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Mini review

# Oxidative stress, the term and the concept

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ABSTRACT

The 30th birthday of a central concept in biomedicine, such as oxidative stress (OS) is a good time for re-evaluation of its contribution to science and particularly to the field of redox biology. In his recent communication, Sies described the history of the concept as well as the benefits and pitfalls of the term OS. In this mini-review, we discuss the problems associated with the still common perception of "bad OS, good antioxidants". Specifically, the term OS is an intuitively understood term originally used to describe an imbalance between pro-oxidative factors and anti-oxidative factors. It has no units, its level is dependent on the way it is measured and there is no correlation between various criteria of OS, which indicates that there are sub-classes (types) of OS (other than the classifications presented by Sies). In spite of these limitations, it is commonly regarded a measure of a person's probability to suffer from oxidative damages and is being held responsible for many diseases and antioxidants are predicted to be good to us. In fact, a "Basal OS" is vital and antioxidants may interfere with the mechanisms responsible for maintaining the oxidative status. We also discuss the linkage of OS to the outcome of antioxidant supplementation and comment on the importance of kinetic studies in evaluation of OS and on the ranking of antioxidants.



## 1. Introduction

The term oxidative stress, as defined intuitively 30 years ago, "has been formulated as a concept in redox biology and medicine" [1–3]. It relates to an excess of prooxidative factors (ROS and RNS) over anti-oxidants. Both ROS and RNS are either free radicals or compounds that yield free radicals upon interaction with various body components. Having an unpaired electron, free radicals are unstable and highly reactive. A free radical reacts with susceptible compounds, including lipids, proteins, and/or DNA. All these reactions result in the formation of a new free radical, hence in a free radical chain reaction that propagates until the chain reaction is terminated by bi-radical quenching. The rate constant of bi-radical-quenching is higher than that of reactions between free radicals and other cellular components so that the steady state concentration of free radicals is low. Nevertheless, the reactions of free radicals with other components are quite rapid and their products cause oxidative damage. Although such damage can result from different interactions with free radicals, the common result of excessive free radical concentrations is that they are likely to always produce damage, which accords with Harman's "Free Radical theory of aging" [4].

In the first decade after the initial definition, the term OS has been "inflationary" over-expressed [3]. Free radicals (and OS) have been held responsible for many diseases, including cardiovascular, neurodegenerative (Alzheimer, Parkinson, and more) and cancer [5,6]. In terms of the original definition of OS, it is either a result of an increase in the formation of free radicals and other oxidants, or a decrease of the concentration of low-molecular-weight antioxidants or in the activity of antioxidative enzymes. Hence, in view of the alleged involvement of OS in a large number of pathologies, antioxidants became a popular source of hope to prevent or cure a large number of pathological situations [5]. Promotion of low molecular weight antioxidants by the respective manufacturers obviously contributed to this trend. In the 30 years since the definition of the term OS, it became clear that OS is not our foe and that antioxidants are not necessarily good. In this mini-review, we discuss the complex, context-dependent term OS and the implications of this complexity.

## 2. Oxidative stress is an ill-defined factor but a useful term for a concept

Two prerequisites should be fulfilled by any factor assayed in a biological fluid (blood, serum, and plasma, urine) to be considered well defined: (i) The concentration of biomarkers used to assay the

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said factor in the tested fluid should reflect the concentration of the biomarker in relevant tissues and (ii). The value of the said factor should be independent of the methods used to assess it, namely that in the case of OS, the results obtained for the different methods of assessing the OS should correlate with each other. Experimentally, the evidence for both these requirements is rather weak. Hence, oxidative stress cannot be defined by any universal index [7] and it remains an ill-defined term. Furthermore, comparisons of OS under different conditions require that the assay used to assess the OS be quantitatively defined in terms of OS units rather than in terms of the specific assay. OS can possibly be expressed in relative terms in comparison to a well-defined standard. To the best of our knowledge, no such criterion has been defined. Two attempts have been made to "classify sub-forms of oxidative stress" either on the basis of its cause [2] or of its intensity [8] but thus far a classification of OS to sub-groups that can be evaluated by well-defined assays is not available for any sub-form.

Of special importance is the association between OS and various diseases. To address this issue requires comparison of the OS in patients of the given disease to the OS in a control group. The difference between patients of any pathology and their matched controls is expressed in terms of two factors: (i) the standard mean difference (SMD), which is a composite index, and (ii) the 95% confidence interval of the SMD, which reflects both the mean difference and the standard deviation. Pooling of results obtained from different studies requires expression of the results in terms of the 95% confidence interval. This expression of the results is particularly important because its use compensates, at least partially, for the use of different methods, different units of measurement and inter-lab differences [9].

## 3. Conceptual modifications

Although the term OS is ill-defined, we tend to believe that 'we know what we mean when we use it'. Moreover, it can be understood by the public (non-scientists), which assists the authorities of many states to enforce laws against toxic compounds. However, the meaning of the term is actually changing with time.

The disappointing results of antioxidants intervention studies put an end to the paradigm of "bad oxidants and good antioxidants" [5]. Free radicals are not enemies: they serve as second messengers, they modify oxidation-reduction (redox) states, they are involved in some enzyme activation and in drug detoxification; they play an essential role in turning food into chemical energy as well as in muscle contraction and in immune responses. In short, they are necessary for life [10,11]. Hence, their level must be tightly controlled by homeostatic mechanisms. Under certain conditions, low-molecular-weight antioxidants may act as pro-oxidants. Each of their effects can therefore be either a result of an increase or of a decrease of the "oxidative stress".

Our understanding of the complex mechanisms responsible for redox homeostasis is limited and the key to gain such understanding is to investigate the real molecular function of each particular situation [3,5,12]. Accordingly, much of the research in the field is devoted to "molecular redox switches governing oxidative stress responses" [3,13] and to the linkage of redox shifts to phosphorylation/de-phosphorylation, as well as to the close linkage of OS and inflammation. Much effort is being invested in multidisciplinary disease-oriented research of the specific pathogenesis of different diseases that have been previously associated with OS. Investigation of the possible contribution of disturbance in cellular redox balance due to oxidative damage to cellular components (i.e., lipids, proteins, and/or DNA) can also help enhance our understanding of changes in the redox homeostasis denoted OS [3,14].

## 4. The lack of correlations between various criteria of OS

The lack of correlation can be a result of the existence of different types of OS, which respond differently to the various assays [7]. Alternatively, any given OS causes time-dependent changes in the concentration of biomarkers. Hence, if the OS is assessed at a given time point, the evaluated value of the OS will depend on the kinetics of formation (and decomposition) of the biomarker. Thus, the lack of agreement between the OS, as evaluated on the basis of different biomarkers, may result from different kinetics of the reactions that caused the changes induced by a given OS in the different biomarkers at a given OS [15]. The use of commercially available kits intended to measure Oxidative Stress of course depend similarly on kinetic factors and therefore does not improve the assessment of OS with respect to the effect of kinetic factors.

Although kinetic factors explain the above lack of some correlations, there are no evidences to rule out the possibility that there are (mechanistically) different "types" (classes) of OS, as suggested by the finding that patients of various pathologies are under OS according to some, but not all the criteria. As an example, our analysis of commonly accepted oxidative stress indices have shown that MDA is the only index that shows a significant difference between CVD patients and controls greater than 1SMD [16]. This can be attributed to a faster production of MDA than of other biomarkers, particularly antioxidant enzymes, because any change in their activity due to a change in the OS will be evident only after a long time [15].

## 5. The linkage of OS to the outcome of antioxidant supplementation

Based on the hypothesis that free radicals are dangerous, a commonly accepted prediction was that antioxidants should prevent the respective pathogenesis. Thus, based on Steinberg's group oxidative modification hypothesis of atherogenesis [17], researchers expected vitamin E to reduce the rate of atherosclerosis. Several meta-analyses concluded that in fact vitamin E supplementation reduces the average longevity [18,19] and quality adjusted life years [20]. This is an example of a reasonable hypothesis that yielded a flawed prediction, because atherosclerosis is a chronically developing multifactorial disease, and if peroxidation is 'merely' the inducer of the process, vitamin E supplementation is not likely to slow it down [21,22].

Yet, some patients gained from the supplementation of vitamin E. The reasonable answer to the question "To E or not to E? How do we tell?" is that people under oxidative stress are likely to gain from supplementation of antioxidants [23]. This of course leads to the question which criterion can be used to "diagnose OS". Witztum proposed monitoring the level of iso-prostanes ("the best general indicator of non-enzymatic lipid peroxidation" [24]) but Roberts did not observe any changes in this possible criterion upon supplementation of less than 1600 (!) units/day, which may be associated with side effects [25]. In short, at present, we lack indices to identify high-risk groups that would theoretically benefit most from antioxidant interventions; and we lack as well a reliable quantitative assay to measure the in vivo effectiveness of such interventions, which means that our knowledge is insufficient to predict who is likely to gain from antioxidants.

## 6. Ex vivo evaluation of the sensitivity of lipids to peroxidation

Rapid peroxidation of lipids, induced either by transition metal ions or by a generator of free radicals, is preceded by a lag

phase due to consumption of antioxidants or, in the case of transition-induced peroxidation, also due to acceleration of the reaction by its products, i.e. hydroperoxides [26–28]. The kinetic profiles of these reactions can be analyzed in terms of rate constants and concentrations based on the values of the lag and the time at which the reaction is maximal [28]. The reaction can be monitored by different methods, the simplest one being continuous monitoring of the absorbance of the formed hydroperoxides at a wavelength of 234 or 245 nm [29]. When monitored spectrophotometrically, the kinetic analysis does not yield information on the specific products. However, continuous monitoring is free of the artifacts introduced by small differences in the time of sampling and if correctly analyzed [29], the continuous monitoring of the reaction is the most appropriate way to compare peroxidation reactions.

## 7. Ranking antioxidants

We consider kinetic studies (by any method) to constitute a reasonable basis for biologically relevant evaluation of antioxidants. Specifically, most assays of the activity of antioxidants are conducted in solutions [30], whereas biologically relevant peroxidation of lipids occurs at water-lipid interfaces, where the kinetics is very different from that of reactions that occur in solutions. Moreover, the effect of an antioxidant depends on its localization with respect to the surface of the assembled lipids (lipoproteins or membranes) as well as on the localization of the metal ion. The effect of an antioxidant is therefore a complex function of its activity and of the detailed structure of the studied system. Hence, if we are interested in the effect of an antioxidant against peroxidation of lipoproteins, we should monitor the peroxidation of the lipoproteins in that system in the presence and in the absence of the antioxidants.

We propose [31] conducting these experiments at different concentrations of the antioxidant and express its activity in terms of the concentration of the antioxidant required to double the length of the lag (high activity = low concentration required). This procedure requires more lab work than other tests but it yields more biologically relevant ranking of antioxidants.

In his 2007 commentary, Azzi raised the question “Oxidative stress: a dead end or a laboratory hypothesis?” [6]. We think that the available data is insufficient to answer this question: the concept OS is a scientific hypothesis that has yet to be tested. If it relates to a state of the redox of the whole body, it has to be shown that the redox potentials in different organs correlate with each other. It also requires that different methods of assessing OS correlate with each other (or relate to different types of OS) and that the OS relates to a redox potential higher than the homeostatic level of redox potential (in contrast to “reductive stress” [32] that may be induced by excessive supplementation of antioxidants). Basic understanding of the complex homeostatic mechanisms is of course essential for testing the OS hypothesis.

By contrast to the concept, since the term OS is ambiguous, unitless and quantitatively defined as a “general term describing a global condition” [3], it should be used only to describe global conditions. Even if the relative value of the OS, as measured by different methods, would have correlated with each other, the OS cannot be used to compare results. This is particularly important when the term OS is used as a criterion to implement antioxidant supplementation. The potency of antioxidants has also to be defined quantitatively and should correlate with pathophysiologic attributes. From this point of view, OS is a dead end. New roads for multidisciplinary basic research are widely open.

## Conflict of interest

None.

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## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.062.

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