Apoptosis in hypertensive heart disease: a clinical approach

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Purpose of review

It is widely accepted that there are two principal forms of cell death, namely, necrosis and apoptosis. According to the classical view, necrosis is the major mechanism of cardiomyocyte death in cardiac diseases.

Recent developments

In the past few years observations have been made showing that cardiomyocyte apoptosis occurs in diverse conditions including hypertensive heart disease, and that apoptosis may be a contributing cause of loss and functional abnormalities of cardiomyocytes in this condition.

Summary

This review will summarize recent evidence demonstrating the potential contribution of cardiomyocyte apoptosis to heart failure in hypertensive patients. In addition, some strategies aimed to detect and prevent apoptosis of cardiomyocytes will be considered.

Keywords

apoptosis, cardiomyocytes, heart failure, hypertensive heart disease

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Abbreviations

ATP	adenosine triphosphate
HHD	hypertensive heart disease
IGF-I	insulin-like growth factor-I
SHR	spontaneously hypertensive rats

Introduction

It has been classically accepted that adult cardiomyocytes are not capable of proliferation and, thus, are resistant to developing apoptosis. Thus, the existence of a balance between apoptotic cell death and cell regeneration in the heart has been denied until recently. In the past decade, however, observations have been made showing that cardiomyocyte apoptosis occurs in diverse conditions [1,2]. One of these conditions is hypertensive heart disease (HHD), here defined by the presence of a greater than normal left ventricular mass in the absence of a cause other than arterial hypertension and by the development of complex changes in myocardial composition that are responsible for the structural remodeling of the myocardium (Table 1). One of these changes is a diminished number of cardiomyocytes due to enhanced cell death, including apoptotic cell death. In fact, cardiomyocyte apoptosis has been shown to be abnormally stimulated in the hypertrophied left ventricle of patients with essential hypertension, no angiographic evidence of coronary artery disease and normal cardiac function [3,4]. In addition, recent findings from our laboratory show that cardiomyocyte apoptosis is increased in patients with HHD and chronic heart failure compared with hypertensive patients with HHD and normal cardiac function [5] (Fig. 1). Thus, it seems that cardiomyocyte apoptosis precedes the impairment in ventricular function and its exacerbation accompanies the development of heart failure in patients with HHD.

Mechanistic links between cardiomyocyte apoptosis and heart failure in hypertensives

Arterial hypertension affects the myocardium at two different stages [6]. In both humans and animal models, pressure overload is characterized by a period of compensation in which left ventricular concentric hypertrophy normalizes systolic wall stress and contractile function is preserved. The period of adaptation, which may last for weeks in rodents and months to years in humans, is inexorably followed by a transition to cardiac failure. This transition is characterized by impaired survival, the onset of chamber dilatation with the failure of further concentric hypertrophic growth to normalize load, and progressive contractile dysfunction. A number of observations suggest that beside changes in the composition of motor unit and cytoskeleton of cardiomyocytes, the transition from hypertrophy to failure relates mainly to alterations in the histological composition of the

Table 1 Main histological components of hypertensive myocardial remodeling

Cellular changes
  Hypertrophy of cardiomyocytes
  Decreased number of cardiomyocytes
  Increased number of fibroblasts
  Phenotypic differentiation of fibroblasts into myofibroblasts
  Presence of abundant inflammatory cells and mast cells

Noncellular changes
  Interstitial and perivascular fibrosis
  Wall thickening of intramyocardial vessels
  Reduced number of capillaries

myocardium as a result of both alterations in the metabolism of extracellular matrix (namely, fibrillar collagen) and cardiomyocyte loss due to multiple mechanisms of death (including apoptosis). Beside reduction in the number of cardiomyocytes, apoptosis may contribute to heart failure through additional pathways [7••,8••] (Fig. 2).

Apoptosis and structural and geometric remodeling

It has been suggested that alterations of the collagen framework in the myocardium may play an important role in the genesis of diastolic dysfunction of hypertensive origin [9•]. This has been supported by the finding that fibrillar collagen deposition in the cardiac interstitium of hypertensive patients increases left ventricular chamber stiffness and compromises left ventricular filling during diastole [10]. The problem concerns whether this type of interstitial alteration occurs through activation of fibroblasts via humoral or mechanical factors in the absence of cardiomyocyte loss, or whether cell death is required for the stimulation of the growth response of the noncardiomyocyte compartment of the myocardium. The observation that fibrosis is associated with cell loss in the left ventricle of hypertensive patients [11] and patients with

aortic stenosis [12] raises questions about the mechanism responsible for the modification of the interstitium with accumulation of fibrillar collagen. As proposed by Anversa *et al.* [13], death of individual cardiomyocytes may stimulate discrete healing processes contributing to the expansion of the interstitium. This proposal is further supported by the experimental finding that failing hearts from spontaneously hypertensive rats (SHR) present colocalization of collagen α_1 type I gene expression to areas of focal cardiomyocyte degeneration [14], suggesting that cardiomyocyte loss is associated with collagen type I production and focal scar formation in these animals during the transition from hypertrophy to failure.

Besides histologic remodeling of the myocardium, cardiomyocyte apoptosis may also contribute to geometric remodeling of the left ventricular chamber. In fact, severe cardiomyocyte apoptosis may lead to side-to-side slippage of cells, mural thinning and chamber dilatation. Thus, wall restructuring secondary to severe cardiomyocyte apoptosis may create an irreversible state of the myocardium, conditioning progressive dilatation and the continuous deterioration of cardiac hemodynamics and ventricular performance with time [15••].
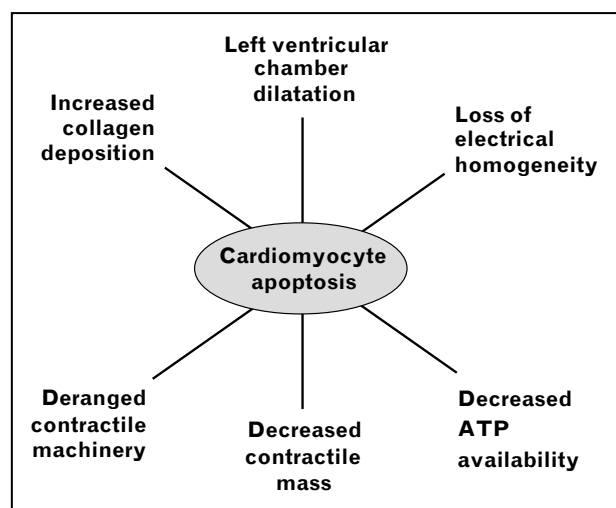
Apoptosis and energy production

It is known that energy metabolism is deranged in the human hypertensive and failing myocardium. Although recent findings suggest that altered myocardial fatty acid metabolism may account for this abnormality in hypertensive patients [16], the possibility also exists that mitochondrial production of adenosine triphosphate (ATP) is diminished in the hypertensive myocardium [17]. In this setting it is interesting to consider that apoptosis is associated with loss of cytochrome *c* from

Figure 1 Cardiomyocyte (CM) apoptosis (left panel) and density (right panel) in patients with hypertensive heart disease and normal cardiac function (non-heart failure) or clinical manifestations of heart failure (HF)
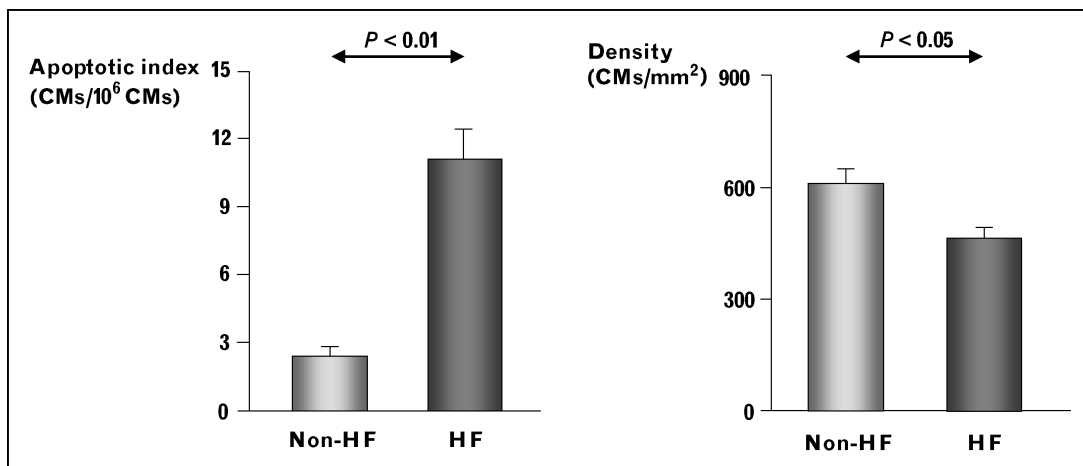
Adapted from [5]

Figure 2 Pathways to heart failure activated by increased cardiomyocyte apoptosis in hypertensive heart disease

the mitochondria and this may halt oxidative phosphorylation and production of ATP, namely in subsarcolemmal mitochondria [18]. Nevertheless, as apoptosis is an energy-requiring process, in conditions of severely diminished ATP availability the mode of cell death would switch to necrosis. One might then predict that apoptotic stimuli do not necessarily induce complete loss of cytochrome c from the mitochondria. Thus, the contribution of the mitochondrial pathway of apoptosis to the energy balance of the hypertensive myocardium remains to be clarified.

Apoptosis and contractile mass
As previously mentioned, increased cardiomyocyte apoptosis and diminished cardiomyocyte number have been found in the failing hearts of hypertensive patients [5••]. This has been confirmed at the experimental level in SHR with heart failure [19]. Thus, apoptosis may be one of the mechanisms involved in the loss of contractile mass and function in HHD. At present, however, there is no information concerning the magnitude of cell loss required to depress cardiac contractility in the hypertrophied human heart when cell death occurs. Although most studies examining apoptosis in the end-stage heart report variable but low apoptosis rates (0.1–0.5% of cardiomyocytes), conservative assumptions about the duration of apoptosis and the constancy of the implied rate of cell death suggest that apoptosis could result in the loss of up to 5–10% of the myocardium per year [20]. Although the progressive nature and poor survival in late-stage heart failure may well be consistent with such a substantial loss of cardiomyocytes, it means that the heart should rapidly disappear. This contention, however, does not consider that the adult heart possesses a stem cell compartment that can regenerate cardiomocytes and coronary vessels [21••]. This does not imply that cell

replication compensates for the extent of apoptotic loss in the diseased myocardium, but allows the hypothesis that inadequate cardiomyocyte division may be a critical event in the evolution of the pathological heart to heart failure [22].

Apoptosis and contractility
Impaired myocardial contractile function may reflect not only a decrease in the number of viable, fully functional cardiomyocytes, but also a decrement in the function of viable cardiomyocytes, or a combination of these mechanisms. It is well known that apoptosis is associated with activation of caspases that mediate the cleavage of vital and structural proteins. Caspase-3 cleaved cardiomyocyte myofibrillar proteins resulting in an impaired force/Ca^{2+} relationship and myofibrillar ATPase activity have been reported [23]. In addition, it has been demonstrated that caspase activation is associated with cleavage of myofilaments, disruption of sarcomeric structure and reduction in contractile force of failing cardiomyocytes, and that blockade of caspase activation improves contractility in failing myocardium [24•]. As apoptosis may not be complete in cardiomyocytes, allowing the cells to persist for a prolonged period within the myocardium, the detrimental effects of caspase activation on systolic function should not be underestimated. This possibility is particularly relevant taking into account that an intense expression of the active form of caspase-3 has been reported in cardiomyocytes from patients with HHD and heart failure [25].

Apoptosis and electrical activity
HHD is associated with increased incidence of both ventricular arrhythmias and sudden cardiac death, namely when heart failure is present [26]. It has been proposed that cardiomyocyte apoptosis may contribute to the development of arrhythmias in the failing heart through two ways [27]. First, in the progress of dying, a cardiomyocyte passes through phases of increased excitability or becomes automatic, at least until it is dead. Second, from a random grouping of several such dead cells, the process of normal activation in that area of heart muscle must be deranged and redirected in a way that would provide a suitable anatomical substrate for re-entrant arrhythmias.

Clinical recognition of cardiac apoptosis
Thus far, methods by which to measure apoptosis were designed for in-vitro analysis. Most of these methods require tissue biopsy specimens to be obtained from the patient to retrieve information of apoptosis *in situ*. This limits the application of these apoptosis detection methods in clinical practice. A noninvasive detection method by which to visualize apoptosis would be ideal to assess its role in disease and to measure treatment outcome in diseases such as HHD.

Imaging studies

Some molecular changes characteristic of apoptosis have been proposed as potential markers to be used in imaging techniques (e.g. nuclear magnetic resonance and radionuclide imaging) to detect previously inaccesible and unrecognized biological phenomena in living cells and tissues undergoing apoptosis [28,29].

For instance, apoptosis activates mechanisms which cause the translocation of phosphatidylserine from the internal to the external leaflet of the plasma membrane [30]. Annexin V is a phospholipid binding protein, which, in the presence of Ca^{2+} ions, specifically and reversibly interacts with the phosphoserine headgroup of phosphatidylserine in the apoptotic cell [31] (Fig. 3). This property has been the driving force for the research of annexin V conjugated with a detectable marker, such as biotin, a fluorochrome, or a radioligand, as a probe to measure apoptosis *in vitro* and *in vivo* in animals and patients [32]. Technetium-99 m labeled annexin V has been successfully used for noninvasive γ-imaging of cardiac apoptosis after acute myocardial infarction, acute myocardial ischemia, acute cardiac allograft rejection and malignant intracardiac tumors [33].

Biochemical assessment

Besides imaging studies, determination of circulating annexin V may be also useful for the biochemical monitoring of the apoptotic process. In this respect, it has been reported in humans that plasma levels of annexin V determined by means of ELISA are increased 8-fold in the early phase of acute myocardial infarction, and immediately decrease after the onset of pain [34]. Other circulating markers of apoptosis are currently under investigation. For instance, it has been recently

Figure 3 The arrow signals an annexin V-positive cardiomyocyte and the arrowhead signals a control nonstained cardiomyocyte
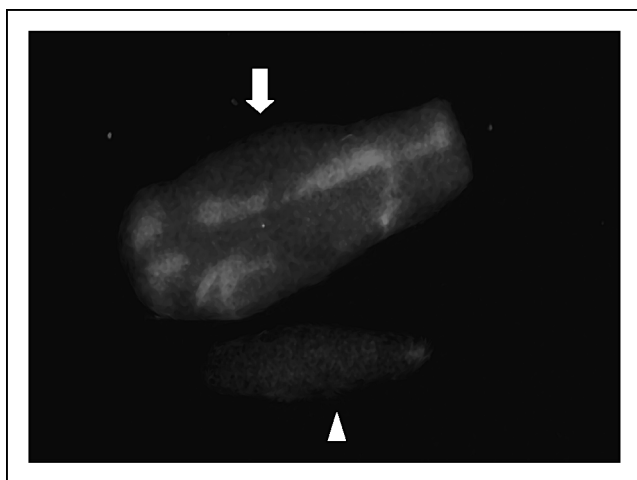
Annexin V staining was detected with tetramethylrhodamine-conjugated extravidine.

reported that during apoptosis cytochrome *c* not only translocates into the cytosol but is secreted to the extracellular medium [35]. Thus, its potential role as a serum marker of cardiac apoptosis in chronic cardiomyopathies, including HHD, remains to be investigated.

Therapeutic modulation of cardiac apoptosis

It has been postulated that the inhibition of cardiomyocyte apoptosis could prevent or slow cardiac failure progression, thus opening new strategies in the treatment of cardiac diseases [36••,37••]. Cardiomyocyte apoptosis may be inhibited by suppressing the systemic and local factors that trigger the process, by directly blunting the intracellular apoptotic pathways or by inducing the survival pathways.

Strategies aimed to blunt triggers of the apoptotic process

The main systemic trigger of apoptosis to be considered in HHD is arterial hypertension itself. The in-vivo effects of antihypertensive drugs on cardiac apoptosis in SHR have been reviewed elsewhere [38]. Collectively, the available findings suggest that the ability of antihypertensive drugs to inhibit cardiac apoptosis is independent of their antihypertensive efficacy, but can be related to their capacity to interfere with the pro-apoptotic actions of humoral factors. This is further supported by clinical findings showing that despite an identical antihypertensive efficacy, the angiotensin II type 1 receptor antagonist losartan, but not the Ca^{2+} channel blocker amlodipine, reduced cardiomyocyte apoptosis in patients with HHD after 1 year of treatment [4].

Oxidant stress is an important local factor inducing cardiomyocyte apoptosis. Thus, antioxidant therapy, whether through administration of additional antioxidants or boosting innate antioxidant mechanisms, has been shown to be a viable approach in reducing cardiac apoptosis in experimental conditions [39•,40•]. In the clinical setting, agents with antioxidant properties such as carvedilol or those that promote antioxidant activity, such as propranolol, are already used to treat patients with heart failure [41]. One ongoing study is testing the effect of oxypurinol, the major metabolite of the xanthine oxidase inhibitor allopurinol, in patients with heart failure [42]. The rationale for this study is that oxypurinol has been reported to substantially improve the cardiac structure and function in SHR with heart failure [43] and that xanthine oxidase has been identified as a source of reactive oxygen species in patients with heart failure [44].

Strategies aimed to interrupt the apoptotic process

The past decade has witnessed tremendous progress in the knowledge of the molecular mechanisms that regulate and execute apoptosis at the intracellular level. Consequently, apoptosis regulators and executors have

Table 2 Potential molecular targets of the apoptotic machinery

At the level of the death receptor pathway
    Blockade of the tumor necrosis factor receptor superfamily of
       death receptors
    Inhibition of adapter proteins
    Inhibition of non-executioner caspases -8 and -10

At the level of the mitochondrial pathway
    Inhibition of the pro-apoptotic Bcl-2 family members
    Stimulation of the antiapoptotic Bcl-2 family members
    Blockade of the release of cytochrome c in the cytosol
    Blockade of the second-mitochondria-derived activator of caspases
    Interruption of the assembly of Apaf-1 and the
       non-executioner caspase-9

At the global level
    Inhibition of the executioner caspases -3, -6, -7
    Stimulation of the members of the inhibitor of apoptosis family

emerged as key targets for the design of therapeutic strategies aimed at interrupting cellular death decisions [36[••],45[••]] (Table 2).

For instance, increased survivin (a member of the inhibitor of apoptosis [IAP] family) myocardial expression in aging SHR with heart failure is associated with reduced cardiomyocyte apoptosis and more favorable echocardiographic indexes of left ventricular remodeling and dysfunction [46[•]]. On the other hand, overexpression of human antiapoptotic protein Bcl-2 decreases cardiac apoptosis and improves cardiac function in transgenic mice after ischemia/reperfusion [47]. By contrast, targeted deletion of pro-apoptotic members of the Bcl-2 protein family in a mouse model of cardiac ischemia/reperfusion attenuated both apoptosis and necrosis [48[•]]. Similar effects have been reported with low molecular weight caspase inhibitors [49[•]]. Interestingly, some of these inhibitory compounds were effective not only in reducing cardiomyocyte apoptosis but also in improving cardiac function and delaying heart failure development [50].

Other studies are focusing on the stimulation of the ubiquitin–proteasome system that contributes to regulation of apoptosis through the degradation of apoptosis-regulatory proteins. This is based on the recent observation that depression of proteasome activities in pressure-overloaded hearts of mice contributes to cardiac dysfunction resulting from cardiomyocyte apoptosis through accumulation of pro-apoptotic proteins by impaired degradation [51[••]].

**Strategies aimed to stimulate cellular survival mechanisms**

An alternative strategy to prevent cardiomyocyte apoptosis is the improvement of cellular survival mechanisms [52[••]]. For instance, cardiotrophin-1 has been shown to protect cardiomyocytes against angiotensin II and oxidative stress-induced cell death via gp130/LIFR and by means of the PI3K/Akt and p42/44 MAPK intracellular cascades [53]. These aspects can be of particular relevance taking into account that molecular and functional alterations of the gp130 survival pathway have been described recently in failing human hearts [54].

Another important cardiomyocyte survival factor is insulin-like growth factor-I (IGF-I). In fact, in-vitro and in-vivo studies have shown that IGF-I decreases experimentally induced cardiomyocyte apoptosis through the activation of signaling pathways similar to those activated by cardiotrophin-1 [55[•]]. Interestingly, in clinical studies with patients with heart failure, IGF-I levels are low and correlate with the severity of left ventricular systolic dysfunction [56].

Novel therapeutic approaches

Several new treatment strategies are currently being explored in chronic heart failure, which, among other effects, have been shown to decrease cardiomyocyte apoptosis either clinically or experimentally. These

Figure 4 Strategies to preserve the population of functional cardiomyocytes in hypertensive heart disease
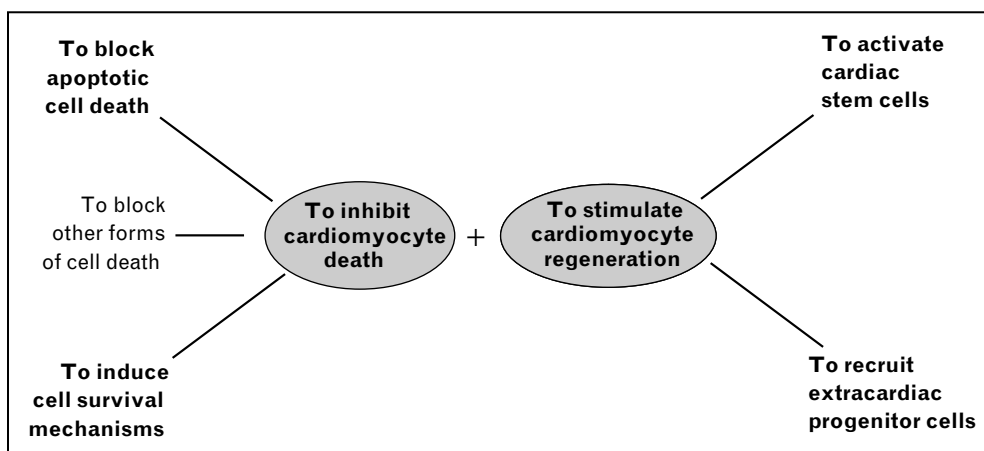
include medical therapy with mineralocorticoid receptor antagonists [57], calcium-sensitizing agents [58], statins [59] and erythropoietin [60], and device therapy aimed to induce cardiac resynchronization [61•].

Conclusion

Numerous hypotheses have been considered to explain the fundamental mechanism(s) for the development of heart failure in patients with HHD. Cardiomyocyte loss and dysfunction due to apoptosis is now being considered as one of the determinants of the maladaptive processes implicated in the transition from left ventricular hypertrophy to heart failure in hypertensive patients. Much work is being carried out regarding the mechanisms and the impact of cardiomyocyte apoptosis on the hypertensive myocardium but several methodological and conceptual issues still remain unsolved. Clarification of these is extremely urgent if one considers that the development of noninvasive tools for the monitoring of cardiac apoptosis and pharmacological strategies aimed to inhibit and/or counteract the apoptotic process could be of particular relevance to protect the cardiomyocyte and prevent the progression to heart failure in patients with HHD (Fig. 4).

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