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Oxidative stress mediated cardiac apoptosis

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Abstract: Cardiovascular diseases are one of the most common diseases all around the world. In both acute and chronic cardiac diseases, apoptosis is the primary pathway of disease nature and according to current studies apoptotic pathway inhibition has an important role in cardiac disease tretament procedures. There are several studies regarding clarification of molecular pathways of cardiac apoptosis in several heart diseases. Oxidative stress could be defined as imbalance between the antioxidant defense systems and high concentrations of reactive species that arose from cellular processes and this mechanism also initiates apoptosis in cardiac cells. In the pathogenesis of apoptosis mediated cardiac diseases, reactive oxygen species (ROS) are the main oxidative stress products that play an important role in complex signaling pathway as secondary messengers. Here, we review the current status of knowledge on ROS induced cardiac apoptosis in heart diseases.

Key words: Cardiac apoptosis, oxidative stress, reactive oxygen species

Introduction

Globally, cardiovascular diseases are the leading cause of morbidity and mortality. Therefore, scientists focused on this research area for many years. Several studies are conducted to clarify underlying mechanisms, which are mainly focused on reasons and process of cell death. Non-myocyte cells demonstrate apoptotic pathways, certain studies declare that the similar pathways exist on cardiomyocytes, too (Wencker et al., 2003; Cook & Poole-Wilson, 1999; Gill et al., 2002).

In recent years, there is an increased interest on apoptosis mediated diseases. Apoptosis is an cellular cascade pathway which occurs under physiological and pathological conditions (Wong, 2011). In biological

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systems, exogenous and endogenous factors trigger apoptosis. These factors include ultraviolet radiation, oxidative stress, and genotoxic chemicals as well as cell damage. Apoptosis plays pivotal role in cell survival, cell turnover mechanisms, cellular development, immune system functions, hormone controlled atrophy, embryonic development, elimination of junk cells and maintains cell homeostasis (Rastogi & Sinha, 2009). Apoptotic pathway is well preserved and it has been shown that pathways can be classified into biochemical and morphological (Saraste & Pulkki, 2000). Cell shrinkage, membrane fragmentation into apoptotic bodies, nuclear fragmentation and chromatin condensation in the nucleus, pyknosis (decreased cellular volume) are typical morphological features of the apoptosis (Wong, 2011; Saraste & Pulkki, 2000). Biochemical marker of apoptosis is intranucleosomal fragmentation of genomic DNA. Cell and nucleus shrinkage are the characteristic onset of apoptosis following karyorrhexis (nucleus breaks up) occurence. Apoptotic cells detach surround tissues and form apoptotic bodies and then eliminate by phagocytosis (Saraste & Pulkki, 2000).

Both, cardiomyocyte death and apoptosis were determined in acute myocardial infraction, myocarditis, arrhythmias, congestive heart failure, ischemic heart diseases. Conversely, cardiomyocyte apoptosis is also essential in homeostasis and its deficiency may cause diseases like Noonan's syndrome. Acquired data were not sufficient for explaining detailed mechanism of apoptosis in cardiomyocytes (Cook & Poole-Wilson, 1999; Haunstetter & Izumo, 2000; Feuerstein & Young, 2000). In neonatal cardiomyocyte culture studies it has been demonstrated that five pathways play crucial role in cardiomyocyte proapototic signaling. These pathways can be listed as 1) redox regulated system that contain oxygen radical activation effect, 2) death domain-Fas/TNF receptor pathway, 3) mitochondrial cytochrome-c stimulated caspase dependent pathway, 4) G-protein coupled reseptor pathway, 5) phospholipase-C biochemical activation pathway (Feuerstein & Young, 2000).

External and internal stress factors cause complex modifications in tissues and functions. However, heart tissue highly adapts to these stress factors through tissue remodeling and functional compensation. This adaptation mechanism might fail under the presence of permanent stress factors (Anilkumar et al., 2009). Oxidative stress is an internal stress

factor for apoptosis and it plays important role in cardiovascular diseases as atherosclerosis, reperfusion injury, hypertension, and heart failure. Permanent oxidative stress causes increased cardiac ROS production. Determination of apoptosis in cardiac cells is an important topic for clarification of cardiovascular disease progressions (Haunstetter & Izumo, 2000). ROS activated transcription factors regulate redox and these factors involve in the basic mechanism of cardiovascular diseases. In studies conducted on animals, the role of oxidative stress has been determined in heart failure and other cardiovascular diseases (Ichihara, 2013). ROS have an important role on the regulation of signal transductions. Increased ROS generation might be a risk factor for several cardiovascular diseases. Therefore, clarifying ROS-generation and intracellular signaling processes will provide an insight for the pathogenesis of cardiovascular diseases (Yoshizumi et al., 2001).

This article aims to summarize the role of oxidative stress in cardiomyocyte apoptotic cell death.

Oxidative stress

Oxidative stress is a term which explains imbalance between oxidants and antioxidants. Several pathological conditions such as cancer, neurological diseases, athetosclerosis, diabetes, hypertension, chronic obstructive pulmonary disease (COPD) and asthma are found to be related with oxidative stress. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase are main antioxidant defense systems of a metabolism in order to eliminate oxidative stress, while ascorbate, α-tocopherol and glutathione, cysteine, thioredoxin, vitamins also take role in defense systems. In normal cellular metabolism, living organisms produce ROS, no matter it is under the influence of environmental factors or not. ROS are chemically reactive molecules which may damage cellular carbonhydrates, nucleic acids, lipids and proteins. Several alterations may occur in molecular functions of these cellular structures. ROS play role in various physiological processes at low and moderate concentrations, while increased ROS production may result adverse conditions (Birben et al., 2012; Al-Dalaen & Al-Qtaitat, 2014; Rahman, 2012, Halliwell, 2007).

Mitochondria, peroxisomes, endoplasmic reticulum, and NADPH oxidase (NOX) complex in cell membranes are major endogenous oxidant sources in the cell. ROS production occurs intracellularly from these endogenous oxidant sources via multiple pathways. ROS are classified into two classes: 1) free radical molecules containing unpaired electrons (Al-Dalaen & Al-Qtaitat, 2014) 2) nonradicals without net charge like H_2O_2 (Sofo et al., 2015). Superoxide anion (O_2-·), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2) are major oxidative stress products that have physiological importance especially for DNA damage. Under normal conditions, ATP production pathway results with oxygen molecule reduction to water. However, a small number of electrons can not complete the reducing process and superoxide radical (O_2-·) production occurs. Superoxide radical may react with cellular enzymes. Superoxides initiates lipid peroxidation process in the cell via its protonated hydroperoxyl (HO_2·) form. The most reactive type of ROS is hydroxyl radical that reacts with proteins, lipids, carbohydrates and DNA (Al-Dalaen & Al-Qtaitat, 2014). In peroxisomes; xanthine oxidase, amino acid oxidase, and NADPH oxidase produce hydrogen peroxide radicals via Haber–Weiss and Fenton reactions in the existence of transition metals such as Fe, Cu, Zn, Al (Al-Dalaen & Al-Qtaitat, 2014; Das et al., 2015). Peroxyl radicals (RO_2·) are produced by lipid peroxidation process through interaction of lipid radicals with oxygen atoms. In the environment, there are several exogenous oxidant sources having critical roles in oxidative stress mechanisms such as radiation, heavy metals, pollutants, ozone, and smoking (Al-Dalaen & Al-Qtaitat, 2014). p53 has a role in maintaining the integrity of the genome and is involved in celular redox homeostasis. ROS interaction with DNA can cause DNA damage and this damage triggers the activation of p53. Increased p53 activity upregulates Blc-2 family proteins which induces cytocrome c release from mitochondria. Therefore. mitochondria are crucial on cellular apoptosis initiation through release of cytochrome c. ROS induced cytochrome c release is regulated by Bax protein which is a member of Bcl-2 family and initiates apoptosis. Apotosis induction with ROS through upregulation of the Fas-FasL system activates caspase-8 and downstream caspases. Mitochondria released cytochrome c interacts with adapter molecule (Apaf1) and initiates mitochondrial apoptosis and this is a critical step for apoptosome formation. The apoptosome is a caspase activating complex inculding Apaf-1, cytochrome c, and caspase-9. The

apoptosome recruits procaspase-9 facilitating autoactivation of caspase-9 which activates effector caspases as a response to apoptotic signals (Chandra et al., 2000; Dhalla et al., 2000; Ott et al., 2007). In contrast, ROS may effect these proteins and blocks apoptosis. Mitochondrial pore oxidation by these reactive molecules may induce cytochrome c release due to differentiated mitochondrial membrane potential. This direct and indirect mechanisms of ROS mediated mitochondrial apoptosis is shown in (Figure 1). We can emphasise that mitochondria is a target and source of ROS (Simon et al., 2000).

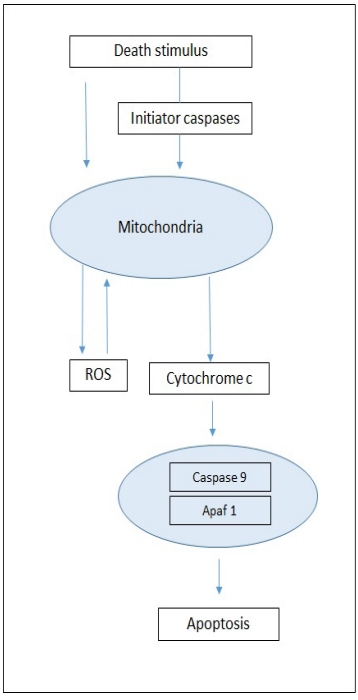

Figure 1. Possible mechanisms of direct and indirect ROS mediated mitochondrial apoptosis (Simon et al., 2000).

In heart failure, cardiomyocyte death by apoptosis is triggered by oxidative cell signaling pathways (Kumar et al., 2002). Oxidative stress may activate by well-defined mitogen-activated protein kinase (MAPK) signaling cascade in cardiac tissue. There are two main MAPK pathways; c-Jun NH2-terminal protein kinase (JNK) and p38 kinase (p38). JNK, p38 and p53 are regulating proteins for ROS mediated apoptosis. JNK and p38

have pro-apoptotic roles whereas extracellular signal-regulated kinase (ERK) has an anti-apoptotic role. MAP kinases take role in balancing cell survival and death in ischemic myocardium. JNK phosphorylates p53 after ROS induction and phosphorylated p53 stimulates apoptosis in cardiomyocytes (Yoshizumi et al., 2001). Qin et al., 2003 shown that cardiomyocyte apoptosis associated with increased oxidative stress, JNK and p38 activity and down regulated ERK activity. Figure 2 demonstrates the mechanism of oxidative stress-induced myocyte apoptosis (Qin et al., 2003).

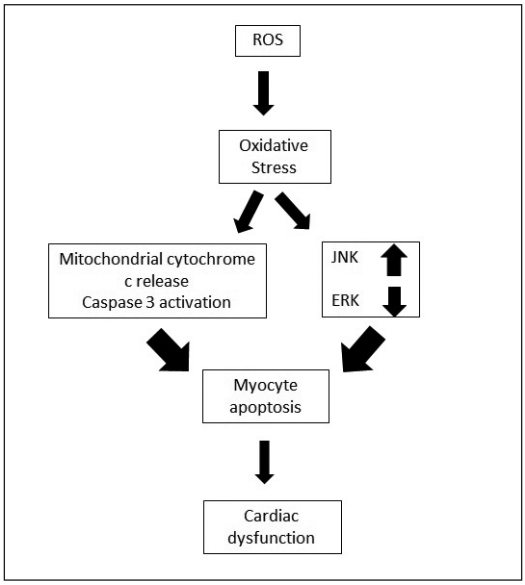

Figure 2. Mechanism of myocyte apoptosis induction by oxidative stress (Qin et al., 2003).

Cardiomyocyte Apoptosis and Oxidative Pathway

Cardiovascular disease progression involves acute or chronic cardiomyocyte loss and apoptosis, which are important components of disease process (Kim & Kang 2010; Khoynezhad et al., 2007). There are several reports about cardiomyocyte apoptosis contribution to cardiac dysfunction in both acute and chronic heart failure. However, this mechanism has not been clearly established yet (Kajstura et al., 1996). One of the main reason of this ambiguity can be based on the selection

of experimental procedures. Most of the invitro apoptosis studies are conducted on dividing and undifferantiated cells. However, adult cardiomyocytes are non dividing, terminally differentiated cells and, when collapsed replacement is uncommon. Therefore, the mechanism of cell death in cardiomyocytes is not well defined (Gill et al., 2002).

There are two mainly-identified apoptosis pathways, they can be listed as the intrinsic (or mitochondrial) and the extrinsic (death receptor) pathways (Gill et al., 2002). Extrinsic pathway is stimulated by Fas death receptor which is a member of tumor necrosis factor (TNF) receptor super family. Intrinsic pathway stimulated via cytochrome-c release from mitochondria with apoptotic signals. Extrinsic and intrinsic pathways are both activate caspase cascades resulting with cell death (Ghobrial et al., 2005). Mitochondria play a key role in cardiac apoptosis. Cardiomyocytes require much energy and mitochondria are the major source of energy in cardiomyocytes (Gill et al., 2002). In the mitochondria mediated pathway; an apoptotic insult induces intrinsic pathway in order to relase cytocrome c in the cytosol. Caspases are members of multigene family and they hydrolyze peptide bonds in aspartic acid residues of carboxyl groups. Cardiac apoptosis is processed by cysteine proteases which are defined as caspases (Khoynezhad et al., 2007; Thornberry & Lazebnik, 1998). There are several animal and human studies have been conducted regarding apoptosis in cardiac cell death. (Mann et al., 2014; Hirota et al., 1999; Koglin et al., 1999; Adams et al., 1998; Huber., 1997; Bartling et al.,1999; Filippatos et al., 1999; Saraste et al., 1999; Frustaci et al., 1999; Narula et al., 1996)

Until now, many evidences have been provided regarding the role of cardiac apoptosis in cardiovascular diseases, however intact mechanism has not been clearly defined yet. There are intrinsic (abnormal blood flow, serum withdrawal, angiotensin II, hyperglycemia and pressure overload) and extrinsic (mechanical forces, neurohormonal activation, oxidative stress, hypoxia, and cytokines) stress factors have been shown in cardiomyocyte apoptosis. These stress factors indirectly cause oxidative stress which effect intracellular signaling pathways (Kumar et al., 2002; Fortuño et al., 2001). Cardiomyocyte apoptosis related to ROS pathway is clarified by different studies. Von Harsdorf et al. (1999) indicated that different ROS inducers triggered cardiomyocyte apoptosis in cardiac

diseases. In this study they used isolated cardiac cell culture model. ROS induction initiated via superoxide anion ($O_2^{\cdot}$) and H_2O_2 and they showed that both these ROS species induced cardiomyocyte apoptosis. It has been shown in this study, apoptosis started via Mch2α induced cytochorome-c release. They indicate that, both H_2O_2 and $O_2^{\cdot}$ induced immediate expression of p53, however H_2O_2 and $O_2^{\cdot}$ could trigger different apoptotic pathways (Von Harsdorf et al., 1999).

Limited studies are found regarding ROS products and their role on cardiac apoptosis mechanisms. Lipid peroxidation pathway initiates with polyunsaturated fatty acid oxidation in cell membrane (Porter et al., 1995). Folden et al (2003) indicated that malondialdehyde, which is the end product of lipid peroxidation, inhibits cardiac contraction via reduction of intracellular Ca+2 transients in myocytes (Folden et al., 2003). Inhibition of myocte contraction occurs via p38 mediated cell death by lipid peroxide activation. Levrand et al have shown that peroxynitrile initiates apoptosis in cardiomyocytes by caspase-3 activation and PARP cleavage in vitro and in vivo (Levrand et al 2006). 8-hydroxy-deoxyguanosine levels widely used for detection of oxidative DNA damaging effect and it is proposed to have a role on cardiomyocyte apoptosis. (Nistri et al., 2015; Qin et al., 2006)

Heart failure

It is estimated that approximately 26 million people suffer from heart failure. In heart failure, heart could not pump enough blood to whole body and may result with life threatening reactions (Ponikowski et al., 2014). Heart failure consists of complex and multifactorial pathogenesis pathway. One of the main biological marker for heart failure is myocyte cell loss, which may occur due to apoptosis or necrosis. The balance between cell death and survival are controlled through genetic/epigenetic pathways (Kumar et al., 2002). Pathogenic, progressive loss of cardiac myocytes may result with heart failure (Kang & Izumo, 2000).

It is speculated that ATP synthesis in mitochondria may linked to oxidative stress induced impairments in energy metabolism. The importance of this mechanism is due to uncoupling energy utilization in vetricule performence which results in heart failure. Increased

ROS formation causes myocyte apoptosis and results with ventricular dysfunction. ROS induced apoptosis in myocyte cells reveals pathological conditions including cardiomyopathy. There are few studies regarding cytochrome-c release initiated apoptosis and the role of caspase activations in left ventricular (LV) dysfunction. However further studies are needed in order to clarify molecular mechanisms of these pathways. It has been demonstrated that upregulated p66shc signaling molecule is related to oxidative stress induced apoptosis in pacing-induced heart failure. p66shc could be activated via several factors like H_2O_2, UV radiation and Epidermal Growth Factor (EGF). In heart failure mitochondria plays a key role in ROS production and apoptosis (Hare, 2001). Also, it has been shown that p66shc phosphorylation by alcohol consumption may induce ROS formation and release of cytochrome c from mitochondria in order to trigger apoptosis. Wang et al (2015) has been shown that protein kinase C-β (PKC-β) inhibition and p66Shc deficiency prevents alcohol induced cardiomyocyte apoptosis (Wang et al., 2015).

Another important oxidative stress related molecule in the mechanism of heart failure is xanthine oxidase (XO) which produce superoxide molecules. It has been shown that XO inhibition restores cardiac ritm and increased myocard contractility (Hare, 2001). Cesselli et al. (2001) demonstrated in dogs that cytochrome-c release stimulates caspase-9 activation in dilated cardiomyopathy. Nitrotyrosine formation and increase of expression p66shc levels induce cytochrome-c release (Cesselli et al., 2001; Krijnen et al., 2002) defined that, long term antioxidant treatment after myocard infarction (MI), reduces oxidative stress, myocyte apoptosis and caspase-3 activity. These results indicates the linkage between oxidative stress and apoptosis in MI (Krijnen et al., 2002).

ROS generation occurs in mitochondria under hypoxic conditions. After then ischemia induces apoptosis results in heart failure. Kuo et al. (2015) revealed that ROS regulating protein expression levels affect apoptosis of cardiomyocytes and fate of the disease. They have shown that under hypoxic conditions, -Lon protease expression is upregulated in cardiomyocytes. However under normoxic conditions stimulation of Lon protease expression results with apoptosis. These results clarifies the mechanism of ROS regulation in apoptosis (Kuo et al., 2015). Myocyte loss through apoptosis in ischemia reperfusion injury has been reported in

acute MI (Sareste et al., 1999) and in the cardiomyopathy patients at the end-stage (Narula et al., 1996). Apoptosis regulatory pathways function like antioxidant pathways while oxidative stress elicites myocardial apoptosis during reperfusion (Hockenbery et al., 1993, Zhao, 2004).

Another research area for scientists is drug induced cardiomyocyte apoptosis mechanism. Apoptosis has major role in drug-related cardiomyopathy development, which results with cardiomyocyte death (Octaviaa et al., 2012; Deavall et al., 2012; Bianchi et al., 2005). It has been reported that cardiomyocyte death occurs after treatment with anticancer drug anthracyclines. ROS induced mitochondrial apoptosis is related with the effects of ROS like lipid peroxidation, adenosine triphosphate synthesis and impairements of mitochondrial oxidative phosphorylation and calcium homeostasis (Shi et al., 2011). Also, doxorubicin (Dox) treatment induced apoptosis occurs with the effect of ROS in mithochondria. ROS related p38 and JNK signalling pathways and p53 accumulation results with cardiomyocyte death (Deavall et al., 2012). Bianchi et al. (2005) demonstrated that intracellular oxidative stress and apoptosis increased in cardiomyocytes of rats under seratonin treatment. It has been discussed that seratonin treatment induces apoptosis via cytochrome-c release, Bax up-regulation and Bcl-2 downregulation (Bianchi et al., 2005).

Based on the several studies conducted on cardiomyocyte apoptosis mechanism pathways, many therapeutic targets have been defined in order to protect cardiomyocytes from cell damage and apoptosis. Table 1 demonstrates possible biological targets and treatment approaches for cardiac cell survival.

Table 1. Possible biological targets for cardiac cell survival and treatment approaches (Hinescu, 2001).

	Metabolic target	***Genetic target***	***Treatment approach***
Apoptosis inducers	Oxygen free radicals Hypoxia Calcium overload	Pro-apoptotic genes	Angiotensin II Norepinephrine TNF***α***
Cell survival inducers	Putative agents preventing/ correcting metabolic disturbances	Anti-apoptotic genes	Losartan ***Beta 1-blockers*** Ab anti- TNF***α***

Diabetic cardiomyopathy

Diabetic cardiomyopathy is one of the leading cardiovascular complications in diabetic patients and increases the risk of heart failure (Zhong et al., 2015). Diabetic cardiomyopathy effects myocardium and associated with defects in cellular organelles. These derangements in cardiomyocytes lead to ventricular hypertrophy and eventually to heart dysfunction and heart failure (Cai & Kang, 2001). Pathogenesis of diabetic cardiomyopathy believed to be multifactorial and involves several mechanisms, including oxidative stress, cardiac inflammation and cardiac cell apoptosis (Hayat et al., 2004).

Myocyte disarray was first described in 1958 by Tersa as the area of abnormal parallel alignment of myocardial cells. Myocyte disarray is a common structural disorganization determined in many cardiac diseases especially for diabetic cardiac diseases. Impaired glucose transporter 1 and 4 (GLUT1, GLUT4) effect intracellular energy metabolism in diabetes. Fatty acid oxidation takes the role of glycolysis and results in ROS production. Sustained mitochondrial ROS production by hyperglycemia results deposition of collagen in myocardium following contractile dysfunction. Therefore, cardiomyocyte apoptosis results myocardial disarray in diabetes (Lasker et al., 2011). In type 2 diabetes, oxidative stress parameters increase due to the symptoms like hyperglycaemia, hyperlipidaemia, hyperinsulinaemia and insulin resistance. Main ROS sources of myocard tissue is defined as mitochondria, NADPH oxidase

or nitric oxide synthesis (NOS). Zhong et al. (2015) showed that down-regulation of ROS level, cardiac inflammation, cell apoptosis, may have therapeutic potential in the treatment of diabetic cardiomyopathy (Zhong et al., 2015).

Oxidative stress induced apoptosis has been well documented, however further investigations needed regarding the role of cardiomyocyte apoptosis in the development and progression of cardiac diseases (Kumar & Jugdutt, 2003).

Conclusion

It has been well known that oxidative stress has toxic effects on several types of cells including cardiac myocytes of heart. Increased ROS production plays crucial role in cardiomyocyte growth mechanism. This indicates that ROS may play role as secondary messenger in cardiac cellular survive or apoptotic mechanisms. Mechanism of mitochondrial ROS generation and ROS triggered cardiac cell death are new approaches for understanding the molecular pathphysiology in cardiovascular diseases.

In cardiovascular diseases, inhibiton of molecules which has a role in ROS mediated apoptosis, blocks cardiomyocyte cell death. This mechanism has a potential for upcoming therapeutic strategies.

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