Free Radicals and Cardiovascular Diseases: An Update

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ABSTRACT

In recent years, a multitude of studies provide comprehenisve evidence that increased production of reactive oxygen species (ROS) are involved in the development and progression of cardiovascular diseases. The ROS are common by-products of many oxidative biochemical and physiological processes. They can be released by mitochondrial respiration, NADH/NADPH oxidase, xanthine oxido-reductase or the uncoupling of nitric oxide synthase (NOS) in vascular cells. ROS mediate various signaling pathways that underlie cardiovascular pathophysiology. In a number of cardiovascular disease conditions, the delicate equilibrium between free-radical generation and antioxidant defense is altered in favor of the former, thus leading to redox imbalance i.e. escalating oxidative stress and increased tissue injury. This review focuses on the updated evidences concerning involvement of ROS in cardiovascular diseases.

Keywords: Cardiovacular diseases, oxidative stress, endothelial dysfunction, review, reactive oxidant species (ROS).

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INTRODUCTION

Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals. There is increasing evidence that abnormal production of free radicals lead to increased oxidative stress on cellular structures and causes changes in molecular pathways that underpins the pathogenesis of several important diseases, including cardiovascular diseases, neurological diseases, cancer and in the process of physiological ageing.[1,2]

Reactive oxygen or oxidant species (ROS) participate in normal cell signaling as mediators that regulate vascular function.[3] In the vascular wall, ROS are produced by all layers, including endothelium, smooth muscle, and adventitia.[4] ROS include free radicals such as superoxide anion (O_2.-), hydroxyl radical (HO.), lipid radicals (ROO-) and nitric oxide (NO). Other reactive oxygen species, hydrogen peroxide (H_2O_2), peroxynitrite (ONOO-) and hypochlorous acid (HOCl), although are not free radicals but they have oxidizing effects that contribute to oxidative stress. ROS has been implicated in cell damage, necrosis and cell apoptosis due to its direct oxidizing effects on macromolecules such as lipids, proteins and DNA. Production of one free radical can lead to further formation of radicals via sequential chain reactions.[5] Under physiological conditions, ROS are produced in low concentrations and act as a signaling molecule that regulate vascular smooth muscle cell (VSMC) contraction and relaxation, and participate in VSMC growth.[3] Under pathophysiological conditions, these free radicals play important roles in various cardiovascular disease conditions.[6, 7]

There is now considerable biochemical, physiological and pharmacological information to establish a link between free radicals and cardiovascular tissue injury. There is accumulating evidence, suggesting that disease conditions are directly or indirectly related to oxidative damage and that they share a common mechanism of molecular and cellular damage. The present review focuses on the recent research-based evidences concerning the involvement of free radicals in cardiovascular diseases and their relationship to specific pathophysiological events.

PHYSIOLOGICAL SOURCES OF ROS IN RELATION TO CARDIOVASCULAR DISEASES

Several mechanisms or pathways are associated with the production of free radicals within cells under physiological conditions. These include mitochondrial respiration, nicotinamide adenine dinucleotide phosphate (NADPH)

oxidases, xanthine oxidoreductase and uncoupled nitric oxide (NO) synthases.

Mitochondrial respiration as a source of ROS

Mitochondrial respiration involves transport of electrons from NADH or flavoprotein-linked dehydrogenases which ultimately result in reduction of oxygen to water, producing ATP in the process. This transport chain involves oxidative phosphorylation of complexes that are both nuclear and mitochondrial DNA encoded. Mitochondria produce significant amounts of cellular reactive oxidant species (ROS) via aberrant O_2 reaction.[8] During electron transport, approximately 2–5% of electrons escape to react with O_2 resulting in the production of ROS. Mitochondral superoxide generation represents a major intracellular source of ROS under physiological conditions.[9] In addition, some elements of the mitochondrial outer membrane such as monoamine oxidases produce NO or H_2O_2 which result in increased free radical stress.[10]

The balance between oxidants and antioxidants commonly termed as redox state of mitochondria also influences the opening of mitochondrial permeability transition pore, which is associated with energy uncoupling and further ROS production and development of disease process.[11] For instance, overproduction of mitochondrial ROS/NO is associated with early atherosclerosis and hypercholesterolemia. Mitochondrial ROS is also linked to vascular cell pathology from hyperglycaemia induced glycation and protein kinase C activation. Mitochondrial source of H_2O_2 play a key role in flow-mediated dilatation in human coronary resistance arteries.[12]

NADH/NADPH oxidase system as a source of ROS

NADH/NADPH oxidases are membrane- associated superoxide-producing enzymes that catalyze the 1-electron reduction of oxygen using NADH or NADPH as the electron donor. NADPH is particularly important in generation of ROS in phagocytic cell systems in response to the presence of foreign organisms in a series of changes known as respiratory burst. NADH/NADPH oxidases are also important sources of endovascular ROS. NADH/NADPH oxidases are the major oxidases in vascular tissue and in cardiac cells.[13]

NADH/NADPH-dependent oxidase activity is also increased in vascular smooth muscle cells by stimulation with the vasoactive agonist angiotensin II. Angiotensin II increases NADH and NADPH driven superoxide production in cultured vascular smooth muscle cells (VSMC) and aortic adventitial fibroblasts.[14] Treatment with exogenous antioxidant enzyme superoxide dismutase (SOD) improves blood pressure and vascular reactivity in rat model of angiotensin II induced hypertension.[15]

Xanthine oxido-reductase system as a source of ROS

Xanthine oxido-reductase catalyzes the sequential hydroxylation of hypoxanthine to yield xanthine and uric acid. Xanthine oxido-reductase exists in two interconvertible forms, either as xanthine dehydrogenase or xanthine oxidase. The first form reduces NAD^+ whereas the latter reacts with molecular oxygen, leading to the production of superoxide anion and hydrogen peroxide.[16] In the purine catabolism, xanthine oxido-reductase catalyses oxidative hydroxylation of hypoxanthine to xanthine, and then from xanthine to uric acid, which is a strong antioxidant and a free radical scavenger. The dual role of xanthine oxidase means that it is an important regulator of cellular redox state.[17]

Under pathophysiological stress conditions, xanthine oxido-reductase is an important cardiovascular source of oxidative stress. Xanthine oxidase generates ROS via purine metabolism pathway and is involved in causing endothelial dysfunction in patients with coronary artery disease and contractile dysfunction in heart failure.[18]

NOS uncoupling as a source of ROS

Uncoupled nitric oxide synthase (NOS) contribute to ROS generation and result in vascular endothelial dysfunction. Endothelial NOS (eNOS) is a cytochrome P-450 reductase-like enzyme that catalyses flavin-mediated electron transport from the electron donor NADPH to a prosthetic heme group. NOS are the major source of endogenous nitric oxide (NO).[19] eNOS can produce both NOS via its oxygenase function and superoxide through its reductase function, the later is dependent on NADPH. This enzyme requires tetrahydrobiopterin (BH-4) bound near this heme group to transfer electrons to guanidino nitrogen of L-arginine to form NO.[19,20] Uncoupling of eNOS contribute to ROS when deficiency of L-arginine or BH-4. In the absence of L-arginine or BH-4, eNOS can produce superoxide and H_2O_2. The product of reaction between NO and superoxide can oxidize BH-4 and this can lead to further eNOS uncoupling.[20, 21]

NO is a major cell signaling molecule involved in a large variety of different physiological processes including

neurotransmission, regulation of vascular dynamics and immune regulation.[22] It is one of the main mediators of endothelium-dependent/derived relaxation (EDR). NO release is induced by either vascular shear stress or by eNOS activation in response to cytokine activation and plays a protective role in suppressing abnormal proliferation of vascular smooth muscle cells (VSMCs) following various pathological situations. NO has been shown to react with and cancelled by reaction with excess ROS directly inactivating it.[22, 23]

Evidence of eNOS contribution to cellular ROS is present in the context of hypercholesterolemia, atherosclerosis, coronary artery disease, ageing and diabetes mellitus. Imbalance between endothelial NO and ROS production is one of the major contributor of endothelial dysfunction which plays an important role in atherosclerosis and cardiac disease.[24, 25]

ROS AND CARDIOVASCULAR DISEASES

Oxidative stress and endothelial dysfunction in atherosclerosis

Endothelial dysfunction is increasingly being accepted as a common trait of essentially all cardiovascular risk factors. One of the key concepts of free radical mediated pathogenesis of cardiovascular disease is endothelial dysfunction, whereby the regulation of vascular wall microenvironment is disrupted.[24] An important element in this concept is that vascular endothelium is an active component of the vasculature, which plays an important role in the regulation of vascular tone, platelet activity, thrombosis, inflammation and atherosclerosis. Endothelium vasoactive tone is maintained by the release of substances like prostacyclins, endothelins and the endothelium-derived relaxation factor nitric oxide (NO) or related compound(s).[26]

Reduction in endothelium-dependent vasorelaxation caused by the reduction in NO bioavailability plays a significant role in endothelial dysfunction. Decreased NO bioavailability disrupts the non-thrombogenic intimal surface and promotes platelet adhesion and aggregation as well as deposition of platelets on the abnormal endothelial surface.[26] The impairment of vasodilatation in response to vasodilator acetylcholine is a measurement of endothelial dysfunction and it correlates with increased local ROS production and reduced superoxide dismutase (SOD) activity.[24]

Endothelial vasodilator dysfunction can lead to paradoxical vasoconstriction effects and occurs in situations with sympathetic activation such as exercise. In the cardiac vasculature this can result in angina pectoris. During increased metabolic demand, vasodilator dysfunction in coronary vessels has been shown to result in ischemia, even in the absence of pathological stenosis.[27]

Atherosclerosis in coronary arteries even at early stages is associated with evidence of endothelium dysfunction. Long term cigarette smoking is an independent risk factor for impaired endothelium-dependent coronary vasodilation, regardless of the presence or absence of coronary atherosclerotic lesions.[28] Studies in the human forearm have demonstrated decreased flow-dependent dilation in chronic smokers. The diminished endothelium dependent relaxation (EDR) was improved with antioxidant vitamin C in chronic smokers, indicating the involvement of ROS in the pathogenesis.[29] Hypertension is also linked to increased vascular oxidative stress in a number of animal models of hypertension. Hypertension leads to impairment of EDR through oxidative stress.[30]

The bioavailability of NOS is more important for coronary artery dilatation than the activity of eNOS and generation of NOS. The bioavailability of NOS is influenced by the amount of ROS present that can transform NO to ONOO- and oxidized tetrahydrobiopterin to dihydrobiopterin which lead to eNOS uncoupling and further ROS production.[31] Upregulation of tetrahydrobiopterin which improves bioavailability of NOS has been shown to improve endothelial function and reduce superoxide production. Supplementation of antioxidant enzyme superoxide dismutase (SOD) has also been shown to improve endothelium dependent vasodilatation of coronary arteries.[32] Treatment with L-arginine, precursor of intracellular NOS, has been found to improve endothelium dependent vasodilation in patients with cardiac risk factors.[33] The significance of mode of NO activity is still under investigation.

Oxidative stress and hypertension

Clinical studies have demonstrated that there is increased ROS production in patients with essential hypertension, renovascular hypertension, malignant hypertension and pre-eclampsia.[34-36] Vascular ROS are produced in endothelial, adventitial and vascular smooth muscle cells (VSMCs) and derived primarily from NAD(P)H by the enzyme NAD(P)H oxidase, a multi-subunit enzyme catalyzing superoxide production.[37] Lipid peroxidation by-products have been shown to be elevated, whereas levels/activities of endogenous anti-oxidant systems have been reported to be impaired in hypertensive subjects.[38]

Among the latter, studies revealed decreases in the activities of SOD and catalase (CAT), as well as an increase in the ratio of oxidized to reduced glutathione.[39]

Generally, antioxidants are not recommended for the prevention or treatment of hypertension. In contrast, dietary approaches are highly recommended, supported by evidence from a trial which demonstrated that subjects consuming high fruit and vegetable diets significantly reduced elevated blood pressure.[40] On the other hand, direct cardiovascular effects of some pharmaceutical agents have been attributed to direct inhibition of NAD(P)H oxidase activity, as shown for angiotensin 1 (AT1) receptor blockers, and to intrinsic antioxidant properties of these agents. Classical antihypertensive agents such as β-adrenergic blockers (carvedilol), angiotensin converting enzyme (ACE) inhibitors, AT1 receptor antagonists, and Ca^{2+} channel blockers may be mediated, partially by decreasing vascular oxidative stress.[41, 42]

Oxidative stress and cardiovascular ischemia

Blood sample from patients with ischaemic heart disease was found to exhibit evidence of oxidative stress.[43] In myocardial ischaemia, hypoxia and reoxygenation induces an increase in free radical production in cardiac tissues and are principal causes of reperfusion injury. ROS produced through reoxygenation lead to direct oxidative damage to cellular components and also through indirect injury via activation of localized inflammation.[1] ROS can also act as signaling messenger in activating biochemical pathways responsible for altering cellular function.[44]

ROS mediated effect in cardiovascular diseases is also reflected in nuclear transcription factor activity. The nuclear transcription factor NFκB activity has been found in myocardial biopsies of patients with unstable angina.[45]

Mitochondrial dysfunction and increase ROS production has also been shown to associate with early atherosclerotic lesion formation. Multiple cell populations in the vascular wall have been shown to both produce and be regulated by ROS signaling.[46] Oxidative free radicals lead to increased oxidation of vascular LDL and increase adhesion molecule expression in endothelial cells, which result in inflammatory cell infiltration and activate matrix metalloproteinases and vascular remodeling.[47] Reactive oxygen species (superoxide and H_2O_2) regulate growth and migration of vascular smooth muscle cells in the atherosclerotic plaque structure.[21] ROS also trigger extracellular matrix remodeling through regulation of collagen resorption resulting in compromised plaque stability.[48]

Oxidative stress and heart failure

Xanthine oxygenase is an important cardiovascular source of ROS. Increase in xanthine oxygenase level and activity was found in heart failure. Upregulation of xanthine oxygenase in patients with heart failure is thought to contribute to mechano-energetic uncoupling.[49]

Nitrosative stress caused by nitrogen free radicals also plays a role in cardiovascular disease. In acute ischaemia, sepsis or heart failure, nitrosative stress is increased due to an increase in the amount of iNOS (NOS2), leading to increased levels of S-nitrosylated proteins. The accumulation of heme NO in heart failure is correlated with venous desaturation.[50] Oxidative stress leads to partial uncoupling of eNOS, resulting in the increased production of superoxide and peroxynitrite species.[51] However, the role of NOS activity in cardiovascular disease is not fully understood yet.

In addition to direct injury, NO/redox imbalance can also impair ion channels within the heart by S-nitrosylation, resulting in functional cardiac impairment.[52] Different NOS isoforms exert varying effects on cardiac physiology. For instance, NOS3 exert its effect on signal transduction at plasmalemmal membrane, inhibiting L-type calcium channel and thus attenuating the β-adrenergic mediated myocardial contractility. On the other hand, NOS1 isoform exerts its effect on sarcoplasmic reticulum, which facilitates calcium cycling and enhancing myocardial contractility stimulated by catecholamines.[53]

Oxidative stress and hyperlipidaemia

Hypercholesterolaemia increases endothelial O_2 production and vascular oxidative stress, which in turn contribute to impaired endothelial damage and atherogenesis. Hypercholesterolaemia was found to be independently associated with increased NADH-dependent superoxide production.[54] It is suggested that superoxide production by eNOS is important in oxidation of LDL during the formation of atherosclerosis in the setting of hyperlipidemia. Endothelial cells, smooth muscle cells, neutrophils and monocytes all have the potential to oxidatively modify LDL, leading to the generation of ROS and lipid peroxidation products. Lipid peroxidation products contribute to tissue damage through direct cytotoxic actions on endothelial cells or via chemical reactions.[55] Acute hypertriglyceridaemia was

found to cause endothelial dysfunction via enhanced oxidant stress.[56]

CONCLUSION

With drastic changes in the life style, increasing number of subjects is at risk of cardiovascular diseases and there is preponderance of evidence for the association of increased oxidative stress with various cardiovascular diseases. The loss of control of free-radical formation right from the mitochondrion can contribute to the pathology of cardiovascular diseases through multiple mechanisms. In recent times, important milestones have been reached with the availability of more overt evidence that shows that cardiovascular disease mechanisms are strongly linked to the production of reactive oxidant species (ROS) and the dysregulation of endogenous oxidant-antioxidants systems/pathways. Recent studies demonstrate that sources of reactive oxidant species, physiological and pathophysiological conditions and cellular oxidant targets determine the characteristic nature of a disease process and resultant outcomes. As these mechanisms are being elucidated, it may be possible to improve the techniques for clinical and pharmacological intervention. However, a better understanding of the ROS-dependent signal-transduction mechanisms, their localization and the integration of both ROS-dependent transcriptional and signaling pathways in cardiovascular pathophysiology is necessary. Further studies involving definitive mechanistic research are needed to achieve more clearer focus on the role of ROS in the pathophysiology of cardiovascular diseases.

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