Current perspectives

Clinical relevance of apoptosis in early and late post-infarction left ventricular remodeling

Antonio Abbate, Giuseppe Biondi-Zoccai, Alessandro Petrolini, Luigi M. Biasucci, Alfonso Baldi*

*Institute of Cardiology, Catholic University, Rome, *Section of Pathologic Anatomy, Department of Biochemistry and Biophysics "F. Cedrangolo", Second University of Naples, Naples, Italy*

Key words:
Coronary artery disease; Heart failure; Myocardial infarction; Ventricular remodeling.

Apoptosis may represent an important pathophysiological mechanism causing progressive myocardiocyte loss and left ventricular dilation, even late after acute myocardial infarction (AMI). This review discusses the role of myocardial apoptosis on the basis of findings from experimental studies in animals and from observational studies in humans with the purpose of assessing the clinical relevance, determinants and mechanisms of myocardial apoptosis and the potential therapeutic implications. A more profound understanding of the impact of myocardiocyte loss on prognosis and of the mechanisms involved may lead to an improved understanding of cardiac remodeling and possibly to an improved patient care. In fact, among the potential modulators of myocardial apoptosis, angiotensin-converting enzyme inhibitors and beta-adrenergic receptor blockers have already been shown to improve the prognosis and symptoms in patients with post-infarction heart failure, and a reduction in myocardial apoptosis could partly contribute to such a beneficial effect. Several other putative factors could also modulate myocardial apoptosis after AMI, and many are currently under intense investigation. In particular, the infarct-related artery patency late after AMI may be a major clinical determinant of myocardial apoptosis and clinical benefits deriving from an open artery (the "open-artery hypothesis"), such as a slowing down of the remodeling process and a reduced arrhythmic risk, could be due, at least in part, to a reduced apoptotic myocardiocyte loss.

(Ital Heart J 2002; 3 (12): 699-705)

Received July 11, 2002; revision received August 26, 2002; accepted August 26, 2002.

Address:
Dr. Alfonso Baldi
Via G. Orsi, 25
80128 Napoli
E-mail: alfonsobaldi@tiscali.it

Acute myocardial infarction (AMI) is characterized by significant early and late mortality. Left ventricular dysfunction and symptomatic heart failure (HF) may complicate AMI in the acute and subacute phases[1]. Acute responses to myocardial ischemia include early excitation-contraction uncoupling ("myocardial stunning"[2]) which may be transient and reversible if an adequate oxygen delivery is re-established. Persistent ischemia leads to AMI which is specifically characterized and defined by the presence of ischemic coagulative necrosis of myocardiocytes[3]. Myocardiocyte loss during AMI, however, may also occur through a different type of cell death, apoptosis. Conventionally, the general term necrosis should be reserved to the changes that occur after cell death regardless of the pathway by which the cells have died[4]. Primary necrosis, better known as oncosis, identifies instead the primitive process of necrotic cell death, associated with cell swelling and blebbing, and followed by local inflammation. Apoptosis, or programmed cell death, represents a completely different means of cell loss, as cells actively pursue an orderly program of suicidal and adenosine triphosphate (ATP)-dependent cell death, through cell shrinkage and chromatin condensation, ultimately leading to cell budding and phagocytosis of cell remnants by nearby cells, usually without any surrounding inflammatory reaction. Nonetheless, even apoptosis-committed cells may ultimately necrotize, thus displaying signs of secondary necrosis.

This review will discuss major findings regarding the pathophysiological role of apoptosis in post-infarction left ventricular remodeling on the basis of experimental animal studies and of observational studies in humans. The determination of the apoptotic rate after AMI may indeed have prognostic and therapeutic implications.

Acute myocardial infarction: early events

During AMI, apoptosis occurs as early as just 2 hours after coronary occlusion, peaks at 4 to 12 hours and persists at a high rate for up to 10 days after AMI[5-8]. Al-

though in AMI apoptosis and necrosis coexist, the former is several times more common than the latter in experimental AMI (a peak value of 43% of apoptotic myocardiocytes at 4.5 hours vs a peak value of 8% of necrotic cells at 24 hours)[5].

Apoptosis is a genetically-programmed cell death and a fundamental physiologic and pathologic mechanism that allows the elimination of normal but no-longer useful cells during embryogenesis or of aged or damaged cells during life[9] (Fig. 1). A delicate balance between survival and death exists in cells in conditions of hypoxia and the initial phases of apoptosis may not always be followed by its completion[4,10]. As stated above, cell rupture, representing the terminal phase of cell death, may indeed affect initially viable cells (oncosis or primary necrosis) or cells already committed to apoptotic cell death (secondary necrosis), as described in detail by Majno and Joris[4]. The balance between apoptosis and primary necrosis depends on the available energy levels as the completion of apoptosis necessitates adequate ATP cellular concentrations[11]. The necrosis of myocardiocytes is usually completed within 24-48 hours following abrupt coronary occlusion. The removal of cellular debris by inflammatory cells starts at 24 hours with features of acute inflammation (interstitial edema and neutrophil polymorphonuclear infiltration) characterizing the first 24-72 hours and macrophage infiltration being prevalent subsequently[12,13]. Myocardiocyte loss, edema and inflammation are associated with an impaired cardiac performance.

Cardiac dysfunction may however occur even days after the initial insult. Infarct expansion has been described as a sudden, necrosis-independent, left ventricular dilation occurring early after AMI and leading to unfavorable hemodynamics and to an increased risk of the mechanical complications of AMI[14]. It may be correlated to the cellular response in AMI and may include inflammatory cell infiltration within the myocardium, fibroblast proliferation and neoangiogenesis, peaking at 4-7 days[12,13]. Olivetti et al.[15] have described side-to-side slippage of myocardiocytes as a possible additional pathophysiological mechanism of infarct expansion. This process is thought to lead to the elongation of fibers and to an absolute reduction in the number of myocytes in transmural sections.

Late post-infarction left ventricular remodeling

Congestive HF after AMI may also occur even later in the clinical course and indeed ischemic heart disease constitutes the most common cause of end-stage HF[16,17]. Post-infarction left ventricular remodeling consists of a progressive chamber dilation, wall thinning and systolic/diastolic dysfunction[16]. This process involves cellular and molecular mechanisms beginning days after AMI and persisting for weeks and months after the initial insult both at the site of infarction (persisting even after complete infarct healing) as well as in the surviving unaffected areas[18]. An initial phase of compensatory concentric hypertrophy is followed by a transition phase of eccentric hypertrophy leading to the end-stage of left ventricular dilation with progressive wall thinning[18]. Left ventricular remodeling is associated with an unfavorable hemodynamic performance and with adverse clinical outcomes such as an increasing rate of symptomatic HF, death due to pump failure and sudden cardiac death during long-term follow-up[16,17]. Several studies have described the presence of apoptosis in end-stage HF[19-22] and, in particular, in post-infarction left ventricular remodeling[23-26]. Myocardiocytes surviving through the acute phases undergo major metabolic rearrangements in order to favor survival in the delicate balance between hibernation and apoptosis[27] (Fig. 1). The term "hibernation" refers to a condition of severe energy deprivation of the myocardium

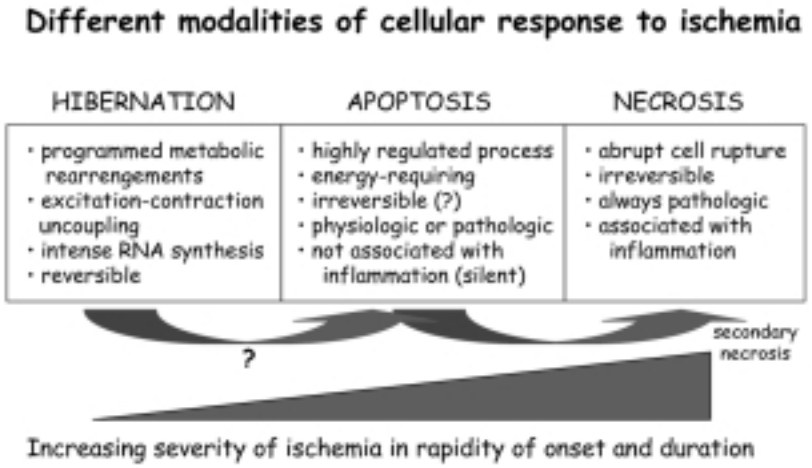

Figure 1. *Hibernation, apoptosis and necrosis represent three possible modalities of the cellular response to stress (such as hypoxia) leading to different solutions. Hibernation is associated with cell survival and reversible metabolic changes. Apoptosis and necrosis (oncosis) both lead to myocardiocyte loss; however, while apoptosis causes silent but persistent loss, necrosis is associated with the abrupt onset and clinical manifestation associated with secondary inflammatory phenomena. From Majno and Joris[4], modified.*

due to chronic or repetitive underperfusion associated with reversible contractile dysfunction[2,28,29].

Underperfused myocardium retains its viability by down-regulating its function, thereby regaining the balance between the request for and the availability of oxygen. Structural and functional alterations occurring in hibernating myocardium include the expression of fetal proteins, suggesting the use of the term of "dedifferentiated" myocardiocytes[29,30]. Under the same experimental conditions of chronic underperfusion, cells with metabolic down-regulation coexist with apoptotic cells[30-33]. Apoptosis therefore determines myocardiocyte loss even in chronic ischemia; however, whether hibernation constitutes a prelude to apoptosis is unknown[27,30] (Fig. 1). With regard to this issue, Elsässer et al.[34] have suggested that myocardial apoptosis may be responsible for the incomplete recovery of cardiac contractility after coronary revascularization.

Experimental and clinical studies

Findings in animal models. In mice and rats left coronary artery ligation is associated with the development of progressive HF after AMI in those animals surviving for at least 12 to 18 weeks after surgery[24-26]. Palojoki et al.[24] have shown that AMI in rats is associated with a persistently elevated apoptotic rate at the border zone up to 12 weeks later and that apoptosis is correlated with the end-diastolic left ventricular diameters as assessed at echocardiography. Similar results in mice were reported by Sam et al.[25] who showed increasing apoptotic rates up to 6 months after surgery (in this case assessed only in remote and unaffected left ventricular regions) and by Prabhu and Chandrasekar[26], with a 30-fold increase in the apoptotic rate in post-AMI (0.6 vs 0.02% in sham-operated rats). A gradual decrease in the apoptotic rate over time has been shown; however, in any case apoptosis was still demonstrable at a low but relevant rate (ranging from 0.02 to 1.10%) up to 3 to 6 months after AMI[24,35].

Likewise, transcatheter coronary embolization with polystyrene latex microspheres causing multiple AMIs in dogs is associated with the occurrence of left ventricular dilation weeks to months after the procedure[36]. In this experimental model of ischemic heart failure, Sharov et al.[37] have shown that 3 to 4 months after the last embolization, a significantly higher number of apoptotic myocardiocytes were found in dogs with post-infarction left ventricular remodeling compared to sham-operated animals. Interestingly, even in this model (up to 4 months after AMI) a statistically significant difference in the localization of apoptotic cells was found, with a nearly 20-fold increased apoptotic rate at the border of infarction scars. This model was subsequently confirmed in two other studies by the same authors[38,39] and elevated apoptotic rates with a preferential localization at the site of infarction scars was confirmed by the same authors in failing human hearts[21]. Multiple coronary embolization was associated with the development of HF and with an increased apoptotic rate even in sheeps[30].

Observational studies in humans. Olivetti et al.[7], in 1996, showed for the first time that myocardial apoptosis was present in the hearts of patients who died within 10 days of AMI. Apoptotic rates of 11.6% (range 1-26%) and of 0.74% were found in the zone bordering the infarction and in the remote regions respectively, leading to significant myocardiocyte loss. Similar qualitative data have been presented by Veinot et al.[8]. Several studies have assessed the process of apoptosis in hearts explanted from patients undergoing cardiac transplantation due to intractable HF. Narula et al.[19], in 1996, have shown significantly increased apoptosis in four hearts of patients with end-stage HF due to idiopathic dilated cardiomyopathy and in 1 out of 3 cases of HF due to severe coronary artery disease (apoptotic rate of 17.3%). Olivetti et al.[20], a few months later, have shown a more than 200-fold increase in the rate of myocardial apoptosis in 8 cases of end-stage ischemic cardiomyopathy vs controls (0.24 vs 0.001% respectively). In the data later presented by Saraste et al.[22], not only was apoptosis increased in cases vs controls, but the apoptotic rates also correlated with the clinical severity of HF with a preferential localization at the site of the infarction scars. Anecdotic data in their paper[22] showed the highest apoptotic rate in the only patient with a previous AMI within 12 months before transplantation (14%). Similar results have been briefly presented by Angelini et al.[40] showing a median of 8.9% of apoptotic myocardiocytes in biopsies taken from patients with ischemic cardiomyopathy.

The first study designed to assess the persistence of apoptotic myocardiocytes late after AMI in humans was published only recently[23]. Baldi et al.[23] have shown data supporting a regional occurrence of myocardial apoptosis at the site of a recent infarction vs remote unaffected areas at the time of autopsy up to 60 days after AMI (25.4 vs 0.7%) (Fig. 2). Although the accurate interpretation of these findings is a limiting factor (as the exact duration of the apoptotic cascade *in vivo* is unknown), these data show that persistent myocardiocyte loss still occurs during the subacute phases of AMI. Moreover, these same authors also reported a strong correlation between the apoptotic rate and macroscopic signs of left ventricular remodeling[23].

Variability in the estimated myocardial apoptosis. The different studies presented showed a wide variability in the estimated apoptotic rates among different series and also among different individuals within each study. Although conclusions should be drawn with caution, these data suggest the existence of a probable modulation of apoptosis in post-infarction left ventricular remodeling.

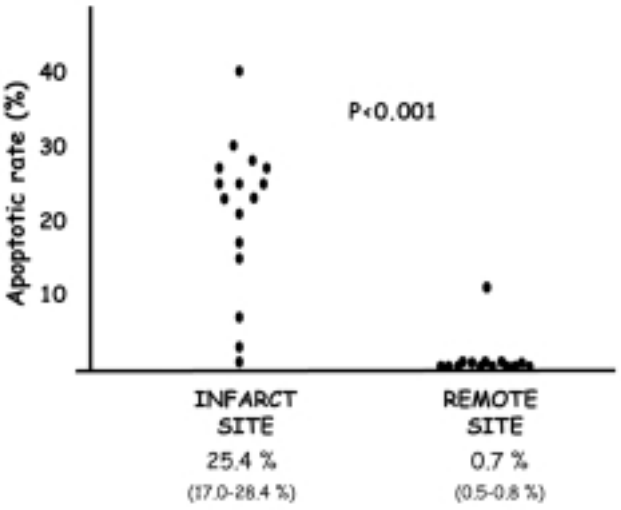

Figure 2. *The regional increase in myocardial apoptosis at the site of infarction late after acute myocardial infarction (25.4%) vs remote unaffected left ventricular regions (0.7%, p < 0.001). From Baldi et al.[23], modified.*

The comparison between different studies is fraught with severe inherent limitations and probably is of limited usefulness since these studies differed in the definitions of the cases, in the selection of the histological samples and in the methods used to assess apoptosis[41,42]. Indeed, one major limitation when comparing animal studies to findings in humans are the selection criteria. Most of the animal studies have included in the analysis only those animals surviving late after AMI and being electively sacrificed. Such a methodology does not consider the fact that approximately 30% of animals die spontaneously[5,6,25]. On the other hand, some studies in humans have selected patients dying, rather than surviving, late after AMI[7,23] and other studies have evaluated living patients at the time of cardiac transplantation or by means of biopsies[19-22,40]. These selection biases may in part explain the differences in the estimated apoptotic rates in the different studies.

Considering in detail the factors associated with increased apoptosis, however, some studies need to be mentioned. Guerra et al.[43] have shown that the female sex is relatively protected from apoptosis in end-stage HF. In post-infarction left ventricular remodeling, a 4-fold higher apoptotic rate in males vs females was present (Baldi A., personal communication), suggesting a role of apoptosis in determining the different pathologic and clinical evolutions of cardiac remodeling in women[44]. As suggested by clinical and pathological data[22,45], apoptotic rates tended to be higher in those patients with more severe left ventricular remodeling. Moreover, one of the major clinical determinants of left ventricular remodeling after an AMI, is the presence of an open or of an occluded infarct-related artery (IRA)[46]. Abbate et al.[47] have shown that potential benefits of the IRA patency after AMI (the "open-artery hypothesis"[48]) may be in part due to a reduced apoptosis at the site of infarction in those cases with a patent artery at the time of death. In their series, an occluded IRA was associated with a 10-fold higher apoptotic rate at the site of infarction vs cases with a patent artery and this association remained statistically significant even after correction for several clinical characteristics such as sex, age, a history of a previous additional AMI and/or HF, transmural AMI, anterior AMI, fibrinolytic treatment, time from AMI to death, trauma as the cause of death and multivessel coronary disease[47]. Interestingly, in previous studies the apoptotic rate at the border of the infarction in AMI in humans with an occluded IRA[7] was as high as 11.6% (range 1-26%), while it was strikingly lower in the hearts of subjects who died after AMI but who were found to have a patent artery at the time of death[49] (median 0.8%) (Fig. 3). These data may in part explain the "open-artery hypothesis"[46,48]. Reduced myocardial apoptosis may be associated not only with a reduced myocardiocyte loss and with a less severe contractile dysfunction but also with a less marked electrical instability. In fact, myocardiocyte loss due to apoptosis occurring in arrhythmogenic right ventricular dysplasia is associated with progressive replacement by fat and fibrous tissue and with death due to ventricular arrhythmias[50].

However, there are many still unresolved issues regarding the role of apoptosis in the pathophysiology of ischemic injury and HF[51]. In particular, the sensitivity and specificity of the methods used to assess myocar-

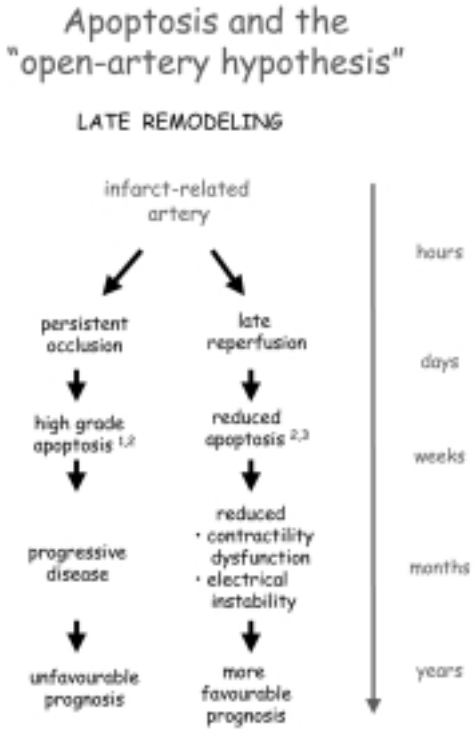

Figure 3. *The long-term beneficial effects associated with the patency of the infarct-related artery ("open-artery hypothesis") may be due to reduced apoptosis at the site of infarction. From Olivetti et al.[7], Abbate et al.[47], and Saraste et al.[49], modified.*

dial apoptosis have been questioned. While the *in situ* end-labeling of DNA fragmentation (TUNEL) is currently the most widely used technique allowing the easy qualitative and quantitative evaluation of apoptosis, many studies suggest that it should not be the sole method used in experimental models[23,52-54]. Other unresolved issues are the uncertainties regarding the time needed for the completion of apoptosis from the stimulus to DNA fragmentation, the reversibility of apoptosis, the causal role of apoptosis in HF and the reliable quantification of the apoptotic rate in order to assess the true impact of apoptosis on myocardiocyte loss.

Therapeutic perspectives

In the last decades significant improvements in the management of patients with HF and, more specifically, those with a previous AMI have been made. Several studies have shown major benefits of ACE-inhibitor treatment in limiting left ventricular remodeling and in reducing morbidity and mortality[55]. Similar results have been shown for beta-blocker therapy[56]. A working hypothesis to explain the benefits achieved with these treatments is a selective reduction of myocardiocyte loss due to apoptosis in ischemic HF.

To this end, Goussev et al.[38] and Sabbah et al.[39] have compared the occurrence of myocardial apoptosis in a dog model of ischemic HF[36] in placebo-treated animals vs animals treated with enalapril or metoprolol respectively. In both cases they have shown, for the therapeutic regimens compared to placebo, a significant reduction in the number of apoptotic myocardiocytes, both at the site of AMI and in remote areas. A 70% reduction in apoptosis was obtained with 10 mg daily of enalapril[38] and treatment with 25 mg of metoprolol twice daily was associated with a higher than 90% reduction in apoptotic rates[39]. Beneficial effects of metoprolol in reducing post-AMI apoptosis by about 50% were also shown in rats[26], and carvedilol was found to be equally effective in rabbits[57]. Whether ACE-inhibitors and beta-blockers reduce apoptosis through a direct or indirect mechanism is unknown. Certainly they favorably affect hemodynamics and the cardiac pre and afterload; however, there is evidence that angiotensin II (via both the AT-I and AT-II receptors) and $beta_1$-adrenergic stimulation activate the apoptotic cascade *in vitro*[58-60]. Angiotensin II and catecholamines may therefore be valid candidates as soluble proapoptotic factors in the context of the neurohormonal derangements in HF.

Caspase inhibition by treatment with ZVAD-fmk (a selective apoptosis-inducing factor blocker) in mice was associated with a promising 70% reduction in TUNEL positive cells[61]. Potential benefits of stem-cell or myoblast transplantation need to be further assessed[62,63]. The occurrence of myocardiocyte regeneration in AMI[64] supports the concept of plasticity of the human heart after ischemic insults, oscillating in a balance between death, survival and regeneration[65].

However, to date the only conservative treatments resulting in definite clinical benefits in humans, presumably in part due to reduced apoptosis, are medical therapy with ACE-inhibitors and beta-blockers and prompt reperfusion of the ischemic myocardium. Clinical trials with caspase inhibitors are on their way[66].

Conclusions

Left ventricular remodeling and HF complicate AMI even weeks to months after the initial insult. Experimental animal models and pathologic studies in humans show that apoptosis may represent an important pathophysiological mechanism in the post-infarction state causing progressive myocardiocyte loss and left ventricular dilation even late after AMI. A more complete definition of the impact of ongoing myocardiocyte loss on prognosis and of the mechanisms involved may lead to an improved comprehension of cardiac remodeling and possibly to an improved patient care.

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