

# Myocytes versus endothelial damage

Frank L. Dini, Enrico Orsini, Mario Marzilli and Giacinta Guarini, Cardiovascular Medicine Division, Cardio Thoracic and Vascular Department, University of Pisa, Italy

Correspondence: Giacinta Guarini, Cardiovascular Medicine Division, Cardio Thoracic and Vascular Department, University of Pisa, Via Paradisa, 2, 56100 Pisa, Italy.  
Tel.: +39 3494544161, e-mail: giacinta\_guarini@yahoo.it

## Abstract

Ischemic injury and reperfusion injury have been largely investigated in vitro and in animal models. This wide research body has elucidated several mechanisms involved in the processes. However, clinical applications of this information are limited and scanty. In the present article we attempt to summarize the different time course and the mechanisms involved in ischemic damage versus reperfusion injury. A better understanding of these complex phenomena may help in a more efficient application of cardioprotective and reperfusion strategies. This in turn could ameliorate the clinical outcome in patients with acute coronary syndromes.

**Keywords:** ischemic injury; reperfusion injury; endothelial damage; cardioprotection

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## Introduction

Reperfusion therapy in acute myocardial infarction (AMI) aims to restore myocardial perfusion in order to salvage jeopardized myocardium. Major advances in interventional techniques and pharmacological adjunctive therapy have made it possible to reopen and maintain patent over 95% of occluded vessels. But, paradoxically, this high recanalization rate has highlighted the limitations of current strategies in regards to restoring adequate tissue perfusion and improving patient prognosis. In fact, although we have observed an overall reduction in mortality, in hospital mortality still approaches 10% and over 25% of patients surviving acute phase are bound to develop heart failure syndrome.

## Ischemic injury

Several studies have conclusively demonstrated that survival in the acute phase of myocardial infarction is directly related to the duration of ischemia and to recovery of tissue perfusion. The salvage of jeopardized myocardium is primarily determined by: 1) total ischemic time, 2) microcirculatory perfusion, and 3) myocytes and non-myocytes (endothelial cells and fibroblasts predominantly) preservation after the ischemia–reperfusion sequence [1,2]. This is consistent with animal models of ischemia and reperfusion described by Reimer et al. [3]. Few changes are detectable in the myocytes during the early phase of ischemic injury, while damage becomes evident and irreversible after 30–40 minutes of ischemia. This transition to irreversibility is associated with new ultrastructural changes [4], including diffuse mitochondrial swelling, the appearance of amorphous densities in the matrix space of the mitochondria, virtual absence of glycogen, marked peripheral aggregation of nuclear protein and the appearance of discontinuities in the cell membrane. This holds true in patients with STEMI, as clearly indicated in a recent work by Agati and al. using cMRI [5]. They observed that patients reperfused

within <90 min had a smaller infarct size, showed less severe microvascular damage and greater myocardial salvage after reperfusion, whereas patients reperfused later (>6 hours) presented with larger infarcts and greater microvascular obstruction and limited, if any, myocardial salvage. They also reported that the area at risk reduced over time only in patients reperfused within 90 min while it progressed to irreversible damage when reperfusion was achieved later. These data clearly confirm that the potential for myocardial salvage decrease dramatically with time and that after 90 minutes of ischemia there is limited benefit, if any, in term of infarct size reduction [2].

### Reperfusion injury

Ultrastructural images obtained at a different time of coronary occlusion (20–80 minutes) in a dog model of coronary ligation and reperfusion, demonstrated that microvascular damage appears after 60 minutes of occlusion and always lags behind myocardial cell injury, appearing as early as 20 minutes after the beginning of ischemia. Microvascular alterations were consistently located in areas of irreversibly damaged myocytes [6]. The extension of non-reperfused area, within the ischemic zone, was larger after severe and prolonged ischemia and after prolonged reperfusion [7,8]. Additional studies have also confirmed that the area of no reperfusion increases with time, suggesting an independent progression of the reperfusion injury [9,10]. Microvascular damage has been identified as one of the key components of the no-reflow phenomenon. The cardiac no-reflow phenomenon was originally described in 1974 by Klöner et al. [11,12]. When canine myocardium was exposed to short periods of ischemia (<40 min of ischemia), no significant changes were noted in small cardiac vessels. However, when ischemia was maintained up to 90 min, dramatic changes appeared in the endothelial cells at capillary level. Electron microscopy studies revealed severe endothelial damage with capillary swelling and blebs causing intraluminal protrusions. Cellular and interstitial edema were also noted. Manciet et al. [13] demonstrated that ischemia for less than 30 min did not have a significant effect on small vessels. However, when arteries were exposed to ischemia of longer duration, capacity for endothelium-mediated relaxation progressively decreased and was totally lost after 120 min of ischemia. With loss of normal endothelial

function the vascular system lost the metabolic regulation capacity, i.e., lost the capacity to dilate in response to myocardial needs. In addition, intracellular edema and endothelial cell swelling occurred during myocardial infarction [14], resulted in compression of capillaries and small arterioles, further increasing resistance to flow through these dysfunctional vessels [13]. Ambrosio et al. [9] studied canine hearts submitted to 90-minute occlusions followed by reperfusion for either 2 minutes or 3.5 hours. The areas of absent capillary filling were larger after 3.5 hours than after 2 minutes of reperfusion and resulted primarily from intracapillary erythrocyte stasis and marked intravascular neutrophil accumulation. Reperfusion injury is a multifactorial process, including the “endothelial trigger” and “the inflammatory amplification” steps [15]. One of major determinants of endothelial dysfunction [16] is the loss of the endothelial cells capacity to release nitric oxide (NO) [17], which occurs within 2.5–5 min following re-establishment of flow. The reduced release of NO from the ischemic-reperfused endothelium occurs after 2.5–5 min of reperfusion, persists for hours, and appears to be related to superoxide radicals production by the abrupt reoxygenation [18]. Thus, a component of the reduced NO release is due to enhanced quenching of NO by superoxide radicals. This is followed by a dramatic increase in polymorphonuclear cells (PMN) adherence to the reperfused endothelium, becoming highly significant at 20 min post-reperfusion. This enhanced leukocyte adherence to the endothelium leads to capillary plugging and edema formation resulting, in turn, in coronary blood flow reduction. Leukocytes present in the coronary microcirculation at the time of reperfusion play a central role in the no-reflow phenomenon [19]. Following PMN adhesion to the endothelium, transendothelial migration of activated PMNs can occur resulting in PMN accumulation in the myocardium. Even if capillary leucocyte trapping is prominent in the area of no-reflow, the effects of leucocytes are probably not limited to mechanical plugging, but may involve complex interactions with endothelium, platelets, and perhaps with myocytes. Polymorphonuclear cells are able to release ROS, proteolytic enzymes, and lipooxygenase products (leukotrienes) that influence platelet and endothelial function. Endothelial cells can modulate leucocyte function by the expression of adhesion molecules—for example, intercellular

adhesion molecule-1 (ICAM-1) or P-selectin—and by release of soluble factors including nitric oxide, prostacyclin, endothelin, and platelet activating factor. Platelets affect polymorphonuclear cell activation by release of thromboxane A<sub>2</sub>, platelet derived growth factor, serotonin, lipooxygenase products, proteases, and adenosine [19].

Latchman et al. [20] have further investigated the specific ways and time of death of endothelial cell (EC) and cardiac myocytes during ischemia and reperfusion in a rat model of ischemia/reperfusion. Apoptosis after ischemia/reperfusion proceeds in a cell- and time-dependent manner. Ischemia alone was not sufficient to complete the apoptotic death of myocyte and non-myocyte cells. Apoptosis of EC was initially visible in small coronary vessels to appear later on also in larger vessels and was associated with a progressively enlarging perivascular cuff of cardiomyocytes apoptosis. After 2 hours of reperfusion, the proportion of apoptotic EC decreased, and apoptotic cardiomyocytes were more homogeneously distributed. This led the authors to suggest that ischemia without reperfusion can initiate the molecular pathway of apoptosis, although reperfusion is required to complete the DNA fragmentation and morphological changes typical of an end-stage apoptotic cell. This requirement for reperfusion in completing the apoptotic program is consistent with previous studies [21,22]. The observation that EC apoptosis precedes that of cardiomyocytes has two important implications. First, mediator(s) generated during the ischemic period, released into the circulation may be necessary to complete the apoptotic process during reperfusion. Second, cardiomyocytes apoptosis may be triggered by the diffusion into the myocardium of soluble apoptogenic mediators derived from damaged EC. Several candidate mediators for this paracrine apoptosis of cardiomyocytes have been postulated, including those that ligate to tumor necrosis factor-alpha or Fas ligand and free radicals. The potential involvement of soluble factors in apoptosis after ischemia/reperfusion injury suggests that strategies based on their scavenging or inhibition may allow endothelial cell rescue and protect the myocardium.

### Summary and conclusions

These observations are consistent with the hypothesis that cellular injury at the microvascular level is already

initiated during the ischemic insult, and that injurious mechanism(s) continues to operate, as a vicious cycle, during the reperfusion process. Therefore the final extent of microvascular damage and myocytes death is determined by the processes developing during the occlusion period as well as by the time at which reperfusion is applied. Ischemic damage needs no longer than 30 to 40 minutes to be established and reperfusion damage occurs within minutes from coronary blood flow restoration. These observations have two major consequences. First, they impose to reperfuse as soon as possible in order to reduce infarct size and second, they mandate the use protective strategies at the time of reperfusion to limit myocardial damage. The potential beneficial effect of these cardioprotective strategies is higher when reperfusion occurs early (less than 2 hours), i.e., when significant amount of viable myocardium is still present. Reperfusion damage contributes little to final infarct size when the myocardium is reperfused later after completing ischemic cell death. •

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