microvascular and endothelial cell dysfunction, altered myocardial metabolism, and activation of neutrophils, platelets, and complement. Reperfusion injury is manifested as stunned myocardium, reversible microvascular injury, and, rarely, lethal myocyte necrosis.
proteolysis has been suggested to underscore reperfusion injury, as has proteolysis of troponin I.\textsuperscript{20,21}

Altered Myocardial Metabolism
Reperfusion of an ischemic myocardium results in altered myocardial metabolism, which in turn may contribute to delayed functional recovery. For example, cardioplegic arrest and aortic cross-clamping during cardiac surgery induce anaerobic myocardial metabolism with a net production of lactate.\textsuperscript{22} Importantly, lactate release persists during reperfusion, suggesting a delayed recovery of normal aerobic metabolism.\textsuperscript{22} Persistent lactate production after ischemia and remains depressed for up to 30 minutes after reperfusion.\textsuperscript{23,24} In addition, the recovery of postischemic myocardial function is dependent on the recovery of PDH activity. These results suggest that persistent anaerobic metabolism may be an important contributor to inadequate postoperative ventricular function; improving the recovery of aerobic myocardial metabolism during reperfusion may serve as an important target for reperfusion injury. Interventions that improve the transition from anaerobic to aerobic myocardial metabolism (insulin, adenosine) facilitate the rapid recovery of aerobic metabolism and left ventricular function after post–cardiac surgery reperfusion.\textsuperscript{25}

Endogenous Protective Mechanisms
The myocardium is the source of endogenous protective mechanisms that are stimulated during reperfusion. These endogenous cardioprotective strategies serve to counter the deleterious mechanisms described above. In many instances, however, they are insufficient to prevent reperfusion injury. The most important endogenous protective mechanisms are adenosine production, opening of ATP-sensitive potassium channels (K\textsubscript{ATP}), and release of NO.\textsuperscript{26} Although the details of endogenous cardioprotection are beyond the scope of this update, it is important to note that these mechanisms have been exploited from pharmacological and therapeutic standpoints (discussed below).

What is the influence of cardiovascular risk factors on reperfusion injury?
Cardiovascular risk factors, including hypercholesterolemia, diabetes, and hypertension, have been reported to increase reperfusion injury. Although the exact mechanisms remain unclear, one recurring theme is that increased oxidative stress and endothelial cell dysfunction may underlie risk factor–mediated exacerbation of reperfusion injury.\textsuperscript{9}
Which pharmacological strategies attenuate reperfusion injury?

Over the past 2 decades, ≈1000 interventions have been studied as potential cardioprotective agents in ischemia and reperfusion injury. We limit our discussion to some of the more contemporary approaches.

Inotropic Stimulation of the Reperfused Stunned Heart

It is important to note that the stunned reperfused myocardium is sensitive to inotropic stimulation.1,2,26 As discussed above, reperfusion injury results in significant desensitization of the myofibrils to calcium; this phenomenon likely is overcome during inotropic stimulation, augmenting contractility. Although inotropic stimulation is not the ideal strategy to counter reperfusion injury, it is effective and is not associated with a worsening of ultimate functional recovery or tissue necrosis. Indeed, transient inotropic support routinely is used for a stunned reperfused myocardium in a variety of settings.

Antioxidants

The central role of oxygen free radicals in the development of reperfusion injury led to a widespread interest in the use of antioxidant therapy to attenuate reperfusion injury. Antioxidants have been tested in several experimental and clinical models with mixed success.26 Despite positive observations in classic models of experimental ischemia and reperfusion, clinical experience with antioxidants has been disappointing. Indeed, therapy with human recombinant superoxide dismutase, designed to attenuate angioplasty-induced reperfusion injury, demonstrated no beneficial effects.27 Although this may be related to cell impermeability, this study cast a shadow on the development of antioxidant strategies for reperfusion injury. It is important to note that the major antioxidant for the cardiomyocyte is glutathione peroxidase, not superoxide dismutase. Vitamin E (alpha tocopherol) is the major lipid-soluble antioxidant and requires prolonged and very high levels of oral treatment to achieve cardiac concentrations that are protective from reperfusion injury.

Sodium-Hydrogen Antiport Inhibition

Inhibition of sodium–hydrogen exchange (Na+–H+) has received much recent attention as a potential cardioprotective factor.28 Ischemia and reperfusion result in marked intracellular acidosis; this in turn activates the sarcolemmal Na+–H+ antiport, which facilitates proton extrusion (in exchange for Na+).3,28 The intracellular hypernatremia that develops results in activation of the sodium–calcium (Na+–Ca2+) exchanger, with resultant increases in [Ca2+]. Indeed, inhibitors of Na+–H+ exchange have been demonstrated to exhibit marked cardioprotection in experimental models of ischemia and reperfusion. More recently, the Na+–H+ inhibitor cariporide was investigated in a large clinical trial involving 11 500 patients (Guard During Ischemia Against Necrosis [GUARDIAN] trial).29 The trial was designed to investigate the potential cardioprotective effects of cariporide in a diverse group of patients receiving reperfusion treatment (unstable angina, non–ST segment elevation myocardial infarction, high-risk PCI, or surgical revascularization). Although the primary end points of death and myocardial infarction were similar between groups, patients subjected to surgical revascularization exhibited a trend (P=0.06) toward improved left ventricular function in the cariporide group. These data suggest that Na+–H+ inhibition may be beneficial in attenuating myocardial stunning after CAGB surgery.

Stimulating Endogenous Cardioprotectants

As discussed above, adenosine is an endogenous cardioprotectant released during ischemia that exerts its beneficial effects via opening of mitochondrial KATP channels through interaction with the A1 and A3 receptors on cardiomyocytes.30 Despite marked beneficial effects of adenosine therapy in experimental models of ischemia and reperfusion, the clinical experience has been limited. Preliminary results of a phase-II clinical trial suggested that adenosine treatment may reduce the requirement for inotropic and/or mechanical support in patients undergoing cardiac surgery.31

Accumulating evidence suggests that endogenous myocardial protection may be mediated via opening of mitochondrial KATP channels. Pharmacological agents that open KATP channels are being evaluated as potential cardioprotective interventions.3

Decreasing reperfusion injury via modulation of nitric oxide bioavailability is an active area of research.32 Nitric oxide may serve to diminish reperfusion injury through improving endothelial function, decreasing platelet and neutrophil activation, and augmenting coronary flow. NO also may exert direct beneficial effects on cardiomyocyte survival (independent of endothelial cells) and may achieve this through the opening of KATP channels.33 It is important to point out that these cardioprotective effects may depend on the magnitude of NO production; excessive NO production may exert marked deleterious effects on functional recovery. Careful dose-ranging studies will be required before developing NO donors for patients receiving reperfusion treatment.

Metabolic Stimulation With Insulin

In an attempt to improve the transition from anaerobic to aerobic myocardial metabolism, the effects of insulin on ischemia and reperfusion injury have been studied. Insulin caused a marked stimulation of PDH activity and prevented the inhibition of PDH activity after reperfusion.24 Furthermore, insulin treatment reduced extracellular lactate release after reperfusion and increased intracellular high-energy phosphate levels. In a randomized, controlled trial comparing insulin cardioplegia versus placebo, insulin produced a more rapid recovery of aerobic metabolism and left ventricular function after reperfusion (cross-clamp release).25

What does the future hold?

The past 2 decades have witnessed several pharmacological interventions designed to limit reperfusion injury. Unfortunately, the success of some agents has been limited to experimental models of ischemia and reperfusion. The lack of a consistent clinical benefit may be related to a variety of factors, including poor clinical trial design, inadequate pharmacokinetic/pharmacodynamic studies, and the complexity...
of the human in vivo model (compared with classic experimental models of reperfusion injury). It is important to distinguish therapeutic strategies for ischemia versus reperfusion, and it is possible that a combination of agents is required to elicit maximum clinical benefit. The GUARD during Ischemia Against Necrosis (GUARDIAN) trial with cariporide provides further insight into this concept. Preclinical evaluation of cariporide indicated a consistent benefit when used as a preischemic therapy (versus a reperfusion strategy). Hence, it is not surprising that in the GUARDIAN trial, the only cohort that exhibited benefit was the CABG cohort, in which cariporide was instituted before the onset of ischemia.3

In the future, we will witness the development and testing of additional cardioprotective strategies. Some of the areas of intense investigation include the use of endothelin receptor antagonists, tetrahydrobiopterin, and statins. Clinical trials employing a combination of preischemic and prereperfusion strategies are currently in progress to develop the optimal pharmacological approach to limit reperfusion injury.

References
