
Lethal Myocardial "Reperfusion Injury"

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Reperfusion of the ischemic tissue is essential for preservation of the remaining viable cells within the jeopardized region. The controversial issue remains, however, of whether or not reperfusion in itself may be harmful. If reperfusion or reoxygenation injury does occur, then a paradoxical situation exists where reperfusion (reoxygenation) may in fact be harmful. Many studies have provided evidence that the insult of ischemia may be intensified by the injury of subsequent reperfusion [1-4]. Simply defined, the injury associated with reperfusion or reoxygenation represents the conversion of normal or reversibly injured cells to a state of irreversible injury due to the reintroduction of oxygen or oxygenated blood to a previously ischemic tissue. Numerous studies in diverse organs, including kidney, heart, skeletal muscle, intestine, and the central nervous system, provide compelling evidence for the existence of a phenomenon whereby reinstating vascular perfusion leads to an accentuation of tissue damage [5]. An examination of whether or not reperfusion/reoxygenation imposes a further risk must consider the extensive, albeit diverse, literature that has developed in a relatively short time involving clear demonstration of reperfusion injury in many organs and tissues.

For the most part, the detrimental effects of reperfusion injury have focused upon the heart, due to the use of coronary artery angioplasty or thrombolytic agents to manage patients with an evolving acute myocardial infarction. In addition, surgical procedures involving the induction of global myocardial ischemic arrest during cardiopulmonary bypass have provided data to support the concept that the phenomenon of reperfusion injury is not just a laboratory curiosity.

Efforts must be directed toward understanding the cellular events associated with the reintroduction of molecular oxygen and/or blood cellular elements to the reperfused region upon which is superimposed the influence of humoral mechanisms controlling the subsequent inflammatory response to ischemic injury. This latter aspect of the overall sequence represents an important contribution to the delayed and protracted extension of cellular injury beyond that associated with the ischemic insult. Multiple cellular-humoral mechanisms are activated during the ischemic period, leading to an orchestrated series of self-limited biological reactions that extend into the reperfusion period. The immediate response to the re-

introduction of oxygen upon the reversal of ischemia or hypoxia involves the rapid intracellular formation of reactive oxygen species, as demonstrated using both direct and indirect methods [6]. The initial burst of free radical formation may be followed by a prolonged sequence of events, characterized by an inflammatory response to tissue injury. There is an abundant literature supporting a major role for intravascular neutrophil sequestration early in reperfusion. The inflammatory cells, through the release of oxidants along with proteolytic enzymes, contribute to extension of tissue demolition in the reperfused region. The factors responsible for initiating and terminating the inflammatory response have not been elucidated fully and are occurrences applicable to most, if not all, organs and tissues subjected to reperfusion after an ischemic insult of sufficient duration.

The duration of the ischemic insult has significance with regard to the final outcome. A brief period of ischemia (5 minutes) does not result in either temporary or permanent injury. Myocardial ischemia of 15 minutes duration, while not resulting in permanent cell injury, is associated with the phenomenon of myocardial stunning. In the latter setting, reperfusion is accompanied by the abrupt and protracted generation of reactive oxygen metabolites. Myocardial stunning can be ameliorated by pretreatment with oxygen radical scavengers. Extending the duration of the ischemic insult beyond 45 minutes results in a progressive and permanent loss of cell viability within the region at risk. In addition, extension of the ischemic interval beyond 45 minutes is associated with local activation of the complement cascade. Complement-derived anaphylatoxins facilitate local recruitment and activation of inflammatory cells. Furthermore, activation of the complement cascade invokes tissue injury directly through assembly of the membrane attack complex that disrupts intracellular control of water and electrolytes. Finally, in the absence of reperfusion, most cells within the myocardial risk zone will be irreversibly injured if ischemia is maintained for 3 hours. There is no question that ischemic myocardial tissue, maintained at normal body temperature, will undergo

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progressive irreversible injury both biochemically and functionally. Ultimately, the cells subjected to prolonged ischemia will assume the characteristic morphologic appearance of cellular necrosis in which an inflammatory process characterized by the regional accumulation of polymorphonuclear leukocytes is a prominent feature, a process requiring 8–12 hours. In the heart, the electrocardiographic manifestations of cell death can be followed with serial electrocardiographic recordings in which evolution of the Q wave signifies the development of cell death coinciding in time with histopathologic evidence of a loss in cellular viability.

There is a marked contrast between the manifestations of ischemic cell death compared with the changes in the presence of reperfusion or reoxygenation occurring 60–90 minutes after the onset of ischemia. Reperfusion is associated with the abrupt loss of contractile function and development of contracture and release of cytosolic enzymes. The obvious and immediate ultrastructural and electrocardiographic changes strengthen the conviction that reperfusion was responsible for the observed “explosive” events.

Most experimental studies have focused on the cellular damage occurring shortly after reperfusion. All too often the experimental observations are terminated within 3–6 hours after reperfusion. The beneficial action of a therapeutic intervention intended to intercept the cytotoxic mediators (reactive oxygen species) responsible for immediate reperfusion injury is negated when the observation period is prolonged (days) into the reperfusion phase. In the latter instance, the selected intervention fails to protect against those events mediated by humoral and/or inflammatory mechanisms. Experimental protocols of limited duration do not permit assessment of the continuing inflammatory process initiated by the abrupt delivery of polymorphonuclear neutrophils to the previously ischemic region. Vascular dysfunction is an additional component of ischemia-reperfusion injury, leading to impaired tissue perfusion (no-reflow phenomenon) and further extension of tissue injury. The loss of vasomotor control as a result of endothelial injury has an added impact on tissue and organ survival.

The concept of myocardial reoxygenation injury was introduced by Hearse [7], who studied the rat isolated perfused heart under conditions of hypoxia and reoxygenation without interruption of coronary perfusion. Reversal of the hypoxic state by the reintroduction of molecular oxygen was associated with an immediate release of cytosolic constituents and impaired relaxation. The abrupt changes could only be accounted for by irreversible cellular injury [7], leading the author to refer to the event as the *oxygen paradox*. Subsequent studies of this phenomenon using *in vitro* and *in vivo* approaches have provided compelling evidence that the cells damaged upon re-

perfusion are in fact viable before reoxygenation [3,8,9].

Despite the evidence, not all studies using the endpoint of myocardial infarct size in models of myocardial ischemia/reperfusion injury have been positive. Thus, the concept of reperfusion injury has yet to be accepted universally. Admittedly, there are inconsistencies among the studies due to multiple factors attributable to differences in experimental protocols, methods for induction of tissue ischemia, duration of the ischemic event, timing of the intervention to be tested, choice of anesthetic agent, degree of residual collateral blood flow, as well as other unrecognized factors that impact upon the outcome. It is not surprising that some investigators embrace the view that reoxygenation or reperfusion of an ischemic area increases the rate of cellular necrosis for cells irreversibly injured as a result of the ischemic insult. Conclusive proof for the existence of reperfusion injury would require experimental evidence indicating that cells that were viable before reperfusion are irreversibly injured upon or soon after the onset of reperfusion [3].

Direct evidence of the conversion of ischemic cells to an irreversibly injured state upon reperfusion is lacking. It has been shown that the binding of a labeled monoclonal antibody to the intracellular protein, myosin, while not occurring during hypoxic perfusion, increased significantly upon reoxygenation of the hypoxic but continuously perfused isolated heart [9]. The antimyosin antibody binds to myocytes that have decreased membrane integrity associated with irreversible injury [10], thereby supporting the concept that reoxygenation of the hypoxic heart is associated with extension of irreversible myocardial damage. Whereas it may be possible to demonstrate a role for reactive oxygen metabolites as mediators of tissue injury, or free radical scavengers as being capable of providing a significant degree of protection under *in vitro* conditions, reproducibility of these observations *in vivo* may be difficult. The complexity of the *in vivo* model due to the multiple cellular and humoral events initiated by the ischemic insult precludes an easy resolution of the problem. However, the lack of concordance among investigators should not lead to a premature conclusion that the phenomenon of reperfusion injury does not exist.

Greater effort should be made to determine the areas of agreement in an effort to characterize the multiple mechanisms involved in the loss of cellular viability associated with tissue ischemia and reperfusion. The one fact upon which most, if not all, will agree is that preconditioning of the myocardium results in tissue salvage when the heart is subjected to an otherwise lethal ischemic insult followed by reperfusion. Does preconditioning affect the phase of ischemic-induced cell injury, or does it modify the component of tissue injury associated with reperfusion? It is dif-

difficult to believe that preconditioning has altered the susceptibility of the tissue to ischemic injury because the protective effect is lost if the ischemic insult is maintained for 90 minutes as opposed to 60 minutes. What changes occur as a result of preconditioning that allow the myocardial cell to survive the otherwise deleterious effects of reperfusion? Current theories suggest a role for adenosine, opening of the ATP-dependent potassium channel, and/or activation of protein kinase C. Acting independently or in concert, these cellular alterations undoubtedly affect other cellular/humoral responses that otherwise would prove detrimental to the involved tissue.

It is time to abandon efforts to disprove the fact that reoxygenation or reperfusion of ischemic tissue is associated with an extension of injury and loss of cell viability. Whereas controversy exists with respect to the clinical importance of the phenomenon in the heart, the experimental data in other organ systems that there are cellular and immunologic mechanisms set in motion by the ischemic insult that facilitate the extension of tissue injury during the phase of reperfusion are convincing. A better understanding of the associated mechanisms is essential for the ultimate development of pharmacologic interventions intended to enhance tissue viability under a wide array of clinical settings in which ischemia is present and must be reversed. The controversy over whether reperfusion injury exists has focused primarily on the heart. It is important to recognize that the biologic phenomenon has been demonstrated in many organs and tissues, and remains an important concern whenever organ ischemia develops as a result of pathophysiology or is induced intentionally as part of patient management. In either case, perfusion must be reinstated. There is an obligation to ensure that reperfu-

sion, while absolutely essential to tissue survival, does not compromise the intended outcome.

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