Oxidative stress, inflammation and endothelial dysfunction: implications in atherosclerosis. Note II.

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Abstract

NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase), with its generically termed NOX isoforms, is the major source of ROS (reactive oxigen species) in biological systems. The oxidant/ antioxidant imbalance in favor of oxidants in the vascular system generates ROS via activation of NADPH oxidase. ROS are small oxygen-derived molecules with an important role in various biological processes (physiological or pathological). Some processes are beneficial and necessary for life under physiological conditions, but they are noxious, harmful under pathophysiological conditions. NADPH oxidases are present in phagocytes and in a wide variety of nonphagocytic cells. The enzyme generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling them to molecular oxygen to produce superoxide anion, a reactive free-radical. Structurally, NADPH oxidase is a multicomponent enzyme which includes two integral membrane proteins, glycoprotein gp91^{phox} and adaptor protein p22^{phox}, which together form the heterodimeric flavocytochrome b558 that constitutes the core of the enzyme. During the resting state, the multidomain regulatory subunits p40^{phox}, p47^{phox}, p67^{phox} are located in the cytosol organized as a complex. The activation of phagocytic NADPH oxidase occurs through a complex series of protein interactions. The increased production of free radicals under pathophysiological conditions is an integral part of the production of cardiovascular diseases and in particular of atherosclerosis. At the onset and progression of atherosclerosis, various non-traditional intercorrelated risk factors contribute such as oxidative stress, inflammation and endothelial dysfunction. Oxidative stress plays a crucial role not only in the formation but also in the evolution and destabilization of lesions. Oxidative stress is closely linked to endothelial damage. Endothelium modulates vascular tone by releasing specific vasoactive substances. At the onset and progression of atherosclerosis contributes to decreasing the bioavailability of NO (nitric oxide) or EDRF (endothelium-derived relaxing factor) with an important role in conserving vasodilation and inhibiting vasoconstriction. Clinical and paraclinical investigations show that inflammatory reactions operate at all stages of atherosclerotic events. According to the theory of oxidative stress, atherosclerosis is also the result of the particularly oxidative changes of LDL (low density lipoproteins) in the arterial wall. Excess ROS can produce peroxinitrite with NO, the cytotoxic oxidant important mediator of LDL oxidation with proaterogenic action.

Keywords: *EDRF, NADPH Oxidase, NOX, Reactive Oxygen Species.*

Introduction

Reactive oxygen species, conventionally seen as harmful end-products resulting from aerobic metabolism, have received special attention due to multiple cellular sources, cellular effects, but especially the consequences that produce them when they are produced in excess.

Among the many sources of ROS in biological systems, including the vascular system, the family of redox enzymes - NADPH oxidases (NOXs) [1]. Reactive oxygen species (ROS) were considered as phagocytic origin initially. Their function as body defense (antimicrobin, antifungal, antibacterial) has been shown. Subsequent research has shown that similar NADPH oxidases (isoforms) have also been described in a wide variety of non-phagocytic (somatic) cells that slowly and sustained generate intracellular O^{2-} in different compartments (intracellular, extracellular). [1, 2, 3] The production of ROS (O^{2-}, H2O2) by NADPH oxidase is achieved through the catalytic subunit of NOX which differentiates it from other oxidases. [4] Although all NOXs isoforms have the same primary function in generating ROS of whatever origin, they differ from several points of view: structural, functional, regulatory factors, component subunit requirements, cellular and subcellular distribution, as well as contributing to the development various pathological conditions, including cardiovascular diseases. [1, 3, 5, 6, 7, 8, 9] While phagocytic NADPH oxidases become active after stimulation to produce superoxide radical anion, NADPH somatic oxides by the catalytic subunit are continuously active as a slow and sustained intracellular source of O2. ROSs resulting from the NADPH oxidase family members activity by the NOX subunit act as intracellular signaling molecules, important regulators of key biological activities such as cell growth, proliferation, differentiation, migration, apoptosis.[10]

Produced in excess (condition of oxidative stress) ROS reacts indiscriminately. They may cause irreversible alterations in most biological molecules which also affects important cellular functions contributing to a wide variety of conditions such as inflammation, endothelial dysfunction, cardiovascular disease, hypertension, diabetes, chronic kidney disease, cardiac hypertrophy, stroke etc. [11, 12] There is a complex pathophysiological process in the development of atherosclerosis involving oxidative stress, inflammation, endothelial dysfunction, NO production, etc. In this context, the paper presents recent data from the literature on the oxidative stress triad, endothelial dysfunction, inflammation that serves as a basis for the development and evolution of the multistage process - atherosclerosis, schematized by Husein et al. (Figure 1) [13]

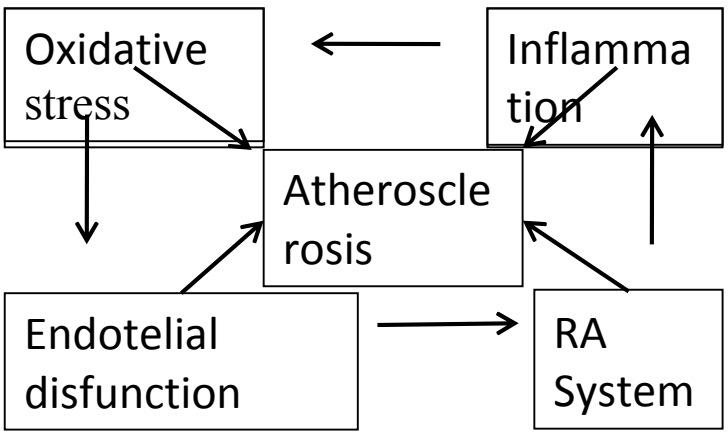

Fig. 1: The relationship between oxidative stress, inflammation, endothelial dysfunction, atherosclerosis, renin-angiotensin (RA)

Oxidative stress in the pathogenesis of atherogenesis

In recent years, a huge amount of data has accumulated that support the direct link between excess free radicals (ROS) that causes oxidative stress (redox status) in different compartments (intracellular, extracellular) and most known diseases, including atherosclerosis. In this regard, it is proven by numerous experimental and clinical studies that oxidative stress is closely linked to various risk factors for atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, smoking.

The importance of ROS in vascular physiology and pathology under oxidative stress conditions is obviously increasing because several vascular sources (endothelial cells, vascular smooth muscle cells, fibroblasts), infiltrating immune cells contribute to the production of ROS mainly via NADPH oxidase non-phagocytic. [14] Both basal and stimulated NOX activity occurs in all segments of the vascular wall (intimate, media, adventitia), where these immune cells can reach.

Into the vascular system are expressed the major isoforms of NADPH oxidase - NOX1, NOX2, NOX4 and NOX5 that differ by distribution, different mechanisms of activation, response to a wide variety of heterogeneous factors (physical, chemical, environmental or biological) and by potential contribution to vascular disease [15, 16], so that NOX as ROS in the vascular system can have both beneficial effects that in the context of physiological conditions maintain major vascular processes as well as harmful effects under overproduction conditions of reactive species derived from oxygen. [16] Oxidative stress caused by risk factors is a major cause of endothelial dysfunction.

Endothelial dysfunction and oxidative stress

The endothelium plays a critical role in regulating vascular function by releasing paracrine factors that maintain vascular tone, inhibits platelets and adhesion of inflammatory cells, promotes fibrinolysis, and limits vascular proliferation. Vasodilatation is mainly mediated by factors such as NO or relaxation factor derived from endothelium derived-relaxing factor (EDRF) and prostacyclin while vasoconstriction is mediated by factors such as endothelin-1, angiotensin II, thromboxane A2, prostaglandin H2. [17, 18, 19]

Endothelial dysfunction (vascular endothelium) is characterized by modifying the properties of endothelial vasodilation consisting of the reduced pro-inflammatory state, prothrombotic status. Endothelium is a selective and metabolically active barrier that plays a crucial role in regulating vascular homeostasis by maintaining a delicate balance between vasodilatation and vasoconstriction. [17, 18]

Endothelial dysfunction, oxidative stress, inflammation and dyslipidemia play a vital and vital role in the development and progression of atherosclerotic lesions. Under physiological conditions, endothelium controls vascular inflammation by releasing NO. Endothelial dysfunction can be regarded as a primum movens of atherosclerotic disease characterized mainly by endothelial overproduction of ROS (as O2) to lower bioavailability of the NO precursor recognized as the start of atherosclerosis and increase reverse angiotensin II in the vascular injury is caused by inducing the generation of oxidative species by activating NADPH oxidase. [12]

Endothelial function is impaired in the early stages of atherogenesis, and is closely related to the atherosclerotic risk factors. [19] NO production is crucial for maintaining normal endothelial vascular integrity. NO as a vasodilator at physiological (nanomolar) concentrations mediates endothelial protective functions by inhibiting neutrophil activation and adhesion, adhesion and platelet aggregation, vascular smooth muscle proliferation, proinflammatory cytokine expression, prevention of development and complications of atherosclerosis. Numerous experimental and clinical studies have shown that oxidative stress is closely linked to various atherosclerosis risk

factors. Oxidative stress caused by risk factors is the major cause of endothelial dysfunction as a common condition predisposing to atherosclerosis. Endothelial dysfunction predisposes to atherosclerotic lesions and has been proposed as an important diagnostic and prognostic factor for coronary syndromes. Endothelium and its major NO product are key regulators of vascular function. The pathogenesis of endothelial dysfunction is multifactorial in which oxidative stress appears to be the common cellular mechanism. [18] Increased production of ROS reduces the production and consequently the bioavailability of NO that results in vasoconstriction, platelet aggregation, and adhesion of neutrophils to the endothelium. Also, the interaction of O^{2-} with NO leads to peroxynitrite, a less effective substance for activating guanilyl cyclase, and thus the bioavailability of NO becomes particularly low.

NADPH oxidize in the regulation of vascular inflammation

Inflammation and oxidation are two closely related basic processes, which support the pathogenesis of most disease states in humans. These two distinct mechanisms (inflammation and oxidation) are constantly reciprocal, with obvious interactions in the vascular wall. [16, 20] Both processes are simultaneously encountered in many pathological conditions [21, 22] including cardiovascular diseases. [23] Traditionally, vascular inflammation begins with activation of the arterial wall endothelium where VCAM-1, ICAM-1 adhesion molecules that express monocytes are expressed, and then migrate through the endothelial layer under the influence of various proinflammatory chemoattractants. [24]

Inflammatory reactions induce ROS production, but the reverse is equally true. Moreover, components of the reaction interact with synergistic effect. Inflammatory reactions are caused by endogenous and exogenous aggressions and are characterized by cellular and vascular events. Inflammation and oxidation are processes involved in the pathogenesis of most human disease states. These distinct mechanisms are reciprocal and have obvious interactions in the vascular wall. [25] Traditionally it is considered that vascular inflammation is initiated at the luminal surface, the layer progresses through the media adventicial. [26] There is evidence suggesting that vascular adventitia is first activated in a variety of cardiovascular diseases and has key role in shaping and evolution of vascular inflammation. [27] Vascular inflammation is part of the defense and tissue repair process and is also involved in many pathological conditions such as cardiovascular disease. Traditionally vascular inflammation begins with endothelial activation and leukocyte extravasation followed by the inflammatory response that spreads from the blood vessels to the environment. [28] The production of ROS by NADPH oxidase is achieved via NOX, which differentiates NADPH oxidase from other oxidases. Increased production of ROS after induction or activation of NADPH oxidase in response to cardiovascular risk factors and inflammation contributes to the development of endothelial dysfunction and cardiovascular disease. Vascular endothelium plays an important role in regulating the inflammatory response after trauma or haemorrhage. [28]

Vascular inflammation is implicated in both local and systemic inflammatory conditions. Endothelial activation and leukocyte extravasation are key events in vascular inflammation. [29] Inflammation, considered as a primary process, plays a fundamental role in all stages of atherosclerosis from initiation, evolution, to thrombotic complications.

There are numerous studies showing that cardiovascular disease (and not only) have an important inflammatory component involved in triggering the disease in atheromatous plaque formation, development and worsening. In other words, chronic inflammation in the body, especially in the cardiovascular system, plays a major role in the development and worsening of atherosclerosis and other cardiovascular diseases.

NOX and atherosclerosis

Pathogenesis of cardiovascular disease is one of the most complex human diseases. A large number of risk factors, physicochemical interactions, cell types, involved biological processes add to the complexity of these diseases, including atherosclerosis. Many arguments prove the role of oxidative stress and inflammation in promoting atherosclerotic cardiovascular disease. [30]

Atherosclerosis is a multifactorial disorder that takes place in several stages and involves large and medium arteries. Preclinical and clinical investigations show that inflammation operates in the pathogenesis of each stage of atherosclerotic events (onset, progression, complexity of lesions). It is recognized that vascular oxidative stress as a key factor for the initiation and evolution of atherogenesis contributes to the destruction of endothelial cell homeostasis [12, 31, 32, 33], which affects the balance between vasoconstriction and vasodilation [12] and initiates a cascade of inflammatory processes both by promoting infiltration of inflammatory cells into the wall both vascularly and indirectly through the induction of cytokines and other inflammatory mediators that eventually lead to structural and functional manifestations of the disease.

Traditionally, vascular inflammation begins with endothelial activation and leukocyte extravasation and ultimately resulting in an inflammatory response that spreads from the blood vessels to the environment. NADPH oxidase, the major source of ROS in blood vessel cells, is an important factor for the onset and development of vascular disease through endothelial dysfunction, inflammation etc. [34]

Thus, inappropriate activation may contribute to the development of cardiovascular diseases such as hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, ischemia-infusion, stroke etc. [35, 36, 37, 38, 39]

Conclusions

Oxidative changes in the arterial wall may contribute to atherosclerosis when the balance between oxidants and antioxidants changes in favor of the increasing of the first. Vascular endothelium also produces paracrine factors that maintain vascular homeostasis; one of these factors is NO. [40]

The link between endothelium-derived NO and vascular health is likely to be due to the pleiotropic effects of NO on the vascular wall. Common to these processes is the increased bioavailability of ROS, low levels of NO (important vasodilator with protective physiological role in the vessels, it is crucial for maintenance of vascular endothelium health and function) and reduced antioxidant capacity in cardiovascular, renal, nervous systems, etc. The NO bioavailability depends on the processes that control NO synthesis and degradation as well as the sensitivity of the target tissue to NO. [41]

Continuously synthesized endothelium has a wide variety of biological properties that maintain vascular homeostasis including modulating vascular dilatation tonus, regulating local cell growth and protecting vessels from injuries of circulating platelets and cells and multiple antiatherosclerotic properties. [27]

High concentrations of ROS increase premature degradation of endothelial-derived NO and NADC production, resulting in endothelial dysfunction. [22] Blocking of NO synthesis with NOS inhibitors results in significant peripheral vasoconstriction and increased blood pressure. In addition to blood pressure control, NO protects vessels thrombosis by inhibiting platelet aggregation and adhesion.

The initial events in atherogenesis is considered increased transcytosis of low-density lipoproteins and their subsequent storage, retention and modification of the endothelium. [27]

It is followed by the infiltration of activated inflammatory cells from the coronary circulation into the arterial wall. Here they secrete ROS and produce oxidized lipids capable of inducing apoptosis of endothelial cells. It is a chronic inflammatory response in the arterial wall, mainly by promoting oxidative alteration and other types of LDL alteration [27, 36, 42], changes known as atherogenic, and at the same time is one of the early events of atherogenesis.

Modified lipoproteins are proinflammatory as they signal the endothelial expression of leukocyte adhesion molecules and chemokines. In addition, direct and indirect evidence supports in particular the contribution of Nox 1, Nox 2 to atherosclerosis. [42, 43] ROS, in particular O^{2-}, is involved in the pathogenesis of each stage of vascular lesion formation in atherosclerosis.

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