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OXIDATIVE STRESS AS A MECHANISM COMMON TO SEVERAL CARDIOVASCULAR DISEASES: A CRITICAL ANALYSIS

ESTRESSE OXIDATIVO COMO MECANISMO COMUM A VÁRIAS DOENÇAS VASCULARES: UMA ANÁLISE CRÍTICA

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ABSTRACT

Oxidative stress is an evolving concept. Much more than a simple imbalance between oxidants and antioxidants, it represents an imbalance of the cellular redox signaling pathways. Redox signaling involves the production of reactive oxygen species, both radicular and non-radicular, as well as non-radicular two-electron oxidation pathways. It is clear that these species can be produced by ancestral and ubiquitous enzymatic mechanisms, therefore they do not represent accidents, and have various beneficial physiological effects. New redox signaling pathways have been described, with emphasis on the role of peroxiredoxins, other protein thiols, and intermediary states of thiol oxidation. These facts have created a need to reassess the definition and scope of the concept of antioxidants. Despite the inefficiency of clinical studies with classic antioxidants, there are new perspectives, which include oxidant-producing enzyme inhibitors, such as NADPH oxidases and mitochondrial pathways. Furthermore, the use of flavonoids and related compounds, capable of activating hormetic pathways of antioxidant protection, is an important route. These facts indicate that the science in the area should enter a new cycle of studies, testing new clinical interventions resulting from these advances.

Keywords: Oxidative stress; Edothelium, Vascular; Antioxidantes.

RESUMO

Estresse oxidativo é um conceito em evolução. Muito mais do que um simples desbalanço entre oxidantes e antioxidantes, representa um desequilíbrio de vias de sinalização celular redox. Sinalização redox envolve a produção de espécies reativas de oxigênio, radicalares e não radicalares, assim como vias não radicalares de oxidação por dois elétrons. Está claro que essas espécies podem ser produzidas por mecanismos enzimáticos ancestrais e ubíquos e, portanto, não representam acidentes, tendo vários efeitos fisiológicos benéficos. Novas vias de sinalização redox têm sido descritas, com ênfase no papel de peroxiredoxinas, outras tiolproteínas e estados intermediários de oxidação de tióis. Estes fatos levaram à necessidade de reavaliar a definição e a abrangência do conceito de antioxidantes. Apesar da ineficácia dos estudos clínicos com antioxidantes clássicos, há novas perspectivas, que incluem inibidores de enzimas, geradores de geram oxidantes, como NADPH oxidases e vias mitocondriais. Além disso, o uso de flavonóides e compostos relacionados, capazes de ativar vias horméticas de proteção antioxidante, é um caminho importante. Esses fatos indicam que a ciência da área deve entrar em um novo ciclo de estudos para testar novas intervenções clínicas resultantes desses avanços.

Descritores: *Estresse oxidativo; Endotélio vascular; Antioxidantes.*

Few scientific concepts have spread as rapidly and ubiquitously as the concept of oxidative stress as a cause of disease. The concept of oxidative stress serves as a metaphor for an excess of oxidants relative to antioxidant defenses, leading to oxidative damage to various molecular components of biological systems, including lipids, proteins, and sugars.[1-3] However, this concept is continuously evolving with significant scientific advances in the field, and whether this term adequately describes the science of the field is now questioned.[3] The objective of this article was to critically analyze the concept of oxidative stress in the context of cardiovascular diseases and significant developments in the field.

A deeper understanding of the mechanisms of oxidative stress has been a key advance in this area. This progress has helped explain various difficulties associated with the inefficacy of previous studies involving classical antioxidants,

opened new perspectives, and introduced new concepts related to cellular signaling mechanisms that can be manipulated therapeutically. In the vascular system in particular, cell biology closely reflects the physiological behavior of an organ and, accordingly, redox signaling in endothelial, smooth muscle, and adventitial cells strongly affects the pathophysiology of the system. In fact, endothelial dysfunction is, fundamentally, redox signaling dysfunction,[3-6] indicating that the inefficacy of clinical trials involving antioxidants is not due to the lack of importance of redox pathways in the cardiovascular system.

A key problem was the lack of positive results from clinical trials involving classical antioxidants in the context of cardiovascular diseases.[5] This is similar to the negative findings in other fields such as cancer, metabolic diseases, and neurodegeneration. These negative findings indicated that the model of cellular redox dysfunction as a mere imbalance between oxidants and antioxidants was inadequately simplistic, forcing researchers to resume the study of basic biochemistry, cellular biology, and physiology of redox processes.

The study of genetically modified cellular and animal models and improvements in oxidant detection methods have shown that oxidant production is a physiological event that does not occur "by accident". In line with this concept, several reactive oxygen species (ROS)-generating enzymatic systems were physiologically and molecularly characterized. One of the main systems, not only quantitatively but also due to its increasing relevance in physiopathology, is the mitochondrial electron transport chain. ROS production by this system has been implicated in several stages of atherosclerosis[7-9], metabolic diseases, inflammation, and cancer. ROSs generated by mitochondrial dysfunction regulate processes such as metabolic adaptation, cell survival, apoptosis, senescence, and autophagy.[9] More recently, the physical proximity and molecular interactions between the mitochondria and the endoplasmic reticulum (ER) have been revealed more clearly. The mitochondria-ER interaction is relevant to the pathophysiology of processes involving the latter, such as ER stress associated with inflammation and dyslipidemia.[10] Another enzymatic system relevant to ROS production is the NOX family of NADPH oxidases.[11-14] These enzymatic complexes produce ROS in specific subcellular compartments. These ROSs ultimately play a role in cell signaling. Nox(s) are implicated in the pathophysiology of various diseases, including cardiovascular diseases[12-13] and particularly complications such as arterial hypertension and vascular remodeling.[15] Nox(s) are also closely related to the physiology and pathophysiology of the ER, and at least Nox4 plays a key role in ROS production during ER stress.[14] Recent studies on the evolutionary origin of Nox NADPH oxidases have identified a well characterized precursor in bacteria.[16]

The existence of ubiquitous and ancestral ROS-generating enzymatic systems and a specific system of enzymatic antioxidants indicates the key physiological role of these intermediates. Indeed, numerous cellular physiological processes depend on controlled ROS production at relatively low fluxes and in specific cellular compartments. Some examples include cell proliferation, migration, autophagy, and cell survival, which are fundamental processes in embryonic development, tissue repair, physiological control of vascular structure, elimination of irreversibly damaged cells, and more.[3] A well-known example that was among the first to be identified is the role of ROS in phagocyte-mediated antimicrobial defense, which involves the enzymatic complex Nox2.[11-14] Another important example is protein folding in the ER, during which hydrogen peroxide (H_2O_2) may be generated by some systems and simultaneously used by other enzymes to promote nascent protein oxidation, forming disulfide bonds essential to the function of most proteins.[14,17] Thus, the involvement of ROS in diseases results from an imbalance of production rather than simply production, i.e., from a localized excess, from a non-physiological metabolic pathway, or from the chemical nature of the species. Regarding the last factor, it should be noted that the term ROS is an abstraction designating unknown chemical species. However, the term ROS encompasses intermediates with vastly different physicochemical characteristics and reactivity, which cannot be regarded as a "single species" from the physiological standpoint.[18,19] For example, H_2O_2 is a non-radical, non-electrically charged, essentially cell membrane-permeable species with pro-oxidant properties that is generally weak in the context of cellular biology. Superoxide ($O_2^{\cdot-}$) is a radical species (it has an unpaired electron in the last layer) generally characterized as a non-membrane-permeable (except for anion channels) and weak redox agent because it is an anion at physiological pH. Nitric oxide is a free radical highly important for vascular regulation that is also poorly reactive with biomolecules, albeit with a much faster and favorable reactivity with $O_2^{\cdot-}$, leading to the production of reactive species such as peroxynitrite and the $NO_2^{\cdot}$ radical, which is capable of oxidizing and/or nitrating biomolecules.

Together, these findings led to the development and characterization of the redox signaling concept.[1-3,18,19] Redox signaling may be understood as the signal transduction of cellular processes consisting of electron transfer reactions involving free radicals or related species, metals active in redox systems (for example: iron and copper, among others), or reducing equivalents. An example of a reducing equivalent is the hydrogen atoms (which have a proton and an electron) donated by reducing substrates such as NADPH, reduced glutathione (GSH), or thiol-proteins (RSH). A key trait of redox signaling is its close relationship with electron transfer kinetics and thermodynamics. Simultaneously, biological factors, such as the nature of enzymatic sources of free radicals and their subcellular compartmentalization and interaction with other proteins, are crucial determinants of redox signals. The distinction between redox signaling and toxic processes is not always obvious, and several beneficial effects of oxidants have been increasingly described, as mentioned above. Therefore, oxidative stress can be redefined as a pro-oxidant imbalance capable of inducing an imbalance of redox signaling, whether or not accompanied by damage to biomolecules.[2,3] Alternatively, oxidative stress may be regarded as a disruption of redox modulation, as proposed by our group.[3] Recent studies have also identified a new phenomenon termed *reductive stress*, in which the accumulation of reduced compounds (including reduced

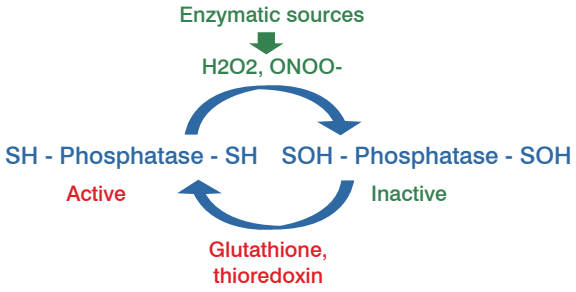
GSH) is toxic to cells, for example by hindering protein folding in a reducing environment, thus inducing ER stress. Reductive stress is responsible for cardiomyopathy in some types of gene mutation.[20]

RECENT ADVANCES IN REDOX SIGNALING

The inefficacy of clinical trials with classic antioxidants and recent advances in this field led to the deeper study of redox signaling mechanisms. Accordingly, results involving two lines of study will be discussed below.

A significant advance in this field has been the characterization of non-radical redox signaling pathways. These signaling pathways involve two-electron oxidants (thus forming no intermediate free radicals). These pathways may involve not only hydrogen peroxide itself but also lipid hydroperoxides, aldehydes, quinones, peroxynitrite, and disulfides.[2,3,18,19,21] These pathways converge into regulatory targets in thiol-proteins (proteins containing thiol [-SH] groups usually associated with the amino acid cysteine) and are controlled by thioredoxins, GSH, and cysteine itself. Non-radical pathways have been increasingly implicated in redox signaling, and it should be noted that these pathways are likely unresponsive to antioxidants specifically targeting radical pathways, such as antioxidant vitamins.[21] Accordingly, thiol proteins have been increasingly studied for their ability to show reversible redox modifications in their structures.[20] Thiol proteins, alongside metalloproteins (not discussed here), are true "redox receptors" aimed at the physiological transduction of cellular signals. These protein families provide specificity to redox signaling, and examples of these target proteins include kinases, phosphatases, transcription factors, receptors, adhesion molecules, and proteases. In this context, new intermediate states of thiol oxidation (for example, sulfonic acid) have been characterized, suggesting previously unproven biochemical specificity[6] (Figure 1). Together with non-protein thiols, such as the GSH system (present in high concentrations in the cytosol [approximately 1–2 millimolar]), they form an integrated signaling network that can be modified by radical intermediaries, albeit without necessarily involving them. Among the most recently studied thiol proteins, peroxiredoxins stand out for their particularity high intracellular concentration and reactivity with hydrogen peroxide, unlike most other thiolproteins.[20, 22] Much more than simple "hydrogen peroxide scavengers", peroxiredoxins are proposed as redox sensors capable of undergoing oxidation by peroxide and of transferring oxidizing equivalents to other target proteins. This is a new concept of great pathophysiological importance.[20] Another key family is thioredoxins. This family includes several subfamilies, including protein disulfide isomerases, a common target of study in our group. This family is involved in protein folding and redox signaling and has important effects on cytoskeleton organization and post-injury vascular remodeling.[15,23-25]

Another important line of study is the identification of molecular antioxidant systems activated by oxidants (Nrf_2 and FOXO) capable of generating cellular protective responses activated by antioxidant signals. These transcription factors



A classical redox signaling model involves the inhibition of protein phosphatase activation by oxidants, such as hydrogen peroxide (H2O2) or peroxynitrite (ONOO-). Maintaining the regulatory cysteines in the reduced state (SH) enables the active enzyme to inhibit the phosphorylation of substrates associated, for example, with cell proliferation and inflammation. The oxidation of the regulatory cysteines to sulfonic acid (SOH) inactivates the phosphatase. Consequently, the mitogenic and inflammatory stimuli persist. This is a normal physiological process of signal transduction. However, intense, persistent, and misplaced oxidant production may generate an excess of mitogenic and inflammatory stimuli, defining a state of oxidative stress. This occurs in arteriosclerosis and in numerous types of cancer."

Figure 1. Redox signaling model.

are also capable of activating genes encoding various antioxidant proteins, potentially allowing mild pro-oxidant stimuli to induce antioxidant defenses.

ANTIOXIDANTS: A REASSESSMENT

The use of antioxidants in disease prevention, herein focused on the cardiovascular system, is an extremely controversial topic. The advances discussed above clearly show that the usual definition and applicability of classical antioxidants must be reassessed. Accordingly, the concept of the antioxidant[1] should be redefined as more than a mere set of interventions able to prevent the generation of or remove oxidative intermediates. Above all, antioxidants should be able to restore the balance of redox signaling.[3] The latter is likely a much more complex task than the former and dependent on fine control mechanisms. Today, it is no longer surprising that strategies involving classical antioxidants have been ineffective.

The results of the most well-controlled and -performed trials have been consistently negative, showing that the use of vitamins, ROS scavengers in general, and related dietary supplements is ineffective.[5,6] The following reasons may explain the negative results of these clinical trials with antioxidants: a) various oxidative processes have beneficial effects; b) available antioxidants are poorly specific, poorly bioavailable at the oxidant generation site, and mostly target radical intermediates that are not necessarily involved in all pathological redox pathways; c) redox processes depend on both oxidation and, in some cases, reduction; d) some antioxidants show concentration-dependent pro-oxidant effects; e) clinical

trials with antioxidants may include selected patients with established vascular disease who are poorly responsive to treatment. It should also be noted that *sensu stricto* vascular remodeling has been quite responsive to redox interventions. This led our group to propose a "biomechanical-redox" model of vascular remodeling.[15]

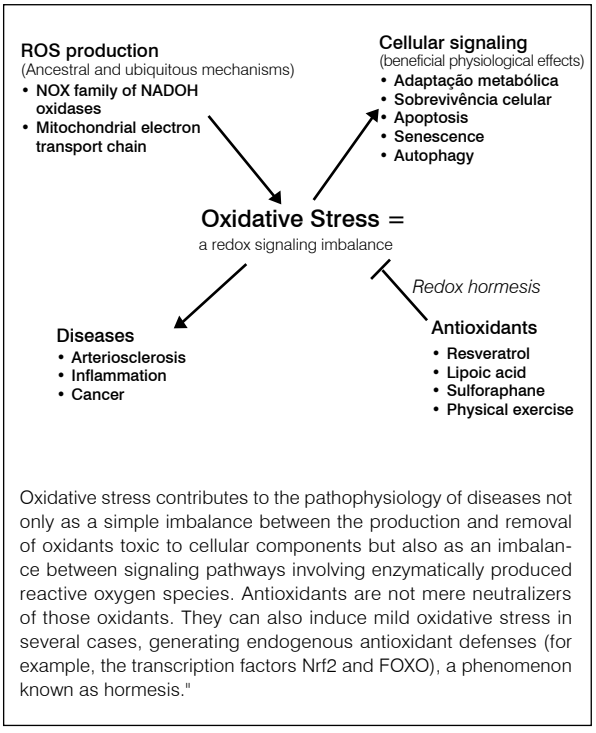
Most importantly, the indiscriminate use of antioxidant supplementation is apparently not risk-free, and at least two recent, well-conducted empirical studies[26,27] showed that common antioxidants may accelerate cancer growth and metastasis.

This discussion refers only to pharmacological supplementation with antioxidants and not to the redox balance modulation provided by diet, moderate exercise, and a healthy lifestyle. Substantial evidence for the protective effects of antioxidants has been reported. However, this protective mechanism is multifactorial and not only dependent on improving redox signaling. Studies on new antioxidant interventions have revealed several possibilities. One of the main strategies is to inhibit the enzymatic production of oxidants through pharmacological or molecular interventions aimed at inhibiting NADPH oxidases or, particularly, modulating mitochondrial function, including through the use of antioxidants specifically targeting the mitochondria. Another line of study focuses on nitric oxide donor compounds.

Furthermore, much recent research has focused on the development of pharmaceuticals that mimic natural products, such as flavonoids and other compounds. Accordingly, an emerging concept is *redox hormesis*, which consists of inducing endogenous antioxidant defenses in response to a nonlethal oxidant challenge.[28] This is apparently the mechanism of action of several natural products, such as flavonoid antioxidants, resveratrol, lipoic acid, sulforaphane, and so on. However, the *in vivo* action of these products is not directly antioxidant but rather hormetic.[28] Physical exercise is, by all accounts, a *redox hormesis* pathway. Indeed, administration of large doses of antioxidants immediately before physical exercise prevents the health-promoting effects of training.[29] Thus, flavonoid intake or physical exercise activate, through direct pathways or an oxidative challenge, respectively, protective antioxidant pathways, ultimately causing an antioxidant effect. These protective pathways include the transcription factors Nrf2 and FOXO, discussed above, which bind promoter sequences in genes encoding several antioxidant proteins.

CONCLUSION

Despite the apparent stagnation of the study of redox processes in cardiovascular diseases caused by the inefficacy of clinical trials with antioxidants, the field remains extremely dynamic and has shown remarkable progress in the understanding of signaling mechanisms. These advances have led to the redefinition and reassessment of some basic concepts (Figure 2). After the return to bench work and basic studies, a new cycle of developments should trigger clinical trials aimed at using the key biological effects of redox processes as a therapeutic tool.



Oxidative stress contributes to the pathophysiology of diseases not only as a simple imbalance between the production and removal of oxidants toxic to cellular components but also as an imbalance between signaling pathways involving enzymatically produced reactive oxygen species. Antioxidants are not mere neutralizers of those oxidants. They can also induce mild oxidative stress in several cases, generating endogenous antioxidant defenses (for example, the transcription factors Nrf2 and FOXO), a phenomenon known as hormesis."

Figure 2. Summary of the main concepts discussed in this review.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this study.

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