Clinical Research

Cardiomyocytic apoptosis and heart failure

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Objective

Heart failure is a major disease seriously threatening human health. Once left ventricular dysfunction develops, cardiac function usually deteriorates and progresses to congestive heart failure in several months or years even if no factors which accelerate the deterioration repeatedly exist. Mechanism though which cardiac function continually deteriorates is still unclear. Cardiomyocytic apoptosis can occur in acute stage of ischemic heart diseases and the compensated stage of cardiac dysfunction. In this review, we summarize recent advances in understanding the role of cardiomyocytic apoptosis in heart failure.

Key Words

patent foramen ovale; elderly; diastolic dysfunction; transcatheter closure

Introduction

It has been shown that apoptosis is a way by which cardiomyocytes are lost. Cardiomyocytic loss contributes to heart failure, especially to the heart failure after acute myocardial infarction. Once left ventricular dysfunction develops in acute myocardial infarction, cardiac function usually deteriorates and progresses to congestive heart failure in several months or years even if no factors which accelerate the deterioration repeatedly exist. Mechanism though which cardiac function continually deteriorates is still unclear. But the deterioration relates to the compensation reactions of heart for providing normal cardiac output: activation of sympathetic nervous system and renin-angiotensin system for rapid compensation, myocardial hypertrophy and left ventricular dilation in the late stage. The compensation usually overdoes, which results in residual myocardium degeneration, necrosis, apoptosis and interstitial fibrosis, and accelerates the deterioration of cardiac function further. Cardiomyocytic apoptosis can occur in acute stage of ischemic heart diseases, the compensated stage of cardiac dysfunction, myocarditis and cardiomyopathy, the roles of many drugs in improving cardiac function were associated with cardiomyocytic apoptosis. Many researches have been performed to explore the effects of apoptosis on heart failure. In this paper, we review some advance in this field.

Cardiomyocytic apoptosis

It is believed that cardiomyocytes can not be lost through apoptosis. In 1994, Gottlieb et al. demonstrated that ischemia/reperfusion could induce cardiomyocytic apoptosis in rabbits. In rats model of myocardial infarction in 1996, Kajstura et al. found that the two-hours ligation of coronary artery could result in cardiomyocytic apoptosis and programmed cardiomyocytic death was the major form of myocardial loss produced by ischemia. In the early stage (four to six hours) of infarction, apoptosis resulted in more cardiomyocytic loss than necrosis, whereas necrotic myocyte cell death followed apoptosis and contributed to the progressive loss of cells with time after infarction. Subsequently Cheng et al. reported that cardiomyocytic apoptosis appeared in three hours after ligation of coronary artery, continued to one month after the ligation. Cardiomyocytic apoptosis appeared not only in the region surrounding infarction but also in the regions remote from infarction. Cardiomyocytic apoptosis was also found in early and late stage of human myocardial infarction. Olivetti et al. reported that the number of apoptotic nuclei was greater in the peri-infarcted region than in that away from infarction. Quantitatively, 12% of myocytes in the border zone showed DNA strand breaks, whereas 1% of cells were undergoing apoptosis in the remote myocardium. Cardiomyocytic apoptosis is a major risk factor of unfavorable LV remodeling and early symptomatic post-infarction heart failure.7

With the advance in cardiomyocytic apoptosis, it was found that not only is apoptosis involved in ischemic heart disease, but apoptosis is involved in myocarditis, myocardiopathy and other myocardial injury.

Sympathetic nervous, renin-angiotensin system and cardiomyocytic apoptosis

Sympathetic nervous system is firstly activated during compensation of cardiac function, then renin-angiotensin system activated, though which contraction force of myocardium and tension of peripheral vessels is increased, blood volume is extended and cardiac function is improved. But over-activation of the two sys-
items also results in cardiomyocytic loss. Norepinephrine, acting via the β-adrenergic receptor, can stimulate apoptosis in adult rat cardiac myocytes in vitro. Cardiomyocytes exposed to norepinephrine (10 micromol/L), or isoproterenol (10 micromol/L) for 24 hours can decrease the number of viable myocytes by approximately 35%. Norepinephrine increases the percentage of apoptotic cells from 5.8±1.0% to 21.0±2.3%. Via β-adrenergic receptor, ischemia/reperfusion can induce cardiomyocytic apoptosis in rabbits.

To determine whether angiotensin II (Ang II) could induce apoptosis of adult ventricular myocytes, these cells were exposed to 10^(-6)M Ang II for 24 h in vitro. It was found that Ang II resulted in a five-fold increase in the percentage of apoptotic cardiomyocytes via AT1 receptor. Ang II could result in a 2.5-fold increase in apoptosis in neonatal ventricular myocytes exposed to 10^(-6)M Ang II for 24 h in vitro. Furthermore, stretch and activation of p53 induce cardiomyocytic apoptosis through upregulation of the local renin-angiotensin system and activation of AT1 receptor. Administration of the angiotensin-converting enzyme inhibitor or AT1 receptor can reduce cardiomyocytic apoptosis that accompanies heart failure, and ameliorate heart failure in the model of the transition from compensated hypertrophy to failure in spontaneously hypertensive rats (SHR).

**Apoptosis after myocardial hypertrophy and ventricular dilation** Myocardial hypertrophy and ventricular dilation are chronic cardiac compensation response to cardiac dysfunction and results of left ventricular remodeling after myocardial infarction. Myocardial hypertrophy and ventricular dilation can also induce cardiomyocytic apoptosis. Expand of ventricular cavity and increase of volume load may passively stretch myocardium cell, which can induce cardiomyocytic apoptosis in vitro. To determine the effects of loading on apoptosis, papillary muscles were exposed to 7-8 and 50 mN/mm² of stretch force, it was found that overstretching produced a 21-fold increase in apoptotic cardiomyocytes. Cardiomyocytic apoptosis can also be detected in samples from patients with dilated cardiomyopathy. In one study of seven patients with cardiomyopathy (four with idiopathic dilated cardiomyopathy and three with ischemic cardiomyopathy), hearts from all four patients with idiopathic dilated cardiomyopathy and from one of the three patients with ischemic cardiomyopathy had histochemical evidence of apoptosis. Pressure overload of left ventricle by aortic coarctation may also induce cardiomyocytic apoptosis, which occurs before compensated myocardial hypertrophy. Myocardial compensated hypertrophy precedes heart failure in SHRs, Li et al. found myocardial hypertrophy was accompanied with cardiomyocytic apoptosis. Apoptotic cells were significantly increased in the SHR with heart failure (38.92 ± 12.79 vs. 8.05 ± 3.98 cells/100,000 nuclei in SHR without heart failure). Captopril treatment of SHR with heart failure reduced the number of apoptotic cells to the level in SHR without heart failure, companied with improvement of cardiac function. Thus increased numbers of apoptotic cells are present in SHR with heart failure, suggesting that apoptosis might be a mechanism involved in the reduction of myocyte mass that accompanies the transition from stable compensation to heart failure in this model.

**Cardiomyocytic apoptosis in heart failure**

Large amount of cardiomyocytic loss result in heart failure and contribute to cardiac insufficiency in myocardial infarction. Progressive deterioration of cardiac function without obvious harmful factors is a main feature of cardiac failure. Whether ongoing loss of cardiomyocytes via apoptosis causes the progressive deterioration of cardiac function still remains to be settled. But many evidences show that cardiomyocytic apoptosis are really detected in animal model of heart failure and in patients with end stage of heart failure. In the study of dogs with chronic heart failure produced by multiple intracoronary microembolizations, evidence for cardiomyocytic apoptosis was based on transmission electron microscopy criteria and on in situ immunohistochemical labeling of nuclear DNA fragmentation. Sharov et al. found different type of classic electron microscopy feature of apoptosis in failing heart and that features of cardiomyocytic apoptosis were observed primarily in regions bordering old infarcts and a few in the regions far from old infarcts. Apoptotic cardiomyocytes were usually surrounded by fibrous tissues. No apoptotic cardiomyocytes was found in normal dog hearts. Olivetti et al. observed myocardium from patients with heart failure with confocal microscopy and found more apoptotic cardiomyocytes in failing heart than in normal heart.

Heart failure is due to progressive deterioration of cardiac function, during which cardiomyocytic apoptosis occurs, and the number of apoptotic cardiomyocytes is related to the progression of heart failure. In the process of cardiac dysfunction with aging, cardiac function deteriorates and apoptotic cardiomyocytes increase with aging. There are ten per million apoptotic cardiomyocytes at three months age of rats, and eighty per million at twenty-four months. Not only are there more apoptotic cardiomyocytes in failing heart than in normal heart, but also cardiomyocytic apoptosis is companied with the transition from compensated cardiac function to heart failure. In patients with dilated cardiomyopathy, apoptotic cardiomyocytes were more numerous in subjects with a rapidly deteriorating clinical course (0.192%, n = 10) than in patients with intermediate (0.093%, n = 6, p = 0.03) or slow (0.026%, n = 5, p = 0.003) progression.

**Mechanism of cardiomyocytic apoptosis** Since hypoxia, ischemia/reperfusion, stretch, activa-
tion of sympathetic nervous and renin-angiotensin system can induce cardiomyocytic apoptosis, but how do these factors activate apoptotic signaling pathway? The research of hypoxia effect on cardiomyocytic apoptosis in vitro has shown that apoptosis is observed in cardiomyocytes as early as 12 hours of hypoxia culture, meantime Fas messenger RNA levels in cardiomyocytes were upregulated by twofold over controls. In the animal model of myocardial ischemia by ligation of coronary artery, after 2 to 4.5 hours of the ligation the expression of Fas in cardiomyocytes increased 131-fold, although the expression of Bcl-2 in cardiomyocytes also increased 18-fold, not enough to counterpart proapoptotic action of Fas, cardiomyocytic apoptosis was the major form of myocardial damage during the time period. Cheng et al. found that cardiomyocytic apoptosis induced by ligation of coronary artery was accompanied by a decrease in the expression of bcl-2 and an increase in the expression of bax. The changes in the expression of these genes were present at 1 and 7 days after coronary artery occlusion. In the rabbit model of myocardial ischemia/reperfusion, it was found that cardiomyocytic apoptosis induced by myocardial ischemia/reperfusion was companied with upregulation of Fas expression and activation of the stress-activated protein kinase (SAPK) and that carvedilol could prevent myocardial ischemia/reperfusion-induced apoptosis in cardiomyocytes possibly by downregulation of the SAPK signaling pathway, by inhibition of Fas receptor expression. But in the research of investigating Fas role in cardiomyocytic apoptosis, we found a tempo-spatial dissociation between the expression of Fas and apoptosis, which appear in different regions of the myocardium: apoptosis in ischemic regions and Fas in the regions surrounding the ischemic myocardium. Cardiomyocyte apoptosis and Fas expression are not synchronized. Cardiomyocyte apoptosis could be detected by the terminal dUTP deoxyxynucleotidy-transferase nick end-labeling (TUNEL) method from three to thirty-six hours after LAD (left anterior descending branch of the left coronary artery) ligation, not at seven days, but Fas expression could be detected by immunohistochemistry from three hours to seven days after the ligation, and by western blots from before the ligation to seven days after the ligation. When cardiomyocyte apoptosis cannot be detected at seven days after LAD occlusion, the expression of Fas is still upregulated. According to the classic pathway of Fas regulating apoptosis, Fas should be expressed at the membrane of apoptotic cells. Our results indicated that cardiomyocytic apoptosis might not be directly related to the expression of Fas during ischemia.

JNK (c-JUN NH2-terminal protein kinase) and ERK (extracellular signal-regulated kinase), two members of mitogen-activated protein kinase (MAPK) family, are involved in stress reaction. It has been demonstrated that activation of JNK and concurrent inhibition of ERK are critical for induction of apoptosis in rat PC-12 pheochromocytoma cells. The dynamic balance between growth factor-activated ERK and stress-activated JNK-p38 pathways may be important in determining whether a cell survives or undergoes apoptosis. In myocardium of Wistar rats, 30 min of continuous ischemia can significantly increase the p46JNK and p55JNK activities (5.9 and 4.2 fold, respectively). Coronary reperfusion can increase both p42ERK and p44ERK activities (3.0 and 2.3 fold), and both p46JNK and p55JNK activities (1.4 and 1.7 fold). Cardiomyocytic apoptosis is considerably more enhanced by reperfusion than continuous ischemia. Activation of p38 MAPK might play an important role in apoptotic cell death. Administration of SB 203580, a p38 MAPK inhibitor, can decrease myocardial apoptosis (14.7±3.2% versus 30.6±3.5% in vehicle, P<0.01) and improve cardiac function both in postischemic heart and in dilated cardiomyopathy hamster heart. The cardioprotective effects of SB 203580 are closely related to its inhibition of p38 MAPK. Administering SB 203580 before ischemia and during reperfusion completely inhibits p38 MAPK activation and exerts the most cardioprotective effects. In contrast, administering SB 203580 10 minutes after reperfusion (a time point when maximal MAPK activation has already been achieved) fails to convey significant cardioprotection. A large myocardial infarction causes a chronic hemodynamic load on the uninjured remote myocardium. Apoptotic cardiomyocytes remote from infracted area is associated with activation of JNK. At four weeks after myocardial infarction, there was 3.8 fold increase in JNK phosphorylation and 4.2 fold increase in JNK kinase activity within the myocardium remote from infracted area, compared with control.

Caspases are a conserved family of proteases that play an essential role in the execution of apoptosis; however, their potential contribution to ischemic cardiomyocytic apoptosis is largely unknown. To examine their role in this process, rabbits were subjected to 30 min of coronary artery occlusion followed by 3 h of reperfusion. Immunoblot analyses revealed that caspases-2, -3 and -7 were proteolytically activated during myocardial ischemia and reperfusion in vivo. Systemic administration of the broad-spectrum caspase inhibitor acetyl-Tyr-Val-Ala-Asp chloromethylketone (YVAD-cmk) partially blocked caspase activation and dramatically reduced the percentage of TUNEL-positive myocyte nuclei in the infract region (3.9±0.8% vs 13.0±2.2% in control animals, P=0.012). Moreover, YVAD-cmk reduced myocardial infarct size by approximately 31% (31.1±3.3% vs 45.3±4.9% in control animals, P=0.032).

Caspase 1 was considered as "proinflammatory" caspase, but recent study indicated that the role for caspase-1-mediated myocardial apoptosis contributed to the progression of heart failure. Transgenic caspase-1 induced...
primary cardiomyocyte apoptosis before structural and molecular signs of myocardial remodeling occurred, and deletion of endogenous caspase-1 was beneficial in the setting of myocardial infarction-induced heart failure.

Mitochondrial cytochrome c death pathway may be involved in cardiomyocytic apoptosis. 53-55 Deprivation of serum and glucose, components of ischemia in vivo, resulted in myocyte apoptosis. Apoptotic myocytes exhibited cytoplasmic accumulation of cytochrome c, indicating release from the mitochondria. Both caspase-9 and caspase-3 were processed to their active forms in serum-/glucose-deprived myocytes. Caspase processing, but not cytochrome c release, was inhibited by a peptide caspase inhibitor, zVAD-fmk, placing the latter event upstream of caspase activation, which resulted the block of cardiomyocytic apoptosis. 54

Myocardial hypertrophy allows the heart to adapt to workload, but how does the hypertrophy culminate in later heart failure? Some researches have answered this question. Cdk (cyclin-dependent kinase) 9 is believed to phosphorylate C-terminal domain (CTD) sequentially and, hence, to mediate mRNA synthesis during the transition from transcript initiation to transcript elongation. Sano M, et al. 56 found that hypertrophy is accompanied by activation of cyclin T/Cdk9. Cdk9 activity was required for hypertrophy in culture, whereas heart-specific activation of Cdk9 by cyclin T1 provoked hypertrophy in mice. At pathophysiological levels, Cdk9 activity suppresses many genes for mitochondrial proteins including master regulators of mitochondrial function peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1), decreases mitochondrial membrane potential, and sensitizes cardiomyocytes to apoptosis. Thus, chronic activation of Cdk9 causes not only cardiomyocyte enlargement but also defective mitochondrial function, via diminished PGC-1 transcription, and a resulting susceptibility to apoptotic cardiomyopathy.

Besides Cdk9, alterations in apoptosis regulatory factors during hypertrophy also contribute to development of heart failure. 57 There were more antiapoptotic changes, including upregulation of Bcl-2 family members and caspases favoring survival, during physiological hypertrophy. However, in pathological hypertrophy, there were more proapoptotic changes, including changes in Fas, the Bcl-2 protein family, and caspases.

Cytosolic Ca2+ in the cells is an important mediator of apoptosis. The change in cytosolic Ca2+ may affect cardiomyocytic apoptosis. 27,29,58-60 Activation of adrenergic receptor and Ang II receptor may induce cardiomyocytic apoptosis via increasing cytosolic Ca2+, but the pathways are different. B-Adrenergic receptor stimulation increases calcium influx via protein kinase A–mediated phosphorylation of voltage-dependent channels. Pretreatment with the L-type calcium channel blocker diltiazem inhibited the norepinephrine-stimulated increase in apoptotic cells. 27 Ang II stimulation is associated with translocation of the epsilon and delta isoforms of protein kinase C (PKC) which is coupled with an increase in cytosolic Ca2+ in the cells. The PKC inhibitor chelerythrine can abolish Ang II-mediated increases in cytosolic Ca2+ and cardiomyocytic apoptosis. 29 Increase in cytosolic Ca2+ further activates Ca2+–dependant caspases or DNase I which induce cardiomyocytic apoptosis.

Cardiomyocytic apoptosis is coupled with stretch-mediated release of angiotensin II. Physical stretch can increase release of angiotensin II, and activate p53 gene in rat adult myocytes in vitro. Stretch-mediated release of angiotensin II is coupled with apoptosis and the activation of p53 which may be responsible for the prolonged upregulation of the local renin-angiotensin system and the increased susceptibility of cardiomyocytes to undergo apoptosis. 31 Angiotensin II may induce apoptosis in myocardium with hypertrophy, administration of the angiotensin-converting enzyme inhibitor captopril, which ameliorates heart failure in SHRs, is associated with a reduction in the exaggerated apoptosis. 32 Angiotensin II has two types of receptor: angiotensin II type 1 receptor (AT(1)) and angiotensin II type 2 receptor (AT(2)), which have different role in apoptosis. AT(1) is believed to be associated with promotion of myocyte growth and cardiac fibrosis in the development of cardiac hypertrophy and heart failure. 33 AT(2) signalling can induce cell apoptosis, but in angiotensin II-evoked apoptosis in cardiomyocytes, the proposed proapoptotic role of AT(2) signalling could not be confirmed. 62

Nitric oxide (NO) is another regulator of apoptosis. Inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) are two synthases which are involved in NO synthesis. The regulation of NO on apoptosis depends on the level of NO production and environmental milieu, which is well illustrated in heart. High levels of NO produced by iNOS could promote apoptosis while basal levels of NO production from eNOS might protect cardiomyocytes from apoptosis, which is detailed in the review by Razavi, et al. 41

**Conclusion**

Cardiomyocytic apoptosis is a mode of myocyte cell death. Facing the overload, heart compensation via activation of sympathetic nervous and renin-angiotensin system, ventricular dilation and hypertrophy can improve heart function in some extent, but over-activation of the two systems and severe ventricular dilation and hypertrophy may induce cardiomyocytic apoptosis and accelerate the process from compensation to heart failure. Cardiomyocytic apoptosis is linked to the deterioration of cardiac function, at least, involved in progression of cardiac dysfunction. The factors, which deteriorate cardiac function, can induce cardiomyocytic apoptosis. Cardiomyocytic apoptosis is in-
increased during the transition from stable compensation to heart failure. The most drugs, which ameliorate heart failure, could decrease the exaggerated apoptosis. Therefore, the identification of apoptotic regulatory pathways that are specific for cardiac myocytes or the better characterization of the time course of myocyte apoptosis in heart disease might provide a good basis for antiapoptotic treatment. Keeping the concerns associated with chronic inhibition of apoptosis in mind, with utilization of noninvasive detection of programmed cell loss in clinical practice, antiapoptotic approaches for heart failure still figure among the most attractive future therapeutic options for heart failure.

References


