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Review article

Antioxidants and vascular health

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ABSTRACT

Oxygen free radicals and other reactive oxygen species (ROS) are common products of normal aerobic cellular metabolism, but high levels of ROS lead to oxidative stress and cellular damage. Increased production of ROS favors vascular dysfunction, inducing altered vascular permeability and inflammation, accompanied by the loss of vascular modulatory function, the imbalance between vasorelaxation and vasoconstriction, and the aberrant expression of inflammatory adhesion molecules. Inflammatory stimuli promote oxidative stress generated from the increased activity of mitochondrial nicotinamide adenine dinucleotide phosphate oxidase, particularly of the Nox4 isoform, with the consequent impairment of mitochondrial β-oxidation. Vascular dysfunction due to the increase in Nox4 activity and ROS overproduction leads to the progression of cardiovascular diseases, diabetes, inflammatory bowel disease, and neurological disorders. Considerable research into the development of effective antioxidant therapies using natural derivatives or new synthetic molecules has been conducted. Antioxidants may prevent cellular damage by reducing ROS overproduction or interfering in reactions that involve ROS. Vitamin E and ascorbic acid are well known as natural antioxidants that counteract lipid peroxidative damage by scavenging oxygen-derived free radicals, thus restoring vascular function. Recently, preliminary studies on natural antioxidants such as goji berries, thymus, rosemary, green tea ginseng, and garlic have been conducted for their efficacy in preventing vascular damage. N-acetyl-cysteine and propionyl-L-carnitine are synthetic compounds that regulate ROS production by replacing endogenous antioxidants in both endothelial and smooth muscle cells. In this review, we consider the molecular mechanisms underlying the generation of oxidative stress-induced vascular dysfunction as well as the beneficial effects of antioxidant therapies.

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1. Introduction

In recent years, considerable research has been conducted on the critical role of reactive oxygen species (ROS) in pathological cellular reactions and in the pathogenesis of vascular diseases, including hypertension, atherosclerosis, diabetes mellitus, and cardiovascular failure [1]. ROS encloses several oxygen-derived molecules, in particular superoxide, hydroxyl radical, and non-radical derivatives, such as H_2O_2 and ozone [2]. Several studies have demonstrated the pivotal role of increased ROS production in the progressive decline of vascular health, which contributes to endothelial dysfunction. The latter is characterized by impaired microvascular vasodilatory capacity, increased capillary permeability, and the expression of intercellular and vascular cellular adhesion molecules (ICAMs and VCAMs), which mediates the recruitment of inflammatory cells [3]. Inflammatory stimuli induce ROS overproduction and increase mitochondrial oxidative stress, leading to the impairment of β-oxidation and vascular function [4]. Mitochondria are not only targets for ROS stimulation but also significant sources of ROS, which under certain conditions may stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to produce ROS themselves [5]. In general, NADPH oxidase, xanthine oxidase, endothelial nitric oxide synthase (eNOS), lipoxygenases, and myeloperoxidase are the enzymes primarily involved in ROS production. The vascular production of ROS is mainly driven by the increased activity of the NADPH oxidase isoforms. [6] The Nox4 isoform is chiefly expressed in the endothelium, whereas smooth muscle cells (SMCs) express Nox1 as well. The Nox1 isoform requires the binding of additional regulatory subunits for its activation [7]. Instead, Nox4 is constitutively active, does not require other cytosolic subunits for its activity, and represents the major endothelial isoform. [8,9] The Nox2 isoform is expressed in circulating leukocytes and monocytes, and in endothelial cells to a lesser extent. Both endothelial cells and SMCs are involved in oxidative stress-induced vascular damage [3,10]. Several studies were aimed at developing effective therapies for counteracting ROS generation by determining the appropriate dosage of natural antioxidants discovering new molecules with antioxidant properties. Antioxidants prevent ROS accumulation by increasing intracellular scavenging or promoting their degradation [11]. Despite their wide use in clinical practice, the molecular basis of the therapeutic efficacy of antioxidants remains largely unexplored. Natural antioxidant molecules, such as vitamin E and ascorbic acid, have been introduced to restore vascular health. Novel natural compounds such as goji berries, thymus, rosemary, green tea, and ginseng are being experimentally investigated for their efficacy, as well as for their ability to prevent vascular damage and reduce the risks of chronic diseases. Among synthetic compounds, N-acetyl cysteine, a derivative of cysteine, and propionyl-L-carnitine, an ester of L-carnitine, have been investigated for their antioxidant properties in counteracting vascular dysfunction [12]. In this review, we analyzed the molecular pathways underlying ROS generation and the mechanisms of action of antioxidants to counteract endothelial dysfunction and to restore vascular health.

2. Biomolecular mechanisms leading to vascular dysfunction

2.1. The role of ROS and oxidative stress

The human body accumulates oxygen free radicals and ROS as terminal products of several physiological and biochemical processes, primarily as the result of aerobic metabolism [13]. Microvascular dysfunction is linked to oxidative stress. When ROS generation exceeds the capacity of antioxidant defense, progressive endothelial dysfunction and apoptosis occur [14]. The main component of intracellular ROS is the superoxide anion ($O_2^{\bullet -}$), which is derived from the reduction of oxygen (O_2), operated by a single electron. The superoxide anion has a high cytotoxic activity, which is quickly converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), and in turn reduced to water by the activity of glutathione peroxidases, peroxiredoxins, and catalase [15]. The major source of intracellular ROS is NADPH oxidase, which has been detected in the plasma membrane, nucleus, and cytoplasmic organelles such as the mitochondria and endoplasmic reticulum [16]. The mitochondrion is the main generator of ROS; excessive production of $O_2^{\bullet -}$ contributes to vascular cell injury, thus favoring hypertension and vasospasm [17]. In addition to NADPH oxidase, other enzymatic systems contribute to vascular ROS production such as xanthine oxidase, uncoupled eNOS, cytochrome P450, and the mitochondrial respiratory chain. Nevertheless, the inhibition of NADPH oxidase activity, particularly of the Nox4 isoform, can counteract the oxidative stress, thus confirming the primary role of NADPH oxidase activity in the generation of endothelial dysfunction [18].

2.2. Nitric oxide and microvascular dysfunction

Nitric oxide (NO) is an important signaling molecule that regulates vascular relaxation, SMC proliferation, insulin secretion, airway tone, peristalsis, leukocyte adhesion, platelet aggregation, angiogenesis, and thrombosis [19]. NO is produced by a family of enzymes called nitric oxide synthases (NOSs), present in many tissues, that catalyze the production of NO from L-arginine [20]. NOSs are homodimers composed of two domains connected via a calmodulin-binding region [21]. Mammals possess three different isoforms of NOSs: endothelial NOS (eNOS/NOS3), neuronal NOS (nNOS/NOS1), and inducible NOS (iNOS/NOS2) [20]. eNOS and nNOS are constitutive isoforms, whereas iNOS is produced in response to inflammatory stimuli (cytokines and tumor necrosis factor alpha (TNF-α)). Under physiological conditions, NO is produced from eNOS by stimuli such as shear stress or factors increasing intracellular calcium concentration, such as acetylcholine, bradykinin, substance P, and thrombin [22]. The increase of intracellular calcium enhances calmodulin binding, thereby activating eNOS. During the development of endothelial dysfunction, iNOS is upregulated in response to cytokines and other inflammatory agents, in parallel to the alteration of expression and/or activity of eNOS. Endothelial NOS is involved in microvascular dysfunction through a process termed as eNOS uncoupling. [23] This process converts eNOS from a NO-producing enzyme to another enzyme that causes overproduction and release of $O_2^{\bullet -}$, which then rapidly reacts with NO to form peroxynitrite ($ONOO^-$), a highly reactive intermediate [24]. Peroxynitrite is a cytotoxic agent that causes oxidative lipid damage, protein S-nitrosylation, and DNA single-strand breakage [25,26]. The mechanisms implicated in eNOS uncoupling are linked to the reduction or absence of substrates such as L-arginine and the NOS cofactor tetrahydrobiopterin (BH4), with the consequent accumulation of endogenous methylarginines [23]. BH4 plays a key role in normal eNOS function, and its reduction contributes to the generation of $O_2^{\bullet -}$ at the expense of NO formation [23]. Multiple conditions lead to BH4 depletion such as hypercholesterolemia, hypertension, and cardiovascular diseases. In these pathological noxae, NO overproduction was linked to the major expression of iNOS and to its production by activated macrophages and neutrophils. Due to its affinity to protein-bound iron, NO overproduction can inhibit key iron-containing enzymes present in catalytic centers, thus increasing iNOS activity and $ONOO^-$ production [24]. In fact, NO can form complexes or remove iron from a range of isolated iron-containing proteins, including ferritin, ribonucleotide reductase, heme-containing proteins, ferrochelatase, and lactoferrin [27]. NO binds avidly to iron in the heme center of soluble guanylate cyclase (sGC) to increase its activity in SMCs, resulting in higher levels of cyclic guanosine monophosphate (cGMP). Consequently, the NO level is altered, which promotes vascular dysfunction [28]. One of the most important examples of iron metabolism is the role of NO in the control of iron-regulatory proteins (IRPs). In fact, NO promotes the RNA-binding activity of IRP-1 (iron-regulatory protein 1), which plays a major role in iron homoeostasis [27]. Moreover, iNOS-dependent regulation by intracellular iron can lead to an autoregulatory loop, with low iron levels inducing iNOS expression [27]. With aging, coronary arterioles are