Review Article

Apoptotic cell death in heart failure associated with diabetes

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Abstract

Diabetes is a serious public health problem. Improvements in the treatment of noncardiac complications from diabetes have resulted in heart disease becoming a leading cause of death in diabetic patients. Several cardiovascular pathological consequences of diabetes such as hypertension affect the heart to varying degrees. Furthermore, hyperglycemia as a sole risk factor may directly cause cardiovascular damage and lead to a complication such as diabetic cardiomyopathy. Alterations in myocardial structure and function occur during the late stage of diabetes. These chronic alterations are believed to result from acute cardiac responses to suddenly increased glucose levels at the early stage of diabetes. In failing hearts, cardiomyocytes degenerate and interstitial fibrosis, which indicates cardiomyocyte loss, becomes more prominent in the myocardium. Various mechanisms may establish an association of diabetes mellitus and heart failure as follows: first, comorbidities such as hypertension may play a role, diabetes also accelerates the development of coronary atherosclerosis, and experimental, clinical studies support the existence of microangiopathy, metabolic factors, or myocardial fibrosis influencing diabetic cardiomyopathy. Apoptosis of adult cardiac muscle cells can have lasting adverse consequences on overall cardiac performance. It is possible that autophagic degeneration may also be one of the mechanisms of myocardial cell death.

Keywords: Apoptosis, cardiac myopathy, diabetes mellitus, heart failure, myocardial cell death, suicidal autophagy

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INTRODUCTION

Diabetes mellitus (DM) comprises a group of metabolic diseases where an individual has high blood sugar, because the pancreas does not produce enough insulin, or that the cells present do not respond to the insulin that is produced.[1] This increased blood sugar levels produce the characteristic symptoms of polyuria, polydipsia, and polyphagia. After food consumption, carbohydrates are broken down into glucose molecules in the gut.[2] Glucose is absorbed into the bloodstream elevating blood glucose levels, and the resulting glycemia stimulates the secretion of insulin from the beta cells of the pancreas. If insulin production and secretion are altered by the disease, blood glucose dynamics will be subjected to changes.[3] Diabetes causes direct stress to the cardiac cells leading to an early precipitating senescence of the heart, a condition termed as diabetic cardiomyopathy.[4] The prevalence of DM in heart failure populations is approximately 20% in comparison to 4%–6% in control populations. Epidemiological studies have showcased an increased risk of heart failure due to poor glycemic control in diabetic populations.[5] Myocardial cell death marks a vital role in the pathogenesis and progression of various

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etiological cardiomyopathies such as ischemia-reperfusion, toxic exposure, and various other chronic diseases, including myocardial infarction and atherosclerosis. Myocardial cell death is a pathogenic feature in the hearts of diabetic patients.

DISCUSSION

Mechanism of heart failure in diabetic patients

DM is associated with a markedly increased risk of coronary artery disease. In the United Kingdom Prospective Diabetes Studies, the risk of myocardial infarction increased as a function of hemoglobin A1C levels.[6] DM is more likely be related to heart failure development by mostly three mechanisms: due to associated comorbidities, by favoring the development of coronary atherosclerosis, or through a specific diabetic cardiomyopathy. Associated comorbidities or risk factors may partly account for the increased risk of heart failure in diabetic patients. These cardiovascular risk factors such as dyslipidemia, hypertension, hypercoagulability, obesity, and inflammation are part of the insulin resistance syndrome and are, at least partly, regulated by nuclear peroxisome proliferator-activated receptors (PPARs); activation of PPAR-gamma improve insulin sensitivity and endothelial function, and lower inflammation and blood pressure.[7] In the Framingham cohort, men and women with diabetes had higher blood pressures and were more obese than nondiabetics; women with diabetes had, in addition, higher low-density lipoprotein-cholesterol values; high-density lipoprotein-cholesterol values were consistently lower in those with DM than in those without diabetes in both sexes.[8] The increased risk of atherosclerosis in diabetic patients may also contribute significantly to the increased risk of HF. Coronary artery disease is the underlying cause of heart failure in approximately two-thirds of patients with the left-ventricular systolic dysfunction.[9] In the study by Haffner *et al*.,[10] the 7-year incidence rate of myocardial infarction in patients with diabetes without prior myocardial infarction at baseline was 20.2% versus only 3.5% in nondiabetic patients without prior myocardial infarction at baseline. This increased risk of atherosclerosis in patients with diabetes has been attributed to diverse mechanisms such as endothelial dysfunction[11] or altered hemostatic factors (higher levels of fibrinogen,[12] plasminogen activator inhibitor-1[13,14] or Von Willebrand factor,[15] or altered platelet function).[16,17] Molecular mechanisms linking hyperglycemia and atherosclerosis have been recently reviewed by Aronson and Rayfield.[18] There are also data to suggest that diabetes may predispose to heart failure development through the existence of a specific diabetic cardiomyopathy.[19] Several hypotheses regarding diabetes-induced heart failure independent of epicardial coronary artery disease have been advanced; these include microangiopathy, metabolic factors, and fibrosis. Intramyocardial microangiopathy has also been observed in patients with diabetes hearts[20,21] combined with functional abnormalities related to endothelial dysfunction, diabetic microangiopathy may explain the reduced coronary blood flow reserve observed in patients with diabetes.[10,22,23] Metabolic factors may also play a role in the development of myocardial dysfunction, hyperglycemia, impaired myocardial glucose uptake, and increased turnover of free fatty acids may all contribute to DM-related myocardial dysfunction.[24,25] Finally, experimental and clinical data also point to a potential role for myocardial fibrosis in diabetic cardiomyopathy; intramyocardial accumulation of collagen is a well-demonstrated consequence of DM.[25] Moreover, the deposition of advanced glycation end-products may result in increased left-ventricular stiffness and consequently to diastolic dysfunction.[26-28] In summary, various mechanisms may induce a specific diabetic cardiomyopathy. Whether this diabetic cardiomyopathy alone may cause HF is, however unknown, another possibility is that these myocardial alterations related to DM may predispose to the development of HF in response to other conditions such as coronary artery disease or hypertension. A synergistic effect may exist between DM and hypertension for the development of myocardial fibrosis.[29-31]

Apoptosis

Unlike necrosis, apoptosis is an active, precisely regulated, energy requiring process which appears to be orchestrated by a genetic program,[32] and hence, the interchangeable use of the terms "apoptosis" and "programmed cell death." Apoptosis plays a crucial role in the regulation of proliferating cell populations in adult tissues and in normal tissue development.[33,34] Cells such as neurons and cardiac myocytes, even though terminally differentiated contain the genes and signal transduction pathways necessary for programmed cell death, and thus, retain the ability to die by apoptosis.[35] In humans and other mammals, adult cardiac myocytes are thought to have, at the best, a very limited capacity for self-renewal,[36] and are intended to survive and actively function for the entire life of the organism. Viewed from this perspective, death of a significant number of adult cardiac muscle cells can have lasting adverse consequences on overall cardiac performance

Cardiomyocyte apoptosis and predisposing factors for heart failure

Myocardial infarction

Myocardial ischemia and infarction represent the major etiologies that underscore the development of congestive heart failure. Cardiomyocyte loss secondary to prolonged

ischemia has long been thought to result from overt necrosis. While this form of cell death remains a primary cause of tissue injury, recent studies have suggested that cardiomyocyte loss after acute myocardial infarction can also be caused by apoptosis.[36-40] Cardiomyocyte apoptosis has been observed in humans following acute myocardial infarction. The observation of a high prevalence of cardiomyocyte apoptosis in the peri-infarct border region in comparison to myocardial regions remote from the infarction is also evident in myocardium of both humans with chronic heart failure secondary to ischemic cardiomyopathy (ICM) as well as in animal models of chronic heart failure produced by intracoronary microembolizations.[41-46]

Ventricular hypertrophy

Heart failure can result from sustained pressure overload as in long-standing hypertension or aortic valvular stenosis. Ventricular hypertrophy is associated with loss of cardiac myocytes that result in focal sites of replacement fibrosis conventionally attributed to necrosis.[47] Recent studies, however, have shown that experimentally-induced left-ventricle (LV) hypertrophy is associated with myocyte apoptosis.[48-50] Studies from other laboratories suggested that cardiomyocyte apoptosis may be important in the transition from compensated hypertrophy to heart failure.[49] In spontaneously hypertensive rats (SHR) with symptoms of heart failure, Li *et al*.[49] showed a near five-fold increase in the number of cardiac myocytes undergoing apoptosis compared to nonfailing SHR rats. In this rat model, the transition to heart failure was accompanied by features characteristic of the heart failure state, including cardiac pump dysfunction,[50,51] myocardial fibrosis,[52] and reduction in the volume fraction of cardiac myocytes.

Ventricular dilatation

Left ventricular chamber enlargement is a characteristic adaptation of the failing heart regardless of etiology. Chronic ventricular enlargement and failure can result from long-standing volume overload as in aortic or mitral valve insufficiency or the development of large conduit vessel arterio-venous fistulas. As with ventricular hypertrophy, LV chamber dilation is associated with the loss of cardiac myocytes that result in focal sites of fibrosis. Recent studies have shown that passive myocardial stretch is also associated with cardiomyocyte apoptosis

Autoimmunity

In inflammatory heart muscle disease, autoimmunity is considered to play a role in the pathogenesis of impaired cardiac performance.[53] Marked depression of cardiac function occurs in patients with dilated cardiomyopathy (DCM) in the absence of extensive loss of viable myocardium. Secretory products of immune cells such as macrophages and other infiltrating cells could well contribute to abnormalities of contraction and relaxation that are seen, for instance, in myocarditis.[53] Pro-inflammatory cytokines such as tissue necrosis factor-α (TNF-α), interleukin (IL)-1, IL-2, and IL-6 are antigen-nonspecific glycoproteins that are synthesized rapidly and released locally by immune cells in response to injury.[54] Cytokines have been shown to reduce the positive inotropic response of isolated cardiac myocytes to adrenergic agonists.[55] TNF-α and IL-1 have also been shown to uncouple agonist-occupied receptors from adenylate cyclase in isolated cardiac myocytes. TNF-α is overexpressed in patients with heart failure regardless of etiology.[56]

Evidence for cardiomyocyte apoptosis in humans with heart failure

Studies in tissues obtained from explanted hearts of patients with an end-stage heart failure have confirmed the presence of cardiomyocyte apoptosis.[56] Four of seven patients in whom heart failure was due to idiopathic dilated cardiomyopathy (IDC) had immunohistochemical evidence of cardiomyocyte nuclear DNA fragmentation by TdT-mediated dUTP nick-end labeling (TUNEL) technique and demonstrated DNA laddering consistent with apoptosis. The study by Peitsch MC *et al*, proved the endonuclease activity in the executionary pathway of the apoptosis and the effectiveness of the TUNEL in identification of the apoptotic activity.[57] In patients with acromegaly-induced cardiomyopathy, Frustaci *et al*.[58] reported a near 500-fold increase in apoptosis of cardiomyocytes compared to that observed in myocardial tissue samples obtained from papillary muscle of patients undergoing mitral valve replacement. Biochemically, apoptosis is characterized by internucleosomal cleavage of DNA by Ca^{2+} and Mg^{2+}-dependent endonuclease whose activity increases during apoptosis.[58] A study showed that deoxyribonuclease I, which is indistinguishable from endonuclease,[59] is significantly increased in myocardium of patients with end-stage heart failure compared to that of myocardium of nondiseased hearts. The above-mentioned studies in human hearts, strongly suggest that apoptosis of cardiomyocytes occurs in heart failure regardless of the predisposing factor.

Molecular triggers of apoptosis in heart failure

The multigene family of Bcl-2-like proteins, some of which such as Bcl-2 itself inhibits apoptosis and others such as Bax which promote apoptosis is one of the best known regulators of the apoptotic process.[60-62] The ratio of Bcl-2 to Bax, the so-called "death switch" is often used as an indicator of apoptosis. An increase in this ratio is used

to signify attenuation of the apoptotic process, whereas a decrease in the ratio is used to signify exacerbation of the apoptotic process. Reported a near doubling of the expression of Bcl-2 in cardiac tissue without changes in the expression of Bax, a situation that favors protection from apoptosis. Another factor involved in apoptosis is the tumor suppressor p53 protein implicated in cell cycle arrest through up-regulation of p21/WAF-1, a cyclin-dependent kinase (Cdk) inhibitor.[62] The p53 protein is believed to induce apoptosis in response to DNA damage[63] and other signals such as increased expression of c-*myc* in a manner independent of cell cycle arrest.[64] A family of cysteine proteases known as interleukin-converting enzymes (ICE) more recently referred to as "caspases" have recently taken a front and center seat as primary regulators of apoptosis. Studies in rats with acute myocardial infarction,[65,66] have suggested that ICE-like proteases modulate apoptosis based on the ability of certain pharmacologic inhibitors, such as z-VAD-fmk, a nonspecific peptide caspase inhibitor, to block the apoptosis process. In a recent study, the expression of caspase-3 was examined in LV tissue obtained from failing human hearts.[67] In this study, caspase-3 was strongly induced in myocytes bordering recent infarcts and to a lesser extent in failing hearts due to DCM. Colocalization of caspase-3 with apoptotic cardiomyocytes has been reported in rats following myocardial ischemia and reperfusion.[67] The release of cytochrome c or the release of "apoptosis-inducing factor" from mitochondria may be an important pathway for the activation of caspases with resulting apoptosis in the failing heart.[68] Cytochrome c release from mitochondria has been shown to precede caspase activation in apoptotic cardiomyocytes during ischemia in the rat.[69] Expression of Bcl-2 appears to prevent activation of the ICE protease cascade,[70] possibly by preventing release of cytochrome c. Mitochondrial abnormalities have been described in patients with heart failure that include structural disruption of the inner membrane, hyperplasia, and reduced organelle size.[71] A cell surface antigen Fas, a member of the TNF family, is also involved in the regulation of apoptosis by acting as a receptor for the ligand Fas ligand (FasL) which induces apoptosis. Recent studies have shown that circulating levels of soluble Fas, a molecule that lacks the transmembrane domain of Fas, and therefore, inhibits apoptosis, is increased in patients with congestive heart failure.[71,72] In contrast to these studies, other studies have reported increased circulating levels of soluble FasL in patients with congestive heart failure.[73] Soluble FasL is a molecule that promotes binding between Fas and FasL and favors apoptosis. Abnormal cell cycle events, cell cycle progression in the face of DNA damage, and forcing cell cycle reentry in terminally differentiated cells are all potent inducers of apoptosis.[74] Cardiac hypertrophy and failure are associated with DNA synthesis in myocytes and with up-regulation of molecular markers of cell cycle progression.[75] An increase in proliferating cell nuclear antigen, a nuclear protein necessary for DNA synthesis, and cell cycle progression[76] was reported in myocardium of dogs with heart failure induced by rapid ventricular pacing. Coordination of events that occur during the cell cycle is also dependent on a series of cyclin-dependent kinases that, as active complexes, appear to be important for the progression from G_2 to mitosis.[77] Progression through G_1 also requires inactivation of several tumor suppressor genes, including p53, p21, p16, p15, and p27 and the retinoblastoma gene Rb, that inhibit the kinase activity of the cyclin/Cdk complexes.[77] Studies by Anversa and Kajstura[77] suggested that adult cardiac myocytes are able to divide and that this capacity increases during cardiac disease, including heart failure. However, the overall frequency of such cell division, if true, remains very low, insignificant. Another possibility is that cardiomyocytes stimulated to divide are driven toward apoptosis. Evidence for this can be found in studies in which DNA synthesis induced in cardiomyocytes transfected with E1A gene, resulted in apoptosis rather cell division.[78-80]

Pathophysiological triggers of apoptosis in heart failure

It is often suggested that apoptosis may be induced by the same agents that produce necrosis with the type of cell death being dependent on the severity of the insult rather than its qualitative nature.[81] Other factors implicated as triggers of cardiomyocyte apoptosis include the formation of oxygen-free radicals,[82] exposure to hypoxia,[83,84] excess levels of angiotensin-II (A-II),[85] excess levels of norepinephrine,and increased levels of specific cytokines such as TNFα. Among these, the role of A-II, norepinephrine, and limited oxygenation of the myocardium has received considerable attention in recent years and for good reason. Enhanced and sustained activity of the renin-angiotensin system and the sympathetic nervous system as well as localized or even global hypoxia, are in many respects characteristic features of the failing heart. Exposure of isolated adult rat cardiomyocytes to A-II was shown to cause a near five-fold increase in apoptosis.[85] When cardiomyocyte were exposed to A-II in the presence of losartan, a selective AT_1-receptor antagonist, apoptosis was completely blocked.[85] Consistent with this finding, we observed an attenuation of cardiomyocyte apoptosis in dogs with microembolization-induced heart failure treated long-term with the angiotensin-converting enzyme (ACE) inhibitor enalapril.[86] ACE inhibition has also been shown to attenuate apoptosis in rats with heart failure. Exposure of isolated adult rat cardiomyocyte to norepinephrine

caused a near two-fold increase in apoptosis.[86] When myocytes were exposed to norepinephrine in the presence of the mixed β_1- and β_2-adrenergic antagonist propranolol, the effect was completely blocked.[87] Consistent with these findings in isolated rat myocytes, we observed a marked reduction of cardiomyocyte apoptosis in dogs treated long-term with the β_1-selective blocker metoprolol.[88] ICE-like proteases have also been shown to be involved in the hypoxia-induced apoptosis in cardiac myocytes.[89] Hypoxic stress a; so leads to an increase in the expression and nuclear accumulation of specific proto-oncogenes such as c-*fos*, c-*jun,* and c-*myc* that have been implicated in the induction of cell cycle progression and apoptosis.[90-92]

Suicidal autophagy – New mechanism for cell death in the diabetic heart

In failing hearts, cardiomyocytes degenerate and interstitial fibrosis, which indicates cardiomyocyte loss, becomes more prominent in the myocardium. However, the precise mechanism of cardiomyocyte degeneration that leads to cell death is still unclear, although it is presumed that lysosomal function and autophagy play an important role because lysosomal activity increases under stress such as hypoxia.[93] Myocardium that had been resected during partial left ventriculectomy performed in patients with DCM was examined. Under light microscopy, some cardiomyocytes had a marked scarcity of myofibrils and had prominent cytoplasmic vacuolization. Atrophic and degenerated cardiomyocytes were often observed adjacent to replacement fibrotic tissue.[94] Immunohistochemistry showed positivity for lysosome-associated membrane protein and a lysosomal catheptic enzyme in vacuoles of various sizes in the cardiomyocytes, and these lysosomal markers were markedly increased in atrophic and degenerated cardiomyocytes. Electron microscopy revealed that degenerated cardiomyocytes had many vacuoles-containing intracellular organelles, such as mitochondria, and were considered to be autophagic vacuoles.[94]

CONCLUSION

Diabetes is a significant risk factor for cardiovascular diseases, with the majority of these complications being attributed to coronary vascular pathology. Diabetes increases both apoptosis and necrosis in human myocardium. Multiple mechanisms and factors involving ventricular myocytes and regarding autophagy, apoptosis and programmed necrosis prove the role of diabetes as a synergistic risk factor in the decline of cardiac performance and contractile impairment after myocardial injury.

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Conflicts of interest

There are no conflicts of interest.

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