Myocyte Cell Death in the Diseased Heart

Piero Anversa, Jan Kajstura

In this issue of Circulation Research, Karwatowska-Prokopczuk et al and Takemura et al report, respectively, that inhibition of the vacuolar proton ATPase (VPATPase) enhances apoptosis in neonatal cardiac myocytes during metabolic recovery and that the progression of the healing process after infarction involves apoptotic death of inflammatory and interstitial cells. We will discuss these 2 studies separately, because the first addresses important mechanisms of myocyte death and survival, and the second provides new information on scarring of the postinfarced heart. Both studies emphasize directly or indirectly through in vitro and in vivo experiments the role of apoptosis in ischemic injury. Two different phases of cardiac damage are examined: myocyte death and reparative fibrosis. In the first article, the recognition that alkalization of myocytes is regulated not only by activation of the Na+-H+ antiport and the Na+-HCO3- symport but also via a third proton-extruding system, VPATPase,1,3 may elucidate some of the critical events affecting the ischemic myocardium. Whether myocardial ischemia occurs in vivo in the absence4,5 or presence6,6 of reperfusion, cell death by apoptosis is the predominant pathological consequence; its etiology, however, remains to be defined. Similarly, the paradoxical beneficial impact of Na+-H+ exchange inhibition during ischemia1 is unclear. The documentation that the ATP-dependent vacuolar proton pump may play a role in hypoxia-induced myocyte apoptosis is significant because it points to alterations in pH, as critical for the transmission of a death signal to myocytes in vivo. Impairment of VPATPase affects the extrusion of protons from the cytoplasm to intracellular acidic complexes, decreasing pH, and abolishes the protective influence of attenuation of the Na+-H+ exchanger on Ca2+ overload.1 The observations by Karwatowska-Prokopczuk et al have been paralleled by results from Lakatta’s laboratory recently published in The Journal of Clinical Investigation.7

Myocyte Death and Ischemic Cardiomyopathy

Let us now attempt to indicate the relevance of these in vitro findings1 in the definition of infarct size in vivo. Tissue and cellular acidosis develops in the presence of ischemia.8 Hydrolysis of ATP, which releases protons, decreases pH.9 ATP levels are reduced by 65% at 15 minutes and by 90% at 40 minutes.10 pH may decrease as much as 1 unit by 10 to 30 minutes.11 Concurrently, the concentration of CO2 and lactic acid in the interstitium increases, resulting in severe acidosis.8 Modifications of pHl and pHd do not occur in a uniform manner. The boundaries of CO2 diffusion are difficult to predict. They are dependent on the size of the ischemic segment and mural thickness and create local differences in pHl and pHd in the tissue. Additionally, the depletion of high-energy phosphates may inhibit VPATPase,12 enhancing electrolyte derangements in the cells. On the basis of these changes in the metabolic properties of the ischemic myocardium, it is tempting to suggest that acidosis is the prevailing mechanism of myocyte apoptosis and of its distribution after myocardial infarction or ischemia/reperfusion injury. Myocyte necrosis is minimal under this setting both in vivo1 and in vitro.1 Moreover, the contention that acidic pH protects myocytes during hypoxia in vitro13 has been applied to the in vivo state; this may have to be reconsidered. The defect in ATP and the depression in VPATPase function in vivo with severe ischemia may counteract the postulated beneficial action of Na+-H+ exchange blockade.

An interesting question raised by the observation that acidosis may be a major etiological factor in myocyte apoptosis is whether gene expression is required or implicated in the initiation and progression of the death process. p53 is upregulated in hypoxia,14 and inhibition of VPATPase is characterized by the induction of p53 and p53-dependent genes such as p21.15 However, the magnitude of apoptosis in the infarcted myocardium is not altered in mice nullizygous for p53.16 Additionally, the expression of Bcl-2 is increased in ischemic myocytes, whereas Bax protein remains constant,4 pointing to the lack of p53 activation under this setting. The Bcl-2 family of proteins constitutes a critical checkpoint in cell death.16 These proteins contain agonists and antagonists of apoptosis, and alterations in their ratio determine the life or death of a cell. p53 is a transcriptional regulator of the bcl-2 and bax genes16; p53 downregulates the apoptotic gene product Bcl-2 and upregulates the proapoptotic gene product Bax. Currently, it is a matter of controversy whether p53 and p53-inducible genes are involved in the modulation of myocyte apoptosis in ischemia.

Cell death, scattered across the preserved portions of the wall, occurs in the failing human heart.17 The number of apoptotic cells is relatively small and in the majority of cases is <0.5%. Similar results have been obtained in animals with ventricular dysfunction and failure (for review, see Reference 18). This distribution of apoptosis differs from that associated with ischemia/reperfusion injury or myocardial infarction. However, defects in blood supply to the myocardium are present in the decompensated heart. Local ischemia leading to decreases in pHl and pHd may develop in discrete regions of the wall, suggesting that a similar phenomenon may be operative here as well. Although this possibility has to be considered, apoptosis affects individual cells, and clusters of...
Dying myocytes are characteristically absent. This tends to exclude alterations in the intramural branches of the coronary circulation in the activation of programmed myocyte cell death. An arteriole of 20 μm in luminal diameter distributes blood to nearly 50 000 myocytes, implying that involvement of vessels of this size should result in ischemia and apoptosis of a large number of cells. It is also difficult to link changes in capillary density with a decrease in tissue oxygenation leading to single myocyte cell death. Ischemia develops when resting coronary blood flow is impaired. Physiological studies have documented reductions in coronary reserve, but baseline flow is rarely affected.

Myocyte loss was introduced in the late 1980s and early 1990s as a potential etiological factor of ventricular dysfunction in the aging heart of animals and humans. However, the interest in myocyte loss exploded nearly 3 to 4 years ago when apoptosis was proposed as a form of cell death in the diseased heart. The number of studies on this subject has increased exponentially, and, currently, little attention is given to myocyte necrosis, the type of cell death that was considered the only one occurring in the myocardium. Even in the old literature concerning prenatal development of the heart, myocyte death is discussed, but apoptosis is not claimed as the mechanism. Currently, very little effort is made to establish whether myocytes die by apoptosis only, by necrosis exclusively, or by a combination. The analysis of cell death in vivo and in vitro is almost invariably restricted to apoptosis, and necrosis is essentially ignored. This is surprising and scientifically wrong. Myocyte necrosis is a major component of the decompensated heart and can affect large groups of cells, resulting in foci of replacement fibrosis, or can occur in a scattered manner across the ventricular wall, mimicking the distribution of apoptosis.

The recognition that loss of myocytes involves apoptosis, necrosis, and apoptosis/necrosis requires the development of methodologies capable of detecting these types of cell death, particularly necrosis. In vitro preparations and in vivo animal studies do not represent a serious problem, because myosin monoclonal antibody can be added to the culture medium or administered in vivo. In vitro, ethidium monoazide bromide (EMB) can be used to reveal small areas of damage in the sarcolemmal membrane, or cells can be exposed to 5-hexadecanoylamino-fluorescein (HEDAF). Additionally, propidium iodide (PI) labeling of nuclei can be used. However, the analysis of myocyte necrosis in the human heart is complicated by the impossibility of injecting myosin antibody in vivo to label dying cells, and EMB, HEDAF, and PI cannot be used in tissue sections of the myocardium. Conversely, myocyte apoptosis can be detected histochemically and morphologically, but whether programmed cell death affects a myocyte independently from necrosis or whether necrosis is present in adjacent or distant myocytes cannot be established. This is a critical issue because apoptosis involves 0.2% of myocytes in end-stage failure, a value that may challenge the significance of cell death in the final stage of the disease. On the other hand, myocyte necrosis may be comparable or may exceed apoptosis, and the combination of necrosis and apoptosis could decrease markedly the number of functioning cells in the heart. These comments are meant to emphasize the need to develop probes able to detect cell necrosis in situ. Of relevance, an accurate quantitative evaluation of the effects of apoptosis and necrosis on the diseased heart would require knowledge of the time necessary for the completion of each form of myocyte death. The rate of myocyte loss could then be calculated, and this information could be highly relevant in predicting the evolution of the overloaded decompensated heart.

Apoptosis and Myocardial Repair

The sequence of structural events involved in healing of the postinfarcted heart has been well characterized in animals and humans. However, the mechanisms by which the various cell populations present in the acute and subacute phases of the repair process are removed from the damaged myocardium are not clear. The study of Takemura et al documents that cell death by apoptosis occurs in all cell types, providing important information on the remodeling of the ventricle after a 30-minute occlusion of the coronary artery and a reperfusion period of 2 days and 2 and 4 weeks. Although it is not surprising that inflammatory cells, consisting mostly of leukocytes, undergo apoptotic death, it is somehow unexpected that myofibroblasts die in the same manner. Myofibroblasts are wound-healing cells with structural characteristics of smooth muscle cells and fibroblasts. They are implicated in wound contraction and, in the infarcted human heart, persist for several years and maintain an orientation parallel to the endocardium and epicardium. This type of alignment, however, is detected only in transmural infarcts. Conversely, myofibroblasts are distributed in the direction of the longitudinal axis of myocytes in small patchy lesions of the wall. Apoptosis occurs in endothelial cells, and this phenomenon may be responsible for the progressive loss of vessels and reduction in blood supply to the healing region. Myofibroblasts counteract, at least in part, local ischemia, remaining a permanent component of the completed scar. The disappearance of the vascular framework with time may induce necrosis of interstitial cells and myofibroblasts as well. The recognition that apoptosis plays a role in tissue repair of the heart is significant, but whether cell necrosis participates in the reduction of proliferating cells and vascular structures in the healing myocardium is unknown. Moreover, the humoral and/or mechanical signals implicated in the activation of the endogenous cell death pathway in myofibroblasts, fibroblasts, macrophages, and endothelial cells remain to be identified. Myofibroblasts isolated from the infarcted myocardium synthesize and secrete angiotensin II, and the scarred noncontracting myocardium is exposed to the physical forces resulting from systolic and diastolic pressure. These 2 events may contribute to the activation of apoptosis. Inflammatory cells and macrophages contain various cytokines and proteases, which, together, may be involved in the initiation of cellular disruption and death.

Conclusion

The 2 studies published in this issue of Circulation Research advance our understanding of the mechanism of cell death in ischemic injury and tissue repair of the myocardium. However, the fundamental question whether apoptotic myo-
cyte cell death alone is a critical etiological factor in the onset and progression of ventricular dysfunction and failure in the human heart remains to be answered. Myocyte necrosis must be reconsidered as a major player in cardiac decompensation. Information concerning the duration of myocyte apoptosis and necrosis is lacking, and this deficiency limits our knowledge on the actual impact of ongoing cell death in the failing heart. Moreover, there is very little understanding of the ability of the same death signal to activate apoptosis in some cells and necrosis in others. The molecular basis underlying the proficiency of cells to die by apoptosis and/or necrosis as well as their capacity to oppose both death stimuli is not clear and constitutes a major challenge for future research in this area of cardiac pathology.

References


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