

Myocyte death and myocyte regeneration in the failing human heart

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(Ital Heart J 2001; 2 (Suppl 3): 12S-14S)

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Myocyte apoptosis has been experimentally implicated in the transition from compensated to decompensated hypertensive hypertrophy and in the acute restructuring of the wall and chamber dilation of the post-infarcted heart^{1,2}. Prevention of cell death attenuates the impact of ischemic damage on ventricular anatomy and performance³. Cell death occurs not only by apoptosis, but also by necrosis and their combination⁴.

Hearts from female and male patients, undergoing cardiac transplantation, were examined and each form of cell death was measured⁵. Internucleosomal DNA cleavage was measured by terminal deoxynucleotidyl transferase (TdT) and *Taq* polymerase assays. *Taq* polymerase yields products with single base 3' overhangs⁶. TdT reaction with a fluorescent probe identifies staggered ends of the cleaved DNA⁷, but does not distinguish between single base and longer 3' overhangs. Levels of apoptosis with these methods were not different in ischemic or dilated cardiomyopathy (Fig. 1). With failure, myocyte apoptosis, measured by *Taq* and TdT, was 2.2-fold ($p < 0.003$) and 2.5-fold ($p < 0.001$) higher in men than in women. With respect to controls, failure resulted in an 85-fold ($p < 0.0001$) and 35-fold ($p < 0.02$) increase in apoptosis in males and females, respectively. During necrosis, the release of endonucleases and exonucleases from lysosomes produces DNA fragments with blunt-ends which are recognized by *Pfu*⁶. With heart failure, myocyte necrosis was nearly 2-fold ($p < 0.001$) higher in men than in women. Moreover, with respect to baseline, this form of cell death increased 13-fold ($p < 0.002$) and 27-fold ($p < 0.0001$) in female and male diseased hearts. In all cases of heart failure, male myocytes showed great-

er levels of DNA diffusion and laddering than female myocytes.

The reduced incidence of cell death in women was apparent, although the disease and cardiac failure were present for a longer time. Higher myocyte death in males was associated with a shorter duration of the myopathy and an earlier onset of heart failure. In women, average time, from beginning of the morbid state to the impairment in function, and from diagnosis to transplantation, was 77 and 24 months, respectively. In men, these intervals were 54 and 14 months. The entire period of the disease at transplantation was 101 and 68 months in females and males. These observations suggest that chronic loss of myocytes plays a critical role in the initiation of ventricular dysfunction and its progression to severe cardiac decompensation. Recent clinical results are consistent with this possibility⁸. The extent of cell death correlates with alterations in ventricular hemodynamics with age⁹, systemic hypertension¹ and ischemic injury⁹ in rats and dogs. Interference with cell death in the surviving myocardium after infarction, or coronary constriction, decreases ventricular loading, chamber dilation and hypertrophy in mice^{3,10}.

Myocyte apoptosis occurs in end-stage cardiac failure^{7,11}. However, apoptosis involves at most 1% of myocytes⁷, a value that may challenge the impact of this phenomenon on the final outcome of the pathologic state. Low levels of apoptosis have been confirmed in the decompensated human heart: 0.18% in males and 0.08% in females. Importantly, myocyte necrosis comprised 1.2% of myocytes in males and 0.5% in females, exceeding apoptosis in both sexes. Although the number of necrotic myocytes was several fold greater than

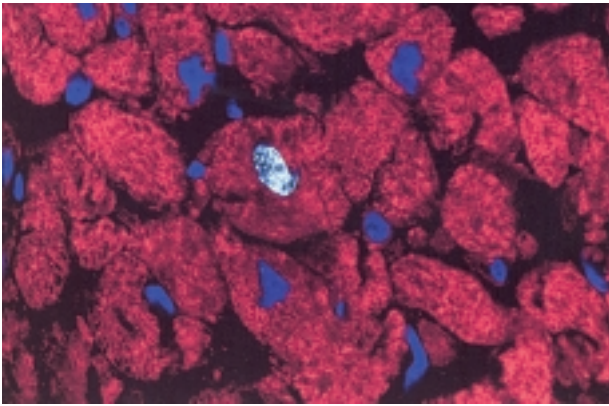


Figure 1. Myocyte apoptosis in a heart affected by dilated cardiomyopathy. Bright fluorescence of the apoptotic nucleus located at the center of the figure corresponds to double DNA strand cleavage with single base 3' overhangs detected by hairpin probe labeling. Nuclei are identified by propidium iodide staining and blue fluorescence while myocyte cytoplasm is recognized by α -sarcomeric actin antibody labeling and red fluorescence.

apoptotic myocytes in the male and female myocardium, the time required for the completion of each form of cell death is unknown. Labeling of DNA strand breaks in myocyte nuclei by TdT or *Taq* corresponds to the early phases of apoptosis and does not provide information on the sequence of events taking place in the cell after nuclear fragmentation. Similarly, the recognition of myocyte necrosis by *Pfu* leaves unanswered the question concerning the duration of the necrotic process. *In vitro* studies in various model systems have shown that apoptosis may be completed in a period ranging from 30 min to 2 hours⁹. However, there is no indication of the time required for myocyte apoptosis. An identical limitation applies to myocyte necrosis; this form of cell death has been claimed to reach its final stage in 1-2 days in infarcted rats⁴. This period is necessary for the cell to be engulfed by surrounding macrophages. Apoptosis may be much faster than necrosis, suggesting that the higher value of myocyte necrosis in the failing heart may not reflect a significant difference in the number of cells dying by these two distinct mechanisms.

A discrepancy exists between the extensive collagen accumulation and the modest reduction in number of ventricular myocytes in the post-infarcted human heart⁹. The magnitude of fibrosis in end-stage ischemic cardiomyopathy would imply a nearly 90% decrease in the total number of left ventricular myocytes. Conversely, decreases of < 30% have been reported. This discrepancy is even more apparent in idiopathic dilated cardiomyopathy in which myocardial fibrosis is associated with preservation of the number of myocytes in the ventricle. This phenomenon can be explained only assuming that myocyte proliferation occurs¹². According to the dogma, myocytes are terminally differentiated and their life span corresponds to that of an individual. Such an assumption contradicts the concepts of cellular aging and the logic of a slow turnover of cells with the

progression of life in mammalian hearts. The latter may be a very likely possibility because the male heart loses 64×10^6 myocytes per year from 17 to 89 years¹³, indicating that cell death occurs with age in the absence of cardiac pathology. We have documented that 14 myocytes per million are in mitosis under normal conditions.

The ability of myocytes to proliferate and replace dying cells is markedly enhanced in the failing myocardium in which 140 myocyte nuclei per million were found in mitosis (Fig. 2). Myocyte apoptosis appears to exceed the level of myocyte proliferation in end-stage cardiac failure⁷. However, a comparison between these two events is impossible because the time required for the completion of the apoptotic process as well as the duration of the cell cycle in myocytes are unknown.

Myocyte proliferation compensates, at least in part, for the massive myocyte death that occurs acutely after myocardial infarction and during the evolution of the ischemic myopathy^{7,11}. Similarly, cell death characterizes idiopathic dilated cardiomyopathy^{7,11}, but cell regeneration maintains the number of myocytes relatively constant in the diseased heart. Myocyte hypertrophy constitutes an additional growth reserve mechanism and cells can nearly double in size in end-stage cardiac failure¹⁴. However, both cellular growth processes are unable to normalize the elevated diastolic load on the myocardium and/or decrease ventricular dilation. Myocytes elongate more than they expand in diameter¹³ and new myocytes may be added in series, providing the structural template for the increase in cavitory volume. If this pattern of cell proliferation is operative in the decompensated heart and the lateral insertion of cells with mural thickening is minimal, myocyte hyperplasia has to be regarded as a contributory factor to ventricular deadadaptation and terminal failure. At present, this is an unresolved issue.

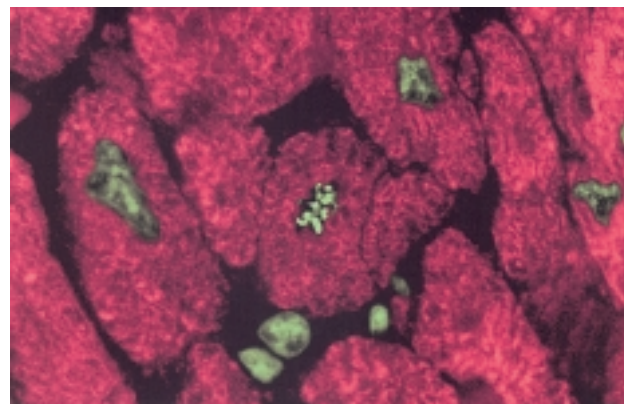


Figure 2. Myocyte mitosis in a heart affected by dilated cardiomyopathy. Chromosomes are identified by propidium iodide staining and green fluorescence while myocyte cytoplasm is recognized by α -sarcomeric actin antibody labeling and red fluorescence.

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