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Mini Review

Is it oxidative or free radical stress and why does it matter?

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Abstract
Although oxidative stress is generally considered to be caused by reactive oxygen species endowed with electrophilic properties, non-oxidizing free radicals also play a role in numerous degenerative diseases. The most biologically active is hydroxyl radical known to be produced *in vivo* under hypoxic conditions. In addition, as shown in this paper, hydroxyl radicals can be generated *in vitro* in the presence of ferric ions without any additional redox agents. This free radical can convert soluble human fibrinogen into an insoluble fibrin-like aggregate. It is argued that this novel phenomenon can explain the *in vivo* association of iron overload with fibrin-like deposits observed in degenerative diseases. In view of the fact that hydroxyl radials are also formed under the reductive conditions, true antioxidants *i.e.* reducing substances, may enhance rather than diminish free radical stress. On the other hand, numerous natural substances, such as polyphenols considered to be antioxidants, can reduce free radical stress by virtue of their direct scavenging of hydroxyl radicals and/or chelation of body free iron. In conclusion, it is suggested in this overview to revise the concept of oxidative stress and introduce a more adequate term of free radical stress.

INTRODUCTION

The concept of oxidative stress is widely accepted by medical community all over the world [1], which has led some people to believe that too much oxygen can be harmful, despite epidemiological evidence to the contrary. Thus, a group of Italian scientists have documented that death rate of all diseases was negatively correlated with vital capacity, which determines the amount of oxygen taken up with breathing [2]. Exaggerated fear of oxygenation is reinforced by studies, which have demonstrated that numerous degenerative diseases are associated with excessive generation of reactive oxygen species (ROS). Regrettably, it is being forgotten that not all ROS are oxidants and that biomolecules can be enriched in oxygen atoms by reactions different than oxidation. For example, the biologically most reactive hydroxyl radical can modify proteins, lipids and nucleic acids by the reductive addition of oxygen-carrying hydroxyl groups [3-7].

However, the widespread use of an oxidizing agent (hydrogen peroxide) for the *in vitro* generation of hydroxyl radicals in the Fenton reaction has reinforced the notion of oxidative stress as a culprit of human pathologies. This simplistic conclusion has not been disturbed by the fact that hydroxyl radicals are also effectively formed under the reducing conditions [8-10].

Iron, hydroxyl radicals and fibrinogen

My interest in biological effects of free radicals stemmed from my over a half century involvement in the research on pathophysiological significance of blood coagulation and fibrinolysis [11, 12]. Although it is well known that the enzymatically generated fibrin is associated with thrombosis, there is no adequate explanation why fibrin-like deposits remain persistent in chronic degenerative diseases. An idea had occurred to me after I have read the paper by Marx and Chevion [13] describing the formation of a fibrin-like aggregate in the presence of transition metal (copper) ions and ascorbic acid. I have then observed that such a fibrin-like aggregate can be generated in physiological solutions by another transition metal iron, without any redox agent whatsoever. Consequently I have

demonstrated that iron-induced fibrous polymer is remarkably resistant to fibrinolytic degradation, a phenomenon that was suggested to explain thrombolytic resistance in patients with heart attacks and strokes [14].

This finding corroborates the results obtained by Eckly *et al* [15] demonstrating that ferric chloride induces thrombosis *in vivo*. Non-enzymatic formation of fibrin-like aggregates may also explain the presence of insoluble fibrin(ogen) antigens in patients not only with atherosclerosis [16, 17] but with other degenerative diseases [18, 19]. It is of interest to note that hydroxyl radicals can also modify other proteins, *e.g.* immunoglobulin G, endowing it with new antigenic properties [20]. Similarly to fibrinogen, the mechanism of action of hydroxyl radicals in this case is based on a limited reduction of intra-molecular disulfide bonds with a subsequent exposure of buried antigenic epitopes (neo-antigens)[21].

Iron overload and degenerative disease

There is a plethora of evidence, albeit not well recognized, indicating the relationship between body iron overload and the degenerative diseases. These pathologic conditions include thrombosis [22], atherosclerosis [23], Parkinson's and Alzheimer's diseases [24], cancer [25], age-related degenerations [26], kidney diseases [27] and neurological disorders [28, 29]. It should be noted that an abundant source of redox active iron is heme, which is released from oxidized hemoglobin [30]. In view of the fact that free iron leads to the formation of hydroxyl radicals *in vivo* [9, 31-33] it can be argued that the presence of fibrin-like deposits found in these pathologies represent modified fibrinogen as recently demonstrated by means of scanning electron microscopy [34]. This notion is supported by the fact that degenerative diseases can be prevented by certain natural products, specifically polyphenols that scavenge free radicals and chelate trivalent iron [35-38].

What is an antioxidant?

Closely associated with the concept of oxidative stress is a definition of an antioxidant [39]. From the electrochemical point of view an antioxidant must be a reducing agent such as ascorbic acid, and/or vitamin E. Yet clinical trials with these substances generally failed to produce the expected health benefits [40, 41]. At the same time other agents, such as polyphenols that are not reducing agents, have been shown to prevent and alleviate many pathological conditions [42-54]. It is important to note that the chemical structure of polyphenols warrants their potential for scavenging hydroxyl radicals by so-called aromatic hydroxylation, first described for salicylic acid and its derivatives [55]. A confusion existing in a definition of an antioxidant is exemplified by a paper of Perron and Brumaghim [56], in which the authors classify polyphenols as antioxidants, despite showing that their effect depends on the iron chelation activity.

It is also worth noting that health beneficial effects of extra virgin oil may be explained by the presence of tyrosol constituting over 30 percent of all olive oil polyphenols [57]. This compound has two ortho positions in its phenol ring available for aromatic scavenging of hydroxyl radicals. Reaction between these free radicals with polyphenols, as well as their capacity to chelate transition metals, was argued to be responsible for medicinal properties of *Micromeria* plant species from Croatia [58]. Methylene blue which effectively shuttles electrons between NADH and cytochrome c, was recently reported by Wen *et al* to act as a neuroprotective agent [59]. Mechanism of action of this heterocyclic compound can also be explained in terms of the neutralization of hydroxyl radicals by virtue of aromatic hydroxylation. In addition, pro-oxidant properties of polyphenols, combined with their interaction with iron, were suggested to contribute to the improvement of health of Mediterranean diet in patients with myocardial infarction [60]. Vitamins B1 and B6, although different from polyphenols, were shown to protect against oxidative stress by virtue of their iron-chelating capacity [61].

There have recently been a series of papers that critically evaluate the concept of antioxidants. For example, Tirzitis and Bartosz [62] suggested that so called antioxidant hypothesis should be considered as an intellectual 'shortcut'. These authors have emphasized that there is a great difference between "antiradical" and "antioxidant" activity, and that true radical scavenging property of a given substance should be tested utilizing hydroxyl radicals generated in the Fenton reaction. This statement is particularly significant in view of the fact that the hydroxyl radical is biologically the most reactive species and that its properties depend on the redox milieu of a given biological system [63]. Moreover, scavenging of hydroxyl radicals by phenolic compounds may simply occur by a chemical addition of a hydroxyl group to their aromatic rings without changing their redox potential. Such is the case with the deoxyribose degradation assay, which is incorrectly classified as a test for measuring antioxidant activity [64]. Obrenovich *et al* [65] in their seminal paper have stated that 'we should not consider all antioxidant compounds as having the same mechanism of action', which is particularly true in view of their iron chelating capacity. Finally, as recently emphasized by Halliwell [66] the *in vitro* tests for evaluation of the effectiveness of antioxidants can lead to artefactual data.

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CONCLUSIONS

One may argue, however, that it does not matter whether a given substance is considered an antioxidant or pro-oxidant, as long as it provides benefits to human health. Yet the problem arises when we start to search for new therapeutic compounds and substances. If we accept the concept of an antioxidant as a reducing agent, than we will miss all those whose mechanism of action is based on the scavenging of hydroxyl radicals and/or iron chelation. Moreover, numerous powerful phytochemical therapeutic agents may never be discovered just because they are being screened with methods using ascorbic acid as a standard antioxidant. Brighelius-Flohe [67] has recently stated: "It is dangerous to classify a xenobiotic as an antioxidant by means of an *in vitro* test and to continue divide chemicals into antioxidants and oxidants." In this context it is important to note that polyphenolic substances have been shown by some researchers to be not antioxidants, but pro-oxidants [68, 69].

According to Prof. Sies, we should not assume that the measuring total antioxidant capacity in a test tube reflects its status in whole body [70]. Relevant to the present article is his statement that 'we also should consider polyphenols in food beyond their antioxidant activity'. In conclusion, it becomes obvious that the concept of oxidative stress is not only inadequate, but have negative consequences to human health, particularly in diseases associated with chronic hypoxia and iron overload. In such situations administration of true antioxidants (reducing agents) may be like trying to extinguish fire with more fuel. Therefore, it is essential that the concept of oxidative stress should be objectively evaluated and properly redefined according to the principles of electrochemistry.

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