

## OXIDATIVE STRESS AND CARDIAC HYPERTROPHY

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### [1] INTRODUCTION

Chronic Cardiac hypertrophy (CH) is enlargement of heart resulting from increased myocyte size which is generally associated with numerous adverse cardiovascular outcomes, including depressed left ventricular ejection fraction, heart failure and overall mortality [1]. A number of cross-sectional studies have shown abnormalities in left ventricular systolic function among those with left ventricular hypertrophy and “diastolic” heart failure [2]. Analysis from the Multi-Ethnic Study in Atherosclerosis (MESA) showed an inverse association of left ventricular systolic function and left ventricular concentricity (LV mass/volume) by quartile [3]. Simultaneously, experimental studies have identified the molecular mechanisms and the key players of the pathology [4]. Oxidative stress has also been identified as one of the major contributing factors towards development of cardiac hypertrophy. In this review we will summarize the evidences supporting the oxidative stress as a cause of cardiac hypertrophy.

#### 1.1. Etiopathology of CH

Cardiac hypertrophy has both genetic as well as post disease etiopathology. Increased wall stress is considered as the trigger factor towards CH. At cellular level, cardiomyocyte hypertrophy is characterized by an increase in cell size, enhanced protein synthesis, and heightened organization of the sarcomere unit [5]. On the basis of molecular changes CH is considered of two types, the first one is physiological CH, mostly seen in athlete’s heart and the other is pathological CH induced by mechanical stress, due to pressure overload or volume overload [6, 7]. In physiological hypertrophy, the increase in cardiac mass is not associated with induction of fetal gene program. It has also been found that there is no collagen deposition in the physiologically hypertrophied myocardium [8]. Hypertension, aortic stenosis, and myocardial infarction cause increased pressure overload over the myocardium to cause pathological CH, while, mitral valve regurgitation causes volume overload. Induction of fetal gene expression in pathologically hypertrophied myocardium leads to myocardial dysfunction [9]. This reactive hypertrophy occurs in response to an extrinsic increase in cardiac work and is distinguished from genetic familial hypertrophic

cardiomyopathy, where the stimulus for hypertrophy is intrinsic to the cardiomyocyte [10].

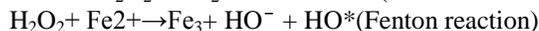
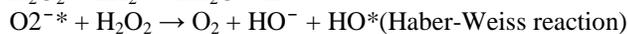
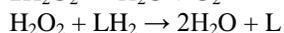
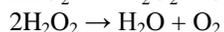
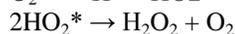
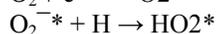
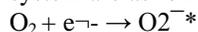
#### 1.2. Oxidative stress and its role in CH

Increased oxidative stress has been recognized as an important mediator in the setting of cardiovascular diseases [11]. Growing evidences support important pathophysiological roles of redox-sensitive signalling pathways in the processes underlying CH [12]. Numbers of studies have found a strong association between development of CH and increased production of reactive oxygen species [13, 14]. In cultured cardiomyocytes, hypertrophy induced by angiotensin II, endothelin 1, tumor necrosis factor (TNF- $\alpha$ ) or pulsatile mechanical stretch has been shown to involve intracellular ROS production which can be inhibited by antioxidants [15]. A recent experimental study reported that ROS production by uncoupled nitric oxide synthase may contribute to the development of left ventricular hypertrophy during chronic pressure overload [16]. The most widely recognized effect of increased oxidative stress is the oxidation and damage of macromolecules, membranes, DNA and enzymes involved in cellular function and homeostasis [17]. The mechanisms involved in regulation of cellular and extracellular events are the activation of key mediators of metabolic regulation by ROS as well as depletion or decreased activity of endogenous antioxidants [18, 19]. Apart from affecting cellular function, they do modulate the extracellular matrix function evident as increased interstitial and perivascular fibrosis [20]. Here we will discuss the sources of ROS generation and their role in modulating specific signalling pathways involved in CH.

#### 1.3. Sources of Reactive oxygen species

Reactive oxygen species (ROS) also termed “oxygen-derived species” or “oxidants,” are produced as intermediates in reduction-oxidation (redox) reactions [21]. ROS are reactive chemical entities comprising two major groups: free radicals (e.g., superoxide [O<sub>2</sub><sup>-</sup>], hydroxyl [OH<sup>-</sup>], nitric oxide [NO<sup>-</sup>]) and non-radical derivatives of O<sub>2</sub> (e.g. H<sub>2</sub>O<sub>2</sub>, ONOO<sup>-</sup>) [22, 23]. A free radical contains one or more unpaired electrons

having capability of independent existence (thus called “free”) which renders them highly reactive and unstable entities. Non-radical derivatives are less reactive and more stable with a longer half-life than free radicals. The various free radicals and non radical species commonly generated in the biological system are as follows:



**Superoxide anion [ $\text{O}_2^{\cdot -}$ ]:** It is an oxygen molecule having a free electron and is generally produced by NADPH-oxidases in different cell types like phagocytes, fibroblasts, and endothelial cells [24]. It is also generated following auto-oxidation of catecholamines, tetrahydrofolates and electron leak from mitochondrial electron transport chain. Superoxide anion has short life, does not cross cell membrane and is readily detoxified by superoxide dismutase. It contributes in formation of highly reactive oxygen species, hydroxyl radical [23].

**Hydrogen peroxide [ $\text{H}_2\text{O}_2$ ]:** It is a reactive oxygen species formed as end product of superoxide detoxification. It is a non-radical entity, readily crosses the cellular and nuclear membrane and is degraded by catalase and glutathione peroxidase [25]. Some of the function of  $\text{H}_2\text{O}_2$  include the upregulation of genes especially those controlled by nuclear factor-kB (NF-kB) transcription factor and the induction of intracellular  $\text{Ca}^{++}$  overload in cardiomyocytes which results in myocardial dysfunction [26].

**Hydroxyl radical [ $\text{OH}^{\cdot}$ ]:** It is the most potent free radical and so short lived. It is generated by two different reactions Haber-Weiss and Fenton reaction involving superoxide anion, hydrogen peroxide, and reduced transition metal ( $\text{Fe}_2^+$ ). Due to its radical nature, it is capable of initiating a free radical chain reaction.

**Nitric oxide [ $\text{NO}$ ]:** It is usually known for its ability to relax blood vessels. However, it also acts as a reactive oxygen species. It is soluble in both aqueous and lipid medium and is generated by enzyme mediated cleavage of arginine to citrulline. Following increased production, it can react with peroxides and form peroxynitrite anion.

#### 1.4. NADPH oxidases

The NADPH oxidase (Nox) enzyme is a family of enzymes which are major source of ROS production in cardiovascular system [27]. It was first identified in neutrophils, where it is normally quiescent but gets activated during phagocytosis and generates high levels of ROS. NADPH oxidases are the only enzymes which are designed for purposeful ROS production

[28]. It is a multi-subunit enzyme that catalyzes superoxide production by the reduction of oxygen using NADPH or NADH as the electron donor. The prototypical NADPH oxidase that is found in neutrophils has five subunits: p47phox, p67phox, p40phox, p22phox (“phox” stands for phagocyte oxidase), and the catalytic subunit gp91phox. Till now there have been seven oxidases reported out of which five oxidases (Nox1-Nox5) called as Nox and two remotely related oxidases Duox1 and Duox2. These different homologs differ in their structure, distribution and mechanism of activation, but all the Nox have the basic similarity in having a cytosolic NADPH binding domain and a heme centre. The oxidase activity occurs when cytosolic NADPH binding domain binds to NADPH, transfers electrons to FAD and the heme centres and finally to oxygen on the outer membrane surface, resulting in superoxide formation.

#### 1.5. NADPH oxidase involvement in LVH

The presence of NADPH oxidases in cardiovascular cells including endothelial cells, adventitial fibroblasts, vascular smooth muscle and cardiomyocytes has been reported. NADPH oxidase in cardiovascular cells continuously generates intracellular ROS and its activity may be significantly enhanced by several different stimuli, e.g. AngII,  $\alpha$ -adrenergic agonists and TNF- $\alpha$  [29-31]. ROS derived from the oxidase also appeared to contribute to the inactivation of endothelium-derived nitric oxide and the consequent left ventricular diastolic dysfunction [32]. In cardiomyocytes, Nox2 and Nox4 are specifically present [33, 34]. In experimental pressure-overload left ventricular hypertrophy induced by aortic banding in guinea-pigs, Li et al have reported increased NADPH oxidase subunit expression as well as activity in both cardiomyocytes and endothelial cells [35]. In subsequent study, the role of the Nox2-containing NADPH oxidase in angiotensin II-induced as well as aortic banding-induced CH was investigated using Nox2-/- mice. Interestingly, Nox2 deficient mice developed less hypertrophy than the wild type mice against Ang II infusion. However, following pressure overload hypertrophy, there was no difference observed between Nox2-/- mice and wild type mice in morphological left ventricular hypertrophy and the associated rises in mRNA expression of molecular markers such as ANF, suggesting involvement of Nox 4 in pressure overload-induced CH. In subsequent study, Nox2 -/- failed to protect against CH induced by infusion of blood pressure increasing dose of Ang II, however protected against fibrosis [36]. However, Nox2-/- mice were protected against pressure overload-induced myocardial dysfunction without having any effect on CH [37]. Studies investigating the role of Nox4 and mutant form of Nox4 (inactive form) reported no change in hypertrophic index however depressed ventricular function was noted [38]. In the same study, adenoviral mediated overexpression of Nox4 in cardiomyocytes resulted in tunnel positive cells, reflecting apoptosis without any change in cell size. These reports do suggest role of Nox2 and Nox4 in cardiac dysfunction subsequent to CH, however their contribution

towards hypertrophy and function alteration is still not unambiguous.

### 1.6. Xanthine oxidase

Increased xanthine oxidase activity has been reported in both clinical and preclinical condition of myocardial dysfunction. However, the enzyme has not been investigated widely for its role in CH. The first study by Xu et al., investigated the effect of Febuxostat, a xanthine oxidase inhibitor, against thoracic aortic constriction induced left ventricular hypertrophy and dysfunction in mice [39]. Febuxostat inhibited the hypertrophic response along with improving the myocardial function. However, the study did not show a direct estimation of xanthine oxidase activity in heart, rather it used serum uric acid level as a marker of xanthine oxidase activity. In another report from the same group, late inhibition of xanthine oxidase did not affect the development of CH [40].

### 1.7. Antioxidant defense system

Antioxidants are the substances that when present at low concentrations relative to an oxidizable substrate, significantly delay or prevent oxidation of that substrate. In normal physiological conditions, the fine balance between ROS generated and antioxidant defense system is maintained in the body. When there is increased production of ROS or impaired endogenous antioxidant defense of the body, the body is called under oxidative stress. To neutralize the excess ROS and to maintain the “redox homeostasis” the antioxidant defense system exists in the intracellular and extracellular compartments and comprises of enzymatic and nonenzymatic types. The major endogenous antioxidants are superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH).

**Superoxide dismutase:** Three isoforms of SOD namely manganese-containing SOD (Mn-SOD), copper containing SOD (Cu-SOD), and zinc containing SOD (Zn-SOD) have been identified in mammalian tissues [41]. Out of these, two isoforms Mn-SOD and Cu/Zn-SOD are present in the heart. Mn-SOD which localizes to mitochondria is responsible for ~70 % of the SOD activity in the heart and ~90% of the activity of the cardiac myocytes [42]. The remaining Cu/Zn-SOD is localized in the cytosol and extracellular spaces respectively. The importance of Mn-SOD is that it plays a critical role in controlling O<sub>2</sub>-generation in mitochondria in myocardium which has been demonstrated by Mn-SOD knockout mice which die due to cardiomyopathy. SOD catalyzes the dismutation of O<sub>2</sub> - into H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>.

**Glutathione:** Glutathione, the major soluble antioxidant, is a tripeptide containing thiol group and is present in cytosol, nucleus as well as mitochondria. Glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GPx), and glutathione transferase and is able to regenerate the important antioxidants, Vitamins C and E

back to their active forms [43]. It can also reduce the tocopherol radical of vitamin E directly or indirectly via reduction of semidehydroascorbate to ascorbate. It scavenges hydroxyl radical and singlet oxygen directly, detoxifying H<sub>2</sub>O<sub>2</sub> and lipid peroxides by the catalytic action of GPx [44]. Glutathione peroxidase reduces H<sub>2</sub>O<sub>2</sub> and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide. The glutathione peroxidase/glutathione system is important in low-level oxidative stress [45].

**Catalase (CAT):** Catalase is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol, which catalyzes the reaction of H<sub>2</sub>O<sub>2</sub> to water and molecular oxygen [46]. Catalase is very effective in high-level oxidative stress and protects cells from H<sub>2</sub>O<sub>2</sub> produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced glutathione peroxidase activity.

### 1.8. ROS and hypertrophic signaling

Involvement of ROS in regulation of cellular function by participating in cell signalling system has been well known [47]. ROS activate a broad variety of hypertrophy signalling kinases and transcription factors [48]. Different signalling pathways are involved in Modulation of myocardial growth, matrix remodelling and cellular dysfunction by various ROS [49].

### 1.9. ROS MAP Kinase pathway

In neonatal rat cardiac myocytes, H<sub>2</sub>O<sub>2</sub> induced activation of mitogen-activated protein (MAP) kinases which was prevented by catalase, but not by superoxide dismutase suggesting that the activation of MAP kinase was via H<sub>2</sub>O<sub>2</sub> [50]. In another study using, exposure of adult rat ventricular myocytes to H<sub>2</sub>O<sub>2</sub> resulted in concentration and time-dependent activation of extracellular signal-regulated kinases 1 and 2, p38, and c-Jun NH<sub>2</sub>-terminal kinase (JNK) MAP kinases [51]. Activation of MAP kinases and ROS generation have been reported following mechanical stretch-induced CH in neonatal rat cardiac myocytes [52]. Hypertrophy induced by phenylephrine and endothelin-1 in adult rat cardiac myocyte resulted in activation of MAP kinase (ERK), which was suppressed by treatment with N-acetylcystein and catalase [53]. Similar findings were reported where alpha-1 adrenergic stimulation of adult rat cardiac myocyte resulted in activation of ERK1/2 and was prevented by inhibiting the NADPH-oxidase [54]. A more direct study by using different concentration of H<sub>2</sub>O<sub>2</sub> reported a concentration dependent response on the activation of MAP kinase pathways and subsequent CH or apoptosis [55].

### 1.10. ROS and NF-κB

NF-κB is another important mediator of CH which has been investigated for its regulation by ROS. Hypertrophy induced in cultured rat primary neonatal ventricular cardiomyocytes by

several hypertrophic agonists, including phenylephrine, endothelin-1, and angiotensin II resulted in nuclear translocation of NF- $\kappa$ B as well as its transcriptional activity was stimulated [56]. In the same study, over expression of NF- $\kappa$ B gene in cardiomyocytes led to the spontaneous hypertrophy of cardiomyocytes. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced CH in isolated rat neonatal cardiomyocytes showed increase in ROS signal in cardiomyocytes over time [57]. In the same study, N-acetyl cysteine, abolished TNF- $\alpha$ -induced NF- $\kappa$ B activation and hypertrophic responses. G-protein-coupled receptor (GPCR) agonist (angiotensin II, endothelin-1, and phenylephrine)-induced CH in isolated rat neonatal cardiomyocytes has been reported to be mediated through NF- $\kappa$ B activation via the generation of ROS [58]. In another study, apoptosis signalling kinase-1 over expression activated NF- $\kappa$ B to stimulate hypertrophy, whereas genetic silencing of apoptosis signalling kinase-1 inhibited hypertrophy induced by angiotensin II, norepinephrine, and endothelin I [59]. In a recent study, the activation of NF- $\kappa$ B by ROS resulting in CH has been reported to be mediated by Akt activation. In transgenic mice having cytosolic overexpression of Cu/Zn-SOD resulted in blunting of hypertrophic response as well as NF- $\kappa$ B activation following thoracic aortic banding [60]. This study further verify the earlier reports and propose a more detailed mechanism of NF- $\kappa$ B activation by ROS and its participation in development of CH.

### 1.11. Evidences of benefits of antioxidants in CH

The strong evidence of the involvement of oxidative stress in CH has generated interest in developing strategies to prevent or reduce oxidative stress by antioxidants. CH induced by Ang II and endothelin was blocked by Tempol, a cell permeable SOD mimetic. Treatment with Tempol prevented the increase in cardiomyocytes size, superoxide generation and gp91phox expression [61]. Dahl salt-sensitive rats fed a high salt diet developed CH which was significantly prevented by Tempol. Interestingly, Affymatrix gene chip assay revealed that approx. 48% of the genes were changed in similar fashion in rats treated with amlodipine (a calcium channel blocker) and Tempol [62]. In GLUT4-knockout mice, Tempol treatment significantly reduced morphological and molecular evidence of CH [63]. In another study, CH induced by transverse thoracic aortic constriction in mice fed on fructose diet, Tempol prevented the hypertrophy, LV remodeling, contractile dysfunction and oxidative stress [64].

Standard drugs being practiced for the treatment of cardiovascular disorder have also been investigated for their

## [II] CONCLUSION

Findings from the experimental studies provide a strong evidence of causative role of oxidative stress in development of

antioxidant potential and some of their superiority to the class has been assigned to their antioxidant potential. Carvedilol, a vasodilator, beta-adrenoceptor antagonist have been reported to reduce the myocardial oxidative stress [65]. Carvedilol prevented hypertrophic changes in stroke-prone spontaneously hypertensive rats, and in pressure overload-induced CH in rats [66, 67]. Similar findings have been reported in the patients with heart failure, where carvedilol improved myocardial function along with reduction in myocardial oxidative stress [68]. Recently we have reported that Ro5-4864, a peripheral benzodiazepine receptor ligand, prevented the development of isoproterenol-induced CH [69]. Along with inhibiting the increase in cardiomyocytes size it also prevented the development of fibrosis and increase in expression of beta-myosin heavy chain. We and others have also reported that U50,488H, a  $\kappa$ -opioid receptor agonist, prevents the development of CH and fibrosis [70, 71]. In our study, we further demonstrated that U50,488H has antioxidant property as it prevented the oxidative stress associated with isoproterenol-induced CH as well as it also prevented the shift in alpha/beta myosin heavy chain [70].

Apart from these synthetic antioxidants, natural products have also been evaluated for their efficacy against CH. Bagchi et al., 2003 reported the cardioprotective effects proanthocyanidines present in grape seed extracts [72]. In subsequent reports, the oligomerized proanthocyanidines from grape seed prevented the isoproterenol-induced CH as well as the associated remodeling. It also inhibited the activation of NF- $\kappa$ B [73]. Similarly, green tea extract has also shown its protective effect against cardiac hypertrophy associated with renal failure [74]. In further studies, involving Ang II-induced CH, green tea extract prevented the increase in expression of gp91(phox) as well as NADPH-oxidase activity thereby reducing the generation of reactive oxygen species [75].

We have reported a protective effect of *Terminalia arjuna*, an Indian medicinal plant against isoproterenol-induced CH [76]. *T. arjuna* prevented the cardiac remodeling associated with CH as well as prevented the shift in alpha/beta-myosin heavy chain protein. Moreover, the decrease in endogenous antioxidants and increased lipid peroxidation observed with isoproterenol-induced hypertrophy was also significantly prevented by *T. arjuna*.

A more focused approach towards investigating the role of antioxidants in CH has been undertaken by tissue specific over expression of endogenous antioxidant enzymes.

CH. Prevention of increase in oxidative stress or reduction of ROS generation alleviates CH. Continuous increase in understanding of molecular pathways being modulated by reactive oxygen species may be helpful in designing and evaluating better therapeutic option/s for CH.

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