Clinical implications of apoptosis in hypertensive heart disease

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THE ESSENTIAL CRITERION in defining hypertensive heart disease is a greater than normal heart mass in the absence of a cause other than arterial hypertension. However, it is now accepted that besides left ventricular hypertrophy, alterations in diastolic and/or systolic cardiac function are frequently present in hypertensive patients, which may evolve to overt heart failure. In fact, as demonstrated in the Framingham study, arterial hypertension is the most common risk factor for congestive heart failure (94). In addition, hypertension has been shown to contribute to a large proportion of heart failure cases in population-based samples (82).

Under a pathophysiological point of view, hypertension affects the myocardium at two different stages (for a review, see Ref. 120). 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 cardiomyocyte loss due to both apoptosis and necrosis (for a review, see Ref. 37), changes in the composition of motor unit and cytoskeleton of cardiomyocytes (for a review, see Ref. 146), and alterations in the metabolism of the extracellular matrix (for a review, see Ref. 148).

Apoptosis is an energy-dependent process by which a specific genetic program leads to the activation of molecular cascades that cause cell death. Apoptosis is marked by the involution of the cell, eventuating in phagocytosis by neighboring cells. By deleting cells, apoptosis plays a physiological role in controlling cell mass and architecture in many tissues, including the myocardium. Because the molecular aspects of cardiac apoptosis have been reviewed extensively elsewhere in recent articles (11, 66, 110), this report focuses on the pathophysiological implications of apoptotic cell death in cardiomyocytes. Thus, besides some considerations on the mechanisms of cardiomyocyte apoptosis in hypertension, its detrimental impact on cardiac function will be addressed. In addition, those noninvasive diagnostic tools currently under evaluation to detect cardiac apoptosis in humans will be underscored. Finally, the available evidence and perspectives of strategies aimed to inhibit cardiomyocyte apoptosis will be considered.

EXPERIMENTAL AND CLINICAL EVIDENCE

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 aging or pathological states of the heart has been denied until recently. In the past few years, observations have been made showing that cardiomyocyte apoptosis occurs in diverse conditions (Table 1) and that cardiomyocyte apoptosis and proliferation are simultaneously present in several situations (for a review, see Ref. 6). Therefore, apoptosis is recognized, increasingly, as a contributing cause of cardiomyocyte loss with important pathophysiological consequences (for a review, see Ref. 11). Recent evidence demonstrates that cardiomyocyte apoptosis is abnormally stimulated in the heart of animals and humans with arterial hypertension (for a review, see Ref. 51).

Experimental Findings

Increased apoptosis has been demonstrated in the hypertrophied left ventricle of both spontaneously hypertensive rats (SHR) (40, 64, 98, 100) and rats with renal hypertension (96) compared with their normotensive control animals. In addition, an increased occurrence of cardiomyocyte apoptosis has been found in the heart from failing SHR compared with nonfailing SHR (64). 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 (106, 119). 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 (32). Thus apoptosis might be a mechanism involved in cardiomyocyte loss that accompanies the transition from stable compensation to heart failure in this model.
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 (57, 150) (Fig. 1). In addition, recent findings from our laboratory indicate that cardiomyocyte apoptosis is increased in hearts from hypertensive patients with congestive heart failure compared with hearts from hypertensive patients with normal cardiac function (A. González, B. López, S. Ravassa, R. Querejeta, J. Díez, and M. A. Fortuño, unpublished data). On the other hand, moderate cardiomyocyte loss has been demonstrated in long-term systemic hypertension with no clinical evidence of heart failure (113, 114). Interestingly, we found a severe loss of cardiomyocytes in failing hearts from hypertensive patients (A. González, B. López, S. Ravassa, R. Querejeta, J. Díez, and M. A. Fortuño, unpublished data). Thus it seems that apoptosis-dependent cardiomyocyte loss precedes the impairment in ventricular function, and its exacerbation may contribute to development of heart failure in hypertensive patients.

Table 1. Conditions in which increased apoptosis of cardiomyocytes has been described

<table>
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<tr>
<th>Condition</th>
<th>Species</th>
<th>Detection Method</th>
<th>References</th>
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<td><strong>In the absence of heart failure</strong></td>
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<tr>
<td>Cardiac aging</td>
<td>Rat, mouse</td>
<td>TUNEL, DNA laddering</td>
<td>3, 48, 78</td>
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<tr>
<td>Injury due to ischemia and reperfusion</td>
<td>Rat, pig, mouse, rabbit</td>
<td>TUNEL, DNA laddering, electron microscopy, annexin V</td>
<td>43, 47, 49, 60, 99</td>
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<tr>
<td>Myocardial infarction</td>
<td>Rat</td>
<td>TUNEL, DNA laddering</td>
<td>24, 77</td>
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<tr>
<td>Pressure-overload hypertrophy</td>
<td>Rat</td>
<td>TUNEL, DNA laddering</td>
<td>142</td>
</tr>
<tr>
<td>Cytokine induced NO production</td>
<td>Rat</td>
<td>Microscopy, TUNEL, PARP</td>
<td>73</td>
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<tr>
<td>In vitro gene transfection of p53</td>
<td>Rat</td>
<td>DNA laddering</td>
<td>121</td>
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<tr>
<td>Cardiac expression of TNF-α</td>
<td>TG mouse</td>
<td>Hematoxyl-eosin staining</td>
<td>22</td>
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<td>TNF receptor knockout + coronary ligation</td>
<td>Mouse, TG mouse</td>
<td>In situ PCR ligation</td>
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<td>Cardiac-specific overexpression of TAK1</td>
<td>TG mouse</td>
<td>TUNEL</td>
<td>156</td>
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<tr>
<td>Gα overexpression</td>
<td>TG mouse</td>
<td>TUNEL, electron microscopy</td>
<td>56</td>
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<tr>
<td>Stretch</td>
<td>Rat</td>
<td>TUNEL, in situ ligation</td>
<td>92</td>
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<td>Genetic hypertension</td>
<td>Rat</td>
<td>TUNEL, caspase 3, annexin V</td>
<td>40, 50, 125</td>
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<td>Angiotensin II-induced hypertension</td>
<td>Rat</td>
<td>TUNEL, DNA laddering, caspase 3</td>
<td>36</td>
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<tr>
<td>Cardiac allograft rejection</td>
<td>TG, mouse, mouse, rat</td>
<td>TUNEL, DNA laddering</td>
<td>12, 42, 86, 139, 140</td>
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<td><strong>In the presence of heart failure</strong></td>
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<tr>
<td>Coronary embolization</td>
<td>Dog</td>
<td>TUNEL, DNA laddering</td>
<td>135</td>
</tr>
<tr>
<td>Coronary ligation</td>
<td>Rat, mouse</td>
<td>TUNEL, DNA laddering, Hoechst staining, azantrichrome staining, TUNEL, DNA laddering, caspase 3, annexin V</td>
<td>14, 97, 102, 131</td>
</tr>
<tr>
<td>Ventricular pacing</td>
<td>Dog</td>
<td>TUNEL, DNA laddering</td>
<td>65, 93, 101</td>
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<td>Pressure overload by aortic coarctation</td>
<td>Rat</td>
<td>TUNEL, DNA laddering, electron microscopy</td>
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<tr>
<td>Gα overexpression</td>
<td>TG mouse</td>
<td>TUNEL, DNA laddering</td>
<td>1</td>
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<td>Overexpression of long-chain acyl-CoA synthetase</td>
<td>TG mouse</td>
<td>TUNEL</td>
<td>26</td>
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<tr>
<td>gp130 knockout + aortic coarctation</td>
<td>TG mouse</td>
<td>TUNEL, DNA laddering</td>
<td>67</td>
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<td>Pressure overload by arterial hypertension</td>
<td>Rat</td>
<td>TUNEL, DNA laddering</td>
<td>98</td>
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<td><strong>In the absence of heart failure</strong></td>
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<tr>
<td>Postnatal morphogenesis</td>
<td>Human</td>
<td>TUNEL, microscopy</td>
<td>75, 76</td>
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<td>Acute myocardial infarction</td>
<td>Human</td>
<td>TUNEL, DNA laddering</td>
<td>9, 69, 115, 134</td>
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<tr>
<td>Cardiac allograft rejection</td>
<td>Human</td>
<td>TUNEL, DNA laddering, annexin V</td>
<td>107, 118, 122</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>Human</td>
<td>TUNEL, caspase 3, electron microscopy</td>
<td>57, 150</td>
</tr>
<tr>
<td><strong>In the presence of heart failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic dilated cardiomyopathy</td>
<td>Human</td>
<td>TUNEL, In situ ligation, DNA laddering, DNase 1 levels, caspase 3, cytochrome c</td>
<td>62, 89, 108, 109, 112, 126, 133, 152</td>
</tr>
<tr>
<td>Acromegalic cardiomyopathy</td>
<td>Human</td>
<td>TUNEL, in situ ligation</td>
<td>54</td>
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<tr>
<td>Diabetes/hypertension</td>
<td>Human</td>
<td>TUNEL, in situ ligation</td>
<td>55</td>
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<tr>
<td>Arrhythmogenic right ventricular dysplasia</td>
<td>Human</td>
<td>TUNEL, electron microscopy</td>
<td>103, 144</td>
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<tr>
<td>Myocarditis</td>
<td>Human</td>
<td>TUNEL</td>
<td>143</td>
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<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>Human</td>
<td>TUNEL</td>
<td>74</td>
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NO, nitric oxide; TAK1, transforming growth factor-β-activated-kinase-1; gp, glycoprotein; TG, transgenic; PARP, poly(ADP-ribose) polymerase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.
POTENTIAL MECHANISMS

Cardiomyocyte apoptosis has been proposed to occur as a result of an imbalance among the factors that induce or block apoptosis (for a review, see Ref. 6). Alternatively, it is possible that apoptosis reflects some intrinsic abnormalities in those factors that act within the cardiomyocyte determining the resistance or the susceptibility of the cell to apoptosis (for a review, see Ref. 51).

Role of Pressure Overload

Mechanical overload secondary to aortic banding has been shown to induce cardiomyocyte apoptosis in the rat (142). Overstretching of isolated papillary muscles in vitro, which mimics an elevation of diastolic stress in vivo, resulted in an increase in cardiomyocyte apoptosis (25, 91). Interestingly, augmented superoxide formation and expression of the cell surface molecule involved in apoptotic death, Pαs, were observed in this condition. The addition of the nitric oxide-releasing drug C87–3754 prevented apoptosis and superoxide anion formation (25). 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 (for a review, see Ref. 130). Thus it is possible that the physical forces may facilitate cardiomyocyte apoptosis in conditions of pressure overload of the heart.

Role of Humoral Factors

Two types of findings suggest that besides the mechanistic factor, local humoral factors may also contribute 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 (40, 50, 52) and in the interventricular septum of hypertensive patients (57). Second, recent studies have shown that the ability of antihypertensive treatment to prevent apoptosis in SHR (40, 50, 52) and to regress apoptosis in hypertensive patients (57) is independent of its antihypertensive efficacy.

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 angiotensin II type 1 (AT₁) receptor with losartan prevents this effect despite the persistence of increased blood pressure (36). Second, an association has been found between enhanced cardiomyocyte apoptosis and exaggerated angiotensin-converting enzyme (ACE) activity in the left ventricle of SHR (40). Finally, chronic treatment with losartan at doses that do not normalize blood pressure is associated with reduction of cardiomyocyte apoptosis in both SHR (50) and hypertensive patients (57).

In vitro studies have shown that angiotensin II binding of AT₁ receptors triggers apoptosis by a mechanism involving activation of p53 protein and a subsequent decrease of the Bcl-2-to-Bax protein ratio, activation of caspase 3, stimulation of calcium-dependent DNase I, and internucleosomal DNA fragmentation (28, 78, 91, 125) (Fig. 2). Although angiotensin II has been shown to induce apoptosis in other cardiovascular cells through stimulation of the AT₂ receptor (for a review, see Ref. 104), recent findings suggest that it is unlikely that this receptor is a strong signal to induce cardiomyocyte apoptosis in vivo (137).

Role of Survival Pathways

The transmembrane signal transducer glycoprotein (gp)130 has been proposed to exert a survival effect in cardiomyocytes, mediating apoptosis-suppressor signals triggered by members of the interleukin-6 cytokine family, including cardiotrophin-1 and leukemia inhibitory factor (LIF) (for reviews, see Refs. 26 and 72). Specific left ventricular gp130 knockout mice develop a rapid dilated cardiomyopathy with massive cardiomyocyte apoptosis in response to mechanical overload (131). Interestingly, an association of diminished expression of both gp130 and LIF proteins with increased cardiomyocyte apoptosis has been found in the heart of SHR (124). It thus can 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 support of this possibility are findings from our laboratory showing that compared with cardiomyocytes isolated from normotensive Wistar-Kyoto rats, cardiomyocytes isolated from SHR exhibit increased susceptibility to the apoptotic effects of angiotensin II (125).

Several data suggest that ischemia is the main perturbation challenging the equilibrium between programmed cell survival and programmed cell death in
the heart (for a review, see Ref. 33). Specifically, a 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 (for a review, see Ref. 53); thus ischemia may inhibit survival pathways and facilitate cardiomyocyte apoptosis in this condition (Fig. 2).

POTENTIAL CONSEQUENCES

During the past few years, there has been accumulating evidence in both human and animal models suggesting that activation of apoptosis is a consistent finding during the development of heart failure (for a review, see Ref. 81) (Table 1). Increased apoptosis may contribute to ventricular dysfunction and progression to cardiac failure through different pathways (Fig. 3).

Decrease of Contractile Mass

As mentioned before, an association between cardiomyocyte apoptosis and cardiomyocyte loss has been found in the failing hearts of both SHR (62) and hypertensive patients (A. González, B. López, S. Ravassa, R. Querejeta, J. Diez, and M. A. Fortunó, unpublished data). However, some difficulties emerge related to the interpretation of the data.

Assuming that apoptosis takes 24 h to be completed, an apoptotic rate of 0.22%, as reported by González et al. (57) using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) technique (57), means that the heart should rapidly disappear. This contention does not consider that, as demonstrated recently in the human heart, cardiomyocytes may proliferate by mitotic division (10, 114) or regenerate from migrated undifferentiated stem cells (123). This does not imply that cell replication compensates for the extent of apoptotic loss in the diseases myocardium, but allows us to hypothesize that inadequate cardiomyocyte division may be a critical event in the evolution of the pathological heart to heart failure.

On the other hand, 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. The 30% loss of cardiomyocytes documented by Olivetti and colleagues (113) in the left ventricle of hypertensive patients did not seem to have reached a critical value for the development of severe ventricular dysfunction and failure. Studies in humans (23, 116, 147) have indicated that occlusion of a major coronary artery, resulting in acute myocardial infarction and a segmental loss of cardiomyocytes, leads to overt failure when destruction in muscle mass involves 40–50% of the cardiomyocyte population of the left ventricle. Whether cardiomyocyte apoptosis, which is diffuse in nature, has to result in loss of nearly 40–50% of the cells before ventricular failure ensues in humans remains an important unanswered question.

Alteration of Contractile Apparatus

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 (for a review, see Ref. 29).

It is well known that apoptosis is associated with activation of caspases that mediate the cleavage of vital and structural proteins. Communal et al. (30)
reported recently that caspase 3 cleaved cardiac myofibrillar proteins, resulting in an impaired force-Ca$^{2+}$ relationship and myofibrillar ATPase activity. In addition, induction of apoptosis in cardiomyocytes was associated with a similar cleavage of myofilaments. Because in cardiomyocytes apoptosis may not be complete, allowing the cells to persist for a prolonged period within the myocardium, the functional consequences of caspase activation should not be underestimated. This possibility is especially relevant when we take into account the fact that overexpression of the active form of caspase 3 has been reported in the heart of hypertensive patients (57).

Compromise of Ventricular Filling

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 (for a review, see Ref. 39). This has been supported by the finding that fibrillar collagen deposition in the cardiac interstitium of SHR (20) and hypertensive patients (41) 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 myocardium. The observation of Olivetti et al. (113) that fibrosis is associated with cell loss in the hypertensive left ventricle raises questions about the mechanism responsible for the modification of the interstitium with accumulation of fibrillar collagen. As proposed by Anversa et al. (4), death of individual cardiomyocytes may be more common than generally believed, and this phenomenon may stimulate discrete healing processes contributing to the expansion of the interstitium.

This proposal is further supported by the finding that failing hearts from SHR present colocalization of collagen $\alpha_1$-type I gene expression to areas of focal cardiomyocyte degeneration (15), 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.

Left Ventricular Chamber Dilation

Increasing pressure loading on the heart induces concentric ventricular hypertrophy, in which wall thickness increases without chamber enlargement. Cardiomyocyte hypertrophy is responsible for this modification in ventricular anatomy after elevation of pressure load on the heart (7). Initially, the increase in cardiomyocyte diameter normalizes the alteration in systolic wall stress generated by augmentation in afterload (61). However, when cardiomyocyte death supervenes, this balanced proportion is not maintained and loading abnormalities occur (8). This event may represent the onset of decompensation, leading to side-to-side slippage of cells, mural thinning, and chamber dilation, and depressed ventricular performance (for a review, see Ref. 5). 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 with time.

DIAGNOSIS

Because of the detrimental effects that cardiomyocyte apoptosis may exert in hypertensive heart disease, recognizing and determining the magnitude of the phenomenon occurrence may be relevant in assessing the clinical outcome of patients with arterial hypertension. Although the most accurate detection of apoptosis is performed in situ using histopathological techniques, it requires an invasive procedure to obtain samples, and the interpretation of the results is controversial. Thus noninvasive protocols for measurement of cardiac apoptosis are being assayed.

Invasive Methods

Most studies on apoptosis in human hearts have been performed in patients undergoing coronary bypass or cardiac transplantation; thus myocardial samples were obtained during surgery (62, 84, 112). In the case of hypertensive patients, transvenous endomyocardial biopsy was the procedure used to obtain myocardial samples for in situ measurement of apoptosis (57, 150). Apoptosis data obtained from tissue sections analysis offer the advantage that the cell type undergoing apoptosis is specified; however, it is obvious that besides the limitations due to the risk of complications, these invasive methods are not useful as routine procedures of diagnosis or for large-scale studies. An additional limitation is that quantification requires analysis of a large number of high-power microscopic fields, because the number of apoptotic cells may be very small, and the analysis should assume that findings observed in biopsies are representative of the whole myocardium.

On the other hand, whether apoptotic cells may be recognized and quantified in situ by the currently employed techniques remains a controversial issue. The most widely technique used for apoptosis quantification in tissue sections is the TUNEL reaction (TUNEL staining or TdT labeling), based on the detection of DNA 3’ ends. However, TUNEL staining is not specific for apoptosis. In fact, using the electron microscopic TUNEL method, it has been shown that positive TUNEL staining is associated not only with apoptotic cardiomyocytes, but also with necrotic (necrotic) cardiomyocytes or even viable cardiomyocytes undergoing DNA repair (83, 111). 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 (34, 35). Taq polymerase-generated probes identify single-base 3’ overhangs in fragmented DNA caused by endonucleo-
ases typical of apoptosis, which are not present in the blunt DNA ends resulting from exonuclease activity in necrotic cell death. The latter are labeled by *Pfu* polymerase-generated probes. With the use of this approach, Guerra et al. (62) reported that cardiomyocyte apoptosis occurs in end-stage cardiac failure at rates 10- to 5-fold lower than those previously reported using the TUNEL method. Nevertheless, as emphasized by Saraste and Pulkki (132), detection of DNA 3' ends should be always accompanied by other confirmation protocols based on different apoptotic features such as caspase activation, nuclear morphological modifications, extracellular cell surface exposure of phosphatidyserine, or the internucleosomal pattern of DNA fragmentation.

**Noninvasive Methods**

Besides the ethical and technical limitations listed above, the use of cardiac biopsy samples makes it very difficult to obtain information about the extent, distribution, or time frame of apoptotic cell death. The need for new methods to gain more understanding of the dynamic pathophysiology of cardiac apoptosis has driven to the development of imaging methodologies for in vivo apoptosis monitorization and quantification.

A pivotal part of the apoptotic concept is the timely removal of the dying cell from the tissue before it causes inflammatory responses by the leakage of intracellular constituents into the surroundings. Clearance of the dying cell occurs through phagocytes, which recognize the death phenotype. Apoptosis activates mechanisms that cause the translocation of phosphatidyserine from the internal to the external leaflet of the plasma membrane (46, 145, 147). Annexin V is a phospholipid-binding protein that, in the presence of Ca²⁺, specifically and reversibly interacts with the phosphoserine head group of phosphatidyserine in the apoptotic cell (138).

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 (Fig. 4) and in vivo in animals and patients (for a review, see Ref. 128). Hence, intra-arterial injection of fluoreoscently labeled annexin V has been used to study the dynamics of cardiac apoptosis in experimental cardiac ischemia, analyzing ex vivo specimens (43) and beating hearts (44). In patients with acute myocardial infarction, apoptosis of cardiac cells has been monitored after intravenous injection of technetium 99m-labeled annexin V, obtaining early and late single-photon emission-computed tomographic images (69). A similar protocol has been employed to detect cardiac allograft rejection (107) and for localizing an intracardiac tumor (68). The use of these procedures to visualize cardiac apoptosis in hypertensive patients remains to be assayed.

Besides imaging studies, 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 eightfold in the early phase of acute myocardial infarction and immediately decrease after the onset of the pain (80). Other circulating markers of apoptosis are currently under investigation. For instance, it has been recently reported that, during apoptosis, cytochrome *c* not only translocates into the cytosol, but is secreted to the extracellular medium (127). Furthermore, patients with hematological malignances exhibit elevated serum levels of cytochrome *c* (127). Because the release of cytochrome *c* from mitochondria has been shown to occur during apoptosis in human heart failure (74), its potential role as a serum marker of cardiac apoptosis in chronic cardiomyopathies, including hypertensive heart disease, remains to be investigated.

NMR spectroscopy and imaging have emerged as powerful noninvasive tools for clinical diagnosis and therapeutic followup. Some biochemical changes characteristic of apoptosis have been proposed as potential markers to be used in NMR techniques (136), and preliminary in
vitro observations indicate that proton NMR may be useful in detecting apoptotic cell death in vivo (13, 18). Current application of this protocols in patients is restricted to monitorization of cancer treatment (17), although its use to cardiac diseases has been proposed (16).

**TREATMENT**

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 (105). 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 (51).

**Antihypertensive Drugs**

The in vivo effects of antihypertensive drugs on cardiac apoptosis in SHR are presented in Table 2. Most reported data indicate that chronic interference of the renin-angiotensin system with either an ACE inhibitor (i.e., quinapril, enalapril, or fosinopril) or an AT1 receptor blocker (i.e., losartan) are effective in preventing cardiomyocyte apoptosis in this model (40, 50, 98, 100, 154). Tea et al. (141) recently reported that treatment of SHR with amlodipine, Tea et al. (141) reported that nifedipine stimulated apoptosis after the first week of administration in treated SHR. Thus the net effect of calcium channel blockade on cardiac apoptosis remains to be elucidated. Whereas in vivo administration of doxazosin is associated with diminished cardiac apoptosis in the rat (129), recent in vitro findings show that doxazosin induces apoptosis in neonatal rat cardiomyocytes by a mechanism that is independent of α1-adrenergic blockade (59). Neither hydralazine nor hydrochlorothiazide modified cardiac apoptosis in SHR, despite a significant reduction of blood pressure (141). Although there is no clear evidence that β-blockers in general modulate apoptosis, in vitro inhibition of cardiomyocyte apoptosis has been reported for carvedilol in a model of ischemia-reperfusion (155). However, no data are available on the antiapoptotic effects of this compound in experimental hypertension.

We (57) recently reported that chronic administration of losartan to patients with essential hypertension reduces both cardiomyocyte and noncardiomyocyte apoptosis (Fig. 1). In contrast, treatment with amlodipine was associated with an increase in cardiomyocyte apoptosis and no changes in noncardiomyocyte apoptosis in hypertensives (57) (Fig. 1). Interestingly, the time-course changes in blood pressure during treatment were similar in the two groups of patients. Collectively, the above findings suggest that the ability of antihypertensive drugs to inhibit cardiac apoptosis in humans is independent of its antihypertensive efficacy but can be related to their capacity to interfere with humoral apoptotic factors, namely, angiotensin II.

**Further Developments**

Studies that gain insight into the pharmacological modulation of cardiomyocyte apoptosis, performed in cell cultures or in experimental models of cardiac disease, are described in this issue (141). The in vivo effects of antihypertensive drugs on cardiac apoptosis are summarized in Table 2. Most reported data indicate that chronic interference of the renin-angiotensin system with either an ACE inhibitor (i.e., quinapril, enalapril, or fosinopril) or an AT1 receptor blocker (i.e., losartan) are effective in preventing cardiomyocyte apoptosis in this model (40, 50, 98, 100, 154). Tea et al. (141) recently reported that treatment of SHR with amlodipine, Tea et al. (141) reported that nifedipine stimulated apoptosis after the first week of administration in treated SHR. Thus the net effect of calcium channel blockade on cardiac apoptosis remains to be elucidated. Whereas in vivo administration of doxazosin is associated with diminished cardiac apoptosis in the rat (129), recent in vitro findings show that doxazosin induces apoptosis in neonatal rat cardiomyocytes by a mechanism that is independent of α1-adrenergic blockade (59). Neither hydralazine nor hydrochlorothiazide modified cardiac apoptosis in SHR, despite a significant reduction of blood pressure (141). Although there is no clear evidence that β-blockers in general modulate apoptosis, in vitro inhibition of cardiomyocyte apoptosis has been reported for carvedilol in a model of ischemia-reperfusion (155). However, no data are available on the antiapoptotic effects of this compound in experimental hypertension.

**Table 2. In vivo effects of antihypertensive drugs on CM apoptosis in spontaneously hypertensive rats**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect of Treatment</th>
<th>Detection Method</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Hydrochlorothiazide</td>
<td>No effect</td>
<td>DNA laddering, radioactive in situ detection</td>
<td>154</td>
</tr>
<tr>
<td>Propanolol</td>
<td>↑ Apoptosis of nonspecific cardiac cell types</td>
<td>DNA laddering, radioactive in situ detection</td>
<td>154</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>↓ Apoptosis of nonspecific cardiac cell types</td>
<td>DNA laddering, radioactive in situ detection</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>↑ CM apoptosis</td>
<td>TUNEL</td>
<td>59</td>
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<tr>
<td>Hydralazine</td>
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<td>Nifedipine</td>
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<td>DNA laddering, radioactive in situ detection</td>
<td>154</td>
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<td>TUNEL</td>
<td>52</td>
</tr>
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<td>DNA laddering, electron microscopy</td>
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<td>154</td>
</tr>
<tr>
<td>Fosinopril</td>
<td>↓ CM apoptosis</td>
<td>TUNEL</td>
<td>52</td>
</tr>
<tr>
<td>Losartan</td>
<td>↓ CM apoptosis</td>
<td>TUNEL</td>
<td>51</td>
</tr>
<tr>
<td>Losartan</td>
<td>↓ CM apoptosis</td>
<td>TUNEL</td>
<td>52</td>
</tr>
<tr>
<td>Losartan</td>
<td>↑ Apoptosis of nonspecific cardiac cell types</td>
<td>DNA laddering, radioactive in situ detection</td>
<td>154</td>
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</table>

CM, cardiomyocyte; ↑, increase; ↓, decrease.
ease other than hypertensive heart disease, are focused in the modification of expression of apoptosis regulators and in the biochemical blockade of apoptosis executors. For instance, overexpression of the human antiapoptotic protein Bcl-2 decreases cardiac apoptosis and improves cardiac function in transgenic mice after ischemia-reperfusion (21). Similar in vitro and in vivo effects have been reported with low-molecular-weight caspase inhibitors (for a review, see Ref. 63). Interestingly, some of these compounds were effective not only in reducing cardiomyocyte apoptosis, but also in improving cardiac function and delaying myocardial failure development (90, 153). These findings suggest that attenuation of cardiomyocyte apoptosis may be partially involved in the protection of cardiac function in some particular conditions.

An alternative strategy to prevent cardiomyocyte apoptosis is the improvement of cellular survival mechanisms. For instance, cardiotrophin-1 has been shown to prevent the cardiomyocyte apoptosis that occurs in conditions of ischemia-reoxygenation (19), likely via activation of the phosphotidylinositol 3-kinase (PI3K)/Akt pathway (88). These aspects can be of particular relevance when we take into account the fact that molecular and functional alterations of the gp130 survival pathway have been described recently in failing human hearts (157).

An important cardiomyocyte survival factor is IGF-1. Several in vitro (70, 151) and in vivo (95, 117, 149) studies have demonstrated that this factor prevents cardiomyocyte apoptosis in different experimental conditions. However, recent data show enhanced cardiomyocyte apoptosis in the left ventricle of patients with acromegaly and increased circulating levels of IGF-1 (54). Furthermore, high levels of circulating IGF-1 have been reported in patients with essential hypertension and hypertensive heart disease (2, 38). Thus the antiapoptotic role of this factor in clinical conditions remains to be established.

Finally, a new approach arises from the field of myocardial regeneration. Kocher et al. (85) demonstrated that an intravenous injection of human bone marrow donor cells to the infarcted myocardium of athymic rats resulted in new blood vessel formation in the infarct bed (vasculogenesis), proliferation of preexisting vasculature (angiogenesis), attenuation of cardiomyocyte apoptosis, and left ventricular remodeling. These data add a new capability to the already impressive potential of stem cells: the power to heal a damaged heart by inducing vasculogenesis in the remaining viable myocardium, thereby attenuating cell death, increasing viability, and restoring heart function.

In conclusion, much work is being carried out regarding the mechanisms and the extent of cardiomyocyte apoptosis in hypertensive heart disease, but many methodological and conceptual issues still remain unsolved. Clarification of these unresolved issues will then allow an estimation of the role of apoptosis in the pathogenesis of heart failure associated with hypertensive heart disease. In parallel with this, the development of noninvasive diagnostic tools should provide the opportunity to establish the true relationship of cardiomyocyte apoptosis with cardiac anatomy and function in patients with hypertensive heart disease. Finally, although some preliminary data suggest that some classes of antihypertensive drugs exhibit an antiapoptotic capacity (i.e., those interfering with the renin-angiotensin system), further studies are required to ascertain whether this property effectively translates into clinical benefit. In summary, although a number of formal proofs are pending, it is conceivable that apoptosis of cardiomyocytes may be an important variable in the clinical evolution of hypertensive heart disease.

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