Cardiovascular Diseases: Electron Transfer, Reactive Oxygen Species, Oxidative Stress, Toxicity, Antioxidants and Arrhythmia

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Abstract

Heart attacks comprise one of the main causes of death in the USA. A main adverse influence in the heart is arrhythmia involving disruption of cardiac electrical function. Another

category of injury is necrosis. Mounting evidence implicates electron transfer (ET), reactive oxygen species (ROS) and oxidative stress (OS) as important factors in cardiovascular diseases. In the generation of ROS, many in vivo enzymes are involved, in addition to exogenous agents. Less attention has been paid to ET functions as the source of ROS. Participation of OS is supported by beneficial effects of antioxidants, but not in all cases. It should be recognized that reaction mechanism is often multifaceted.

Key words: cardiovascular diseases, reactive oxygen species, oxidative stress, toxicity, antioxidants, arrhythmia

Abbreviations: ROS, reactive oxygen species; OS, oxidative stress; ET, electron transfer; AO, antioxidant

Introduction

Atherosclerosis is the leading cause of death world wide, representing one-third of total deaths in the industrial countries. In the USA, coronary atherosclerosis, cerebrovascular artherosclerosis and other cardiovascular diseases cost the health care providers billions of dollars annually [1-3].Based on global trends, the World Health Organization projects that, by 2020, approximately half of all deaths in developed countries will be due to cardiovascular disease (CVD). In developing nations, like, India and China, 35-53% of CVD deaths are in people of younger than 70 years of age [4].

In the past several decades, a plethora of evidence implicates oxidative stress (OS) as a mechanism with a central role in the pathogenesis of varieties of diseases and toxicities, such as CVD, cancer, neurodegenerative diseases like Alzheimer's and Parkinson's, hepatitis, reproductive toxicity, neurotoxicity, ototoxicity, and eye toxicity(Fig. 1).
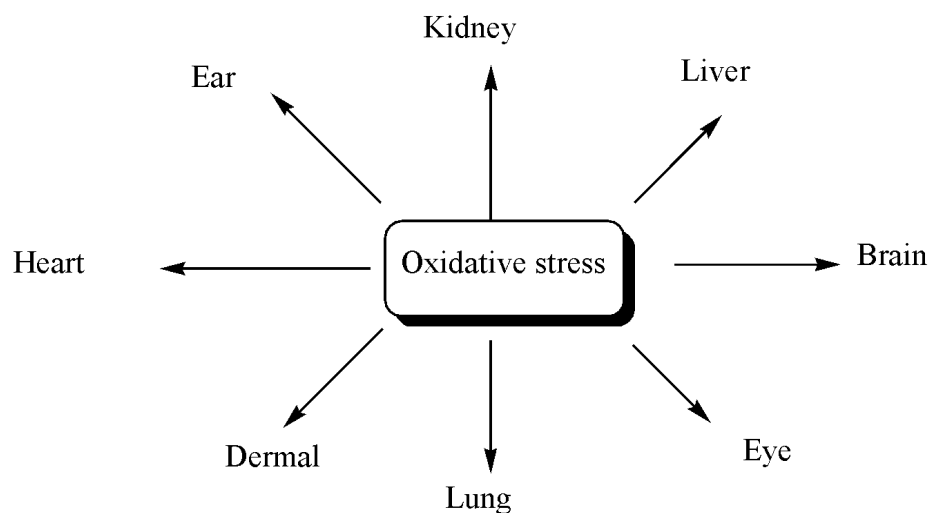

Fig.1. Oxidative diseases and toxicity

OS is a result of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced within the cell endogenously by various enzymes or influenced by exogenous materials. The most potent of the ROS/RNS are the free radical species, which have an unpaired electron and capable of reacting with most of molecules by abstraction or insertion. These free radical reactions can bring about permanent changes to the molecular structures of compounds having specific functions in the cell, leading to altered physiological properties. By nature, these

free radicals have short life times and mobility within the cell is limited. The common ROS and RNS species are shown in Table 1 [5,6].

Table 1 Reactive species

Reactive Oxygen Species (ROS)

Radicals	Nonradicals
Superoxide, $O_2^{\bullet-}$	Hydrogen peroxide, H_2O_2
Hydroxy, $OH^{\bullet}$	Hypochlorous acid, $HOCl$
Peroxyl, $RO_2^{\bullet}$	Hypobromous acid, $HOBr$
Alkoxyl, $RO^{\bullet}$	Ozone, O_3
Hydroperoxyl $HO_2^{\bullet}$	Oxygen, O_2

Reactive Nitrogen Species (RNS)

Radicals	Nonradicals
Nitric oxide $NO^{\bullet}$	Nitrous acid, HNO_2
Nitrogen dioxide, $NO_2^{\bullet}$	Peroxynitrite, $ONOO^-$
	Peroxynitrous acid, $ONOOH$

Of all these reactive species, hydroxyl radical appears to be the most potent. This radical, produced from superoxide anion radical generated by enzymes, reacts at a diffusion-controlled rate with almost all adjacent molecules in the living cells. Fortunately, the migrating ability of this species is limited. On the other hand, reactive H_2O_2 which is a non-radical reactive species produced from superoxide, has a longer biological life span, and is

very diffusible within and between cells causing oxidative damage to molecules in its path. This property makes hydrogen peroxide an excellent cell signaling agent.

Another aspect of mobility of these ROS and RNS is the subject of electron transfer (ET) agents, which has received limited attention in the literature over the years. The electron in motion is a charged entity which is accompanied by an electrical field which could play a role. Certain molecules can donate an electron to ET agents. Common ET agents are conjugated iminium, quinones, metal compounds, nitrobenzenes and nitrosobenzenes (Fig. 2).
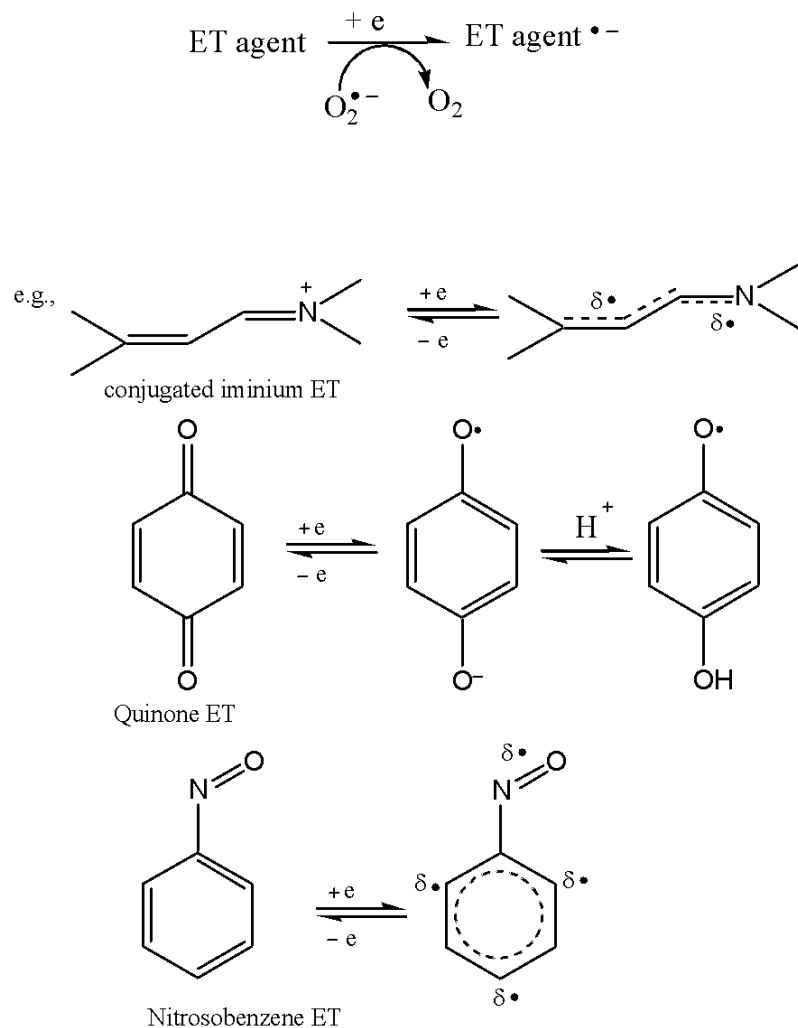
Fig 2. Common ET agents

These ET agents bearing the single electron can diffuse through cells to sites away from where they were generated and have the ability to create more ROS and RNS at a new site. Evidence also suggests that the ROS could behave as intercellular signaling molecules. The present review mainly presents the most recent literature in relation to ROS, OS, AO and toxicity in cardiovascular diseases. The various sources of ROS are addressed,

in addition to their extensive involvement in cardiovascular diseases. There is extensive literature documenting the prevention or amelioration of harmful OS by AOs. On the other hand, conflicting data exist.

Sources of ROS and their role in cardiovascular disease

A variety of enzymatic and non-enzymatic sources of ROS exists in blood vessels. Hence, ROS and RNS produced by these sources play a crucial role in the functioning of vascular physiology of cardiovascular disease (Fig. 3). The main ROS and RNS produced in the vessel walls are superoxide radical anion ($O_2 \bullet^-$), hydrogen peroxide (H_2O_2), hydoxyl radical ($OH\bullet$) and nitric oxide ($NO\bullet$). A plethora of evidence exists for the involvement of ROS/RNS in CVD, such as atherosclerosis, hypertension, and ischemia, as set forth in this review and prior ones referenced herein [7-13].

Enzymes within the cell are primary sources of ROS/RNS. Superoxide is produced by one electron reduction of oxygen by several different enzymes, such as NAD (P) H oxidase, xanthine oxidase, cyclooxygenase and cytochrome P450 within the cell (Fig. 3). In addition to these, a number of external mediators also contribute to the ROS generation, such as ionizing radiation, heavy metals, like Hg, Pb, Cd, Cr, Fe and Cu, metal-complexes, nano-particles, cigarette smoke, pollutants from automobiles, fossil burning furnaces, various drugs and certain types of other chemical compounds.

Mitochondria

An important non-enzymatic source of ROS is the mitochondrion. The electron carriers NADH and $FADH_2$ situated in the inner mitochondrial membrane assist the oxidative phosphorylation of ATP and superoxide generation. Evidence shows that the lack of oxygen supply by either hypoxia or ischemia disrupts the mitochondrial electron transport chain, resulting in ATP depletion, overload of Ca^{2+}, membrane depolarization and cell death [7, 14]. Experimental models involving ischemia/reperfusion or hypoxia in vivo and vitro, suggested that injury in the myocardium is caused by ROS from mitochondrial respiration [7, 14]. A small amount of electron leak from the transport chain leads to superoxide via reaction with oxygen [15].

Xanthine oxidase (XO)

XO enzyme is involved in the purine degradation pathway. The reaction generates uric acid and superoxide. The enzyme is localized in the plasma, endothelial cells, liver and the small intestine. Excess ROS production, during hypoxia, enhances XO release into the plasma. The significant increased XO activity in the vascular tissue leads to endothelial dysfunction in hypercholestrerolemic rabbits and atherosclerotic humans; allopurinol or oxypurinol improved vasodilatation [7, 8]. Studies revealed XO inhibitor allopurinol improved cardiac function in ischemic/reperfused rat heart and in human trials during coronary bypass surgery [7, 8].

NADH/NADPH oxidase and NOX enzymes

NADPH oxidase is a multi-subunit enzyme that catalyzes superoxide production. Found in phagocytic cells, it plays a major role via superoxide in host defense against infection. The NAD(P)H oxidases also include the NOX family of enzymes. NADH/NADPH oxidase induces the formation of superoxide production in all types of cells in the vasculature, endothelium, smooth muscle, and adventitia [7]. A study showed that angiotensin II induced superoxide formation through NADH/NADPH in the vasculature leading to AngII-induced hypertrophy and endothelial dysfunction [7]. The oxidation of LDL is well described in artherosclerosis and a number of enzyme systems including NADPH and mitochondria are involved in this process [9]. A recent study showed that ROS produced by NADPH oxidases and NOX enzymes have a role in the endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis and angiogenesis [10]. NOX family of NAD(P)H oxidases has been implicated in many forms of hypertension [11, 12].

A new vascular peroxidase 1 (VPO1) has been recently identified as a member of the peroxidase family. VOP1 utilizes NOX-derived H_2O_2 to produce hypochlorous acid, a strong oxidant which promotes OS [16]. NOX/VOP1 pathway-mediated OS plays an important role in myocardial ischemia-reperfusion injury, endothelial apoptosis and smooth muscle proliferation.

Nitric oxide synthase (NO)

Another source of superoxide is nitric oxide synthase, which is a cytochrome P450 reductase-like enzyme that requires cofactor, tetrahydrobiopterin, for transfer of electrons to

L-arginine to form NO. However, under certain circumstances, the uncoupling of this enzyme can lead to O_2 reduction and the production of superoxide. Impairment of the vascular endothelium is caused by disruption of the protective NO signaling pathway, which results in decreased relaxation responses of blood vessels and vascular wall thickening and prothrombotic state, leading to endothelial dysfunction [13]. Increased ROS production reduces the bioavailabilty of NO, resulting in vasoconstriction, platelet aggregation and adhesion of neutrophils to the endothelium [8]. NO also reacts with superoxide anion at diffusion-limited rates to form the potent ONOO,⁻ which can lead to lipid, protein and nucleic acid oxidation. Experimental data indicated peroxynitrite impaired vascular function and decreased cardiac performance [7]. Other examples of NO involvement in CVD are presented in a 2001 review [7].

Myeloperoxidase

This enzyme, produced by activated phagocytes, uses H_2O_2 for the production of more powerful oxidative substances. The enzyme is involved in NO consumption, modification of LDL by oxidation and reaction with L-arginine for the production of NO synthase inhibitors. This enzyme through NAD(P)H interaction forms HOCl which leads to atherosclerotic lesions [8].

Lipoxygenases

These enzymes are involved in creating biologically active lipids, such as prostaglandin, thromboxanes and leukotrienes, which participate in inflammatory reactions and

which increase the permeability of vessels. Studies show that 15-lipoxygenase induces LDL oxidation, which leads to plaque formation in the vasculature [8].
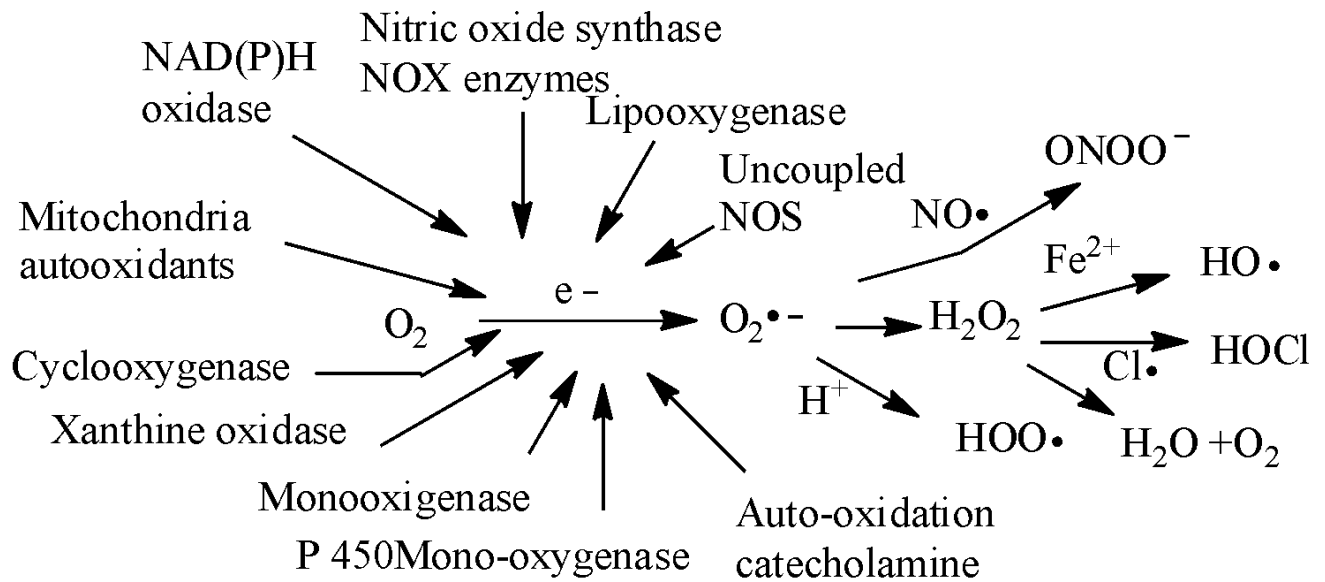

Fig. 3. Generation of ROS

Others

A review deals with oxygen, OS and hypoxia, in connection with heart failure [17]. The article details ROS in relation to ischemic syndrome, lipotoxicity, ion channels and calcium flux. Another recent review highlights the importance of ROS in vascular biology and focuses on the potential role of OS in human hypertension [10]. A study showed that free fatty acids play a

role in OS-induced elevated blood pressure [18]. Smoking and alcohol consumption were shown to increase lipid peroxidation and cellular damage in coronary, artery disease patients [19]. A review deals with lipid biosynthesis and its implications on cardiovascular disease [20]. Atherosclerosis is a progressive multifaceted inflammatory disease affecting large- and medium-sized arteries. Typically, this involves the formation and build-up of atherosclerotic plaques by vascular degradation. A review deals with the elastin-driven degradation and its signaling pathways [21]. Degradation of native elastin, the main extracellular matrix protein responsible for resilience and elasticity of arteries, leads to the production of elastin-derived peptides. These peptides have been proposed to participate in LDL oxidation and calcification of the vascular wall.

A recent review summarizes the status of redox-sensitive signaling in brain Ang II-dependent cardiovascular diseases, hypertension and heart failure [22]. Data suggest increased OS and decreased membrane fluidity in erythrocytes of coronary artery disease [23].

A study revealed that OS and high-density lipoprotein cholesterol are associated with coronary artery calcification in patients with rheumatoid arthritis [24].

Blocking aldosterone receptors in patients with cardiovascular diseases reduced their mortality [25]. The observation of reduced NO bioavailability in hyperaldosteronism and NF-kβ activation implied the generation of OS by aldosterone. Data suggest that increased flux via aldose reductase in diabetic increases injury after myocardial infarction, accelerates atherosclerostic lesion formation, and promotes restenosis via increased ROS generation [26].

Increased ROS have been proposed as a possible cause of cardiac dysfunction in diabetic patients. A report suggests activation of Ca^{2+}/calmodulin-dependent protein kinase II induced by impaired intracellular Ca^{2+} metabolism may stimulate ROS production in the diabetic heart [27]. Rho-kinase plays a critical role in various cellular functions. Mitogen p38-activated protein kinase is involved in the inflammatory cytokine response to immune challenge. Findings suggest Rho-kinase and p38 MAPK pathways may play a major function in ventricular hypertrophy [28].

Reactive Oxygen Species and Oxidative Stress: Recent Reports

There is extensive literature linking ROS-OS with various cardiovascular diseases. This section deals with reports from part of 2013. Some studies dealing with ROS-OS can be found in the AO section. Many of the cardiovascular diseases, such as atherosclerosis, heart failure and hypertension are linked to enhanced levels of ROS, and decreased AO function [29]. The review emphasizes involvement of OS in cardiovascular illness. In heart failure, data indicate a connection between OS and the cardiac operation in heart failure [30]. A vital role is played by kinases oxidant sensing and signaling which are connected to phosphorylation [31]. Redox regulation by kinases is involved in cardiovascular operation. Hypoglycemia and diabetes are both related to cardiovascular malfunction [32]. Various pathways play a role, all of which are related to ROS. The ROS damage cellular constituents and modulate cell signaling. Hyperglycemia can lead to OS and endothelial dysfunction which play central roles in vascular diseases. OS brings about changes in vascular cells. There is involvement of ROS in atherosclerosis and apoptosis. Conflicting evidence exists concerning the beneficial role of AOs

in the diabetic cardiovascular afflictions. In a related report, the widespread occurrence of cardiovascular disease among diabetic patients indicates participation of OS [33]. The review addresses the role of sirtuins in modulating proteins related to signaling and OS. Evidence indicates an association of OS and the mitochondria with heart failure [34]. Myeloperoxidase plays a role in cardiovascular disease via OS and inflammation [35]. The heme protein yields oxidant products which play a role in the immune system. Nickel-induced OS and resulting inflammation are crucial aspects in variability of heart rate [36]. Sleep apnea appears to be involved with cardiovascular problems, including stroke, heart failure and arterial hypertension [37]. These conditions appear to involve OS and inflammation. The OS appears to arise from NADPH oxidase and other enzymes that generate ROS. Inside cardiomyocytes, ROS/RNS and autophagy have pathological influences [38]. Cross talk between the two aspects occurs within cardiovascular illness. Mitochondria are also involved in OS in cardiomyocytes. The complicated interplay is involved in many pathologies, including those in the cardiovascular system. Evidence supports an important relationship between obesity and cardiovascular illness [39]. Certain proteins and kinases are also involved in regulating OS and redox levels. Possible therapeutic aspects are addressed. A study was made of OS and damage to DNA in patients with heart failure and circulating blood leucocytes [40]. The implanted patients showed increased OS and damage to DNA. A growing health problem worldwide is presented by cardiovascular disease [41]. Extensive evidence points to OS as a key factor. The review addresses therapy based on prevention of OS. A related report emphasizes the importance of OS as a factor in

cardiovascular pathology [42]. Activation of AO responsive signaling pathways protects constituents from oxidative damage. MG 132 (tripeptide derived from amino acid leucine) is a drug that prevents cardiovascular damage. Various vascular diseases are influenced by OS [43]. Enhanced ROS is importantly involved in cardiovascular pathology. The review discusses involvement of RNAs in ROS production with participation vascular illnesses in which redox imbalance plays a role.

A long chain hydroxyl unsaturated carboxylic acid enhances generation of superoxide which aggravates myocardial injury induced by ischemia-reperfusion [44]. In a study of hypobasic hypoxia, there exists regulation of ROS and NO which is controlled by AO proteins [45]. Low high-density lipoprotein is linked to enhancement of lipid peroxidation as a mechanism associated with enhanced cardiovascular disease [46]. A report deals with signaling by ROS in cardiovascular injury, together with the influence of AOs [47]. Zinc levels are linked to inflammation and lipid peroxidation in heart disease [48]. In treatment of patients suffering from myocardial infarction, attention should be paid to the harmful effects of oxidation products, such as peroxides and aldehydes [49].

An article deals with the role of angiotensin (Ang) in OS and ROS in cardiovascular diseases. The action mode entails NADPH oxidases that generate ROS, with similar action from mitochondria [50]. A related report discusses the critical role of mitochondrial ROS in heart disease involving Ang and oxidases [51]. These events may play a role in atherosclerosis. Use of

AOs may be helpful. Excessive Ang is a factor in cardiovascular pathology [52]. The Ang properties may reflect involvement of ROS and redox signaling. AOs are helpful in the Ang-induced heart pathologies. A study deals with OS and inflammation in patients with cardiovascular problems [53]. Hypertension, which is related to OS, is well established as a contributor to cardiovascular illness [54]. In a study with the rat heart during aging, various ET sites in mitochondria were found to increase ROS production and oxidative destruction [55]. Heart transplant patients demonstrate reduced OS after everolimus therapy [56]. Data indicate that lower SOD and high uric acid may be linked to cardiovascular death in hemodialysis [57]. Hypothyroidism produces OS in the heart which is countered during pregnancy [58]. Data show that ROS control various processes connected to cardiac functioning by various networks of genes [59]. An investigation deals with changes during aging involving OS and energetics in the hippocampus and heart of rats [60]. Possible therapy for cardiovascular illness was addressed involving OS and inhibition of NO synthase [61]. A review puts focus on OS in the cardiovascular system and CNS with emphasis on disease associated with aging [62]. Tocotrienol reduces OS in the heart and AO homocysteine in the plasma in rats [63]. Evidence supports participation of OS, including inflammation, in atherosclerosis [64]. In kidney malfunction, dislipidemia plays a role in impairment of OS, cardiovascular disease and inflammation [65]. OS was found to play a role in cardiovascular illnesses in the obese [66]. Inhibition of cycloxygenase and NO synthase affects OS in the heart of rats [67]. A report finds

vascular peroxidase 1 as a promotor of OS in the cardiovascular system [16]. Data demonstrate a role for OS in obesity [68].

Antioxidants

The prior sections present extensive data supporting an important role of ROS-OS in cardiovascular illnesses. It is not surprising to find widespread evidence for prevention or amelioration by AOs. The literature covered is from 2012 and part of 2013. Some AO material is also present in the ROS-OS section. Prior reviews present beneficial effects of AOs, as well as disappointing results. The imbalance between ROS/RNS and AOs lead to OS when the former predominate. Cells manifest potent AO defenses against ROS through enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH) [69]. External sources of AO supplementation, such as vitamin E, C, flavonoids, phenols and carotenes, can also suppress the ROS [70].

Vitamin and antioxidant supplements were shown to prevent CVD through suppression of OS [71]. α-Tocopherol acts as an AO to alleviate methotrexate-induced OS in rat heart [72]. A study revealed intake of diet with high total AO capacity has preventive effects on CVD risk [73]. Resveratrol and other stilbene derivatives were shown to modulate NO bioavailability during OS [74].

In diabetic cardiomyopathy animal models, cobalt-protoporphyrin treatment improved both cardiac function and coronary flow by inhibiting OS, and restoring eNOS/iNOS expression balance, thereby improving both endothelial function and insulin sensitivity [75]. A

report deals with antioxidant enzymes as potential targets in cardioprotection and treatment of CVDs [76]. Extracellular superoxide dismutase showed protective action against development of atherosclerosis, hypertension, and heart failure involving in vivo experiments.

In a clinical investigation involving patients with coronary heart disease and dyslipidemnia, treatment with atorvastatin exerted antioxidative effects in 90% of the patients and it normalized parameters of lipid peroxidation [77]. Rat models with myocardial infarction on a diet of soy protein showed increased antioxidant enzyme activity and lower lipid peroxidation [78]. Diet rich in olive oil phenolics was shown to prevent OS in the heart of mice by modulating Nrf2-dependent gene expression [79]. LDL-oxidation plays a key role in pathogenesis of atherosclerosis and cardio vascular heart diseases through the initiation of plaque formation. Lycopene was shown to act as an AO in decreasing this effect [80]. Interleukin-33 (IL-33) has been linked to chronic heart failure in animal studies. Serum levels of interleukin-33 were elevated in chronic heart failure patients whereas its activity was reduced [81]. Authors suggest IL-33 may exert AO effects.

Epidemiological studies with large numbers of men, women and diverse populations have been supportive of the hypothesis that AOs prevent artery disease, peripheral vascular disease, and other CVD-related disease through the prevention of low density lipoprotein oxidation. However, interventional trials have been controversial, with some positive findings, many null findings, and some suggest the AO being even harmful. A report involving smaller

interventional studies with carefully chosen populations, such as those under high levels of OS, have yielded largely positive results [82].

Evidence points to prevention by edaravone which could lead to therapy for heart failure, and atherosclerosis, among other illnesses, arising from OS or apoptosis [83]. The compound guards against inflammation and ischemia in the heart. The targets may be radicals in these diseases since edaravone is well known as scavenger of radicals. The potent AO provides neuroprotection in brain ischemia and other cardiovascular dysfunction. Apocynin ameliorates atherosclerosis, apparently by countering superoxide [84]. The AO is a member of the phenol class which is well known to exhibit AO properties [70]. Drugs that posses AO properties are used in treating cardiovascular illnesses [85]. This class demonstrates better success than those with similar receptor affinity, but lacking AO action.

Cardiovascular illness is the main cause of disease and death globally [86]. Evidence supports involvement of ROS in the adverse conditions, contributing to aatherosclerosis and diabetes. Involvement of OS in vascular disease is buttressed by data illustrating the therapeutic effect of AOs. Addition of the AOs vitamins C and E to the diet decreased the prevalence of arterial disease [87]. Vitamin E also elicited a positive effect in combating cardiac ischemia-reperfusion injury. Thus, alleviation of OS appears ro exert beneficial, cardiovascular effects. However, controversy exists concerning the protection provided by vitamin E.

Flavonoids, prevalent in healthy diets, are polyphenols that display AO properties and contribute to lessening of cardiovascular illnesses [88]. Apigenin, a member of this AO class,

reduces inflammation and counters adverse mitochondrial OS [88]. Inflammation is associated with the presence of ROS. The flavonoid lessened apoptosis by opposing production of ROS. Mechanistic aspects of flavonoid AO action have been addressed [70]. Heart failure is appreciably increased by diabetes [89]. Diabetic cardiomyopathy is linked to advanced glycation end products (AGEs) which give rise to OS. In rats, diabetes produce cardiac changes associated with OS and inflammation which are mediated by glyoxalase.

Ginger has been used in folk medicine to treat cardiovascular diseases, including hypertension [90]. A possible mechanism entails opposition to lipid peroxidation, which may be associated with the AO properties of the polyphenol (related to vitamin E). In a related article, wild blueberries which are replete with polyphenols, such as flavonoids, anthocyanins and phenolic acids, are used in countering cardiovascular diseases [91].

Arrhythmia

This cardiac condition involves irregular heartbeat in which the electrical activity is abnormal, either too fast or too slow. The seriousness can vary from minor to life threatening. Antiarrhythmic drugs are available to treat the condition.

Supplementation of fish oil in animals was found to lessen vulnerability of the heart to arrhythmia induced by ischemia-reperfusion [92]. Desferrioxamine provides protection against the heart condition, and is known to posses AO properties [93]). Exposure to particulate matter (PM) in vivo and in vitro produced ventricular arrhythmia [94]. Introduction of PM induced ROS which was prevented by AO thiol. Oxidative stress could be involved, as well as cell signaling

by kinase. Carbon monoxide was found to increase arrhythmia after stress [95]. The OS changed Ca homeostasis. The end result could be rapid death. The drug carvediol appears to inhibit arrhythmia in rabbits possessing cardiomyopathy [96]. The effect may be associated with a decrease in ROS.

Conclusion

Cardiovascular diseases pose a serious threat worldwide. Knowledge at the basic, molecular level concerning cause and prevention should aid in measures to prevent and cure. Extensive literature demonstrates involvement of ROS and OS in the diseases. There is scant recognition that ET agents play an important role in ROS formation. Although extensive reports support prevention or alleviation by AOs, other studies proved disappointing. More data concerning fundamental aspects are needed from future research. A prior review in 2005 provides the involvement of ET-ROS-OS in the mechanism of cardiovascular toxins [97]. The present review provides an update of recent advances based on the unifying mechanism of Et-ROS-OS.

Acknowledgement

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