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RESEARCH ARTICLE

Oxidative Stress: Cannot be Measured, Localized, Prevented, or Treated.

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ABSTRACT

Oxidative stress refers to an imbalance between the production of reactive oxygen species and the ability of the body to detoxify or repair the resulting damage. However, it will be shown that the term "oxidative stress" is often used instead of the correct "oxidative damage". The term "eustress" has been used for describing beneficial signaling by small amounts of reactive oxygen species, but it will be shown that reactive oxygen species signaling can also promote cancer cell growth. The term "oxidative distress" has been created to describe the negative effects produced on cells, organs, and the entire body by large amounts of reactive oxygen species. However, if the reactive oxygen species are used to kill infectious microorganisms, the result is beneficial. Measurements of oxidative stress in body fluids or tissue specimens are a measure of oxidative damage potentially occurring simultaneously in different cells, tissues, and organs; they only provide a sum of non-separable events, possibly with opposite effects. There is no officially approved therapy to prevent or treat oxidative stress or oxidative damage. This implies that while oxidative stress issues are already a complex challenge for basic biological sciences, in a clinical setting oxidative stress is only a term of convenience with no diagnostic or therapeutic value. A critical appraisal of oxidative stress terminology, quantification, and therapeutic attempts is presented.

Introduction

The term "oxidative stress" appeared first in the biological literature in 1970 [1]. The concept of biological oxidative stress was further elaborated in "Oxidative stress: a concept in redox biology and medicine"[2] defining it as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and molecular damage" [2]. During this period, when free radicals were considered potential primary agents in many diseases, oxidative stress became a welcomed concept, connecting the damaging role of free radicals with the protective role of both endogenous and exogenous antioxidants. Subsequently, many articles on "oxidative stress" have been published. A search on PubMed as of January 19, 2024, revealed 311,596 articles using this term, surpassing the popularity of terms like mitochondria (255,028), lysosome (98,870), Golgi (55,240), or Double Helix (8,742), and closely trailing behind vitamin (471,625). A search combining oxidative stress and disease yielded 129,232 articles, representing 57% of those with the keyword Alzheimer's (225,134) and 43% of those related to "Heart Infarction" (296,415). The described modulation of the concept of oxidative stress can be defined with the term "chameleon-like," which is used metaphorically to describe something or someone that is adaptable, changeable, or capable of blending into different environments or situations. Such flexibility can explain the remarkable success of the term "oxidative stress" in biological and medical sciences. However, in biology and medicine, values must be measured and quantified to have a scientific meaning, and this rule must also apply to oxidative stress. The purpose of this paper is to critically analyze the scientific values present in the concept of oxidative stress. In the paragraphs below, the efforts that have attempted quantification, localization, prevention, and treatment of an event allegedly at the basis of a large number of human diseases will be described.

Quantify oxidative stress and oxidative damage.

Initially, oxidative stress was perceived as an event linked to excessive free radical generation or insufficient elimination, focusing on remedying it through antioxidants rather than measuring it. However, extensive trials involving antioxidant supplements with free radical scavenging abilities failed to demonstrate significant human benefits. This led to a new hypothesis, suggesting that oxidative stress arises from the disruption of thiol redox circuits, which typically contribute to cellular signaling and physiological regulation [3]. Whether defined as an imbalance between oxidants and antioxidants favoring the latter or as redox disruption caused by oxidants [2], there is a need to measure the rates of oxidant production versus elimination. To this end, various methods have been devised to comprehend oxidative stress's role in human diseases. Although measuring disease-associated oxidative stress is crucial, it may not definitively answer whether oxidative stress is a cause or a consequence of the disease [4].

Various techniques can be employed (referenced from https://www.cellbiolabs.com/oxidative-cellular-stress), tailored to different sample types such as cells, tissues, blood, urine, and food samples. The majority of tests focus on assessing the damage caused by radicals or excessive oxidants [5], including DNA/RNA damage indicators such as 8-Hydroxyguanosine (8-OHG), 8-Hydroxydeoxyguanosine (8-OHdG), Abasic (AP) sites, BPDE DNA Adduct, Double-strand DNA breaks, and the Comet Assay[6] for general DNA damage. Additionally, some techniques examine products resulting from lipid damage due to radical or oxidant impact, such as lipid peroxidation markers like 4-hydroxynonenal, 8-isoprostane, malondialdehyde (MDA), and TBARS [7]. Protein modifications[8] are also considered, involving oxidation/nitration, protein carbonyl content (PCC), 3-nitrotyrosine, advanced glycation end products (AGE), advanced oxidation protein products (AOPP), protein adducts, methylglyoxal adducts, protein radicals, and s-glutathione adducts [9]. It's important to note that these techniques assess the damage resulting from oxidative events rather than the dynamic concept of oxidative stress. Some alternative methods aim to measure the molecules responsible for damage, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrogen peroxide, and nitric oxide [10]. However, these measurements face challenges due to the rapid and uncontrolled decay of the molecules in question. Efforts have been made to gauge the antioxidant function of enzymes like catalase, glutathione, superoxide dismutase, or complex mixtures through Oxygen Radical Antioxidant Capacity (ORAC), Hydroxyl Radical Antioxidant Capacity (HORAC), and Total Antioxidant Capacity (TAC). Criticisms have been raised against these techniques, particularly the concept of "total antioxidant capacity (TAC)," which originated in chemistry and was subsequently applied to biology, medicine, nutrition, and epidemiology. It is suggested that a critical appraisal is needed due to significant limitations that hinder meaningful application to in vivo conditions [11]

Body fluid analyses cannot determine cellular, tissue, and organ locations of oxidative stress.

The location of oxidative stress (measured as damage from samples of urine, serum, cerebrospinal fluid, or tissue samples as oxidative damage) is hard to define in a biological system. In fact, oxidative damage can result from various events in different cellular compartments, tissues, or organs. Consequently, assessing overall oxidative damage in biological fluids doesn't provide insights into where it occurred and whether it occurred at a singular or multiple sites. Furthermore, the consequences of oxidative events, such as molecular damage or changes in signaling pathways, depend on the location of the damage, making it challenging to deduce diagnostic or prognostic value from global measurements [12]. The locations of the production of reactive oxygen or nitrogen species, under physiological conditions, are mitochondria, cell membrane-associated NADPH-oxidases, and lipoxygenases; they generate superoxide or hydrogen peroxide[13]. The latter can interfere with redox signaling networks [14] and diffuse over longer distances compared to superoxide. It can also oxidize thiol residues in protein tyrosine phosphatases, leading to increased tyrosine phosphorylation on target proteins. The production of reactive oxygen species (ROS) is initiated by NADPH oxidases, enzyme complexes associated with cell membranes. Various isoforms of NADPH oxidases [15]are found in plasma membranes and phagosomes, contributing to immune responses and cell signaling. Their activity is tightly regulated to maintain physiological ROS concentrations. Activation can occur in response to stimuli such as bacterial products and cytokines, with regulation by hormones and factors involved in vascular remodeling and disease. Other sources of ROS include cytochromes P450, xanthine oxidase, peroxisomal oxidases, and lysyl oxidase. The diverse localization of oxidative event generators in the body emphasizes the limitation of total body measurements for understanding potential pathologies.

Distinction between good and bad oxidative stress

The term "eustress" is derived from the ancient Greek "εὖ," meaning good, and is countered by the negative "distress," from the Latin "dis-," meaning apart or away [16]. Lower levels of oxidants can participate in regulating various biochemical reactions, such as hydroxylation, carboxylation, peroxidation, or the reduction of ribonucleotides. Additionally, they play crucial modulatory and regulatory roles in intercellular signal transduction. Although the intricate regulation of reactive oxygen species production is physiologically essential, it does not necessarily induce stress [17,18]. Studies indicate that low levels of reactive oxygen species contribute to redox signaling, positively influencing physiology and health [18,19]. However, these low levels, under different conditions, may also support cancer survival [20,21]. At higher intensities, the production of reactive oxygen species can damage biomolecules, resulting in potentially harmful outcomes and diseases known as "oxidative distress." Nevertheless, this heightened oxidative state may also have positive effects, such as inducing apoptotic death in cancer cells [22]. Large quantities of reactive oxygen species play a beneficial role in phagocytosis due to their microbicidal function. Similarly, localized intense production of reactive oxygen species is physiologically essential for T4 synthesis in the thyroid. Inhibiting these intense oxidative events may lead to disease.

In conclusion, the distinction between low and high oxidative events as either positive or negative cannot be universally applied. Oxidative eustress and oxidative distress are abstract concepts, not chemical situations, with the same actors—reactive oxygen species—in all cases. Reactive oxygen species can either have a regulatory function (seen as good or bad) or a damaging function (seen as bad or good). Despite potential topographical differences, stress events, whether causing damage or regulation, are exclusive to specific locations, occurring simultaneously in various and multiple areas.

Oxidative stress (oxidative damage) cannot be prevented.

Can oxidative stress (as discussed above, what can be measured is oxidative damage) be prevented? Shielding against the peroxidation of membrane and lipoprotein lipids ideally involves antioxidants. However, translating in vitro findings about an antioxidant to its effectiveness in cells, organs, animals, or populations requires proof. An antioxidant displaying efficacy in vitro may have contradictory, pro-oxidant, or supplementary properties in an integrated system. Examples illustrating this are discussed below. In vitro, research suggests estrogen's role in preventing lipoprotein oxidation and offering antioxidant neural protection. Nevertheless, in vivo, estrogens primarily act through receptor-mediated signaling, not weak antioxidant effects [23]. While retinol exhibits in vitro antioxidant traits [24], its in vivo function links to binding to opsin for vision.

Melatonin is claimed to have antioxidant functions, but its in vivo concentrations are too low to exert antioxidant effects. Genistein, a soy isoflavonoid, inhibits certain enzymes but is not an antioxidant. Curcumin's anti-inflammatory effects are seen in vivo, because it regulates NF-κB, MAPK, AP-1, JAK/STAT and other signaling pathways, and inhibiting the production of inflammatory mediators[25]. Natural antioxidant intake faces challenges due to low bioavailability, and small amounts may not regenerate after oxidation. Tea polyphenols EGCG, EGC, and (2)-epicatechin likely contribute to tea's benefits, but their availability to provide antioxidant effects is limited due to high conjugation. Simply introducing an antioxidant doesn't guarantee activity; flavonoid-rich foods cause a plasma antioxidant burst, mainly driven by increased uric acid, not flavonoids themselves [26]. Flavonoids and their metabolites may not function as major in vivo antioxidants, but they can reach concentrations to exhibit pharmacological activity. The controlled production of reactive oxygen species in physiological contexts has specific targets, and excess antioxidants might disrupt vital signaling pathways [27].

The antioxidant properties of vitamin E have been well documented in vitro and vitamin E has been for a long time considered one of the most important physiologically active antioxidants, needed to prevent all diseases allegedly based on oxidative stress. At the basis of vitamin E antioxidant properties is its ability to quench fatty acid peroxyl radicals in membranes and lipoproteins, where the hydrophobic molecule dissolves, by becoming an α-tocopheroxyl radical itself. The potential damage by α-toco-peroxyl radicals and the rapid consumption of the vitamin would be avoided by α-toco-peroxyl radicals' reduction by a suitable reducing agent. Thus, the combination of vitamin E, absorbed from nutrients in small amounts and ascorbic acid (or lipoic acid) would protect membranes form losing their structure and lipoproteins to become oxidized and atherogenic. Such a mechanism has been demonstrated in numerous in vitro systems. Experiments in cell cultures, in erythrocytes, in hepatocytes, in lipid bilayers, in micelles, and in low-density lipoproteins have shown that ascorbate saves cellular α-tocopherol through its recycling. However, supplementation in humans has provided less-clear results. Based on the assumption that increased lipid peroxidation is associated with accelerated atherogenesis, supplementation with the combination of vitamin E and ascorbate should provide protection. In a study [28], supplementation of individuals susceptible to accelerated atherosclerosis with α-tocopherol resulted in a near doubling of its plasma concentrations, but additional supplementation with ascorbic acid did not significantly increase the basal level of vitamin E. This suggests that there is no in vivo consumption of vitamin E and ascorbic acid is not providing a recycling/rescuing effect. The National Academies, USA, [29] have stated that "the extent to which vitamin E is recycled in humans and which antioxidant species are preferentially used for recycling is not known". α-Tocopherol quinone in humans is produced as an oxidation derivative of vitamin E; its presence has been taken as evidence that vitamin E protects as antioxidant against the oxidation of biological molecules induced by reactive oxidation species [30]. This study has shown that vitamin E oxidation products can be measured in vivo, but vitamin E oxidation does not mean that it has an antioxidant function resulting in protection against supposed lipid, protein or DNA damage associated diseases. Absurdly, it can be argued that DNA can also be oxidized, its oxidation products measured, and the oxidative damage repaired [31], but this does not imply that the function of DNA is that of an antioxidant. A study administering high-dose RRR-α-tocopherol to coronary artery disease patients showed a significant reduction in plasma oxidative damage biomarkers but no notable impact on carotid intima-media thickness over a 2-year treatment period. This suggests a dissociation between measured oxidative damage and the pathological situation, implying that vitamin E may not effectively protect against diseases supposedly rooted in oxidative stress [32]. Furthermore, the general notion that antioxidants can protect against various diseases lacks substantial evidence, and medical doctors are not obligated to prescribe antioxidants.

Excessive antioxidant intake may even have adverse health effects [33]. Despite high expectations for antioxidants as health-supporting agents, meta-analyses of clinical studies reveal that antioxidant supplementation does not consistently confer expected health benefits and, in some cases, is associated with increased mortality. Large-scale trials involving β-carotene, vitamin A, and vitamin E showed no beneficial effects on mortality and, in some instances, increased all-cause mortality, linking excess carotenoids and vitamin E to cancer growth and other health issues [34]. In terms of kidney disease, there is currently insufficient evidence to recommend increased vitamin E intake for patients with Chronic Kidney Disease [35].

Mounting evidence indicates that antioxidant supplementation can attenuate the benefits of

endurance training, hindering mitochondrial biogenesis, cellular defense mechanisms, and insulin sensitivity mediated by reactive oxygen species[36] [37]. While signs of oxidative damage are associated with certain diseases, a clear cause-and-effect relationship has not been established in clinical settings [38] . Randomized clinical trials found no significant differences in overall cardiovascular events with vitamin E supplements, and human trials do not support their use in preventing most cancers, age-related macular degeneration, or cataracts. Additionally, high doses of alpha-tocopherol supplements may lead to hemorrhage and increase the risk of prostate cancer [39].

Oxidative stress (oxidative damage) cannot be treated.

Can oxidative stress/oxidative damage be effectively treated? After delving into the transient nature of oxidative stress and the more tangible concept of oxidative damage, the pertinent question arises: Can the harm caused by oxidative damage be remedied? The potential pharmacological use of antioxidants is currently being explored, with several clinical trials underway. These approaches encompass the elimination of $O_2^{\bullet-}$ before its reaction with •NO to form $ONOO^-$, the removal of hydrogen peroxide before it forms •OH, boosting glutathione (GSH) levels using precursors, stimulating the synthesis of antioxidant enzymes—particularly through NRF2 activation—inhibiting NOXs, enhancing mitochondrial antioxidant defenses, supplementing dietary antioxidants, and inhibiting aberrant redox signaling [38]. However, it's noteworthy that, as of now, none of these approaches are recommended or even under consideration for clinical use.

Conclusion

In conclusion, while the theoretical definition of oxidative stress is clear, describing it as "an imbalance between oxidants and antioxidants in favor of the oxidants," practical considerations introduce complexities. The distinction between good (eustress) and bad oxidative stress becomes untenable, where physiologically relevant stress can lead to negative consequences and oxidative distress may be viewed as either beneficial or harmful. The measurements of oxidative stress made until now are essentially a measure of damage, not an imbalance that could cause harm. Subtle physiological changes in local reactive oxygen species levels are challenging to measure due to their highly localized nature and diverse signaling properties. Distinguishing between good and bad oxidative stress, or wanted and unwanted, is inherently difficult and cannot be separately quantified. Originally, the notion was that soluble antioxidants, such as vitamins and biofactors, could directly scavenge reactive oxygen species and prevent their negative effects. However, evidence suggests that these antioxidants do not prevent diseases attributed to oxidative stress. Moreover, concerns arise regarding the absorption and targeting of soluble antioxidants. Attempts to increase antioxidant doses resulted in adverse effects, raising questions about their general efficacy. The paradigm has shifted to emphasize the greater value of the endogenous antioxidant system, primarily composed of superoxide dismutase, catalase, and glutathione peroxidase. However, manipulating these enzymes' activities presents substantial risks due to the intricacies of cellular oxidant and antioxidant networks. Finally, the concept of oxidative stress, with its nuances of eustress and distress, serves as an intriguing pathophysiological hypothesis. Utilizing the oxidative stress notion in complex systems and clinical studies is deemed a superficial cover for unknown mechanisms in disease pathogenesis.

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