Reactive Oxygen Species in Cardiovascular Disease

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Abstract

Based on the 'free-radical theory' of disease, researchers have been trying to elucidate the role of oxidative stress from free radicals in cardiovascular disease. Considerable data indicate that ROS and oxidative stress are important features of cardiovascular diseases including atherosclerosis, hypertension, and congestive heart failure. However, blanket strategies with antioxidants to ameliorate cardiovascular disease have not generally yielded favorable results. However, our understanding or reactive oxygen species has evolved to the point that we now realize these species have important roles in physiology as well as pathophysiology. Thus, it is overly simplistic to assume a general antioxidant strategy will yield specific effects on cardiovascular disease. Indeed, there are several sources of reactive oxygen species that are known to be active in the cardiovascular system. This review will address our understanding of reactive oxygen species sources in cardiovascular disease and both animal and human data defining how reactive oxygen species contribute to physiology and pathology.

Keywords

reactive oxygen species; biomarkers; clinical trials; cardiovascular disease

Introduction

In the beginning of 20th century, 'The rate-of-living hypothesis' was derived from observations that animals with higher metabolic rates were characterized by shorter life spans, implying that a species metabolic rate ultimately determines its life expectancy. In 1956, Denham Harman proposed a 'free-radical theory' that endogenous oxygen radicals were generated in cells over time resulting in cumulative cellular damage targeting DNA, protein, lipids and other components of the cell [1]. Since cardiovascular disease is a manifestation of aging, researchers have attempted to elucidate the relation between cardiovascular disease and oxidative stress caused by free radicals.

Clinical studies of antioxidants in vascular disease

Initially, these studies focused on how antioxidants may influence the clinical course of atherosclerosis and cardiovascular disease. Numerous clinical trials have been performed to

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examine the potential for preventing cardiovascular disease using antioxidant therapies (please see Table 1). The term, cardiovascular disease encompasses the major clinical endpoints related to the heart and vascular system including myocardial infarction (MI; heart attack), ischemic heart disease (MI and angina), stroke, and peripheral arterial disease (ischemia of the limbs). The most common manifestations of cardiovascular disease are MI and stroke. Thus, a predominance of studies investigating antioxidant status focus on cardiovascular disease (CVD) with combined endpoints of stroke and MI, whereas some focus on MI using the term coronary heart disease (CHD).

Primary prevention of vascular disease

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. Observational studies of vitamin C in the primary prevention of CVD have been conflicting with only some suggesting a benefit with the consumption of vitamin C supplements [2–8]. Large-scale randomized trials evaluating vitamin C indicate no effect on the primary endpoint of CVD and no meaningful impact on all-cause mortality [9, 10]. In contrast, observational studies of vitamin E supplementation predominantly found that vitamin E was associated with a lower risk of CHD [6, 7, 11, 12]. These findings were of considerable interest, but had to be interpreted with caution as observational trials are subject to unintended bias and confounding. Thus, several randomized, double-blind, placebo-controlled trials were conducted to investigate the impact of vitamin E supplementation on CVD and none have found a benefit for the primary prevention of CVD [9, 13–16]. In fact, evidence from some of these trials found that vitamin E treatment was associated with increased heart failure [17] and hemorrhagic stroke [9]. Beta carotene has also been investigated and randomized trials of this antioxidant failed to show any effect on the primary prevention of CHD or the risk of death from CVD [13, 18–20]. Thus, it has been difficult to demonstrate that antioxidant supplementations (vitamin C, E and beta carotene) have an impact on the primary prevention of CHD or CVD.

Secondary Prevention of vascular disease

Secondary prevention refers to inhibiting manifestations of CVD in those patients who already have the disease. Since the risk of a second cardiovascular event (MI, stroke, angina) is highest in patients that have already had a first event, established prevention measures (*e.g.* cholesterol lowering, smoking cessation, etc.) are most effective in secondary prevention. Thus, if antioxidant therapy were to be of benefit, it would be expected to be most effective in secondary prevention. However, treatment of postmenopausal women with CHD using vitamins C and E showed no benefit on CHD and even demonstrated excess death compared to placebo [21]. Similarly, in women with CHD or at high risk for CHD, treatment with vitamin C, E, and beta carotene, either alone or combination, did not show any benefits on cardiovascular events [22]. With regards to vitamin E alone, there are two trials that suggested a benefit. The Cambridge Heart Antioxidant Study (CHAOS), demonstrated a benefit for vitamin E in patients with coronary disease, but no impact on mortality [23], and in patients with kidney disease the administration of vitamin E significantly reduced the incidence of cardiovascular events [24]. However, in other large scale randomized trials, vitamin E administration alone or in combination had no effect on cardiovascular outcome [25]. With beta carotene, there is no convincing evidence of a benefit on angina [26], and it may be harmful for patients with previous MI because of increased cardiac death [27]. Thus, on balance, these results lead the conclusion that there is no consistent evidence of benefit from vitamin C, E and beta carotene for the secondary prevention of CHD. This conclusion is also supported by meta-analyses looking at all the trials collectively [28].

Other synthetic antioxidants have been studied in human clinical trials including probucol and succinobucol. Probucol was originally developed as a lipid lowering agent and then also noted to be a potent antioxidant that prevents endothelial dysfunction [29, 30] and (low density lipoprotein) LDL oxidation [31]. Pretreatment with probucol significantly reduced the arterial response to injury compared to placebo [32–34], suggesting promise for the treatment of atherosclerosis. However, in one large clinical study probucol did not have a significant impact on femoral artery atherosclerosis [35]. Moreover, probucol is no longer available for clinical use due to prolongation of the QT interval and potential pro-arrhythmic effects. Succinobucol is a probucol derivative with antioxidant efficacy[36] that lacks QT-interval prolonging effects. However, a large scale randomized clinical trial with this drug added to conventional treatments had no effect on cardiovascular death, resuscitated cardiac arrest, MI, stroke, unstable angina, or coronary revascularization [37].

On balance, these trials have not demonstrated clinical efficacy of antioxidant treatment to ameliorate the clinical manifestations of atherosclerosis. There have been multiple reasons proffered, including the notion that the effects of ROS are complex, making outcome predictions difficult, and that the types of antioxidants, their dosage, and their duration of action have been inadequate [38–41]. It is also known that genetic factors influence the response to antioxidants [42–44], raising the possibility that only some patients will benefit from antioxidant treatment and that we lack the ability to identify this subset of patients. This latter point deserves particular attention. During the time of many clinical studies, there was not an agreed upon manner to identify patients who suffer from an excess of vascular oxidative stress that would be most likely to benefit from antioxidant treatment. In fact, we do not really know what proportion of CVD patients might fit into this category. As a consequence, we could be treating large numbers of patients with only a small fraction likely to benefit. Thus, in the future it may make more sense to measure oxidative stress markers, such as F2-isoprostanes that have been used successfully clinically [45], as a means to target appropriate patients and document appropriate antioxidant efficacy.

Another compelling explanation for the mixed performance of general antioxidant treatment in mitigating cardiovascular disease is the incomplete knowledge of how reactive oxygen species (ROS) impact both physiology and pathophysiology. There is now overwhelming evidence that ROS have a role as second messengers for cellular signal transduction, and the balance between oxidizing and reducing species is known to be an important component of cellular homeostasis (Figure 1) [46, 47]. Thus, the premise that one can selectively scavenge "pathological" vs. "physiological" ROS with specific antioxidants is likely not valid. Consequently, it may be more fruitful to understand the mechanisms whereby ROS are produced and the source(s) of ROS than to focus on a strategy directed at ROS scavenging.

Selected sources of reactive oxygen species (ROS) in cardiovascular disease

A variety of enzymatic and non-enzymatic processes can generate ROS in mammalian cells. Some of the most important sources are the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, lipoxygenase, uncoupled nitric oxide synthase (NOS) and myeloperoxidase (MPO) and these systems are depicted in Figure 2. There is evidence linking each of these sources with CVD pathology (Table 2) and, as a consequence, we will discuss each in turn below.

Mitochondrial respiratory chain

Mitochondria have long been known as an important source of ROS generation. Of the entire electron flux through mitochondria, more than 97% is utilized to reduce cellular

oxygen to water [48]. Estimates from experiments in isolated mitochondria estimate a 2 – 3% leakage of electrons to form $O_2^{\bullet-}$ that is largely dismutated to H_2O_2 by manganese superoxide dismutase (Mn-SOD) in the mitochondrial matrix [48]. The importance of removing mitochondrial $O_2^{\bullet-}$ is emphasized by observations that animals null for the Mn-SOD allele exhibit perinatal lethality due to cardiac dysfunction [49] and cardiac-specific Mn-SOD deletion produces progressive congestive heart failure with specific molecular defects in mitochondrial respiration [50]. It is also important to realize that Mn-SOD generates H_2O_2, another ROS with pathophysiologic importance as overexpression of peroxiredoxin-3 (a mitochondrial H_2O_2 scavenger) prevents heart failure after experimental MI in mice [51]. Collectively, data indicate that mitochondria are an important source of ROS that has implications for the cardiovascular system.

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase

The NADPH oxidases are a family of multiple-subunit complex enzymes that generate $O_2^{\bullet-}$ via one electron reduction of oxygen using NADPH as an electron source. Interested readers are directed to a recent excellent review of this enzyme family [52]. In brief, the prototypical NADPH oxidase (Nox2) consists of two membrane-bound subunits (gp91 and p22) and three or more cytoplasmic subunits (p40, p47, p67, G protein Rac1 or 2). Genetic defects of gp91, p22, p47, or p67 are the basis for the clinical syndrome of chronic granulomatous disease, because Nox2-derived ROS are necessary for host defense such as the killing of microorganisms by neutrophils and monocytes. There are a total of 7 Nox isoforms that are also present in non-phagocytic cells, including vascular endothelial cells, smooth muscle cells, fibroblasts and cardiomyocytes [53]. Data generated from experimental animals indicate roles for Nox isoforms in cardiac fibrosis [54], preconditioning [55], cardiac remodeling post-MI [56] and angiotensin II -dependent cardiac hypertrophy [57–60]. Within the vasculature, evidence indicates that Nox isoforms may contribute to atherosclerosis [61–63], aortic aneurysm formation [64], and the response to arterial injury [65]. With regards to physiologic responses, Nox-derived ROS have been linked to endothelial cell migration and ischemia-induced angiogenesis [66]. Thus, there is ample evidence for Nox-derived ROS in modulating both vascular physiology and pathophysiology.

Xanthine oxidase

Xanthine oxidase and xanthine dehydrogenase are forms of the same enzyme, known as xanthine oxidoreductase. This enzyme is widely distributed in mammalian tissues and with particularly high expression in capillary endothelium [67]. Both forms of xanthine oxidoreductase catalyze the conversion of hypoxanthine to xanthine and xanthine to uric acid, however only the oxidase form generates $O_2^{\bullet-}$ and H_2O_2 [68]. The enzyme typically exists in the dehydrogenase form, but under certain stressful conditions, such as hypoxia, the oxidase isoform predominates [69]. Xanthine oxidase has been implicated as a source of ROS after reperfusion of ischemic tissue in several organs [70–72]. However, genetic evidence is lacking since xanthine dehydrogenase-null mice are runted and die by 6 weeks of age from renal dysplasia [73]. As a consequence, most of the data implicating xanthine oxidase relies on pharmacologic inhibition via allopurinol or oxypurinol, or by administration of a tungsten-rich, molybdenum deficient diet to experimental animals, which leads to an inactive enzyme [74] and is associated with reduced atherosclerosis [75]. In addition to ROS production, xanthine oxidase may act as a NO_3^- and NO_2^- reductase to generate nitric oxide (NO) under hypoxic conditions [76–79]. In addition, xanthine oxidase expression and $O_2^{\bullet-}$ production are upregulated by NADPH oxidase [80], indicating that factors regulating NADPH oxidase may also have influence on xanthine oxidase [81].

Lipoxygenases

The lipoxygenases are non-heme, iron-containing enzymes that catalyze the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids [82]. Certain lipoxygenase isoforms are thought to promote atherosclerosis by generating ROS and oxidatively modified lipids, like oxidized low density lipoprotein (Ox-LDL) [83]. The 12/15-lipoxygenase (12/15-LO; also known as the leukocyte-type 12-lipoxygenase and 15-lipoxygenase-1) and 5-lipoxygenase (5-LO) are most studied because of their expression pattern in inflammatory cells and endothelial cells [84]. Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes that are playing an important pathophysiological role in inflammatory diseases. Reactive oxygen species are generated as a byproduct during the biosynthesis step of leukotrienes by lipoxygenases[84]. In human atherosclerotic lesions, 5-LO is present in macrophages, foam cells, mast cells and dendritic cells [85] and 12/15-LO is widely distributed to including blood vessels, the brain, and the kidneys [86].

There is considerable genetic evidence that lipoxygenases can participate in cardiovascular physiology and pathophysiology. Mice lacking 12/15-LO exhibit decreased atherosclerosis in a number of models [87–90]. Consistent with these observations, 12/15-LO has been implicated in a number of events important for atherosclerosis such as cytokine production [91] and the adhesion of monocytes to endothelial cells [92–94]. The 5-LO has been implicated in atherosclerosis susceptibility in mice [90], suggesting that the lipoxygenases represent a source of ROS that is relevant to CVD.

Given the available data in animal models, lipoxygenases have been investigated with regards to human cardiovascular disease [95]. During the period from early to advanced atherosclerosis, the quantity of 5-LO positive cells are increased in human atherosclerotic plaque specimens [96] and elevated 5-LO activity has been linked to plaque instability [97]. In human abdominal aortic aneurysm tissue, 5-LO is expressed in macrophage-rich adventitial areas and associated with intraluminal thrombus [98]. Thus, targeting the 5-LO pathway could potentially reduce CHD or abdominal aortic aneurysm. To this end, a recent study reported that a potent 5-LO inhibitor reduced leukotriene production and coronary plaque burden (by CT scan) in patients after acute coronary syndrome [99]. Since this study is preliminary and the observation period is relatively short (6 months), these results require confirmation in larger scale trials of longer duration. Another drug of interest is an inhibitor of 5-LO activating protein (FLAP), known as DG031. This compound was shown to reduce biomarkers of cardiovascular risk in patients with genetic variants of the gene for 5-LO activating protein [100].

Nitric oxide synthases (NOSs)

The NOS enzymes are a family that catalyze the conversion of L-arginine to L-citrulline with the production of NO. There are three NOS isoforms that are termed neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) based upon the tissues in which they were first described (reviewed in [101]). In most cardiovascular tissues in which they are expressed, nNOS and eNOS are constitutively present, but in distinct subcellular locations. In contrast, iNOS is usually absent, but induced by pro-inflammatory mediators [102]. The bulk of evidence linking NOS production of ROS pertains to the eNOS isoform. Active eNOS exists as a homodimer with each monomer consisting of reductase and oxygenase domains [103]. The production of NO via eNOS involves electron transfer from the cofactor NADPH to flavin adenine dinucleotide and flavin adenine mononucleotide to heme. This electron transfer affords oxidation of L-arginine is oxidized to form NO and L-citrulline, with the assistance of tetrahydrobiopterin (BH_4) as a cofactor. The tight coupling of electron flow through eNOS to L-arginine is dependent upon adequate levels of cofactors and, under specific circumstances, eNOS may become "uncoupled" and reduce molecular

oxygen rather than transfer electrons to L-arginine, there by generating $O_2^{\bullet-}$ as an important source of ROS [104]. The most labile eNOS cofactor is BH_4 and, as a consequence, it is also the most common *in vivo* mechanism for eNOS uncoupling. Although all NOS isoforms are capable of uncoupled NO production, it is most often described with eNOS.

The data for eNOS uncoupling *in vivo* spans animal and human data. With regard to the former, platelets contain eNOS and platelets isolated from eNOS-null mice produce less $O_2^{\bullet-}$ than wild-type platelets [105]. Cholesterol-fed animals also exhibit eNOS uncoupling that is linked to the amount of BH_4 in vascular tissues [106]. We know that these findings are clinically relevant, as arterial segments from humans with diabetes [107] or atherosclerosis [108] exhibit eNOS uncoupling. The uncoupling is reversible, however, as treatment with 5-methyl tetrahydrofolate (5-MTHF; the active form of folic acid), which increases intracellular BH_4 levels, also decreases vascular superoxide production [109]. Although the substantial long-term folic acid and vitamin B12 supplementation did not have beneficial effects on vascular outcomes [110], 5-MTHF has a direct anti-atherogenic effects by decreasing vascular superoxide generation and augmenting NO bioavailability in the human vascular wall [109, 111]. The methyl tetrahydrofolate reductase gene polymorphism which affected plasma or circulating 5-MTHF exerts a direct effect on vascular BH_4 levels, NO bioavailability, and eNOS coupling in human vessels *in vivo* [112].

Thus, it appears the impact of eNOS on the vasculature can depend upon whether there are appropriate levels of cofactors to support eNOS function. Data on atherosclerosis support this contention as eNOS-null animals on an apolipoprotein E (ApoE)-null background exhibit more atherosclerosis than ApoE-null mice alone [113], whereas overexpression of eNOS in ApoE-null mice shown increased atherosclerotic lesion formation with elevated levels of vascular $O_2^{\bullet-}$ [114]. These findings were explained by a relative deficiency in BH_4 with eNOS overexpression leading to eNOS uncoupling. Consistent with this hypothesis, overexpression of GTP-cyclohydrolase I (the rate-limiting enzyme for BH_4 synthesis) prevents eNOS uncoupling [115] and reduces atherosclerosis and ROS levels [116, 117].

In the heart, eNOS is not only expressed in the endothelium, but also in cardiac myocytes. In general, evidence supports cardiac eNOS as a protective enzyme. Genetic eNOS deletion increased long-term mortality and attenuated left ventricular (LV) function in mice with MI [118]. In the case of pressure overload induced by aortic constriction, eNOS-null mice exhibited more severe LV hypertrophy, LV dysfunction, and myocardial fibrosis than in wild-type mice [119]. Consistent with eNOS as a protective factor, eNOS overexpression protects against MI and ischemic reperfusion injury compared to the wild-type phenotype [120, 121]. Cardiac-specific overexpression of eNOS also attenuates the LV hypertrophy induced by coronary artery occlusion [122] and eNOS-null mice with eNOS restored only in the myocardium showed less LV hypertrophy and dysfunction caused from pressure overload than mice with global eNOS deletion [123]. As one might expect given the deleterious effects of eNOS uncoupling, BH_4 replacement therapy rescued pressure overload-induced LV hypertrophy, fibrosis, and cardiomyocyte dysfunction after aortic constriction and these effects were associated with less oxidant stress and recoupled eNOS [124]. Thus, functional eNOS in either the endothelium or the cardiac myocyte appears to promote adaptive responses in the heart.

The two other NOS isoforms, iNOS and nNOS have also been investigated in the cardiovascular system, although the contribution of uncoupling is less clear. Several studies performed in iNOS-null mice on the ApoE-null background show a significant reduction in atherosclerotic plaque formation [125–127]. In an experimental MI model, iNOS-null mice showed increased contractility and decreased mortality compared to wild-type mice [128]. In an aortic constriction model, iNOS-null mice demonstrated much less hypertrophy,

dilatation, fibrosis, and dysfunction of the LV, than wild-type mice [129]. Conversely, myocardial overexpression of iNOS in mice demonstrated cardiac fibrosis, cardiomyocyte death, cardiac hypertrophy, and LV dilatation. These mice developed overt heart failure and most of them died suddenly from atrioventricular block and asystole [130]. One possible mechanism may be an iNOS-mediated oxidation of BH_4 needed for eNOS-mediated NO production [131].

With regards to nNOS, nNOS-null mice on the ApoE-null background demonstrated promotion of atherosclerosis formation [132]. One potential explanation for the above observations is that nNOS inhibits xanthine oxidoreductase activity and in nNOS-null mice the production of ROS is increased [133]. With regards to the heart, nNOS-null mice with acute MI showed severe LV remodeling and a higher mortality rate than wild-type mice [134, 135]. Conversely, cardiomyocyte-specific nNOS overexpression produced better preservation of LV function with pressure overload than wild-type mice [136]. Deletion with both the nNOS and eNOS genes produces spontaneous concentric left ventricular hypertrophy associated with interstitial fibrosis, impairment of LV diastolic properties, and high mortality [137].

Myeloperoxidase

MPO is a member of the heme peroxidase family of enzymes. This enzyme is expressed in neutrophils and monocytes, and it generates reactive species that can oxidatively modify lipids and proteins [138, 139]. The MPO catalytic cycle typically involves the consumption of H_2O_2 to produce a range of oxidizing products. Amongst the MPO-derived oxidants *in vivo*, HOCl is the most abundant as physiologic chloride concentrations (~150 mM) favor its formation [140–142]. One notable feature of HOCl as an oxidant is the fact that it is a two-electron oxidant and reacts poorly with one-electron antioxidants such as vitamin E. Thus, if HOCl were an important oxidant in the progression of atherosclerosis its chemical properties could provide an explanation for the poor track record of antioxidant supplementation in treating and preventing atherosclerosis.

There is considerable evidence that MPO produces oxidation during the course of atherosclerosis. Enzymatically-active MPO is abundant in monocyte-derived macrophages of human atherosclerotic lesions [143] that are co-localized with lipoproteins that show evidence of HOCl-mediated oxidation [144]. Oxidation from MPO has also been implicated in the transition of stable atherosclerotic plaques to a type more "vulnerable" to plaque rupture. The mechanism relates to MPO-generated HOCl that can lead to matrix metalloproteinase activation and lipid peroxidation in the artery wall [143, 145], two processes linked to forming unstable atherosclerotic plaques [146]. In addition to producing reactive chlorinating species in humans, MPO catalyzes the oxidation of NO in human plasma in a manner that can reduce the bioavailability of NO [147, 148]. These data, combined with observations that MPO-derived HOCl can induce endothelial dysfunction [149] and eNOS uncoupling [150], indicate that MPO significantly disrupts normal vascular homeostasis.

Efforts to model the impact of MPO on atherosclerosis have been difficult, in part, because murine atherosclerosis, unlike the human condition, does not demonstrate lesional MPO accumulation. Thus, the effects on animal atherosclerosis have been mixed. In low density lipoprotein receptor (LDL-R)-null mice, populating the bone marrow with MPO-null cells increases atherosclerosis [151], whereas populating the bone marrow with cells over expressing human MPO also increased atherosclerosis compared to wild-type bone marrow [152]. More recently, several studies with "humanized" MPO in murine models have supported the notion that MPO promotes atherosclerosis [152, 153]. Collectively,

differences between human and murine MPO have made the appropriate modeling of human disease in mice challenging.

In contrast to the animal studies, human studies have been much more consistent with regards to the role of MPO on CVD and its outcomes. Patients with reduced MPO levels appear to have reduced CVD [154]. Leukocyte MPO levels are associated with the extent of coronary atherosclerosis [155] and circulating MPO levels are a good indicator of the presence and extent of coronary artery disease (CAD) [156, 157]. Finally, the activity of atherosclerosis is also predicted by MPO in apparently healthy individuals [158]. Thus, data from biochemical, animal, and human studies support a role for MPO and MPO-derived oxidants in the clinical course of atherosclerosis.

The precise mechanism for these observations is not known. However, MPO has recently been determined to have a significant impact on high density lipoprotein (HDL) and the process of reverse cholesterol transport. Apolipoprotein A-I (Apo A-I), the major protein component of HDL, contains a specific binding site for MPO [159, 160]. This HDL-bound MPO retains enzymatic activity and modifies Apo A-I in a manner that further enhances Apo A-I binding affinity for MPO [161]. The oxidation of Apo A-I also impairs the HDL particle capacity to promote cellular cholesterol efflux by ATP-binding cassette transporter 1 [160] and oxidized Apo A-I cannot activate lecithin:cholesterol acyltransferase, an enzyme important for HDL maturation [162]. Thus, MPO has the capacity to inhibit reverse cholesterol transport and HDL function, thereby promoting both atherosclerosis and the clinical activity of CVD.

Detection of ROS in cardiovascular disease

One strategy to determine the role of ROS in CVD involves looking for experimental evidence of oxidative reactions in clinical populations. A major challenge in monitoring ROS in biologic systems is the highly reactive nature of the compounds in question. Fluorescence probes have been designed that can detect individual ROS [163] and electron spin resonance probes exist that can provide information about the activity and location of free radical reactions [164]. However, these tools are limited in human and animal applications due to technical considerations [165]. As a consequence, there has been considerable effort directed at developing techniques to assess either the downstream consequences of ROS (*e.g.* oxidation products) or enzyme levels important for ROS reactions (*e.g.* myeloperoxidase). The levels of these biomarkers, therefore, can provide at least indirect evidence for ROS action in CVD.

Oxidized low density lipoprotein (Ox-LDL)

LDL contains lipid species that are subject to oxidation in the presence of several ROS known to exist in vascular wall [87, 166, 167]. Oxidative modification of LDL is known to be a feature of the atherosclerotic process [168] as Ox-LDL is taken up by macrophages via scavenger receptor pathways to form cholesteryl ester-rich foam cells and endothelial cells become dysfunctional, in part, by taking up Ox-LDL via the lectin-like oxidized LDL receptor-1 [169]. The presence of Ox-LDL has been confirmed in atherosclerotic plaques using immunohistochemical staining for modified apolipoprotein B-100, the protein moiety in LDL [170]. Because the formation of Ox-LDL produces many immunogenic epitopes, it has been possible to detect the presence of Ox-LDL via immunoassays of plasma or circulating plasma auto-antibodies. Several clinical studies (Table 3) revealed that elevated Ox-LDL, auto-antibodies against Ox-LDL or malondialdehyde modified LDL particles are strongly associated with atherosclerosis and CAD including acute coronary syndrome [171–177]. In healthy individuals, circulating levels of Ox-LDL are independent predictors of atherosclerosis detected by ultrasound techniques or the occurrence of clinical CAD [178,

179]. Based upon this literature, Ox-LDL levels have been proposed as a biomarker of CVD risk beyond LDL-cholesterol concentrations [179]. However, more information is needed about how Ox-LDL compares to other, more established, biomarkers of CVD activity before one can conclude with confidence the ultimate utility of Ox-LDL levels in predicting atherosclerosis disease activity.

MPO

MPO can be measured in both inflammatory cells and as a circulating enzyme and it is known to participate in both atherosclerosis and its clinical sequelae. In human atherosclerotic lesions, MPO and hypochlorite-modified proteins are co-localized [144, 180]. Macrophages in eroded or ruptured plaques are rich in MPO compared to macrophages in fatty streaks that contain little MPO [181]. These data suggest that MPO predicts the clinical activity of atherosclerosis and there is data that tests this prediction (Table 3). Indeed, in human study participants, there is a strong inverse association between MPO serum concentrations and brachial artery flow-mediated dilation, another predictor for the clinical activity of atherosclerosis [182]. As one might expect, this enzyme has been the subject of a number of epidemiological studies across a wide range of patient populations that clearly indicate MPO is an important risk marker for CAD, even in patients with unstable angina and MI [155, 156, 158, 183–191]. If one accepts the notion that MPO is associated with atherosclerosis-related CVD, it follows that reduced MPO levels should predict a lower occurrence of CVD. Indeed, epidemiological studies have indicated that individuals with total or near total deficiency of MPO are less likely to develop CAD [154]. Individuals who have specific MPO gene promoter polymorphism that decrease MPO expression have reduced CAD manifestations, whereas subjects with polymorphisms increasing MPO expression exhibit increased CAD [192, 193]. Thus, there is ample evidence that measurements of MPO in humans provide important information about the likelihood of CAD and its clinical sequelae.

Although the data outlined above provide a compelling case for MPO in the clinical prediction of atherosclerosis, there are a few caveats that bear mention. For example, MPO mass is typically measured in the clinical studies, whereas its activity has not been well studied. It is also important to note that specific cut-off values for MPO levels have not been firmly established. The relative merits of different types of analytical specimens also remains to be established as plasma MPO levels are sensitive to heparin dosing [194, 195], neutrophil activation [196], and the method of collection [197]. Thus, before MPO can be used routinely in clinical practice for CVD risk stratification, there will need to be considerable standardization of its sampling.

Plasma F2-isoprostanes

The F2-isoprostanes are a non-enzymatically generated oxidative products of arachidonic acid that may be derived from esterified fatty acid sources such as membrane phospholipids [198] or circulating LDL [199, 200]. Once formed, these compounds may circulate as the free form, or remain esterified in phospholipids and may be found in both plasma and in urine [201, 202]. Because F2 isoprostanes are structurally stable end products *in vivo*, they are cumulative and serve as good markers of oxidative stress [203]. The reliability of isoprostanes as *in vivo* markers of lipid peroxidation makes them an effective method of quantifying both ROS impact and the biologic activity of antioxidants [204].

The production of F2-isoprostanes can occur through the action of several cell types known to be involved in atherosclerosis including monocytes [205] and these oxidation products have been localized within foam cells and atherosclerotic plaques in human specimen [206, 207]. Consistent with this production of isoprostanes during atherosclerosis, several clinical

studies have established a link between CAD and isoprostane levels (Table 3). Elevated levels of plasma F2-isoprostanes is associated with the extent and the severity of CAD [208]. Increased levels of urinary isoprostanes are a sensitive and independent risk marker of CAD and are known to be increased in patients with unstable angina [209, 210] and free F2-isoprostane and choline levels are useful prognostic indicators of future (30 day) cardiac events [211]. Isoprostanes also appear to be reliable markers of ischemic tissue injury. For example, increased levels of F2-isoprostanes have been noted after ischemia/reperfusion induced by percutaneous coronary intervention [212]. Furthermore, Pericardial levels of F2-isoprostanes increase with the functional severity of congestive heart failure and are associated with pathologic cardiac remodeling [213]. Plasma levels of F2-isoprostanes are also increased in patients with chronic heart failure in relation to disease severity and the degree of cardiac dysfunction [214].

The data outlined above suggest that F2-isoprostanes could be used as a clinical tool for the prediction of cardiovascular events. However, before any biomarker can be used clinically, there are certain requirements that must be met [215] and one of these criteria is the ease of assay performance. Until the performance of isoprostanes is relatively facile and reproducible in non-expert hands, it is unlikely they will serve as a clinical tool. Moreover, few studies to date with isoprostanes have been sufficiently diverse to ensure that any findings with this biomarker are broadly applicable. Thus, there is considerable work that still needs to be done before the clinical utility of isoprostanes is secured.

Other specific measures of oxidative damage

In addition to those outlined above, there are other measures of ROS action that have been applied to clinical populations as biomarkers of CVD (Table 3). On those whole, these biomarkers are less mature in their development and, as a consequence, have less literature supporting their use. For example, lower plasma concentration of ascorbic acid (a well known endogenous antioxidant) predict the presence of an unstable coronary syndrome [216]. Bilirubin is an effective antioxidant [217] and has been consistently shown to be inversely related to CVD [218–220]. There are now several reports indicating that urinary levels of biopyrrins, oxidative metabolites of bilirubin, may be useful in predicting subsequent cardiac events after reperfusion in acute MI and that biopyrrins are elevated in heart failure [221, 222]. The cellular antioxidant enzyme, glutathione peroxidase-1 (GPX-1) activity was decreased in CAD patients and lower GPX-1 activity predicted the incidence of cardiovascular events at five years [223]. Cellular DNA is a target of ROS-mediated damage and 8 hydroxyl-2′-deoxyguanosine is a well-characterized product of DNA damage. The urinary levels of 8 hydroxyl-2′-deoxyguanosine in acute MI have been used to predict subsequent cardiac events [224] and patients with dilated cardiomyopathy exhibited significantly elevated serum levels of 8 hydroxyl-2′-deoxyguanosine compared with control subjects [225].

In considering new biomarkers as tools for predicting CVD, it is important to realize there is already a rich literature in cardiovascular risk prediction. We know that LDL cholesterol, HDL cholesterol, smoking, diabetes, advancing age, and hypertension are all informative with regards to an individual patient's risk of developing the two major manifestations of CVD, heart attack and stroke. Moreover, there are numerous scoring systems used for risk prediction that quantify the individual contributions of the risk factors outlined above. Thus, it is rather difficult for any one (oxidative stress) biomarker to add significant information to these existing risk prediction models. Nevertheless, the development of oxidative stress biomarkers has helped establish that ROS and oxidative events are part of the pathophysiology of CVD. It remains to be seen if consideration of these ROS-sensitive biomarkers can add clinically important information to patient care [226].

Studies of ROS involving the myocardium

In addition to the vascular component of cardiovascular disease, considerable morbidity and mortality also results from pathology in the myocardium related to ischemia/reperfusion injury and heart failure. The former being an important manifestation of heart attack and stroke, whereas the latter is a consequence of poor myocardial function. Thus, there have been attempts to determine the impact of ROS on the heart directly. In the paragraphs below, we will focus on studies that have provided insight into how ROS impact heart function independent from events in the vasculature.

Ischemic reperfusion injury, arrhythmia and infarction

Since MI involves interruption of the blood flow to the myocardium, contemporary therapies for MI include using mechanical (balloon angioplasty) and pharmacological (thrombolysis) strategies to restore blood flow. It has long been known that restoration of blood flow affords the recruitment of inflammatory cells and the activation of cellular injury responses that can produce additional cardiac damage and complications, a process known as reperfusion injury [227–229]. This injury can be manifest as dysfunction and/or death of myocardial and vascular tissue. Moreover, myocardial damage can also produce dysfunction of the cardiac electrical system leading to arrhythmias. The pathophysiology of reperfusion injury has been linked to changes in energy balance, cellular architecture, and the activity of leukocytes, platelets, and the complement system. Also among the features of reperfusion injury is a burst of free radical formation that may continue for hours [230] and has been attributed to multiple sources including xanthine oxidase, activated neutrophils, electron leakage from mitochondria, catecholamine oxidation, cyclooxygenase and lipoxygenase [231]. This increased ROS flux has been shown to damage myocytes, impair contractile function, and contribute to capillary leakage [231, 232]. Thus, there is considerable evidence that reperfusion-induced ROS production can contribute to cardiac pathology. As a consequence of the evidence outlined above, there has been investigation into the implications of suppressing the ROS flux associated with reperfusion injury.

One strategy for mitigating ROS-dependent reperfusion injury relates to the release of free iron, an element known to be released from tissues during ischemia and reperfusion [233]. Free iron supports the oxidation of lipids [234, 235], particularly in the presence of ROS [236]. Moreover, metal-catalyzed oxidation reactions have been linked to cardiac dysfunction during ischemia and reperfusion [233]. Thus, it not surprising that metal chelation has been tested as an antioxidant strategy in preventing ischemia/reperfusion injury. Treatment with the iron chelator, deferoxamine, has been shown to limit reperfusion injury [237] and reoxygenation-induced myocyte death [238]. Consistent with these findings, clinical trials of deferoxamine in cardiac bypass patients show reduced myocyte necrosis [239] and improved cardiac function [240]. Thus, iron chelation limits reperfusion injury in the clinical setting, supporting the notion that ROS are important for cardiac reperfusion injury.

One agent that deserves mention is edaravone. This compound prevents lipid peroxidation and is thought to function via free radical scavenging [241]. There have been two small studies examining this agent before reperfusion in patients with acute MI that demonstrated decreased incidence of reperfusion arrhythmia and reduced cardiovascular events in long term follow-up [242, 243]. Consistent with this activity, edaravone is now approved in Japan for the treatment of acute ischemic stroke [241]. It is important to realize, however, that the trials in the heart have been of a small scale and without blinding. Therefore, more data will be needed before this agent, currently unavailable in the US, can be recommended as a treatment for reperfusion injury.

Another interesting therapeutic strategy is the use of high oxygen tensions that can diminish reperfusion injury [244]. It is though the effect is mediated, in part, by reducing the formation of lipid peroxide radicals [245]. This concept was evaluated in two clinical trials [246, 247] and the results demonstrated that supersaturated oxygen therapy was associated with a reduction in infarct size.

There are a number of therapeutic challenges in devising strategies to prevent reperfusion injury and many of these have been outlined in excellent reviews [248, 249]. In brief, challenges lie in the multiple mechanisms that contribute to the consequences of MI and reperfusion injury and it is naïve to believe any one strategy can target all of these mechanisms. Moreover, it is exceedingly difficult to target ROS-mediated events *in vivo*, particularly if they are occurring within the cell. Moreover, the therapeutic window is extremely narrow. Most agents are very effective when administered *before* the injury and their efficacy diminishes as a function of time after injury. In the clinical realm, we rarely have the luxury of knowing when a patient is likely to suffer an MI or ischemic stroke, so pre-injury administration is unlikely. Nevertheless, as we develop a more thorough understanding of the mechanisms related to reperfusion injury, we may come upon the requisite knowledge needed to design new therapies that are not as time sensitive with respect to the ischemic insult.

Myocardial hypertrophy, cardiomyopathy and heart failure

Space constraints preclude a detailed discussion of the pathophysiology of heart failure here, but it is a term used to describe the syndrome that results from poor contractile function of the left ventricle, the cardiac chamber responsible for pumping blood into the systemic circulation. Upon the failure of the left ventricle, the heart filling pressures increase (*i.e.* congestion) and patients develop symptoms of breathlessness. Poor cardiac performance also leaves vital organs underperfused. We know from many years of study, that heart failure involves not only some acute injury to the myocardium (*e.g.* infarct, genetic abnormality, pressure overload from hypertension, etc.), but also a maladaptive healing process that can exacerbate the injury. There is evidence for oxidative stress in all of these facets of heart failure development [250, 251]. It is clear ROS may contribute to myocyte injury resulting from ischemic reperfusion injury (as above) [252], anthracycline cardiotoxicity [253], reduction of endogenous antioxidants in the myocardium [254–258], and the remodeling response [259]. For example, mice over expressing the endogenous antioxidant enzyme glutathione peroxidase are protected against LV dilation, dysfunction, and death after MI [260]. Overexpression of heme oxygenase-1, an antioxidant enzyme that metabolizes heme and has anti-apoptotic effects, prevented pathologic LV remodeling with decreasing oxidative stress in an MI model [261]. Antioxidant therapies for heart failure in animal models also suggest a benefit from reduced generation of free radicals that: i) limits myocyte damage [262]; ii) improves cardiac function after reperfusion [263, 264]; iii) attenuate remodeling and cardiomyopathy [265–270].

There are several biomarkers that also specifically support the increase of oxidative stress in patients with heart failure (please see table 3) [211, 213, 214, 222]. NADPH oxidase activity in myocardium is increased in the failing human hearts [271] and electroparamagnetic resonance studies with spin trapping directly demonstrate an increased levels of $O_2^{\bullet-}$ in human failing hearts [272]. Serum uric acid levels are increased as a function of heart failure disease severity, and independently predict survival in chronic heart failure patients [273]. The uric acid levels are thought to reflect, in part, xanthine oxidase activation and this may also predict the incidence of heart failure [274]. Consistent with this contention, xanthine oxidase activity in the myocardium is increased in patients with chronic mitral regurgitation with normal LV function and may contribute to myofibrillar degeneration and contractile dysfunction [275].

The finding that xanthine oxidase activity may be increased in the setting of heart failure has prompted additional studies. Intracoronary infusion of allopurinol, a xanthine oxidase inhibitor, increases myocardial efficiency in heart failure patients [276] and systemic allopurinol reduces markers of oxidative stress and improves endothelial function in patients diagnosed with heart failure [277], independent of serum uric acid levels [278]. While these small scale studies demonstrate that xanthine oxidase contributes to some elements of heart failure physiology (*e.g.* endothelial dysfunction and impaired myocardial efficiency), they do not tell us if these effects translate into improved patient outcomes. To address this issue, 405 patients were enrolled in a randomized, multicenter trial (OPT-CHF) of oxypurinol in the treatment of heart failure [279]. Oxypurinol, a drug similar to allopurinol, did not have any impact on heart failure morbidity, mortality, or quality of life in that study [279]. The most obvious explanation is that xanthine oxidase, while contributing to some aspects of heart failure physiology, does not contribute to the clinical outcome of patients. Other possible reasons for the discrepancy between physiologic and outcome studies are: a) differences between allopurinol and oxypurinol; b) the fact that the OPT-CHF dose of oxypurinol is only equivalent to a low dose of allopurinol [280]; and c) that many more patients might be needed to detect clinical outcome differences compared to the modest number of patients needed to detect physiologic changes to xanthine oxidase inhibition.

Conclusion

Overall, there are considerable data linking oxidative stress and ROS to the physiology and pathophysiology of CVD. Initial attempts to ameliorate manifestations of CVD with simple antioxidant strategies have not proven helpful, likely because ROS have important and diverse physiological roles. As our understanding of how the source(s) of ROS are regulated and how specific ROS interact with their target(s), we have developed a greater understanding of how ROS modulate cardiovascular pathophysiology. It seems clear that our ability to impact distinct ROS-sensitive pathways will require even more understanding of the specific molecular targets and effective therapeutic strategies will likely exploit factors that dictate ROS specificity.

Acknowledgments

List of Abbreviations

5-LO	5-lipoxygenase
5-MTHF	5-methyl tetrahydrofolate
12-LO	12-lipoxygenase
12/15-LO	12/15-lipoxygenase
AII	angiotensin II
AAA	abdominal aortic aneurysm
ACS	acute coronary syndrome
Apo A-I	apolipoprotein A-I
ApoE	apolipoprotein E
AV	atrio-ventricular

BH$_4$	5,6,7,8-tetrahydrobiopterin
CAD	coronary artery disease
CHD	coronary heart disease
CVD	cardiovascular disease
DCM	dilated cardiomyopathy
eNOS	endothelial nitric oxide synthase
GCH	GTP-cyclohydrolase
GPX-1	glutathione peroxidase-1
HDL	high density lipoprotein
HF	heart failure
iNOS	inducible nitric oxide synthase
LDL	low density lipoprotein
LDL-R	low density lipoprotein receptor
LV	left ventricular
MI	myocardial infarction
Mn-SOD	manganese superoxide dismutase
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
Ox-LDL	oxidized low density lipoprotein
PC	preconditioning
PROBE	prospective randomized open blinded end-point
RCT	randomized controlled trial
ROS	reactive oxygen species
TAC	transverse aortic constriction

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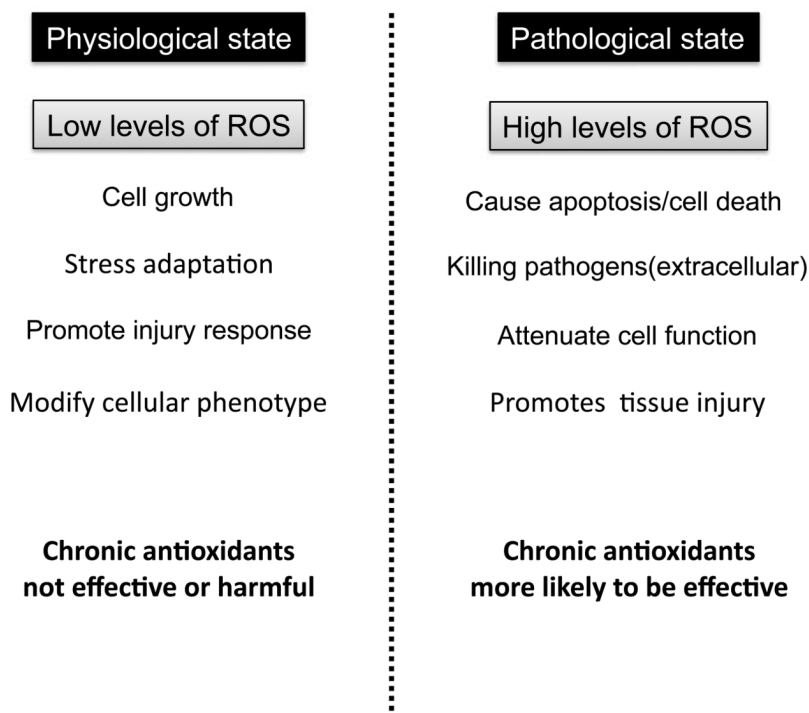
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Physiological state	Pathological state
Low levels of ROS	**High levels of ROS**
Cell growth	Cause apoptosis/cell death
Stress adaptation	Killing pathogens(extracellular)
Promote injury response	Attenuate cell function
Modify cellular phenotype	Promotes tissue injury
Chronic antioxidants not effective or harmful	**Chronic antioxidants more likely to be effective**

Figure 1.
Roles of ROS in physiological vs. pathological states. Physiological states are characterized by low levels of ROS that have been implicated in cell growth, stress adaptation, injury responses, and various modifications in cellular phenotype. In contrast, pathological states typically exhibit unregulated high levels of ROS that are linked to cellular apoptosis, killing of pathogens, impairement of cellular functions and ongoing tissue injury.
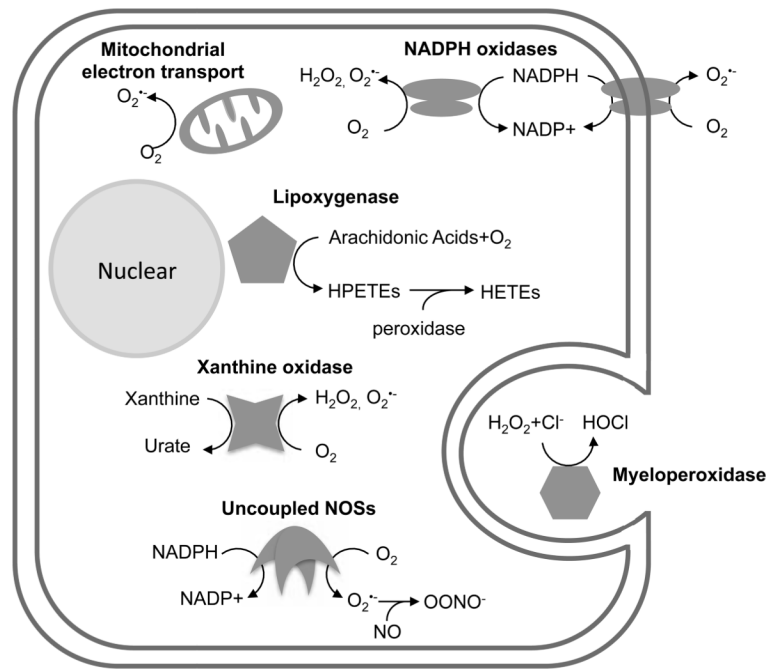
Figure 2.
Scheme depicting selected sources of ROS that are known to have implications for cardiovascular disease. Complete explanations are contained in the text.

Table 1

Selected clinical studies of anti-oxidant therapy associated with cardiovascular disease

Anti-oxidant	Study design	Study subjects	Findings	Interpretation	Reference
Primary prevention					
Vitamin C	Prospective Cohort		associated with lower cardiovascular disease risk	Effective	[2–8]
	RCT		no effect on composite cardiovascular events	Non-effective	[9, 10]
Vitamin E	Prospective Cohort		associated with lower cardiovascular disease risk	Effective	[6, 7, 11, 12]
	RCT		no benefit to prevent cardiovascular events, but increase HF	Non-effective or harmful	[9, 13–17]
Beta carotene	RCT		no impact on cardiovascular death or MI	Non-effective or harmful	[9, 18–20]
Secondary prevention		*Study subjects*			
Vitamin C	RCT	women with CAD	increase mortality	Non-effective or harmful	[21, 22]
	RCT	CAD patients	reverse endothelial dysfunction	Effective	[281]
	Prospective cohort	variant angina	attenuate abnormal vasomotor reactivity	Effective	[282]
Vitamin E	RCT	CAD patients	reduce CAD events	Effective	[23]
	RCT	hemodialysis patients	reduce cardiovascular events	Effective	[24]
	RCT	patients after MI	no benefit to prevent cardiovascular events	Non-effective	[25]
	PROBE	variant angina	reverse endothelial dysfunction	Effective	[283]
	RCT	patients with HF	no improvements in prognostic or functional indexes of HF	Non-effective	[284]
Beta carotene	RCT	patients after MI	increase CAD risk	Harmful	[27]
Probucol	RCT	patients after PCI	prevent restenosis	Effective	[32–34]
Succinobucol	RCT	patients after ACS	no benefit to prevent cardiovascular events	Non-effective or harmful	[37]
Edaravone	PROBE	patients with acute MI	decrease infarct size, RI and cardiac event	Effective	[242, 243]
Coenzyme Q10	PROBE or RCT	patients with HF	no improvement of outcome or important cardiac function	Non-effective	[285]
Allopurinol	RCT	patients with HF	reverse endothelial dysfunction	Effective	[278]
Oxypurinol	RCT	patients with HF	no clinical improvements	Non-effective	[279]

Table 2

Source of ROS and transgenic or knockout mice model related to cardiovascular disease

Source of ROS	Product	Tg or KO mouse model	phenotype or related cardiovascular disease	Reference
Mitochondrial respiratory chain	$O_2^{\bullet-}$	Mn-SOD -null	Cardiomyopathy (lethal in neonatal)	[49]
		heart/muscle specific Mn-SOD -null	Cardiomyopathy, heart failure	[50]
		peroxiredoxin3 Tg	Reduced LV remodeling and HF after MI	[51]
NADPH oxidase	$O_2^{\bullet-}$	Nox2-null	Attenuated cardiac remodeling and PC effect	[54–56]
		gp91phox-null	Attenuated AII induced hypertrophy but not TAC	[58–60]
		p47phox-null / ApoE-null	Reduced atherosclerosis and AII induced AAA	[61, 62, 64]
		Nox2-/y / ApoE-null	Reduce atherosclerosis	[63]
		gp91 phox-null / ApoE-null	No significant effects on atherogenesis	[286]
		cardiomyoccyte-specific Rac1-null	Attenuated AII-induced hypertrophy	[57]
Xanthine oxidase	$O_2^{\bullet-}/H_2O_2$	Xanthine oxidase-null	Inhibitors attenuated IR injury *	[73, 74]
Lipoxygenase	$LOO^{\bullet}, LO^{\bullet}$	5-LO+/− / LDL-R-null	Reduced atherosclerosis	[90]
		12/15-LO-null / LDL-R-null	Reduced atherosclerosis	[89]
		Leukocyte-12-LO-null / Apo E-null	Reduced atherosclerosis	[87, 88]
eNOS	$O_2^{\bullet-}, NO^{\bullet}, O$	eNOS-null	Increased mortality after MI	[118]
			Promoted LV remodeling after TAC	[119]
			Prevented LV remodeling after TAC	[287]
			Attenuated or worsened IR injury	[288–291]
		eNOS-null / ApoE-null	Promoted atherosclerosis	[113]
		eNOS Tg	Reduced IR injury infarct size after MI	[120, 121]
		endothelium-specific eNOS Tg / ApoE-null	Promoted atherosclerosis	[114]
		endothelium-specific GCH Tg / ApoE-null	Reduced atherosclerosis	[116]
		endothelium-specific eNOS/GCH Tg / ApoE-null	Reduced atherosclerosis	[117]
		cardiomyocyte-specific eNOS Tg	Attenuated compensatory hypertrophy after MI	[122]
		eNOS-null / cardiomyocyte-specific eNOS Tg	Prevented LV remodeling after TAC	[123]
iNOS	$NO^{\bullet}, OONO-$	iNOS-null	Decreased mortality after MI	[128]
			Prevented LV remodeling after TAC	[129]
		iNOS-null / ApoE-null	Reduce datherosclerosis	[125–127]
		cardiomyocyte-specific iNOS Tg	Increased HF, AV block and sudden death	[130]

Source of ROS	Product	Tg or KO mouse model	phenotype or related cardiovascular disease	Reference
			No significant difference compared to wild type	[292]
			Prevented I/R injury and reduced infarct size	[293]
		cardiomyocyte-specific iNOS Tg / myo-null	Developed HF	[294]
nNOS	NO•, OONO	nNOS-null	Promoted LV remodeling after MI	[134, 135]
		nNOS-null / ApoE-null	Promoted atherosclerosis	[132]
		nNOS-null / eNOS-null	Increased mortality and hypertrophy	[137]
		cardiomyocyte-specific nNOS Tg	Prevented LV remodeling after TAC	[136]
MPO	HOCl, •OH	MPO-null	Promoted LV remodeling after MI	[295, 296]
	Tyr•, Cl$_2$, NO$_2$•	MPO-null / LDL–R-null **	Promoted atherosclerosis	[151]
		human-MPO-Tg / LDL–R-null **	Promoted atherosclerosis	[152]

Abbrebiations: AAA = abdominal aortic aneurysm; AII = angiotensin II; AV = atrio-ventricular; GH = GTP cyclohydrolase; HF=heart failure; IR = ischemia/reperfusion; LV = left ventricular; PC = preconditioning; TAC = transaortic constriction;

*
XO-null mice did not show significant change in heart, but several animal studies with xanthine oxidase inhibitors (e.g. allopurinol) attenuated I/R injury

**
bone marrow transplantation model

Table 3

Biomarkers of oxidative stress measured in human clinical studies related to cardiovascular disease

Biomarker	Indicates	Disease	Reference
Oxidized or malondialdehyde LDL	lipid peroxidation	CAD↑, ACS↑, HF↑	[171–177, 297]
Myeloperoxidase	potential for MPO oxidation	CAD↑, ACS↑, acute MI↑	[155, 156, 158, 183–191]
F2-isoprostanes	lipid peroxidation	CAD↑, ACS↑, HF↑	[208–214]
Ascorbic acid (Vitamin C)	water-soluble antioxidant level	ACS↓	[216]
Biopyrrins	bilirubin oxidative metabolites	acute MI↑, HF↑	[221, 222]
Glutathione peroxidase 1	capacity to detoxify ROOH species	CAD↓	[223]
8 Hydroxyl-2′-deoxyguanosine	oxidative damage in DNA	acute MI↑, HF↑	[224, 225]
Total antioxidants status	total capacity of antioxidants	CAD↓	[298]