Clinical Perspectives on Lethal Reperfusion Injury

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“Lethal reperfusion injury” refers to the death of a population of potentially viable cells in an ischemic territory subsequent to restoration of blood flow. This is distinct from ischemic cell death, and from nonlethal manifestations of reperfusion, such as reperfusion arrhythmias and myocardial stunning. In the experimental setting, it can be extremely difficult to determine whether the death of a given population of cardiac myocytes is due to ischemia or reperfusion. Experimental evidence for the existence of lethal reperfusion injury is based on an understanding of intervention in the mechanisms that might lead to the death of viable myocytes—the inflammatory response, free radical–induced injury, microvascular injury, and impaired reflow.

Myocardial Edema and Inflammation

Animal studies of myocardial water content in ischemic and reperfused myocardium suggest that tissue edema is an early feature of reperfusion. Tissue water content increases by up to 45% after 48 minutes of coronary occlusion and 2 hours of reperfusion in a pig model [1], although this extent of tissue edema is not observed in all species. Tissue edema may be induced by rapid reperfusion because of movement of normo-osmotic fluid from the intravascular compartment into the hyperosmotic interstitial space. This, in turn, may lead to cell swelling and potentially cell death. Hyperosmotic reperfusion using mannitol, which distributes freely in the extracellular space, leads to a significant reduction in myocardial edema. This is supported by the experimental observation that gradual restoration of blood flow to ischemic tissue (and therefore gradual osmotic equilibration) induces less tissue edema than abrupt reflow. In the clinical setting, the use of hyperosmotic solutions during cardioplegia has led to enhanced postischemic functional recovery.

Early clinical studies of the effects of antiinflammatory therapy for reduction of infarct size were flawed because of poor understanding of the mechanisms underlyng myocardial injury and remodeling. The use of nontargeted antiinflammatory steroid therapy in myocardial infarction led to deleterious effects such as myocardial rupture [2]. If antiinflammatory therapy is to be effective in reducing reperfusion-induced myocyte death, it needs to be targeted at processes that are specific to reperfusion injury.

In acute myocardial infarction, tissue inflammation is accelerated early in the reperfusion phase; the processes involved also have the potential to injure viable myocytes. Early postischemic cardiac lymph is strongly chemotactic, and neutralization of the C5a complement fraction reduces its chemotactic action. Neutrophil localization in postischemic myocardium is accompanied by the production of autacoids such as leukotriene B4, which are also chemotactic, and the induction of P-selectin surface expression, which may induce neutrophil trapping. Abrupt restoration of blood flow can result in a sudden influx of neutrophils during early reperfusion in response to these and other tissue factors. Although experimental evidence exists for reduction of infarct size by the administration of the leukotriene B4 receptor antagonist LY223882 before reperfusion, as yet there is no conclusive evidence from human studies that chemotactic blockade is of clinical benefit [3].

After trapping, neutrophils may exert a negative effect on viable tissue through two principal mechanisms—release of proteolytic enzymes and production of reactive oxygen species. In vivo, neutrophils adhere to cells that express specific ligands: intercellular adhesion molecule-1 (ICAM-1) and integrins CD11b/CD18 (Mac-1) [4]. ICAM-1 expression is known be induced in viable myocytes at border zones of myocardial necrosis. It is in these zones that neutrophil aggregation is most intense, suggesting that viable myocytes are at risk through direct injury and because of impaired microvascular flow. A potential therapeutic target has been identified through the observation that ICAM-1 upregulation is interleukin-6 (IL-6) dependent; the therapeutic effects of inhibiting the action or production of IL-6 have still to be determined.

Free Radical–Induced Injury

Reperfusion is associated with a sharp increase in free radical activity [5]. Free radicals may be produced by auto-oxidation of catecholamines, through the prostaglandin pathway, and by leakage of radicals from mitochondria. Activated neutrophils also produce free

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radicals through the NADPH oxidase pathway. Although superoxide radicals and hydrogen peroxide may be produced by the action of xanthine oxidase on hypoxanthine in other reperfused tissues, inhibition of xanthine oxidase is unlikely to be of clinical benefit in myocardial reperfusion because of its very low tissue concentration in the myocardium. The injurious potential of free radicals may depend on local levels of endogenous antioxidants. Determination of the pathological and clinical relevance of free radical–induced myocyte injury involves examination of the effects of manipulating the oxidant-antioxidant balance in vivo and in vitro.

Administration of the antioxidants superoxide dismutase and catalase during reperfusion has been reported to reduce infarct size in dogs [6], but several negative reports have also been published. Administration of exogenous antioxidants may not achieve the local concentrations required to confer a protective effect, particularly if they are administered after the onset of ischemia rather than prior to it. Furthermore, if antioxidant therapy is to have a sustained protective effect, these agents may need to be administered for several days after reperfusion since oxidant stressors (particularly neutrophil-derived free radicals) are produced over a sustained period. Initial experimental evidence suggests that induction of endogenous antioxidant production through hyperthermic stress or genetic manipulation may have the potential to reduce reperfusion-induced cell death during planned procedures such as open-heart surgery [7]. It is important that future studies of antioxidant protection and preconditioning satisfy criteria for distinguishing ischemic injury from reperfusion injury [8].

**Microvascular Injury and Impaired Reflow**

Restoration of coronary flow after a period of ischemia does not always result in full restoration of perfusion in an at-risk territory. Impaired reflow is established at the time of reperfusion, associated with coagulative necrosis of the myocardium. Importantly, more marked reduction in flow occurs later during reperfusion, involving zones with initially normal perfusion [9]. Endothelial injury and capillary occlusion with platelets and neutrophils have been observed, and tissue edema may lead to capillary compression [10]. Tissue blood flow may progressively diminish, leading to further compromise of potentially viable myocytes. Potentially harmful consequences of this are a reduction in endothelial nitric oxide production (and a consequent vasconstrictor response) and depletion of endogenous free radical scavengers. Clinical studies of impaired reflow are limited by difficulty in detecting this phenomenon in vivo, and there is as yet no known clinical therapy to minimize microvascular injury. Impaired reflow may be partly induced by oxidant stress and intense neutrophil localization secondary to chemotactic factors and ligand expression; amelioration of impaired reflow is likely to necessitate manipulation of these variables.

**Conclusions**

Experimental evidence suggests that lethal reperfusion injury does exist. The magnitude of such injury in clinical settings of reperfusion is unknown. Reperfusion induces tissue edema and osmotic stress, which can lead to sarcolemmal disruption, and intense neutrophil migration is seen in infarct border zones where viable myocytes are found. Free radical release is intense during reperfusion and may be focused at sites of neutrophil activation. Microvascular damage is likely to be induced by similar mechanisms, leading to microvascular compromise and further tissue ischemia. Therapies directed at reducing neutrophil chemotaxis merit further experimental evaluation, and clinical antioxidant trials are already in progress. Reperfusion injury may also be of relevance during planned interventions such as coronary angioplasty and cardiac surgery, where both pathological and clinical myocardial dysfunction may result. The use of hypotensive solutions during cardioplegia, and preconditioning of the endogenous myocardial oxidant-antioxidant balance by heat stress or short periods of ischemia, are also potential areas for further clinical investigation.

Clinically, there is no doubt that the benefits of reperfusion outweigh the adverse effects, and clinicians should not be deterred from using reperfusion strategies in acute coronary syndromes. However, limitation of infarct size is a key objective in the development of new adjuvant therapies for the treatment of myocardial infarction. Targeting of these therapies to specific reperfusion injury mechanisms should prevent the deleterious effects seen in the methylprednisolone trial, which demonstrated the potential of therapeutic interventions to interfere with normal repair mechanisms [3]. At present, therapies that manipulate the injurious mechanisms described in this article have not seriously impacted on clinical practice. To determine whether treatments and interventions designed to reduce lethal reperfusion injury are of definitive clinical benefit requires further experimental and clinical evaluation.

**References**

