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The Role of Oxidative Stress and Apoptosis in the Pathogenesis of Heart Failure

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Abstract

Apoptotic cell death has been implicated in cardiovascular pathologies such as congestive heart failure, myocardial ischemia, cardiomyopathy and atherogenesis. A growing body of literature suggests apoptosis induced by oxidative stress plays a key role in the pathogenesis of heart failure. Our preliminary results suggest that there is increased expression of antiapoptotic Bcl-2 proteins and decreased expression of antiapoptotic protein Mcl-1 in ischemic heart disease patients as compared to the control group. This increase in Bcl-2 expression is accompanied by a decrease in expression of proapoptotic Bax proteins when compared to controls. We believe that irrespective of proapoptotic or antiapoptotic protein influences, the ratio of Bcl-2/Bax proteins plays a major role in determining cardiomyocyte apoptosis. An enhanced Bcl-2 expression in ischemic heart disease patients suggests a possible compensatory mechanism of cell protection. The precise trigger of the apoptotic process in cardiomyocytes is not yet clear but our preliminary data suggest that apoptosis and oxidative stress may play a critical role. It is plausible that alterations caused by reactive oxygen species (ROS) in the molecular functions of cellular structures may contribute to ischemia, apoptosis and ultimately to heart failure. Further studies are required to establish a possible link between oxidative stress and apoptosis. The conclusive evidence and precise mechanism of apoptosis in the pathogenesis of ischemic heart disease may open abundant therapeutic avenues to prevent cardiac dysfunction.

Keywords

Apoptosis; Oxidative stress; Heart failure; Heart disease; Pathogenesis

Abbreviations: CHF: Congestive Heart Failure; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase

Introduction

Apoptosis is a highly regulated and programmed cellular cascade pathway whereby the cell takes part in its own demise. Apoptosis is crucial for cell survival and essential for development, tissue homeostasis and immunity [1]. Apoptotic cell death, a strongly preserved process, has been implicated in cardiovascular conditions such as congestive heart failure [2], myocardial ischemia [3,4], cardiomyopathy [5] and atherogenesis [6,7]. Congestive heart failure (CHF) is a chronic progressive cardiovascular condition that affects approximately 26 million people worldwide [8]. Major risk factors include ischemic, hypertensive, toxic and inflammatory diseases. In heart failure, the heart loses its ability to pump sufficient blood to all organs, resulting in life threating consequences.

Regardless of the cause, important components of heart failure include cardiomyocyte death and apoptosis [1,9,10]. Non-myocyte cells demonstrate apoptotic pathways – some studies suggest that similar pathways exist in cardiomyocytes [11-13]. Apoptosis of cardiomyocytes in heart failure has been shown to be associated with increased ventricular wall stress. In turn, this stress may trigger further apoptosis of cardiomyocyte cells in the heart [14]. Adult cardiomyocytes are non-dividing, terminally differentiated cells; therefore, the mechanism of apoptosis in cardiomyocytes is not well defined [11]. Here we review studies suggesting apoptosis induced by oxidative stress plays a key role in the pathogenesis of heart failure.

Oxidative stress and cardiomyocyte apoptosis

There is mounting evidence to suggest that increased oxidative stress may promote cardiomyocyte apoptosis [15]. Stress factors can cause modifications in heart tissue and its functions. Although the heart can adapt to stress factors in the short term, these adaptive mechanisms eventually fail under permanent stress [16]. One of the internal stress factors is oxidative stress, as has been previously shown to be important in cardiovascular disease. Oxidative stress in the heart acts as a stimulus for the activation of the mitochondrial apoptotic pathway, one of the pathways for the apoptotic process. This stimulation results in cytochrome c activation and its release from the mitochondria. This process may cause cardiomyocyte apoptosis resulting in decreased myocardial contractility and eventual heart failure (Figure 1).

Reactive oxygen species (ROS) are chemically reactive molecules which may damage cellular carbohydrates, nucleic acids, lipids and proteins. ROS generation occurs in mitochondria under hypoxic conditions and plays a role in various physiological processes at low and moderate concentrations. Increased ROS production has been found to result in adverse conditions [17-20]. Von Harsdorf et al. explored signaling pathways in ROS-induced cardiomyocyte apoptosis and suggested an important pathophysiological role for ROS in cardiac diseases characterized by apoptotic cell death [21]. Kuo et al. identified that ROS production levels affect the apoptosis of cardiomyocytes [22]. Ichihara determined the role of the oxidative stress in heart failure [23]. Similarly, in rat [24] and rabbit [25] cardiomyocytes, oxidative stress induced by ischemic reperfusion injury has been shown to promote apoptosis. From these studies, it is plausible to infer that alterations caused by ROS in the molecular functions of cellular structures may contribute to ischemia, apoptosis and ultimately to heart failure.

Apoptotic genes and mitochondrial apoptotic pathways

Apoptosomes recruit procaspase-9, facilitating the autoactivation of caspase-9 which then activates effector caspases as a response to apoptotic signals [26-28]. In contrast, ROS may affect these proteins and blocks apoptosis. Mitochondrial pore oxidation by these reactive

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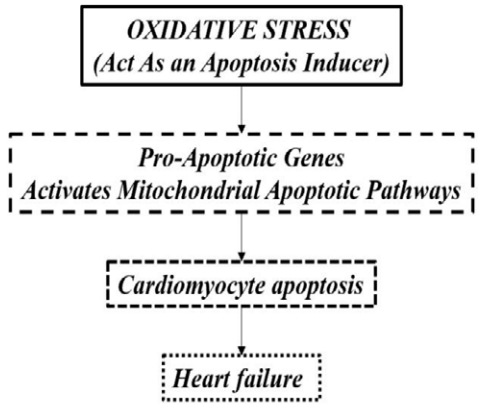

Figure 1: Oxidative stress and apoptotic changes leading to heart failure.

molecules may induce cytochrome c release due to differentiated mitochondrial membrane potentials. Pro-survival cell guardians including Bcl-2, and the pro-apoptotic effector proteins Bax (Bcl-2 associated X protein) and Bak (Bcl-2 antagonist/killer) [29] play important roles in the apoptotic pathway. The Bcl-2 protein family determines the commitment of cells to apoptosis. Olivetti et al. observed a nearly two-fold increase in the expression of anti-apoptotic Bcl-2 in failing hearts as compared to controls [30]. In contrast, expression of the Bax protein, which is a member of the BCL-2 family and initiates apoptosis, remained constant. Narula et al. demonstrated DNA laddering, the hallmark of apoptosis, in myocardial samples of patients with dilated cardiomyopathy [15]. It has been reported that serotonin treatment induces apoptosis via cytochrome-c release, Bax up-regulation and Bcl-2 downregulation [31]. These findings further illustrate the importance of reviewing current evidence on the association between oxidative stress and cardiac apoptosis and assess markers of oxidative stress and apoptosis in patients with ischemic heart disease.

The apoptotic process involves a complex cascade of signaling pathways involving the family of cysteine proteases called caspases and Bcl-2 family of proteins [32]. The effector caspases play a central role in apoptotic cell death. There are at least 14 mammalian members of the caspase gene family that have been discovered [33]. In the apoptotic process, caspases have a dual role, functioning in cell disassembly (effectors) and initiating the disassembly of receiving proapoptotic signals (initiators) [34]. The Bcl-2 family includes pro-apoptotic proteins Bax [35], Bak [36], Bcl-xS [37], Bad [38] and antiapoptotic proteins Bcl-2 [39,40], Bcl-xL [37] and Mcl-1 [41].

Discussion and Conclusion

The Bcl-2 family of proteins plays an important mediatory role in the regulation of apoptosis. The regulation of apoptosis by the Bcl-2 family is brought about by the ability of members of the Bcl-2 family to interact with one another, forming homo - and hetero-dimers. The ratio of antiapoptotic Bcl-2 to proapoptotic Bax protein determines if the cell survives or perishes due to the apoptotic process [42]. The ratio of Bcl-xL to Bcl-xS proteins also plays a role in determining the susceptibility of a cell to apoptosis [37]. The genes for regulatory proteins of the Bcl-2 family may have a tissue-specific expression [43] and the proteins may function in a tissue-specific manner [38]. It has been suggested that if Bax homodimerization is a dominant feature, the result is cell death, whereas if Bcl-2-Bax heterodimerization predominates, the cell survives [44]. Our preliminary results suggest that there is an increased expression of antiapoptotic Bcl-2 proteins in ischemic heart disease patients than the controls [45]. This increase in Bcl-2 expression is accompanied by a decreased expression of proapoptotic Bax protein as compared to controls. We also observed the Bcl-2/Bax ratio was increased in the disease group compared to the controls. We believe that irrespective of proapoptotic or antiapoptotic protein influences, the overall ratio of these proteins plays a major role in determining cardiomyocyte apoptosis. These results though consistent with the findings of Olivetti et al. who reported an increased Bcl-2 in heart failure group as compared to controls [30], differed in their finding of an unchanged Bax expression in the control and heart failure group. This difference may be due to the variation in the sample characteristics. Olivetti et al. studied explanted hearts from heart failure patients [30], while our study included heart tissue from subjects with ischemic heart disease. It is possible that the apoptotic process follows a distinct pattern in various diagnostic subgroups of cardiovascular disease. Olivetti et al. suggested that the increased Bcl-2 expression in the heart failure group may be a compensatory response to maintain cell survival [30]. The study by Olivetti et al. comprised mainly of patients in advanced stage of heart failure, the compensatory phenomenon of Bcl-2 increase may not necessarily occur in only heart failure patients as suggested by our data, which consisted of patients with ischemic heart disease. There may be some conditions in which apoptosis occur irrespective of the expression of Bcl-2. It has been suggested that Bcl-2 may not confer protection from cell death in all types of apoptosis [46,47] and a high Bax/Bcl-2 ratio prevents Bcl-2 from protecting against cell death [35]. Chittenden et al. observed the partial blocking action of Bak, a Bcl-2 homolog, on the antiapoptotic effect of Bcl-2 [36].

Along with an increased Bcl-2 expression, we also observed a decreased expression of antiapoptotic protein Mcl-1 in the disease group as compared to the control group [45]. Bingle et al. suggested a dual role of antiapoptotic and proapoptotic action of Mcl-1 [48]. This study suggested, that the human Mcl-1 could undergo differential splicing and an exon skipping that yields an internally deleted, death inducing gene product. Bae et al. suggested that the fate of Mcl-1 expressing cells could be regulated through alternative splicing mechanisms and interactions of the resultant anti- and proapoptotic gene products [49]. Therefore, the compensatory mechanism hypothesis proposed by Olivetti et al. may explain our finding of a decreased Mcl-1 expression in the disease group [30]. In the myocardium, as in other tissues, antioxidant enzymes protect cells by maintaining superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) at low levels. Oxidative stress refers to an imbalance between oxidants and antioxidants. Three known types of superoxide dismutase (SOD) are present in mammalian tissues [50]. Apoptosis induction with ROS through upregulation of the Fas-FasL system activates caspase-8 and downstream caspases. Mitochondria released cytochrome c interacts with an adapter molecule (Apaf1) and initiates mitochondrial apoptosis – a critical step for apoptosome formation. The apoptosome is a caspase-activating complex including Apaf-1, cytochrome c, and caspase-9. Mitochondria are a target and source of ROS [51]. In heart failure, cardiomyocyte death by apoptosis is triggered by oxidative cell signaling pathways [52]. Cardiomyocyte apoptosis related to ROS pathway is clarified by different studies. Von Harsdorf et al. indicated that different ROS inducers triggered cardiomyocyte apoptosis in cardiac diseases. In this study, they used isolated cardiac cell culture models. ROS induction initiated via superoxide anion (O_2^-) and H_2O_2 and they showed that both these ROS species induced cardiomyocyte apoptosis. It was reported that apoptosis starts via Mch2α induced

cytochrome c release. Both H_2O_2 and O_2^- induced immediate expression of p53, however, H_2O_2 and O_2^- may trigger different apoptotic pathways [21]. Puma induced apoptosis is associated with regeneration of superoxide and H_2O_2 which is Bax dependent [53]. This is confirmed by the presence of antioxidants that prevent Puma-dependent apoptosis. On the other hand, Bax inactivation confers a resistance to Puma-dependent apoptosis [54]. ROS are critical activators of the mitochondrial permeability transition pore, inducing apoptosis and/or necrosis [55].

An enhanced Bcl-2 expression in ischemic heart disease patients suggests a possible compensatory mechanism of cell protection. The precise trigger of the apoptotic process in cardiomyocytes is not clear – a strong role for oxidative stress is suggested. Our preliminary data suggest that apoptosis and oxidative stress may play a critical role in the pathogenesis of ischemic heart disease. Further studies are required to establish a possible link between oxidative stress and apoptosis. The conclusive evidence and precise mechanism of apoptosis in the pathogenesis of ischemic heart disease may open abundant therapeutic avenues to prevent cardiac dysfunction.

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Table 1: 2014 IMWG Diagnostic criteria for multiple myeloma [2].

Diagnostic criteria for multiple myeloma		
1	Clonal bone marrow plasma cells ≥ 10% or a biopsy-proven bony or extramedullary plasmacytoma	
2	Any one or more of the following myeloma defining events:	
	a. Evidence of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder:	Hypercalcaemia: Serum calcium > 0.25mmol/L above the upper limit of normal or > 2.75mmol/L
		Renal insufficiency: Creatinine clearance < 40ml/min or serum creatinine > 177umol/L
		Anaemia: Haemoglobin > 2g/dL below the lower limit of normal or haemoglobin < 10g/dL
		Bone lesions: One or more osteolytic lesions on skeletal radiography, CT or PET-CT
	b. Any one or more of the following biomarkers of malignancy:	Clonal bone marrow plasma cells percentage ≥ 60%
		Involved: Uninvolved serum free light chain ratio ≥ 100
		>1 focal lesions on MRI

Table 2: R-ISS Staging system for multiple myeloma [16].

Staging system for multiple myeloma		
Stage I	All of the following: a. Serum albumin ≥ 35g/L b. Serum β_2 microglobulin ≤ 3.5mg/L c. Serum LDH < upper limit of normal d. No high-risk cytogenetic abnormalities	5 Year OS of 82%
Stage II	Not fitting stages I or III	5 Year OS of 62%
Stage III	All of the following: a. Serum β_2 microglobulin ≥ 5.5mg/L b. LDH > upper limit of normal OR presence of high-risk cytogenetic abnormalities: del 17p t(4;14) t(14;16)	5 Year OS of 40%