Cardiomiocyte apoptosis in hypertensive cardiomyopathy

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Abstract

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. However, in the past few years, observations have been made showing that cardiomyocyte apoptosis occurs in diverse conditions and that apoptosis may be a contributing cause of the loss and functional abnormalities of cardiomyocytes with important pathophysiological consequences. In this regard, although a number of formal proofs are pending, it is conceivable that cardiomyocyte apoptosis may be an important variable in the clinical evolution of hypertensive cardiomyopathy. This review summarizes recent evidence demonstrating that cardiomyocyte apoptosis is abnormally stimulated in the heart of animals and humans with arterial hypertension. In addition, the potential mechanisms of cardiomyocyte apoptosis in hypertension and its detrimental impact on cardiac function will be addressed. Finally, the perspectives of strategies aimed to detect and modulate apoptosis of cardiomyocytes in hypertensive cardiomyopathy will be considered.

Keywords: Apoptosis; Arterial hypertension; Cardiomyocytes; Heart failure

1. Overview of apoptosis

Cell death occurs by necrosis or apoptosis [1]. These two processes have distinct histologic and biochemical characteristics. In necrosis, the stimulus of death (e.g., ischemia) is itself often the direct cause of demise of the cell. In apoptosis, by contrast, the stimulus of death activates a cascade of events that orchestrate the destruction of the cell. Unlike necrosis, which is a pathologic process, apoptosis is part of normal development (physiologic apoptosis); however, it also occurs in a variety of diseases (pathologic apoptosis).

1.1. The apoptotic process

Apoptosis is a form of cell death that results in loss of cell volume, plasma membrane blebbing, nuclear condensation, chromatin aggregation, and endonucleocytic degradation of DNA [2,3]. Apoptotic death occurs as a consequence of the activation of cell surface death receptors (e.g., tumor necrosis factor receptor superfamily of proteins, TNFR, and Fas receptor) by extracellular ligands (e.g., TNF and Fas Ligand, FasL) [4,5] and/or the activation of mitochondrial-related pro-apoptotic mechanisms in response to unfavorable changes in the intracellular environment [6,7].

After ligand binding, death receptors interact with a variety of death domain adaptors, such as FADD,
TRADD, RIP, and Daxx, which act downstream of the activated death receptor to activate a cascade of cell signaling pathways (e.g., MAP kinase, NF-kB and Akt) that regulate gene expression and the phosphorylation status of proteins (e.g., Bcl-2 proteins and IAP proteins) [8,9], which, in turn, modulate the activity of different families of proteases (e.g., caspases, cathepsins and calpains) involved in the execution of the final steps of the apoptotic process [10–12]. Whereas the stimulation of some MAP kinase pathways is associated with pro-apoptotic effects (e.g., JNK and p38), activation of other MAP kinase pathways is accompanied by anti-apoptotic effects (e.g., ERK) [13]. On the other hand, stimulation of NF-κB and Akt pathways mediates anti-apoptotic effects [14,15]. The balance between negative and positive regulation by these signaling pathways is thought to be essential for the determination of cell fate.

In addition to their established role in energy production, mitochondria are actively involved in the regulation of apoptosis. In essence, changes in the internal environment of the cell may modify the permeability of the outer mitochondrial membrane through changes in membrane potential, thus facilitating the release into the cytosol of a number of highly lethal mitochondrial substances that can initiate apoptosis. One of these is the small electron transporter cytochrome c that forms an apoptosis-promoting complex, the apoptosome, with procaspase-9 and its cofactor apoptotic protease-activating factor-1 (Apaf-1), thus activating caspase-9 and, subsequently, caspase-3 and other caspases [16]. It appears that caspase activation not only requires proteolytic cleavage of the enzymes themselves, but removal of inhibitory influences (e.g., IAPs) is also necessary. In this setting, mitochondria can also release a specific inhibitor of IAPs: second mitochondria-derived activator of caspases (Smac), also known as direct IAP-binding protein with low pi (Diablo) [17].

In recent years, major progress has been made in understanding that whether apoptosis is initiated through the death receptor pathway or through the mitochondrial pathway, interactions between the two pathways may occur [18]. For example, the pro-apoptotic protein Bid is cleaved by caspase-8 in response to FAS and TNF receptor activation [19,20]. Following cleavage, the C-terminal fragment of Bid translocates and binds to the mitochondria, leading to the release of cytochrome c and activation of downstream caspases. Another example is that of the apoptosis signal-regulating kinase 1 (ASK1), which is a member of the MAP kinase family, which activates JNK and p38 activates and executes apoptosis mainly by mitochondria-dependent caspase activation, probably involving the phosphorylation and inactivation of the anti-apoptotic protein Bcl-2 that prevents the release of cytochrome c [21]. Thus, Bcl-2 family proteins are considered as mediators linking the death receptor signals to the mitochondria-dependent death signals [22,23].

1.2. Apoptosis in the cardiomyocyte

A number of different experimental models have been used to demonstrate the ability of the cardiomyocyte to activate and die by apoptosis in response to a range of stresses, including work overload, hypoxia, neuro-humoral overstimulation, free radical stress, viral infection and toxic insults [24]. Although no single model perfectly represents the conditions in the adult human heart or the biochemical properties of the adult human ventricular cardiomyocyte, these studies have shown that these cardiomyocytes possess the necessary machinery for apoptosis.

In fact, Fas receptor and TNFR-1 are expressed in cardiomyocytes and are induced by various types of oxidative and toxic stresses [25,26]. Complex alterations in the levels of Bcl-2 proteins occur in various forms of myocardial stress, including mechanical deformation secondary to hemodynamic loading [27], hypoxia–reoxygenation [28], and nitric oxide (NO) exposure [29]. These studies show that the levels of both pro-apoptotic and anti-apoptotic Bcl-2 proteins can increase in response to myocardial stress and are associated with changes in mitochondrial membrane potential [30]. The release of cytochrome c has been shown in cardiomyocytes in response to serum and glucose deprivation, redox stress and constitutive activation of Gαq protein signaling [31–33]. Factors that signal through Gαq-coupled receptors, including α1-adrenoceptor agonists, angiotensin II, and endothelin-1, are potent hypertrophic stimuli; however, sustained, high level activation of Gαq signaling can be accompanied by cardiomyocyte apoptosis due to activation of the mitochondrial death pathway [33]. Interestingly, activated JNKs are detected in the mitochondria of adult myocytes subjected to hypoxia, and are associated with the apoptotic response [34], thus suggesting that some MAP kinases may directly activate the mitochondrial apoptotic machinery in these cells. On the other hand, activation of various combinations of MAP kinases occurs in response to ischemia–reperfusion, β-adrenergic stimulation, NO and anthracycline exposure and has been implicated in survival signaling in cardiomyocytes [35–37]. Finally, the ability of some hypertrophic agents (e.g., insulin-like growth factor-1, IGF-1, and cardiotrophin-1) to promote cardiomyocyte survival correlates with activation of the Akt pathway [38,39].

It is clear, therefore, that, although there is the potential to induce cardiomyocyte cell death, stimulation of either the TNFR or the FAS receptor does not necessarily do so. Both receptors have the potential to activate NFκB, which is cytoprotective in many systems, but activation of caspases might be expected to occur within the time required for NFκB-dependent transcription/translation to take effect. Thus, although NFκB may mediate cardiomyocyte cytoprotection, this is likely to be a longer-
term effect. Components of the death receptor pathway, including caspase-8, are expressed in cardiomyocytes, but there are also significant levels of antagonist proteins such as cFLIPs [40]. Since receptor-mediated cell death is highly dependent on the protein–protein interaction, the level of expression of these different proteins significantly influences the outcome for the cell. Under normal conditions, high expression of inhibitory proteins (such as cFLIPs) in the heart may prevent apoptosis, but in response to cellular stresses, the expression of such proteins may be altered. For example, cFLIP is downregulated in terminally failing human hearts [41] and in apoptotic grafted myocytes [40], which may be expected to increase the sensitivity to death receptor-induced apoptosis. Such a mechanism may account for the apparent anomaly in that overexpression of FasL in transgenic mice is insufficient to induce apoptosis [42], whereas ischemia/reperfusion-induced apoptosis is decreased in animals with a defective Fas receptor [43]. Overall, it seems probable that the death receptors do play a significant role in cardiomyocyte apoptosis, but the precise role depends on the physiological setting. Therefore, it has been proposed that the death receptor signaling to apoptosis may prove particularly significant in later phases of cardiac diseases in which death receptors and their ligands have been upregulated and protective pathways have become attenuated [44].

Evidence is accumulating that cells exposed to death signals actively try to protect themselves through a number of mechanisms. When successful, such mechanisms may keep the cell alive, a factor especially important in terminally differentiated cells like the cardiomyocytes. Some of the known mechanisms include release of endogenous anti-apoptotic factors to block the actions of cytochrome c [45], or a change in PARP and/or ATP levels [46] to modulate the apoptotic pathway. In this setting, it has been reported that, although there is continuous evidence of caspase-8 activation and mitochondrial cytochrome c release in cardiomyopathic hearts, cardiomyocytes prevent downstream activation of caspase-3, and lose DNAases to preserve nuclear integrity and defer widespread loss of cytoplasmic proteins [47–49].

2. Cardiomyocyte apoptosis in hypertensive cardiomyopathy

Although apoptosis of cardiomyocytes has been detected in different cardiac disease states (Table 1) and its occurrence has been reported to be of pathophysiological significance in these conditions (reviewed in Refs. [75–79]), only recently has a critical role for cardiomyocyte apoptosis been suggested in hypertensive cardiomyopathy (defined here as a greater than normal left ventricular mass in the absence of a cause other than arterial hypertension).

2.1. Experimental findings

Increased apoptosis has been demonstrated in the hypertrophied left ventricle of spontaneously hypertensive rats (SHR) [80–83], rats with renal hypertension [84], rats with angiotensin II-induced hypertension [85] and Dahl salt-sensitive hypertensive rats [86] compared with their normotensive control animals. In addition, an increased occurrence of cardiomyocyte apoptosis has been found in the heart from failing SHR as compared to non-failing SHR [81]. The SHR is a genetic model of hypertension in which early hypertrophic adaptation to hypertension and subsequent transition to severe heart failure and premature death occur [87,88]. The transition from compensated hypertrophy to heart failure in SHR is accompanied by numerous structural and functional changes, including a reduction in the relative cardiomyocyte mass [89]. Thus, apoptosis might be a mechanism involved in cardiomyocyte loss that accompanies the transition from stable compensation to heart failure in this model.

2.2. Clinical findings

Cardiomyocyte apoptosis has been shown to be abnormally stimulated in the hypertrophied heart of patients with essential hypertension, no angiographic evidence of coronary artery disease and normal cardiac function [90,91] (Fig. 1). In addition, recent findings from our laboratory indicate that cardiomyocyte apoptosis is increased in hearts from hypertensive patients with chronic heart failure compared with hearts from hypertensive patients with left ventricular hypertrophy and normal cardiac function [92]. In fact, increased cardiomyocyte apoptotic index and active caspase-3 expression was found in hypertensive failing hearts compared with hypertensive hypertrophic hearts and normotensive hearts in this study (Fig. 1). On the other hand, moderate cardiomyocyte loss has been demonstrated in long-term systemic hypertension with no

Table 1
Clinical conditions in which increased apoptosis of cardiomyocytes has been described in humans

<table>
<thead>
<tr>
<th>Condition</th>
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<tr>
<td>In the absence of heart failure</td>
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<tr>
<td>Postnatal morphogenesis</td>
<td>[50,51]</td>
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<tr>
<td>Acute myocardial infarction</td>
<td>[52–56]</td>
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<tr>
<td>Cardiac allograft rejection</td>
<td>[57–59]</td>
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<tr>
<td>In the presence of heart failure</td>
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<tr>
<td>Ischemic cardiomyopathy</td>
<td>[47,60–65]</td>
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<tr>
<td>Idiopathic dilated cardiomyopathy</td>
<td>[47,60–68]</td>
</tr>
<tr>
<td>Acromegalic cardiomyopathy</td>
<td>[69]</td>
</tr>
<tr>
<td>Diabetes/hypertension</td>
<td>[70]</td>
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<tr>
<td>Arrhythmogenic right ventricular dysplasia</td>
<td>[68,71,72]</td>
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<tr>
<td>Myocarditis</td>
<td>[73]</td>
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<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>[68,74]</td>
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Fig. 1. (A) Histological sections of myocardial biopsy specimens showing positive immunostaining (in brown) for the active form of caspase-3 in a normotensive subject, a hypertensive patient with normal cardiac function, and a hypertensive patient with heart failure. Values represent the percentage of the sample surface stained for caspase-3 as assessed with an automated image analysis system (magnification ×100). (B) The bars represent the cardiomyocyte apoptotic index of the three patients included in (A).
clinical evidence of heart failure [93,94]. Interestingly, we have found a severe loss of cardiomyocytes in failing hearts from hypertensive patients compared with hearts from hypertensive patients with left ventricular hypertrophy and normal cardiac function [92]. Thus, it seems that apoptosis and cardiomyocyte loss precedes the impairment in ventricular function and its exacerbation accompanies the development of heart failure in hypertensive patients.

3. Pathogenetic mechanisms

Cardiomyocyte apoptosis has been proposed to occur as a result of an imbalance between the factors that induce or block apoptosis [95]. Thus, arterial hypertension may represent a condition in which inducers of cardiomyocyte apoptosis predominate over suppressors of cardiomyocyte apoptosis [96].

3.1. Inducers of apoptosis

Mechanical overload secondary to aortic banding has been shown to induce cardiomyocyte apoptosis in the rat [97]. Overstretching of isolated papillary muscles in vitro, which mimics an elevation of diastolic stress in vivo, resulted in an increase in cardiomyocyte apoptosis [98,99]. Interestingly, augmented superoxide formation and expression of Fas receptor were observed in this condition. The addition of the NO-releasing drug C87-3754 prevented apoptosis and superoxide anion formation [98]. Therefore, the induction of superoxide seems to be a relevant factor in overstretching-induced cardiomyocyte apoptosis. On the other hand, mechanical stretch causes release of humoral factors from cardiomyocytes that may induce apoptosis in these cells [100]. Thus, it is possible that physical forces may facilitate cardiomyocyte apoptosis in conditions of pressure overload of the heart.

Three types of findings suggest that, besides the mechanical factor, local humoral factors may contribute critically to cardiomyocyte apoptosis in arterial hypertension. First, increased apoptosis has been found not only in the hypertrophied left ventricle but also in the right ventricle of SHR [80,101,102], and the interventricular septum of hypertensive patients [90]. Second, recent studies have shown that the ability of antihypertensive treatment to prevent apoptosis in SHR [80,101,102] and to reduce apoptosis in hypertensive patients [90] is independent of its antihypertensive efficacy. Third, apoptosis was not detectable in the left ventricle of patients with valvular aortic stenosis and increased left ventricular systolic pressure [103].

Several arguments suggest that angiotensin II may be one of the humoral factors potentially involved in cardiomyocyte apoptosis in hypertension. First, cardiomyocyte apoptosis increases in angiotensin II-infused hypertensive Sprague–Dawley rats and blockade of the type 1 angiotensin II (AT₁) receptor with losartan prevents this effect despite the persistence of increased blood pressure [85]. Second, an association has been found between enhanced cardiomyocyte apoptosis and exaggerated angiotensin converting enzyme (ACE) activity in the left ventricle of SHR [80]. Finally, chronic treatment with losartan at doses that do not normalize blood pressure is associated with a reduction of cardiomyocyte apoptosis in both SHR [94] and hypertensive patients [90].

In vitro studies have shown that angiotensin II binding of AT₁ receptors triggers apoptosis by a mechanism involving stimulation of p38 MAP kinase activity, activation of p53 protein and subsequent decrease of the Bcl-2-to-Bax protein ratio, activation of caspase-3, stimulation of calcium-dependent DNase I, and internucleosomal DNA fragmentation [98,104–107] (Fig. 2). Although angiotensin II has been shown to induce apoptosis in other cardiovascular cells through stimulation of the AT₁ receptor [108], recent findings suggest that it is unlikely that this receptor is a strong signal to induce cardiomyocyte apoptosis in vivo. In fact, apoptosis is not increased in the heart of transgenic mice overexpressing AT₁ receptors in the myocardium [109]. In addition, Diep et al. have reported that blockade of AT₁ receptors with losartan is accompanied by normalization of cardiac apoptosis in rats with angiotensin II-induced hypertension that exhibit increased expression of AT₁ receptors in the heart [85].

In the heart, aldosterone promotes hypertrophy and fibrosis. Recently, De Angelis et al. [110] have reported that aldosterone induces ventricular cardiomyocyte apoptosis in vivo and in cultured cells. The effect of aldosterone seems to be directed to the cardiomyocyte and is mediated by mineralocorticoid receptors, since it is abolished by spironolactone in primary culture of these cells [110]. Interestingly, cardiomyocyte apoptosis induced by infusion of angiotensin II is reduced by 50% in hearts of rats pre-treated with spironolactone, suggesting that the proapoptotic effect of the peptide could be due, at least in part, to stimulation of aldosterone synthesis in vivo [110].

3.2. Suppressors of apoptosis

It has been proposed that natural adaptation of cardiomyocytes to chronic injury (such as arterial hypertension) is associated with upregulation of protective factors and/or downregulation of death factors (as mentioned before) [111]. For instance, besides hypertrophic and apoptotic pathways, biomechanical stress of the heart that occurs in conditions of pressure overload can also induce survival pathways in the cardiomyocyte [112]. There is normally a balance between hypertrophic and survival signals on one hand, and apoptotic signals on the other hand, and cell death occurs in response to a persistent shift in this balance [113].

The transmembrane signal transducer gp130 molecule
has been proposed to exert a survival effect in cardiomyocytes, mediating apoptosis-suppressor signals triggered by members of the interleukin-6 (IL-6) cytokine family, including cardiotrophin-1 (CT-1) and leukemia inhibitory factor (LIF) [114]. In support of this, it has been shown that left ventricular gp130 knockout mice develop a rapid dilated cardiomyopathy with massive cardiomyocyte apoptosis in response to mechanical overload [115]. Interestingly, an association of diminished expression of both gp130 and LIF proteins with increased cardiomyocyte apoptosis has been found in the heart of SHR [116]. It can thus be hypothesized that inhibition of the gp130 signaling pathway in arterial hypertension decreases the survival capability of cardiomyocytes and makes them more susceptible to apoptotic factors. In accordance with this possibility are findings from our laboratory showing that, compared with cardiomyocytes isolated from normotensive Wistar Kyoto (WKY) rats, cardiomyocytes isolated from SHR exhibit increased susceptibility to the apoptotic effects of angiotensin II [107]. Several data suggest that ischemia is the main perturbation challenging the equilibrium between cell survival and apoptosis in the heart [117]. Specifically, diminished energy availability may inhibit cell survival mechanisms and facilitate cell death. Coronary hemodynamic alterations and structural and functional alterations of intramyocardial arteries are common in hypertensive heart disease [118], thus ischemia may inhibit survival pathways and facilitate cardiomyocyte apoptosis in this condition.

4. Pathophysiological impact

From a pathophysiological point of view, hypertension affects the myocardium at two different stages [119]. 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 the transition to failure relates mainly to changes in the composition of the motor unit and cytoskeleton of cardiomyocytes [120], alterations in the metabolism of the extracellular matrix [121] and cardiomyocyte loss due to multiple mechanisms of death, including apoptosis [122]. Besides a reduction in the number of cardiomyocytes, apoptosis may contribute to heart failure through different pathways [48,123–125] (Fig. 3).

4.1. Compromising contractile function

As mentioned before, increased cardiomyocyte apoptosis and diminished cardiomyocyte number have been found in the failing hearts of both SHR [82] and hyperten-
Fig. 3. Mechanisms activated by the apoptotic process in the cardiomyocyte that may contribute to a deterioration of cardiac function and alter the electrical activity of the myocardium in hypertensive cardiomyopathy.

sive patients [92]. Thus, apoptosis may be one of the mechanisms involved in the loss of contractile mass and function in hypertensive cardiomyopathy. However, at present, 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) [47, 60, 62, 63, 126, 127], conservative assumptions concerning 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 [24]. Although the progressive nature and poor survival in late-stage heart failure may well be consistent with such a substantial loss of cardiomyocytes, it indicates that the heart should rapidly disappear. However, this contention does not consider that, as demonstrated recently in the human heart, cardiomyocytes may proliferate by mitotic division [94, 128, 129] or regenerate from migrated undifferentiated stem cells [130]. 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 [131].

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 [132]. It is well known that apoptosis is associated with activation of caspases that mediate the cleavage of vital and structural proteins. Communal et al. [133] have reported recently that caspase-3 cleaved cardiac myofibrillar proteins, resulting in an impaired force/Ca$^{2+}$ relationship and myofibrillar ATPase activity. In this regard, Laugwitz et al. [134] have demonstrated that caspase activation is associated with cleavage of myofilaments, a disruption of sarcomeric structure and a reduction in the contractile force of failing myocytes, and that blockade of caspase activation improves contractility in the failing myocardium. Since, in cardiomyocytes, apoptosis may not be complete, 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 especially relevant taking into account that overexpression of the active form of caspase-3 has been reported in the heart of hypertensive patients [90].

Cytochrome c plays a major role in ATP production through mitochondrial oxidative phosphorylation. It has thus been hypothesized that the release of cytochrome c from mitochondria during the occurrence of the apoptotic process should interfere with cardiomyocyte energy production and lead to functional impairment [48, 123]. The potential relevance of this mechanism is shown by the observation that both the ATP content and the capacity of the mitochondria for oxygen consumption and oxidative phosphorylation are significantly reduced in the failing heart compared to the normal heart despite the absence of myocardial ischemia [135, 136].

4.2. Facilitation of cardiac 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 [137]. This has been supported by the finding that fibrillar collagen deposition in the cardiac interstitium of
SHR [138] and hypertensive patients [139] increases left ventricular chamber stiffness and compromises left ventricular filling during diastole. 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 mycardium. The observation that fibrosis is associated with cell loss in the left ventricle of hypertensive patients [93] and patients with aortic stenosis [103] raises questions concerning the mechanism responsible for the modification of the interstitium with accumulation of fibrillar collagen. As proposed by Anversa et al. [140], the death of individual cardiomyocytes may be more common than generally believed, and this phenomenon may stimulate discrete healing processes contributing to expansion of the interstitium. This proposal is further supported by the finding that failing hearts from SHR present the colocalization of collagen α1 type I gene expression to areas of focal cardiomyocyte degeneration [141], suggesting that cardiomyocyte loss is associated with collagen type I production and focal scar formation in the SHR during the transition from compensated 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 dilation [142]. 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 [143–146].

4.3. Impairment of myocardial energy production

Energy metabolism has been shown to be deranged in the human hypertensive and failing myocardium [147,148]. Although recent findings suggest that altered myocardial fatty acid metabolism may account for this abnormality in hypertensive patients [148], the possibility also exists that mitochondrial production of ATP is diminished in the hypertensive myocardium [149]. In this setting it is interesting to consider that apoptosis is associated with loss of cytochrome c from the mitochondria and this may halt oxidative phosphorylation and the production of ATP, namely in subsarcolemmal mitochondria [150]. Nevertheless, since apoptosis is an energy-requiring process, in conditions of severely diminished ATP availability the mode of cell death would switch to necrosis. Then, one might 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.

4.4. Triggering of ventricular arrhythmias

Cardiomyocyte apoptosis may lead to the development of arrhythmias, potentially resulting in sudden cardiac death [151]. An arrhythmogenic effect by apoptosis may be mediated in two ways [51]. 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.

The potential clinical relevance of this mechanism is shown by the observation that hypertensive cardiomyopathy is associated with an increased incidence of both ventricular arrhythmias and sudden cardiac death [152].

5. Further developments

Because of the detrimental effects that cardiomyocyte apoptosis may exert in hypertensive cardiomyopathy, to recognize and prevent or limit the magnitude of this phenomenon may be relevant in both assessing and modifying the clinical outcome of patients with arterial hypertension. Thus, methods for the measurement of cardiac apoptosis and strategies for the treatment of this lesion are currently being investigated and should be applied to the study of the hypertensive heart.

5.1. Accurate in situ identification of apoptotic cardiomyocytes

The most widely used technique for apoptosis quantification in tissue sections is the terminal deoxynucleotid nick-end labeling (TUNEL staining or TdT labeling) reaction based on the detection of DNA-3’ ends. However, TUNEL staining is not specific for apoptosis. In fact, it has been shown that positive TUNEL staining is associated not only with apoptotic cardiomyocytes but also with oncocytic (necrotic) cardiomyocytes or even viable cardiomyocytes undergoing DNA repair [153,154]. Therefore, because the rate of apoptosis is generally very low in normal hearts as well as in diseased hearts, a high false-positive rate severely limits the interpretation of TUNEL-positive cells.

This problem was partially avoided by the development of the Taq and Pfu labeling techniques that specifically identify apoptotic DNA fragments [155,156]. Using this approach, Guerra et al. [60] have reported that cardiomyocyte apoptosis occurs in end-stage cardiac failure at rates 10- to five-fold lower than those previously reported using the TUNEL method. Nevertheless, as emphasized by Saraste and Pulkki [157], the detection of DNA-3’ ends should always be accompanied by other confirmation
protocols based on different apoptotic features such as caspase activation, assessment of caspase substrates (e.g., poly(ADP-ribose)polymerase), nuclear morphological modifications, extracellular cell surface exposure of phosphatidyserine or the internucleosomal pattern of DNA fragmentation.

5.2. Clinical recognition of cardiac apoptosis

Apoptosis activates mechanisms which cause the translocation of phosphatidyserine from the internal to the external leaflet of the plasma membrane [127,158,159]. Annexin-V is a phospholipid binding protein, which, in the presence of Ca$^{2+}$, specifically and reversibly interacts with the phosphoserine headgroup of phosphatidyserine in the apoptotic cell [160]. This property has been the driving force for the research into 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 [161]. Technetium-99m-labeled annexin-V has been successfully used for the non-invasive gamma imaging of cardiac apoptosis after acute myocardial infarction, acute myocardial ischaemia, acute cardiac allograft rejection and malignant intracardiac tumours [53,162].

Besides imaging studies, the determination of circulating annexin-V may also be 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 eight-fold in the early phase of acute myocardial infarction, and immediately decrease after the onset of pain [163]. Other circulating markers of apoptosis are currently under investigation. For instance, it has recently been reported that, during apoptosis, cytochrome c not only translocates into the cytosol but is secreted to the extracellular medium [164]. Thus, its potential role as a serum marker of cardiac apoptosis in chronic cardiomyopathies, including hypertensive cardiomyopathy, remains to be investigated.

Some biochemical changes characteristic of apoptosis have been proposed as potential markers to be used in nuclear magnetic resonance (NMR) techniques [165], and preliminary in vitro observations indicate that proton NMR may be useful in detecting apoptotic cell death in vivo [166]. The current application of these protocols in patients is restricted to the monitoring of cancer treatment, although its use in cardiac diseases has been proposed [167].

5.3. Therapeutic strategies to prevent and reduce 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 [168]. Cardiomyocyte apoptosis may be inhibited by suppressing the local factors that trigger the process, by directly blunting the intracellular apoptotic pathways or by inducing the survival pathways [96].

The in vivo effects of antihypertensive drugs on cardiac apoptosis in SHR have been reviewed elsewhere [125] (Table 2). Collectively, the available findings suggest that the ability of antihypertensive drugs to inhibit cardiac apoptosis is independent of its 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, losartan but not amlodipine reduced cardiomyocyte apoptosis in patients with hypertensive cardiomyopathy after 1 year of treatment [90].

As mentioned previously, oxidant stress is an important factor inducing cardiomyocyte apoptosis. Thus, antioxidant therapy, whether through administration of additional antioxidants or by boosting innate antioxidant mechanisms, has been shown to be a viable approach in reducing cardiac apoptosis in experimental conditions [169,170]. 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 hypertensive cardiomyopathy [171]. With the knowledge that these antioxidants function in part by inhibition of apoptosis, the development of more potent therapeutic agents may be possible. On the other hand, recent data suggest that activation of mitochondrial ATP-sensitive

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<th>Antihypertensive agent</th>
<th>Effects on apoptosis</th>
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<td>Cardiomyocytes</td>
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<td><strong>Diuretics</strong></td>
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<tr>
<td>Hydrochlorothiazide</td>
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<td>Doxazosin</td>
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<td><strong>Vasodilators</strong></td>
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<td>Hydralazine</td>
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<td>Nifedipine</td>
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<tr>
<td>Amlodipine</td>
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<td><strong>ACE inhibitors</strong></td>
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<td><strong>AT$_1$ antagonists</strong></td>
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<td>Losartan</td>
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Adapted from Ref. [125]. ACE, angiotensin converting enzyme; AT$_1$, angiotensin II type 1 receptor. ?, Data not provided.

*Cells are mostly fibroblasts.
potassium channels, either by the vasodilator diazoxide [172] or by the antianginal drug ncorandil [173], inhibits apoptosis induced by oxidative stress in cultured rat neonatal cardiomyocytes.

Some experimental studies have focussed on the modification of apoptosis regulators and the blockade of apoptosis executors. For instance, overexpression of human anti-apoptotic protein Bcl-2 decreases cardiac apoptosis and improves cardiac function in transgenic mice after ischemia/reperfusion [174]. Similar in vitro and in vivo effects have been reported with low molecular weight caspase inhibitors [175]. Interestingly, some of these compounds were effective not only in reducing cardiomyocyte apoptosis but also in improving cardiac function and delaying myocardial failure development [134].

An alternative strategy to prevent cardiomyocyte apoptosis is the improvement of cellular survival mechanisms. For instance, cardiotorphin-1 has been shown to prevent cardiomyocyte apoptosis that occurs in conditions of ischemia–re-oxygenation [176], likely via activation of the PI3K/Akt pathway [39]. These aspects may be of particular relevance taking into account that molecular and functional alterations of the gp130 survival pathway have recently been described in failing human hearts [177].

An important cardiomyocyte survival factor is insulin-like growth factor-1 (IGF-1) [178]. However, recent data show enhanced cardiomyocyte apoptosis in the left ventricle of patients with acromegaly and increased circulating levels of IGF-1 [69]. Furthermore, high levels of circulating IGF-1 have been reported in patients with essential hypertension and hypertensive cardiomyopathy [179,180]. Thus, the anti-apoptotic role of this factor in clinical conditions remains to be established.

6. Conclusions

Numerous hypotheses have been considered to explain the fundamental mechanism(s) for the development of heart failure in patients with hypertensive cardiomyopathy. Besides contractile disturbances of cardiomyocyte function and interstitial and perivascular fibrosis, cardiomyocyte loss is now being considered as one of the determinants of the maladaptive processes implicated in the transition from compensated to decompensated left ventricular hypertrophy. Recent findings show that cardiomyocyte apoptosis is abnormally stimulated in the hypertrophic heart of animals and humans with hypertension. Much work is being carried out regarding the mechanisms and the impact of cardiomyocyte apoptosis in hypertensive cardiomyopathy, 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 promote the inhibition of the apoptotic process could be of particular relevance to prevent the progression to heart failure in patients with hypertensive cardiomyopathy.

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References

C  ook SA, Sugden PH, Clerk A. Regulation of bcl-2 family proteins

J  ames TN, St Martin E, Willis 3rd PW, Lohr TO. Apoptosis as a

I  manishi T, Murry CE, Reinecke H et al. Cellular FLIP is expressed

K  uhara K, Saito Y, Kishimoto I et al. Cardiotrophin-1 phospho-

L  atif N, Khan MA, Birks E et al. Upregulation of the Bcl-2 family

M  a XL, Kumar S, Gao F et al. Inhibition of p38 mitogen-activated

A  oki H, Kang PM, Hampe J et al. Direct activation of mitochondrial

A  dams JW, Pagel AL, Means CK et al. Cardiomyocyte apoptosis


