Oxidative Stress and Inflammation in Cardiovascular Diseases: Two Sides of the Same Coin

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Abstract

Globally, the major cause of long-term disability and death is an "epidemiologic transition" from infectious diseases and malnutrition complications to non-communicable chronic diseases like cardiovascular disease (CVD), cancer and diabetes. CVD accounts for major global mortality. Imbalance due to the generation of reactive oxygen species (ROS) levels above normal baseline levels and decreased antioxidant defence reserve makes the cardiovascular system (cardiac and vascular cells) susceptible to oxidative stress and damage. Growing evidences support the notion that oxidative stress plays a crucial role in the development and progression of CVD by altering normal functions such as inactivation of nitric oxide (NO) leading to endothelial dysfunction, intracellular Ca^{2+} overload and others. Oxidative stress also mediates inflammation through various signalling cascades such as the activation of inflammatory transcription factors (TFs) namely NF-κB, AP-1 and Nrf-1. A vicious cycle of oxidative stress-mediated inflammation and inflammation-induced oxidative stress makes the CVD-related complications worse. Therefore, it is also very important to clearly understand the role of enzymatic sources of ROS,

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mechanisms underlying pathological conditions and link between oxidative stress and inflammation during each stage of CVD. The present chapter will elucidate the role of oxidative stress and inflammation in CVD development and progression. It is important to find the remedial measures, to develop the efficient biomarkers and to design the therapeutic strategies for CVD in the near future.

Keywords
Cardiovascular diseases • Reactive oxygen and nitrogen species (ROS/RNS) • Inflammation • Oxidative stress

1 Introduction

Cardiovascular diseases (CVDs) are disorders involving the heart and the blood vessels or both. CVD includes acute coronary syndrome, angina, arrhythmia, cardiomyopathy, congenital heart disease, coronary heart disease, heart failure, inflammatory heart disease, ischemic heart disease, rheumatic heart disease and valvular disease, brain-related cardiovascular diseases (such as cerebrovascular disease), haemorrhagic stroke/ischemic stroke and peripheral circulatory system-related cardiovascular diseases [deep vein thrombosis, hypertensive heart disease, peripheral artery disease (PAD) and pulmonary embolism] [1]. More than 80 % of the deaths from CVD occur in low- and middle-income countries, and the economic burden due to CVD remains high in developing countries [2]. Many modifiable (environment, diet and exercise) and non-modifiable (genetic, gender, age, early menopause and ethnic group) causal risk factors are involved in CVD [3]. Current preventive and treatment strategies are inadequate to prevent and cure CVD. This raises an urgent need for effective therapeutic strategies which in turn require extensive fundamental understanding of key processes involved in the development of CVD.

The human heart is an obligate aerobic organ with very high metabolic energy demand and relatively lower levels of antioxidant defence as compared to other organs. The metabolic energy generation involves the use of oxygen thereby resulting in significant production of reactive oxygen species (ROS) which in turn is implicated in processes affecting cardiac function. Atherosclerosis and hypertension are the most common causes of CVD. Atherosclerosis is the thickening of the arterial wall due to Ca^{2+} and cholesterol, which reduces the elasticity of arterial wall, reduces the blood flow and thus increases blood pressure. It is due to chronic inflammatory response elicited by the accumulation and rupture of white blood cells (T cells and macrophages) in response to cholesterol-carrying oxidised low-density lipoprotein (oxLDL) molecules, which prompts endothelial cells to produce adhesive substances that snag monocyte/macrophage precursor cells from the blood [4].

The conventional view of CVD as altered lipid storage is now changing, as the role of key mechanistic pathways of oxidative stress and inflammation in the initiation, development and progression of CVD is becoming clearer. Studies advocate the involvement of oxidative stress and inflammation in endothelial dysfunction and atherosclerosis. Blood vessels consist of three main layers—the outer layer is the connective tissue and provides structure to the layers beneath, the middle layer is the smooth muscle and controls the blood flow, and the inner lining is the thin layer of endothelial cells. The underlying cause of all vascular diseases is the dysfunction of endothelial cells that occur much before the appearance of clinical symptoms. Endothelial cells prevent entry of harmful blood-borne substances into the smooth muscle of the blood vessel. The oxidative damage to the inner layer can result into the modulation of endothelial permeability,

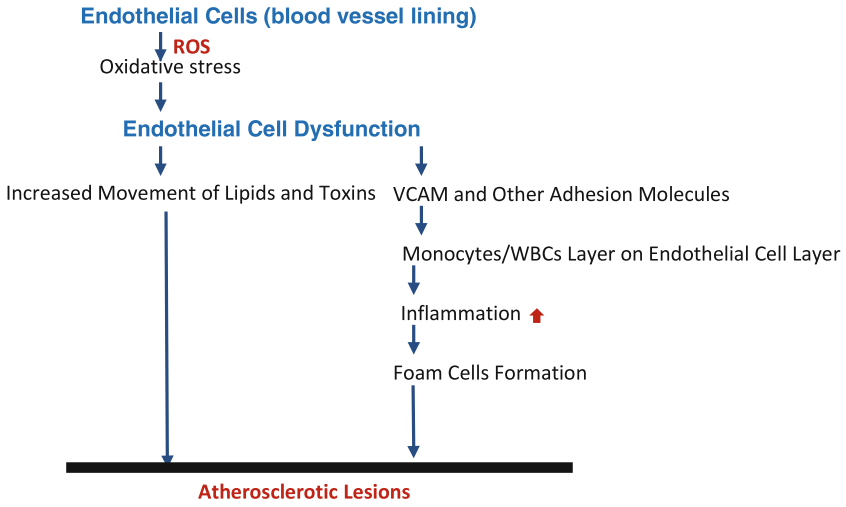

Fig. 1 Effect of oxidative stress on endothelial cell functions. Oxidative stress makes the endothelial cells dysfunctional by altering their permeability, thus allowing the entry and movement of lipids and toxins across the endothelial layer and smooth muscle cells into sub-endothelial space. It also stimulates the expression of vascular cell adhesion molecule (VCAM) and other adhesion molecules, which leads to the recruitment of monocyte/WBC layer on endothelial layer that leads to inflammation-induced foam cells and atherosclerotic lesion formation

thus allowing the movement of lipids and toxins across the endothelial layer and smooth muscle cells into the sub-endothelial space [5]. The normal endothelium resists adhesion by white blood cells. However, exposure to risk factors may result in the expression of vascular cell adhesion molecule (VCAM-1) on the surface of endothelial cells. These adhesion molecules mediate adhesion of monocytes to endothelial cells [6]. These monocytes then mature into macrophages which then form the cholesterol-laden foam cells in arterial wall forming "atheroma" plaques. Exploration of the role of inflammation and oxidative stress in CVD will not only increase our understanding of diseases but also have applications in risk stratification and the development of targeted therapeutics to control these diseases [7] (Fig. 1).

2 Pathophysiological Agents Associated with CVD

2.1 Role of ROS in CVD

ROS are highly reactive free radicals derived from molecular oxygen (O_2) that can readily oxidise other molecules. ROS have vital role in normal physiological cellular signalling pathways in various cells of the cardiovascular system (CVS) and other systems. However, pathological levels of ROS can alter structure and vital functions of cellular proteins, lipids and nucleic acids. The site and extent of ROS production have important consequences and thus determine the ultimate cell/tissue fate (Fig. 2). ROS can be formed in the heart, vascular tissue, splenocytes and blood leukocytes through the action of specific oxidases and oxygenases (xanthine oxidase, NADPH oxidase and NOX) and peroxidases (myeloperoxidase); through the Fenton reaction; and as by-products of the electron transport chain (ETC) of the mitochondria [8]. Further, cyclooxygenase, lipoxygenase and cytochrome P-450 enzymes produce ROS during arachidonic acid metabolism [9]. Nitric oxide (NO) is produced by the enzymatic activity of nitric oxide synthases (NOSs), which oxidises L-arginine, transferring electrons from NADPH [10]. The endothelium has been identified as a major source of ROS in the human blood vessels, and endothelial function is closely linked to the homoeostasis of ROS formation within the vascular wall [11]. The human system has enzymatic and nonenzymatic systems to get rid of increased ROS. Imbalance

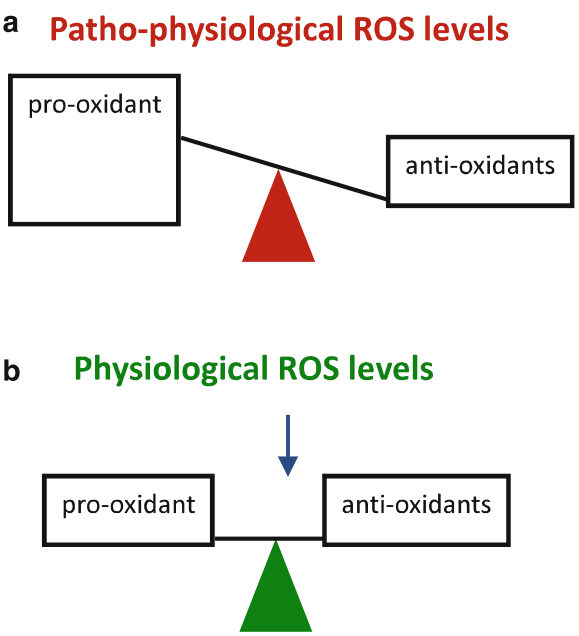

Fig. 2 (**a**) Pathophysiological ROS levels result as a consequence of imbalance between pro-oxidants and antioxidants; and (**b**) where as physiological ROS levels cause/result in balance between pro-oxidants and antioxidants

between ROS production and antioxidant defence of the cell can result in oxidative stress which in turn may cause tissue injury and diseased condition [12], which is summarised in Table 1.

2.2 Role of Mitochondrial Electron Transport Chain in CVD

The mitochondrial electron transport chain is the major endogenous source of ROS. In the mitochondria, the partial reduction of O_2 occurs as a result of leakage of electrons from the ETC, contributing one, two or three electrons to form $O_2^{\cdot}$, H_2O_2 or HO·, respectively. As much as 2–4 % of the reducing equivalents escape the respiratory chain, leading to $O_2^{\cdot-}$ formation. $O_2^{\cdot-}$ is dismutated by manganese superoxide dismutase (MnSOD) to H_2O_2 that may then be converted to highly reactive and deleterious HO· radicals. Generally, the leakage of electrons at CI flavoprotein generates $O_2^{\cdot-}$ in mitochondrial matrix while CIII ubisemiquinones (UQ–) generated at Q1 (UQ−1) and Q*o* (UQ−0) sites release $O_2^{\cdot-}$ in the matrix and intermembrane space of the mitochondria, respectively. Superoxide anions can give rise to other reactive species such as peroxynitrite ($ONOO^-$), H_2O_2, OH^- radicals and hypochlorous acid (HOCl). Increased $O_2^{\cdot-}$ levels are the result of electron loss from ETC or diminished activity of mitochondrial ROS scavengers such as MnSOD [8]. Monoamine oxidase (MAO) present in outer mitochondrial membrane contributes to oxidative stress via generation of NO or H_2O_2, thus contributing to oxidative stress [34]. Altered mitochondrial DNA as result of oxidative stress affects vital mitochondrial functions, leading to the production of more ROS [35].

The heart is highly dependent on the mitochondria for the energy required for its contractile and other metabolic activities. The mitochondria represent 30 % of the total volume of cardiomyocytes and provide >90 % of the cellular ATP energy through oxidative phosphorylation [36]. It is now documented that myopathic heart sustains mitochondrial dysfunction at gene, protein and biochemical levels. Global microarray profiling of gene expression has identified alterations in several of the mitochondrial function-related transcripts in the myocardial biopsies of humans [37] and experimental animals [38]. Further studies documented a decline in the activities of respiratory complexes and NADH-ubiquinone reductase (CI), ubiquinol-cytochrome c reductase (CIII) and ATP synthase (CV) complexes in diseased hearts [39].

A study showed that MnSOD-deficient mice developed progressive congestive heart failure with specific molecular defects in mitochondrial respiration. Studies have revealed the role of peroxiredoxin-3, a mitochondrial H_2O_2 scavenger, in the prevention of heart failure after experimental myocardial infarction (MI) in mice [40]. Numerous studies have shown that physiological stimuli such as vasoactive agents angiotensin II [41], epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and tumour necrosis factor-α (TNF-α) that are involved in pathogenesis of vascular diseases can lead to the production of mitochondrial ROS. Studies have also shown

Table 1 Major sources of ROS and their respective functions in different cell types of CVS

Cell type	Major source of ROS	Functions	References
Cardiomyocytes	*Mitochondrial enzymes*		
	1. P66shc	Catalyses electron transfer from cytochrome c to oxygen	[13–16]
	2. Nicotinamide adenine dinucleotide phosphate oxidases 2 and 4 (NOX2 and 4) isoforms	Associates with subunit $p22^{phox}$ for its activation upon stimulation by G protein-coupled receptor (GPCR) agonists and TGF-β, generates superoxide and H_2O_2	[17–21]
	3. Monoamine oxidases (MAOs)	MAO metabolises serotonin, releases H_2O_2	[22]
Endothelial cells	1. Nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4)	Constitutively active at low level, levels increase in response to pressure overload, generates H_2O_2, triggers further ROS generation	[19]
	2. Endothelial nitric oxide synthase (eNOS)	Generates NO, which affects cell functions by post-translational modification of effector proteins or by stimulating guanylate cyclase	[23–25]
	3. Heme oxygenase I (HO-1)	Upregulation of HO-1 in the endothelium protects it against inflammation via enzymatic degradation of the pro-oxidant and pro-inflammatory molecule heme and via the generation of its anti-inflammatory products bilirubin and CO	[26]
Vascular smooth muscle cells (VSMCs) and fibroblast cells	1. NADP(H) oxidase	Produce $O_2^{-\cdot}$, leads to the production of peroxynitrite, involved in the growth response of VSMCs and fibroblasts, VSMC migration and cell apoptosis	[27–30]
	2. Mitochondria	ANG II-stimulated mitochondrial reactive oxygen species production in rat cardiac fibroblasts is accompanied by a reduction in the expression of the mitochondrial antioxidant peroxiredoxin-3 (Prx-3)	[31]
Infiltrating immune cells	1. Myeloperoxidase	Neutrophils and monocytes secrete myeloperoxidases and NOXs which initiates lipid peroxidation via tyrosyl radical and nitrogen dioxide	[32, 33]
	2. NADP(H) oxidase	NOXs which initiate lipid peroxidation via tyrosyl radical and nitrogen dioxide	

that mitochondrial ROS in hypoxia induces signalling which in turn is closely associated with inflammation. Mitochondrial H_2O_2 is linked with flow-mediated dilatation in human coronary resistance arteries [42].

In our recent study, in which we utilised genetically modified mice, we found that chagasic MnSOD transgenic mice equipped with a variable capacity to scavenge mitochondrial and cellular ROS had low inflammatory infiltrate and showed significant myocardial remodelling when compared to the chronic chagasic wild-type mice [43]. Collectively, it is indicative that the mitochondria are an important source of ROS that has implications for the cardiovascular system (CVS).

2.3 Role of Oxidases and Oxygenases in CVD

Numerous oxidases and oxygenases expressed in different cell types and locations within the cell contribute to the formation of ROS. By definition, oxidases reduce O_2, whereas oxygenases (oxidoreductases) transfer O_2 to substrates. ROS produced by activated phagocytes such as macrophages and neutrophils from NADPH oxidase and/or by myeloperoxidase activity is termed as "oxidative burst" [44, 45]. This ROS production is critical to antimicrobial function, contributing either directly or indirectly to the killing of intracellular organisms. NADPH oxidase, produced by many types of phagocytes, reduces O_2 to $O_2^{\cdot-}$ [46]. Subsequently, $O_2^{\cdot-}$ and HOCl further can react to form HO· [47].

2.3.1 NADPH Oxidase (NOX)

The NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase, NOX) is a membrane-bound enzymatic complex that remains present as a transmembrane complex and transfers electrons to oxygen across the biomembrane that converts into superoxide anion, referred as respiratory burst, that serves as the first line of host defence against microbes [48]. The prototypic NOX (gp91phox), renamed as NOX2, was first identified in phagocytes (neutrophils, macrophages). Presently, seven mammalian NOX homologues have been identified, namely, NOX1–NOX5 and dual oxidases 1 and 2 (DUOX1 and DUOX2). In the cardiovascular system, NOX1, NOX2, NOX4 and NOX5 have been identified. NOX1 is expressed mainly in vascular smooth muscle cells (VSMCs). NOX2 and NOX4 are expressed in endothelial cells, cardiomyocytes, fibroblasts and VSMC [49]. NOX5 has been reported in human endothelial cells and smooth muscle cells but is absent in rodents [50]. It has been found that some NOX family members preferred NADPH or NADH, but some can't discriminate between NADPH and NADH. NOX2 generally remains present in phagocytic cells such as neutrophils and macrophages [48] and helps vascular endothelial growth factor (VEGF) to induce angiogenesis mediating through NOX3 [51]. NOX4 protects the vasculature against inflammatory stress [52]. NOX-derived ROS plays a physiological role in the regulation of endothelial function and vascular tone and a pathophysiological role in endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis, angiogenesis, rarefaction, important processes underlying cardiovascular and renal remodelling in hypertension and diabetes [53, 54]. It has been reviewed that monocyte/macrophage extravasation through NOX into the vessel wall is a critical step in the development of atherosclerosis. Upon activation, NOX complex of monocytes produces a burst of superoxide anion. This superoxide anion develops oxidative stress at the inflammatory sites. ROS thus generated activate an enzyme that makes the macrophages adhere to the arterial wall by polymerising actin fibres [54].

In a study by Liu and co-workers, antioxidant as a peptide inhibitor of NOX has been shown to reduce blood pressure and forestall macrophage accumulation in rats during angiotensin II infusion [55]. Similarly, work on chagasic cardiomyopathy showed that NOX-dependent ROS is a critical regulator of the splenic response (phagocytes, T cells and cytokines) which effects the heart-infiltrating phagocytes and $CD8^+$ T cells resulting in cardiac remodelling [56].

2.3.2 Xanthine Oxidase

Both xanthine oxidase and xanthine dehydrogenase, derived from xanthine oxidoreductase (XOR), produce H_2O_2 and $O_2^{\cdot-}$ while metabolising hypoxanthine and xanthine to uric acid [57]. ROS due to XOR has been implicated in endothelial dysfunction, atherosclerosis, hypertension and heart failure. Further inhibitors of xanthine oxidase such as febuxostat, allopurinol and oxypurinol showed diminished ROS, improved contractile function and myocardial efficiency [58]. Understanding of the mechanism(s) of action of XOR in CVD development can lead to the development of therapies targeting XOR. Enhanced xanthine oxidase has been shown to be associated with vascular dysfunction in animal models of hypercholesterolemia [59]. Overexpression of

renin and angiotensinogen has been linked with increased xanthine oxidase activity in endothelial malfunction in transgenic rats [60].

2.3.3 Lipoxygenase

Lipoxygenase (LO) is an important enzyme involved in the conversion of arachidonic acid to leukotrienes which are pro-inflammatory lipid mediators [61]. The role of leukotrienes (LTs) as mediators in asthma is well known [62]; consequently, leukotriene inhibitors are used for treatment of asthma. Now, researchers are trying to elucidate the role of 5-lipoxygenase and leukotrienes in other chronic inflammatory diseases including atherosclerosis [63]. 5-LO is abundant in monocytes/macrophages, dendritic cells, mast cells and neutrophilic granulocytes of 5-LO^{+} cells markedly increased in advanced lesions.

Lipoxygenase or cyclooxygenase metabolised products such as eicosanoids and LTs are associated with several pathogenesis in human beings such as cancer, CVD, asthma and others [62, 64]. LTs play important role in immunity as well as inflammation [63]. Lipoxygenase induces/attracts various leukocytes including macrophages, circulatory monocytes, mast cells and foam cells. But lipoxygenase remains non-functional until 5-lipoxygenase-activating protein (FLAP) is not associated with it. Lipoxygenase products like leukotriene B4 (LTB4) is the most powerful inflammatory product, while the products of 12/15-LOs have both pro-inflammatory and anti-inflammatory responses. This inflammatory response promotes atherosclerosis, abdominal aortic aneurysm and myocardial infarction/reperfusion injury via increased leukocyte chemotaxis and vascular inflammation, enhanced permeability and subsequent tissue/matrix degeneration. Some recent studies have shown that LTB4 is a signal-relay molecule secreted by neutrophils [61] which promotes atherosclerotic initiation by inflammation through various mechanism(s) including the release of pro-inflammatory cytokines IL-6 and TNF-α.

The expression of 5-LO by activated macrophages in symptomatic plaques leads to LTB4 accumulation and enhanced synthesis and release of matrix metalloproteinases (MMPs) that can promote plaque rupture [65]. From studies on animal models, it has been observed that 12/15-LOs play a crucial role during late-phase inflammation and atherosclerosis by fixing the interactions between monocytes and endothelial tissues in vivo [66]. It has been found that 15-LOs in monocytes generate superoxide that leads to the oxidation of LDL [67]. This 15-LO protein has been observed to be localised in atherosclerotic lesions in rabbit and humans [68, 69].

2.3.4 Heme Oxygenase-1

Heme oxygenase-1 (HO-1) catalyses the oxidation of heme to generate carbon monoxide, biliverdin and iron. These reaction products of HO-1 have potent anti-inflammatory and anti-oxidative functions. Although HO-1 is expressed at low levels in most tissues under normal basal conditions, it is highly inducible in response to various pathophysiological stresses. The role of HO-1 in inflammation and several CVDs such as atherosclerosis, myocardial infarction, graft survival after heart transplantation and abdominal aortic aneurysm has been reported. HO-1 is emerging as a great potential therapeutic target for treating CVD [70]. Experimental evidence from various cell culture and animal models suggests an association of HO-1 with the complex sequence of events that cause atherosclerosis [26]. It has been demonstrated that HO-1-deficient mice develop cardiac abnormalities, thus suggesting its role in CVD [71, 72].

2.3.5 Myeloperoxidase

Myeloperoxidase (MPO) is a heme peroxidase enzyme abundantly expressed in monocytes, macrophages and activated neutrophils. It utilises co-substrate to generate other ROS/RNS [73]. MPO oxidises tyrosine to tyrosyl radical and also produces HOCl during phagocytosis from H_2O_2 and Cl^{-} by neutrophil respiratory bursts to kill bacteria and infectious pathogen [74]. Therefore, MPO is an enzyme that plays an important role in innate immune system. The oxidants produced by MPO are also associated with CVD in the coronary circulation or in peripheral arterial

vasculature. High plasma MPO is reported to be a risk factor for early adverse cardiac events in patients with chest pain [73].

In the vascular system, MPO remains present in endothelial cells attached with cytokeratin 1 [75]. It has been elucidated that MPO-generated oxidants are one of the major causes of vascular damage during inflammation [76] and also act as biomarker of vasculitis [77], cardiac dysfunction and left vascular ejection fraction (LVEF) and a risk marker in acute coronary syndrome [78]. MPO functions as a survival signal for neutrophils and thereby contributes to prolonged inflammation [79]. The resultant inflammatory response induced by MPO leads to endothelial dysfunction, atheroma initiation and propagation, subsequent complications of plaque rupture, thrombosis and ventricular remodelling [80] which makes MPO a major player contributing to CVD.

Elevated plasma MPO levels lead to inflammatory diseases like dermatitis herpetiformis, systemic inflammatory response syndrome and anti-neutrophil cytoplasmic autoantibody (ANCA)-mediated glomerulonephritis [76]. MPO suppresses the vascular dilation and promotes smooth muscle cell proliferation, which is a major reason of endothelial dysfunction [76]. Chronic inflammatory process leads to oxidative damage of the arterial wall and its subsequent outcome is atherosclerosis [81]. The foam cells (hallmark of atherosclerosis) formed by the accumulation of cholesterol and lipids during atherosclerosis arise from oxidised LDL by MPO [40]. High levels of oxLDL are associated with increased risk of future myocardial infarction [82].

MPO, a major granule enzyme in neutrophils, accounts for 5 % of the total neutrophil proteins and is responsible for the production of oxidant HOCl [61]. The release of ROS and HOCl by neutrophils may cause damage to important biological structures, such as proteins carbohydrates, lipids and nucleic acids, and may enhance inflammatory responses. Dityrosine-containing protein cross-linking products, designated as advanced oxidation protein products (AOPPs), are formed by HOCl-induced chlorination of amines and constitute an excellent marker of MPO activation. AOPPs are found in the extracellular matrix of human atherosclerotic plaques, and increased levels of AOPP have been described as an independent risk factor for coronary artery disease [57] and in several infectious inflammatory diseases [83].

2.4 Role of Nitric Oxide in CVD

During aerobic respiration, ROS are generally produced, while in hypoxic condition, NO are produced and that in turn form RNS. Some more reactive species have been noticed such as reactive aldehydes, i.e. malondialdehyde (MDA) product of lipid peroxidation [84]. NO synthase is an enzyme that helps in the synthesis of NO from L-arginine in various types of cells and tissues. In mammals, there are three distinct types of isozymes of NOS, neuronal (nNOS or NOS-1), inducible (iNOS or NOS-2) and endothelial (eNOS or NOS-3) [84]. iNOS and nNOS are soluble and found predominantly in the cytosol, while eNOS is membrane associated. eNOS and nNOS are constitutively expressed while the expression of iNOS is activated during infection [85].

NO is a soluble gas that delivers signalling as paracrine hormones in vasorelaxation, vascular haemostasis, neurotransmission and cytotoxicity [86]. It protects blood vessel from injurious consequence of platelets and cells circulating in the blood. The NOS catalyses an NADPH- and O_2^--dependent oxidation of arginine to generate NO and citrulline, with the formation of N-hydroxyarginine (NOHA) as an intermediate [87]. iNOSs in the macrophages are different from the others, as it is Ca^{2+} independent. Different NOSs synthesise NO in response to different stimuli such as eNOS synthesising NO in a vascular endothelial cell in response to acetylcholine, nNOS synthesising NO in a neuron in response to glutamate, and iNOS synthesising NO in macrophages following its induction by IFN-γ [88].

In the endothelium vessels, some major functions such as blood pressure, platelet aggregation, leukocyte adherence and vascular smooth

muscle cell mitogenesis are regulated by eNOS. eNOS is acutely activated by agonists of diverse G protein-coupled cell surface receptors and by physical stimuli such as haemodynamic shear stress and varying oxygenation. Mutational studies have proved that unlike other NOS isoforms, eNOS shows N-myristoylation which targets its localisation to the plasma membrane [89]. Diminished NO availability contributes to systemic and pulmonary hypertension, atherosclerosis and airway dysfunction [90]. It has been seen that C-reactive protein (CRP), a prototypic marker of inflammation, decreases the eNOS level in endothelial tissues leading to CVD and atherogenesis [91].

From pharmacological studies in cultured cells, it has been observed that increased cAMP can exert opposite effects on the endotoxin- or cytokine-induced expression of NOS-2, being either stimulatory or inhibitory in macrophages; stimulatory in adipocytes, smooth muscle, skeletal muscle and brain endothelial cells; and inhibitory in pancreatic, liver and brain glial cells. The regulation of NOS-2 gene transcription appears to be the primary mechanism of action of cAMP and, whether it is stimulatory or inhibitory, hinges on the cell-specific regulation of transcription factors (TFs) including CREB, NF-κB and C/EBP. cAMP must therefore be considered a modulator rather than a suppressor of NOS-2 expression [92].

2.5 Role of Fenton Reaction in CVD

Besides oxidases and oxygenases, the "Fenton reaction" is another mechanism of ROS formation which forms the basis for CVD. The reaction results in the Fe^{+2}- or Cu^{+}-mediated conversion of H_2O_2 to HO· [41]. The relationship between iron and CVD was proposed in 1981 by Jerome Sullivan. Since then, numerous epidemiologic studies have been conducted to test this hypothesis. Increased iron levels in the body after menopause in women and adolescence in men are associated with the development of atherosclerosis and ischemia [93]. Further, reduction of iron in the body via phlebotomy may be used in the treatment of CVD. The administration of deferoxamine, a potent iron chelator, resulted in a decrease in myocyte necrosis in a random study of ischemia/reperfusion in dogs, thus indicating the role of Fenton reaction [94].

Study on the association between oxidative stress markers and iron nutrition status in humans revealed significantly higher concentrations of serum ferritin than control group. Also these subjects showed significantly lower levels of the transferrin receptor than control group. Further, significantly higher levels of oxidative stress markers including heme oxygenase activity, oxLDL and thiobarbituric acid reactive substances were reported in individuals with metabolic syndrome than in the control group. DePalma and group found a positive correlation between ferritin levels, inflammatory biomarker interleukin-6, CRP levels and mortality in patients with symptomatic PAD. This study suggested the role of iron-induced oxidative stress in the initiation and development of inflammation in PAD patients. Further, statins were found to suppress ferritin levels which were in turn associated with improved clinical outcomes [95]. On the other hand, the increased intake of vegetables, fruits, tea and coffee is associated with lower levels of oxidative stress.

3 Role of Oxidative Stress and Inflammation: Development and Progression of CVD

3.1 Oxidative Stress in CVD

Various studies have demonstrated the role of oxidative stress in the development and progression of CVD [96, 97]. It is well known that imbalance between raised ROS levels and antioxidant systems creates oxidative stress, which makes the cells prone to the damage. Antioxidant defence systems such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) scavenge ROS and inhibit degradation of NO. Prostacyclin and endothelium-derived

hyperpolarizing factor (EDHF) and NO are vasodilators which help in maintaining vascular homoeostasis via regulating vascular tone, reactivity and vascular smooth muscle cell proliferation [98, 99]. Oxidative stress inactivates NO inducing decreased NO bioavailability and further production of ROS species such as $ONOO^-$. Decreased NO bioavailability leads to imbalance of vessel wall, impaired endothelial-dependent vasodilation and thus endothelial dysfunction. Studies have suggested the role of ROS-mediated accelerated inactivation of NO in endothelial dysfunction in CVD [100]. Apart from reduced NO bioavailability, endothelial dysfunction also results from lipid peroxidation and inflammatory responses. ROS-mediated altered endothelial permeability prompts the entry of LDL into subendothelial space and its subsequent oxidation to form oxLDL. oxLDL molecules are seized by macrophages and transformed macrophages (i.e. foam cells) and are then added to the atherosclerotic lesion development [101]. Elevated abnormal ROS also activates inflammatory TFs such as NF-κB, AP-1 and Nrf1 involved in immune system and inflammatory processes [102].

Clinical studies have also demonstrated the role of oxidative stress in the development and progression of CVD. Oxidative stress-mediated increased lipid peroxidation has been shown to correlate with the severity of heart failure and has been found to be reduced by free-radical scavenger [103–105]. Elevated free-radical activity and low SOD/GPx levels have been reported in patients with congestive heart failure [106]. It has been observed that risk factor-stimulated enzymatic sources generate ROS leading to hypertension [107, 108]. Altogether, these studies advocate for the role of oxidative stress in CVD. Further investigations delineating the role of enzymatic sources of ROS and oxidative stress can help to develop specific therapeutic strategies to prevent the development and progression of CVD.

3.2 Inflammation in CVD

Till now, it is not well understood whether inflammation is responsible for CVD or not. But it has been found that many heart diseases and stroke are related to inflammation and it also promotes atherogenesis (formation of atheromatous lesions in arterial walls) that can directly lead to CVD. In Atlanta, on March 14 and 15, 2002, a workshop titled "CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice" was held, whose main purpose was to illustrate some potential markers in pro-inflammatory or inflammatory condition [109].

As discussed earlier, oxidative stress can enhance inflammation, and this inflammation promotes many diseases along with coronary diseases including the initiation and progression of atherogenesis characterised by atherosclerotic plaque, plaque rupture and thrombosis [2]. Collagen and some other factors strengthen the formation of a tough cap over plaques. Inflammatory mediators can weaken this cap by inhibiting collagen synthesis and also by enhancing the production of collagen-breaking enzymes. Inflammation is therefore responsible for not only the initiation of atherosclerosis but also the promotion of other complications. Macrophages, another potent mediator of inflammation, in the later stage of plaque rupturing, break down the clotting factors [10]. oxLDL in endothelial tissue initiates a cascade of events to cholesterol-laden macrophages and accumulates them in the arterial wall during atherosclerotic plaques [10]. CRP is a traditional inflammatory marker observed during inflammation and also independently (genetically). Its high levels have been linked with incidences of CVD and coronary heart diseases through IL-6 [110, 111]. Inflammation raises cytokine IL-6 level that has been also found to relate with MI and CVD [112]. A well-recognised signalling molecule such as NF-κB also contributes in inflammation and atherosclerosis [113].

Some common physiological behaviour such as hypertension is also associated with an increased risk of inflammation and, consequently, development of carotid heart disease [7]. Several inflammatory cytokines have been shown to contribute to cardiac dysfunction under various pathophysiological conditions associated with heart failure, including I/R injury, MI, atherosclerosis, hypertrophy and acute viral myocarditis [114].

Cytokines and chemokines implicated in the progression of heart failure include TNF-α, IL-1, IL-6, IL-8, IL-13, IL-18, IFN-γ, cardiotrophin-1, monocyte chemotactic peptide-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α), anti-inflammatory mediators transforming growth factor beta (TGF-β), IL-10 and other pro-inflammatory mediators [114]. Previously, most of the studies related to inflammation and CVD were performed in experimental animals, and there was no proof that inflammation contributes to human heart diseases until, recently, when studies by various groups started to report the role of inflammation in heart diseases. A correlation was found between inflammation, oxidative stress and persistent platelet activation in android obese women [115]. A review summarising the results from clinical studies indicated the role of vascular inflammation in CVD, and a positive correlation between upregulated inflammatory markers and cardiovascular risk has been reported [116].

All of these observations support the idea that antioxidant depletion and inefficient scavenging of ROS, resulting in sustained oxidative stress, are of pathological importance in human CVD. The reexpression of foetal genes (ANP, BNP, α-actin and β-MHC) is a hallmark of hypertrophic remodelling, and a considerable body of evidence shows the redox regulation of various signalling cascades and remodelling responses in cardiac diseases of various aetiologies. Current evidence supports the involvement of the following pathways: (i) ERK-1/ERK-2 [117] and the small GTPase Ras [118] in response to adrenergic agonist and angiotensin II stimulation [119, 120], (ii) MAPKs in pressure-overload hypertrophy and (iii) NF-κB and apoptosis signal-regulating kinase 1 (ASK-1) in response to angiotensin II infusion. ASK-1 is the upstream of p38 MAPK and JNK in the MAPK signalling cascade, and both of these have been shown to be activated by NOX/ROS [121]. The inhibition or scavenging of free radicals has been shown to modulate the ERK signalling and hypertrophic responses in neonatal and adult cardiomyocytes. Besides ROS, experimental studies have shown that the inflammatory cytokines (e.g. TNF-α, IL-1β, and MCP-1) also promote myocardial hypertrophy and contribute to the development and progression of heart failure [122]. Several recent studies have supported the concept that cytokines produced by T cells and other inflammatory cells contribute to hypertension. More recently, it has been found that the novel, pro-inflammatory cytokine IL-17 contributes to hypertension. This cytokine is produced by TH_{17} cells, a subset of $CD4^+$ cells, which are distinct from TH1 and TH2 cells [123].

3.3 Relationship Between Oxidative Stress and Inflammation

ROS enhances inflammation directly via the activation of certain inflammatory TFs such as AP-1, NF-κB and Nrf2 [102, 124], modifying the expression of gene coding for chemokines and adhesion molecules, causing accumulation of inflammatory cells [125–127]. On the other way, ROS-mediated oxidative injury augments endothelial permeability, which prompts the lipoproteins to enter sub-endothelial space, gets oxidised and intensifying inflammation [5]. These oxidised lipoproteins also interact with Toll-like receptors (TLRs) to foster vascular disease [128]. Inflating inflammatory cells further release ROS, strengthening the oxidative environment and continuing the series of events of oxidative stress-inflammation-oxidative stress.

There is substantial evidence to show that ROS modulate T-cell function and can affect T-cell polarisation and cytokine secretion [114]. Exogenously generated ROS cause apoptosis and suppression of T-cell proliferation and production of IL-2. Of note, T cells also produce ROS endogenously via a NOX2-based NADPH oxidase, promoting a TH2 phenotype.

Inflammation and oxidative stress are involved in atherosclerosis right from initiation through development to thrombotic stage. Therefore, clearance of the clogged vessels via surgery and standard medical treatments cannot be the prime treatment option. Instead, inflammatory processes and oxidative stress pathways involved in cardiac diseases need to be targeted [109]. Reckoning with the studies advocating the

relationship between oxidative stress and inflammation in the matter of CVD and the general fact that overall level of cellular ROS is determined by the relative rate of generation and the rate of reduction by antioxidants, enzymatic and nonenzymatic antioxidants, scavenging myocardial ROS can decrease inflammation and thus can demote CVD.

4 Are There Ways to Prevent CVD?

Numerous clinical trials have been performed to examine the potential for preventing CVD using antioxidant therapies. Some antioxidant studies have focused on the primary prevention of CVD, meaning the prevention of CVD in patients that do not already have the disease. β-Carotene, vitamin C and vitamin E have been investigated and randomised trials of this antioxidant failed to show any effect on the risk of death from CVD. Thus, it has been difficult to demonstrate that antioxidant supplementation has significant impact in CVD.

Secondary prevention refers to inhibiting the manifestations of CVD in those patients who already have the disease. Because the risk of a second cardiovascular event (MI, stroke, angina) is high in patients that have already had a first event, established prevention measures (e.g. cholesterol lowering, smoking cessation and others) are the most effective in secondary prevention.

4.1 Enzymatic Antioxidants

Enzymatic antioxidants are expressed in response to ROS production and display function as catalyst in reactions that convert specific ROS to different and, presumably, less harmful species. The principal enzymatic antioxidants are SOD, CAT, peroxiredoxin (Prx) and GPx [129]. SOD converts $O2^{-}$ to H_2O_2. MnSOD, the mitochondrial isoform, makes up to ~70 % of the SOD activity in heart and 90 % in cardiomyocytes. The remaining fraction consists primarily of cytoplasmic CuZnSOD with <1 % extracellular SOD (ECSOD). The importance of MnSOD in regulating $O_2^{\cdot-}$ in the myocardium is demonstrated by the fact that $MnSOD^{-/-}$ mice die soon after birth with dilated cardiomyopathy [130]. GPx (isoforms GPx1–GPx5), using glutathione (GSH), reduces H_2O_2 or ROOH to H_2O or alcohols (ROH), respectively. GPx1 and GPx3 are the most abundant intracellular isoforms and GPx4 is a mitochondrial isoform. Unlike MnSOD mice deficient in GPx develop normally and show no marked pathological changes under normal physiological conditions and exhibit a pronounced susceptibility to myocardial ischemia-reperfusion injury [131]. CAT, located in peroxisomes, is highest in the liver and erythrocytes and converts H_2O_2 to H_2O and O_2. Prx reduces peroxides, including H_2O_2 and alkyl hydroperoxides (ROOH).

The importance of removing mitochondrial $O_2^{\cdot-}$ is emphasised by observations that animals null for the MnSOD allele exhibit perinatal lethality due to cardiac dysfunction, and cardiac-specific MnSOD deletion/depletion produces progressive congestive heart failure with specific molecular defects in mitochondrial respiration. It is also important to realise that MnSOD generates H_2O_2, another ROS with pathophysiological importance, as overexpression of Prx3 (a mitochondrial H_2O_2 scavenger) prevents heart failure after experimental MI in mice [132].

4.2 Nonenzymatic Antioxidants

The role of glutathione (GSH) in maintaining cellular redox state is complex. GSH cooperates with GPx in the detoxification of H_2O_2 to $2H_2O$. In addition, GSH participates in reactions with glutathione S-transferase (GST) to bind ROS such as attachment of NO to form S-nitrosoglutathione adducts. Glutathione reductase (GR) functions to regenerate antioxidant capacity, converting from glutathione disulphide (GSSG) to GSH. Vitamins and other chemical antioxidants play an important role in the control of ROS cascades. Vitamin E (α-tocopherol) is active in membranes where it functions to reduce ROS and lipid peroxy radicals. Vitamin C (ascorbate) serves predominantly as an antioxidant in

plasma due to its water solubility. It functions by reducing α-tocopherol lipid peroxide radicals to normal form [129]. Uric acid, found in extracellular fluids, detoxifies HO· metal ions (Fe^{+2} or Cu^{+}) contributing to ROS-mediated peroxidation of lipids via the Fenton reaction that produces H_2O_2. Additionally, a study by Ku and group has shown that the relationship of vitamin D (calciferol) deficiency with diabetes, hypertension, inflammation and increased cardiovascular risk and also analysed the association between vitamin D supplementation and the reduction in CVD [133].

4.3 Phytochemicals

Consumption of fruits and vegetables has been associated with lower risk of CVD [134], and their cardioprotective role is not attributable to any of the macro- and micronutrients, thus indicating role of other plant components in CVD. Plant sterols, flavonoids and sulphur-containing compounds are three categories of compounds in fruits and vegetables that may have important roles which prevent the cardiac diseases in some way. Non-nutritional bioactive compounds including isoflavones, diosgenin, resveratrol, quercetin, rosmarinic acid, catechin, sulphoraphane, tocotrienols and carotenoids comes under these three classes of compounds and are proven to reduce the risk of CVD and aid in cardioprotection [135, 136]. These compounds further need to be characterised as their mechanisms of action are not yet understood. Apart from these, vitamins, phytoestrogens and trace minerals may also have roles in cardioprotection. Most of the CVD consist of multiple events. Hence, a single cardioprotectant may not be enough to combat the CVD. These phytochemicals nowadays are greatly used in various pharmacological medicines in curing of CVD as well as cardioprotective due to their properties and mechanisms involved including antioxidative, anti-hypercholesterolemic, anti-angiogenic, anti-ischemic, inhibition of platelet aggregation and anti-inflammatory activities that reduce the risk of CVD.

Low to moderate consumption of red wine is associated with decreased incidence of CVD [137, 138]. Resveratrol and quercetin, polyphenols present in red wine, are anti-proliferative, anti-mitogenic, anti-platelet and anti-inflammatory [139]. The therapeutic action of resveratrol has been reported in animal models of arterial injury [140]. Resveratrol increases vascular NO production through an oestrogen receptor modulation and also inhibits NF-κB activation [141]. Quercetin and other wine polyphenols have been shown to prevent cardiac cells from apoptosis, oxidative stress and endothelial dysfunction both in vitro and in vivo studies [142].

Organosulfides, present mainly in garlic and onion, possess antioxidant and anti-inflammatory properties. The efficacy of experimental and clinical effects of garlic preparations and constituents in CVD complications have been well studied [143]. Garlic extract prevents oxidative stress via NOX and lipid peroxidation in experimental model of metabolic syndrome [144]. The systolic blood pressure decrease is associated with intake of garlic extract and allicin in fructose-induced hyperinsulinemic, hyperlipidemic and hypertensive rats [144, 145]. The action of garlic extracts against hypertension may be exerted via prostaglandin-like effects [146] or by increasing the bioavailability of NO or scavenging oxidants [147] or by inhibiting angiotensin-converting enzyme in vitro [148]. The effect of garlic against hypertension has been demonstrated in high blood pressure human cases in randomised controlled trials and meta-analysis trial [149, 150]. Garlic exerts anti-inflammatory action via the inhibition of the expression of intercellular cell adhesion molecule 1 (ICAM1) through the down-regulation of AP-1 and c-Jun N-terminal kinase (JNK) pathway [151]. The mechanism and role of garlic and its active constituents in cardioprotection need to be verified in humans through more experimental and clinical studies, so that they can be utilised as better therapeutic agents.

Higher intake of fruits and vegetables is linked with a decrease in risk of MI in prospective cohort study of women, thus increase in consumption of fruits and vegetables may protect against CVD. Increased total flavonoid and

flavone intake is associated with lower risk of mortality due to CVD in large cohort of 38,180 men and 60,289 women, while long-term supplementation with β-carotene has been proved ineffective in preventing CVD in a randomised, double-blind, placebo-controlled trial [152]. Additionally, the combination of β-carotene and vitamin A showed no positive effect on the risk of CVD [153]. Tea and coffee consumption is associated with the risk of CVD [154]. Phytochemicals present in tea and coffee may exert their cardioprotective action through the regulation of vascular tone by influencing endothelial function, enhanced reverse cholesterol transport, ameliorated glucose metabolism, restrained foam cell formation, immunomodulation, inhibition of oxidative stress and effects on platelet function or by altering gene expression [155]. Further research is required to identify the role of constituents of tea and coffee in CVD [155].

Another phytochemical that showed the capability of having therapeutic potential in the treatment of CVD is cannabidiol (CBD). CBD is an abundant constituent of *Cannabis sativa*, which have been reported to have anti-inflammatory effect in various disease models including multiple sclerosis in humans. A study investigated the effects of CBD on cardiac dysfunction, elevated oxidative stress and amplified inflammatory cell signalling pathways in a mouse model with type I diabetic cardiomyopathy. It was observed that CBD remarkably attenuates myocardial dysfunction, oxidative stress, cardiac cell death, inflammatory and other interrelated cell signalling pathways. It was also found to inhibit high-glucose-mediated elevated ROS production as well as NF-κB activation in primary human cardiomyocytes [156].

5 Conclusions and Future Perspectives

Till date CVD remains the principal cause of long-term disability and death worldwide. Numerous studies have been performed, but its efficient prevention and treatment management are still failing off. For better understanding of the pathophysiology of CVD, for identifying specific biomarkers and for determining effective therapeutic targets, it is important to resolve the complexity of biochemistry of involved important pathophysiological factors such as oxidative stress and inflammation, displaying association between, which promotes the development and progression of CVD.

Oxidative stress is the disturbance of physiological balance between oxidants (ROS/RNS generation) and antioxidant defence systems. Oxidative stress is the fundamental mechanism of cell damage, and ROS is the damaging factor leading to the onset and progression of CVD. Increased ROS production in the vascular wall promotes endothelial dysfunction, infiltration and accumulation of inflammatory cells. ROS are also involved in redox activation of certain TFs responsible for expression of genes for inflammatory responses. Elevated levels of pro-inflammatory cytokines in circulation such as CRP, TNF-α and IL-6 have been shown to be associated with CVD. Accumulating evidence shows that increased ROS-mediated oxidative damage coupled with downstream inflammatory pathways augments pathological complications associated with CVD.

Therapeutic interventions involving diet, nutrition and pharmacology may target enzymatic sources of ROS, activates antioxidant defence systems in vasculature and can prevent oxidative stress and inflammation in CVD. Evidences from studies suggest that antioxidants hold the potential to act as therapeutic intervention against CVD by reducing ROS generation, thus attenuating oxidative stress and downstream inflammatory processes. Results from various studies also indicate that phytochemicals may have therapeutic potential against CVD, by reducing oxidative stress and oxidative stress-mediated inflammation. The motive of this book chapter was to make understand the role of excessive oxidative stress and chronic inflammation in CVD and to summarise the recent scenario displaying the perspectives of antioxidant and anti-inflammatory therapeutic interventions in CVD (Fig. 3). Future studies aimed at inferring the biochemical and molecular links concerning

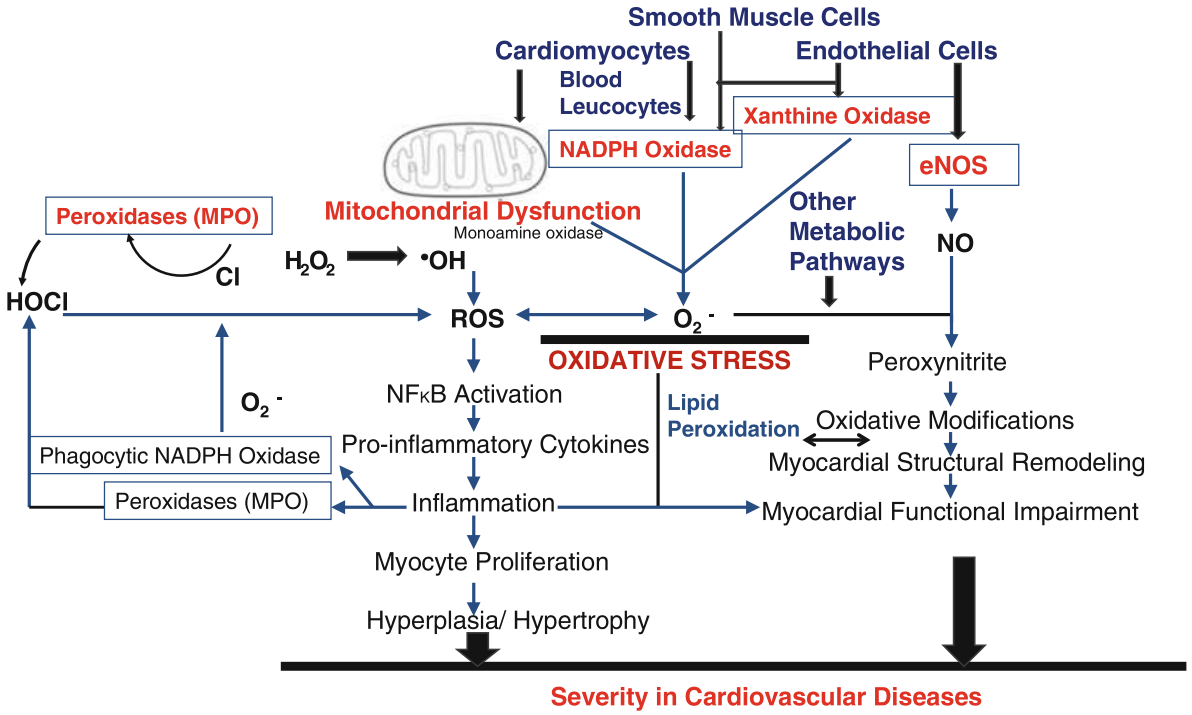

Fig. 3 Role of oxidative stress and inflammation in the development and progression of CVD. Specific enzymes of cells of the cardiovascular system, such as NADPH oxidases, xanthine oxidases and myeloperoxidases (MPO) of cardiomyocytes and endothelial cells, generate pathophysiological levels of ROS/RNS which cause oxidative stress-induced damage, promote endothelial dysfunction and accumulation of inflammatory cells and further involved in the activation of inflammatory response signalling pathways. Altogether leads to the severity of CVD

oxidative stress and inflammation and mechanisms underlying phytochemical-induced cardioprotection in CVD need to be elucidated in CVD to develop more new effective therapeutic interventions.

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