Pathophysiology of ischemia-reperfusion injury: experimental data

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Introduction

Early reperfusion has clearly been shown to be the most effective means to prevent cell death after coronary artery occlusion. It is now widely accepted that the prompt reopening of the occluded vessel, either by mechanical (coronary angioplasty or bypass surgery) or pharmacological means (thrombolytic drugs), should be performed as soon as possible in patients with acute myocardial infarction[1-3]. However reperfusion of the ischemic myocardium may not always be completely beneficial, and there is now evidence that it may initiate a cascade of events that partially counteract the beneficial effects of blood flow restoration. This phenomenon has been termed "reperfusion injury"[1,4].

Reperfusion injury may affect various aspects of myocardial and endothelial function, with different and complex pathophysiological consequences[5,6]. Several key issues in the understanding of reperfusion injury are now clarified. First, ischemic changes are the necessary pre-requisites, but are not in themselves sufficient. Second, analyses after reperfusion do not distinguish cell death caused by ischemia from cell death caused by reperfusion. Third, while reperfusion-induced alterations have been well documented in experimental studies, a clear demonstration of their occurrence in the clinical practice has not been unequivocally obtained (Table I)[3]. Therefore, the only valid criterion to attribute cell damage to reperfusion injury is by demonstrating that modifications of reperfusion conditions prevent cell death or dysfunction.

This paper will review the mechanisms of reperfusion injury and highlight the ample opportunities that such knowledge may open for new therapies of acute myocardial damage initiated by ischemia, highlighting the importance of experimental models to design new intervention strategies.

Table I. Possible effects of postischemic reperfusion on the heart: main available evidence in experimental and clinical studies.

Effects of reperfusion	Experimental data	Clinical evidence
Oxygen radical generation	+++	?
Membrane lipid peroxidation	++	+–
Release of oxidized glutathione	+++	?
Neutrophil activation	+++	+
Contractile impairment	+++	+++
Electrical instability	+++	+++
Myocyte death (necrosis or apoptosis)	+++	?
Microcirculatory alterations	+++	++
Endothelial dysfunction	+++	++
Changes in gene expression	++	?
Osmotic overload	+++	?

From Ambrosio and Tritto[3], modified.

Mechanisms of reperfusion injury

The term "ischemia-reperfusion injury" encompasses a list of events including: reperfusion arrhythmias, microvascular damage, reversible myocardial mechanical dysfunction, and cell death (due to apoptosis or necrosis). These events may occur together or separately[6-10]. Oxidative stress, intracellular calcium overload, neutrophil activation, and excessive intracellular osmotic load have all been proposed to explain the pathogenesis and the functional consequences of the inflammatory injury that is well documented in the ischemic-reperfused myocardium (Fig. 1).

Increased oxidative stress. An increase in the formation of reactive oxygen species during ischemia-reperfusion and the adverse effects of oxyradicals on myocardium have been well established by both direct and indirect measurements[3,11]. Although several experimental studies have demonstrated the cardioprotective effects of antioxidants[12-14], larger clinical studies have so far failed to confirm such earlier results[15,16]. It has now become evident that some of the endogenous antioxidants, such as glutathione peroxidase, superoxide dismutase, and catalase, act as a primary defense mechanism, whereas others, including vitamin E, may play a secondary role in attenuating the ischemia-reperfusion injury[11]. The importance of various endogenous antioxidants in reperfusion injury is evident from the decrease in their activities occurring at the time of myocardial damage, and from the inhibition of detrimental cardiac changes during ischemia-reperfusion injury upon their exogenous administration[3,11]. The effects of an antioxidant thiol-containing compound, N-acetylcysteine, and of ischemic preconditioning were shown to be similar in preventing changes in hearts subjected to ischemia-reperfusion[11].

Inflammatory changes. The inflammatory process characterizing early and late periods of reperfusion is an important aspect of changes leading to tissue damage. Neutrophils feature prominently in the inflammatory component of postischemic injury. This occurs because ischemia-reperfusion prompts a release of oxygen free radicals, cytokines and other pro-inflammatory mediators that activate both the neutrophils and the coronary vascular endothelium[10,17]. Activation of these cells promotes the expression of adhesion molecules on both neutrophils and the endothelium, which recruit neutrophils on the endothelial surface and initiate a specific cascade of cell-cell interactions. This leads first to the adhesion of neutrophils to the vascular endothelium and, subsequently, to their transendothelial migration and direct interactions with interstitial matrix and myocytes[18-22]. This specific series of events is a prerequisite for the full expression of reperfusion injury, including endothelial dysfunction, microvascular collapse, impairment of blood flow (the "no-reflow" phenomenon), myocardial infarction and apoptosis[3]. Pharmacological therapies can target the various components in this critical series of events. Effective targets for pharmacologic agents include: a) inhibiting the release or accumulation of pro-inflammatory mediators; b) altering neutrophil or endothelial cell activation and c) attenuating adhesion molecule expression on the endothelium, neutrophils and myocytes[17]. Monoclonal antibodies directed towards adhesion molecules or their ligands (P-selectin, L-selectin, CD11b-CD18), and towards complement fragments or their receptors attenuate neutrophil-mediated injury (vascular injury, infarction). Clinical application of these new agents has however limitations in the immunogenicity of the peptide reagents first used[17,23-26]. Humanized antibodies and non-peptide agents, such as oligosaccharide analogs to sialyl-Lewis^X (one of the main selectin ligands) may prove effective in this regard[27]. Both nitric oxide (NO) and adenosine, two fundamental regulators of coronary flow and endothelial function, exhibit a wide range of effects against neutrophil-mediated events. These agents can therefore be used to tackle several critical points in the ischemia-reperfusion response, and offer greater benefit than agents acting at one single point in the pathogenetic cascade[28-31].

The intense inflammatory response following reperfusion has been implicated as a factor not only in the extension of tissue injury[10], but also in tissue repair. Myocardial injury initiates a cascade of cellular and humoral responses that ultimately facilitate tissue repair. The early generation of complement-derived chemotactic factors does not depend upon reperfusion, but reperfusion of the infarcted myocardium accelerates other cellular and cytokine responses. This acceleration enables the early entry of neutrophils into the viable border zone surrounding the myocardial infarction, where myocyte ICAM-1 has been induced, thus providing the potential for post-reperfusion injury[32]. However, it also accelerates the influx of mononuclear leukocytes, with subsequent production of anti-inflam-

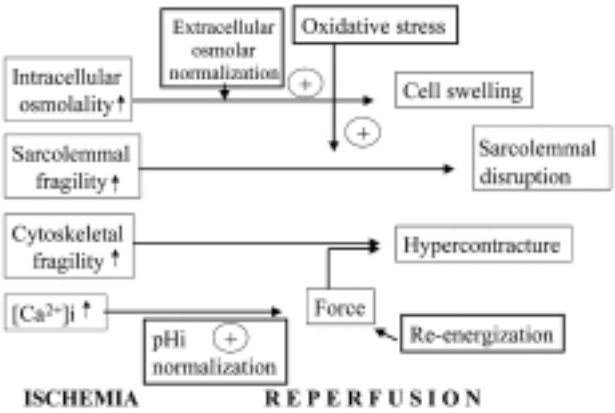

Figure 1. *Schematic representation of the main factors contributing to rapid lethal cardiomyocyte injury during reperfusion.*

matory cytokines and factors promoting the maturation of macrophages, the influx of mast cells, and the production of fibrogenic and angiogenic mediators. These processes probably explain the positive role of reperfusion in myocardial tissue repair[2]. Despite these observations, the modulation of an excessive inflammatory response remains critical in preventing overall reperfusion injury.

Metabolic changes. A metabolic protection of the myocardium subjected to ischemia-reperfusion also appears to be an important factor in limiting reperfusion damage[33]. Major metabolic changes occurring during the early hours of myocardial infarction include the increased secretion of catecholamines and the production of circulating free fatty acids. Under normal conditions, the myocardium depends on aerobic metabolism, with free fatty acids as the preferred energy source. During ischemia-reperfusion, free fatty acid levels are strongly increased, and exert toxic effects on the myocardium, manifested by increased membrane damage, arrhythmias, and decreased cardiac function[34]. These detrimental metabolic effects might be significantly reduced by the administration of glucose-insulin-potassium solutions at the time of reperfusion[35,36]. Rationales for glucose-insulin-potassium administration are the stimulation of myocardial K^+ reuptake by insulin's stimulation of Na^+, K^+ ATPase, the provision of glucose for glycolitic adenosine trisphosphate production, and the reduction of intracellular osmotic load and cell swelling during reperfusion.

Changes in endothelial function. Alterations of endothelial function are pivotal in the development of reperfusion damage and the no-reflow phenomenon. Here the enhanced release or increased bioavailability of NO appears to be central. Besides its well known vasodilatory effects, NO is known to reduce post-ischemic hyperpermeability[37,38], to decrease platelet adhesion and aggregation[39], and to reduce leukocyte adherence and emigration[40]. On the one hand, NO has been reported to exert beneficial effects by inhibiting inositol-1,4,5-trisphosphate, by reducing calcium overload, by inducing protein kinase C translocation, and by inhibiting neutrophil-associated injury[17,41]. On the other hand, NO also reacts with superoxide to form peroxynitrite, which is considered a strong cytotoxic agent. Because of this, the role of NO in ischemia-reperfusion damage and myocardial dysfunction remains controversial. Several investigations have reported that the administration on NO donors prevents reperfusion injury[42]. Accordingly, approaches to remove NO by pharmacological inhibition of NO-synthases (NOS), and transgenic endothelial and inducible NOS (eNOS and iNOS) knockout mouse models have shown an exacerbation of reperfusion injury[41]. Comparisons among experimental studies are difficult because of differences in agents and study design, and because of the multiple effects of NO during ischemia and reperfusion, partially depending on concentrations. It is conceivable that the biological role of eNOS and iNOS are different in ischemia-reperfusion conditions: an increase in the basal NO production in the picomolar range by an augmentation of eNOS activity would likely prevent deterioration and/or restore endothelial function in the coronary microcirculation. Conversely, the burst of NO production in the nanomolar range that occurs during reperfusion by an increase in iNOS activity would promote lipid peroxidation and cell damage[3,17].

Several pharmacological approaches have been proposed to restore a valid endothelial function in ischemic conditions, including angiotensin converting enzyme inhibitors, angiotensin II type-1 receptor antagonists, and calcium-antagonists[43]. Recently, 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitors (statins) have been suggested as a novel additional therapeutic approach in acute coronary syndromes[44,45]. In isolated working rat hearts, we have recently shown that the acute administration of the HMG-CoA reductase inhibitor simvastatin, given soon before myocardial ischemia, reduces myocardial dysfunction, vascular endothelial and myocardial damage. This was shown by the reduction in postischemic microvascular hyperpermeability, ultrastructural changes in endothelial cells and cardiomyocytes, and changes in microcirculatory resistances occurring after ischemia-reperfusion in the isolated working rat heart[46,47]. Concurrent with these beneficial effects, simvastatin partially prevented eNOS reduction induced by ischemia-reperfusion, likely occurring through post-transcriptional mechanisms. Also simvastatin, however, prevented most of ischemia-reperfusion-related iNOS induction. The action of simvastatin on the enzymes catalyzing NO production appears to be causally linked to the here-described reported beneficial effects, since totally abolished by the simultaneous treatment of the heart with the NOS inhibitor N^G-nitro-L-arginine methyl ester.

Conclusions

The understanding of the pathophysiology of ischemia-reperfusion damage is one of the fundamental challenges to design novel therapeutic approaches in acute coronary syndromes. Currently, however, it is still not easy to dissociate the myocardial effects on ischemia from those on reperfusion by agents experimentally proven to be beneficial. Contrary to *in vivo* situations where the effects of a treatment on mechanisms of ischemia cannot be easily dissociated from the effects on tissue sensitivity to ischemia or on reperfusion damage, several experimental models, like the isolated working heart, can be very useful to this purpose. These models allow to elucidate mechanisms of reper-

fusion damage, and the establishment of potential functional, morphologic or biochemical targets for interventions. The experimental approach thus opens ample opportunities for the study of new therapeutic strategies, or for the re-evaluation of the mode of action of already established therapies. The ultimate success of some of these interventions in humans will be itself the proof of the occurrence and relevance of ischemia-reperfusion damage.

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