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Oxidative stress in cardiovascular disease: myth or fact?

Claudio Ceconi,[a,*] Antonella Boraso,[b] Anna Cargnoni,[b] and Roberto Ferrari[c]

[a] *Department of Cardiology, Spedali Civili di Brescia, Piazzale Spedali Civili, 1, 25100 Brescia, Italy*
[b] *Fondazione S. Maugeri, IRCCS, Cardiovascular Pathophysiology Center, Gussago (Brescia), Italy*
[c] *Chair of Cardiology, University of Ferrara, Ferrara, Italy*

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Abstract

Oxidative stress is a mechanism with a central role in the pathogenesis of atherosclerosis, cancer, and other chronic diseases. It also plays a major role in the aging process. Ischemic heart disease is perhaps the human condition in which the role of oxidative stress has been investigated in more detail: reactive oxygen species and consequent expression of oxidative damage have been demonstrated during post-ischemic reperfusion in humans and the protective role of antioxidants has been validated in several experimental studies addressing the pathophysiology of acute ischemia. Although an impressive bulk of experimental studies substantiate the role of oxidative stress in the progression of the damage induced by acute ischemia, not a single pathophysiologic achievement has had a significant impact on the treatment of patients and randomized, controlled clinical trials, both in primary and secondary prevention, have failed to prove the efficacy of antioxidants in the treatment of ischemic cardiovascular disease. This dichotomy, between the experimental data and the lack of impact in the clinical setting, needs to be deeply investigated: certainly, the pathophysiologic grounds of oxidative stress do maintain their validity but the concepts of the determinants of oxidative damage should be critically revised. In this regard, the role of intermediate metabolism during myocardial ischemia together with the cellular redox state might represent a promising interpretative key.

Keywords: Reactive oxygen species; Coronary artery disease; Clinical trials; Antioxidants

Oxidative stress involves any condition in which oxidant metabolites (e.g., oxygen radicals) can exert their toxic effects because of increased production or altered cellular mechanisms of protection. The effects of oxidative stress can be evidenced by cellular accumulation of peroxides (e.g., lipid peroxides) or by-products, such as malondialdehyde (MDA),[1] and by oxidized glutathione. Oxygen itself has a radical nature and can be called a diradical, but it does not exert any major reactivity. The term ''oxygen free radicals'' is frequently, but mostly wrongly, used to indicate all reactive intermediates including also molecular forms that are not radicals, e.g., hydrogen peroxide. For this reason, the term ''reactive oxygen species'' (ROS) appears more appropriate to indicate their effects on the organisms. ROS are constantly formed in living organisms and the presence of defense mechanisms is established.

In 1969, the interest in this specific field was stimulated when McCord and Fridovich [1] identified superoxide dismutase (SOD), an enzyme that removes superoxide radicals by catalyzing a dismutation reaction to hydrogen peroxide and oxygen. The challenge of understanding oxidative stress mechanisms and protection inspired so great an interest in many fields of biological research that resulted in more than 100,000 full papers, as documented in the PubMed of the last 20 years. Interest has not yet been worn out but, on the

* Corresponding author. Fax: +39-030-2522362.
E-mail address: cceconi@libero.it (C. Ceconi).

[1] *Abbreviations used:* GSH/GSSG, reduced/oxidized glutathione; NADPH/NADP$^+$, nicotinamide adenine dinucleotide phosphate redox couple; NADH/NAD$^+$, nicotinamide adenine dinucleotide redox couple; prot-SH, protein thiols; prot-SSG, protein mixed disulfides; ATP, adenosine triphosphate; glucose 6-P, glucose 6-phosphate; HMS, hexose monophosphate shunt, MDA, malondialdehyde, ROS, reactive oxygen species; TBA, thiobarbituric acid; SOD, superoxide dismutase.

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contrary, is still increasing with time following an exponential pattern.

This short review will indeed focus on the increasing interest in oxidative stress and its implications specifically with regard to the cardiological field and the perspective of treatment of heart diseases, e.g., infarction and failure.

Reactive oxygen species: metabolic production and detoxification

In a physiologic environment, various ROS are formed by different generating systems, whereby they exert their physiologic actions.

The bulk of oxygen reduction in most cells, such as in the heart, occurs by the mitochondrial cytochrome oxidase pathway. Oxygen radicals, moreover, are involved as key intermediates in metabolic reactions both spontaneous or enzymatically driven.

Some unstable radicals may also intervene in biological intracellular pathways, for example vascular relaxation, thus exerting crucial physiologic roles. In the vascular endothelium, nitric oxide (NO) radicals are produced from L-arginine by the nitric oxide synthase (NOS) family enzymes and cause relaxation of vascular smooth muscle. NO radicals are also observed in granulocytes and macrophages [2,3] where they react with superoxide anion to form hydroxyl radical.

Phagocytic cells also synthesize hypochlorous acid through oxidation of chloride ions by hydrogen peroxide and the reaction is catalyzed by myeloperoxidase. These ROS are extremely important for the phagocytic function.

ROS production, on the other hand, can cause extensive damage. In fact, while NO has a physiologic role in neurotransmission, blood pressure regulation, and vasodilation [4,5], an excessive NO radical production causes lipid peroxidation and depletion of cellular energy via disruption of mitochondrial enzymes and nucleic acids [6,7]. The reaction of ROS with NO may lead to functional damage in the endothelium. Furthermore, when in contact with superoxide anion NO radicals forms peroxynitrite, a highly toxic and reactive nitrogen-based ROS [8], and trigger apoptotic cell death, especially in the nervous system [9].

In living cells, damage can be done by ROS when tissues are exposed to high-energy radiation. The γ radiation, for example, causes one of the oxygen–hydrogen bonds in water to split, thus generating two radicals, the hydrogen and the hydroxyl radicals. Hydroxyl radicals are the most reactive radicals known to chemistry, with a rate constant that is close to the theoretical value for bimolecular reactions. They interact therefore immediately with any biological molecule to produce cellular damage [10,11].

Among the several oxidative effects the most "popular" is lipid peroxidation, that occurs when a strong oxidant is generated close to cell membranes. This, after combination with oxygen through peroxyl radical formation, leads to lipid hydroperoxidation with membrane disruption and highly cytotoxic products such as MDA.

Organisms have developed primary systems to protect themselves against generation and damage by ROS. These mechanisms of detoxification in the heart have been profoundly studied and the most active are enzymes such as SOD, catalase, and glutathione peroxidase [1,12–14].

Secondary systems are also present. α-Tocopherol, or vitamin E, is found in cell membranes and circulating lipoproteins and appears to be the most important lipid-soluble chain-breaking antioxidant in vivo in humans [15,16].

Cells also contain systems to specifically contrast oxidative damage in DNA [17], proteins [18], and lipids [19].

Oxidative damage by ROS has been documented in a number of experimental studies from subcellular to organs to in vitro and in vivo models [20–26]. In vivo production of ROS has been indirectly demonstrated in experimental hypoxic and ischemic hearts, via measurement of oxidized glutathione and by the use of antioxidants or enzymes [27–36]. Direct detection via electron spin resonance coupled to spin-trapping agents has also been shown [37–40].

Oxidative stress has been evidenced for the first time in human heart during by-pass surgery, where oxidized glutathione has been detected by measuring the arterovenous difference [41,42]: oxidized glutathione accumulation was found to negatively correlate with functional recovery. Also in other human studies, such as after myocardial infarction or in heart failure, oxidative stress was involved in the pathogenesis and/or progression of the disease and identified through determination of oxidized glutathione or release of inflammatory mediators and breath penthane [43–50].

Oxidative stress: myth or fact?

Numerous clinical trials have been designed on the pathophysiologic hypotheses identified in the experimental studies carried out in the previous years. ROS are involved in the development and progression of various cardiac diseases [41–45] and oxidative stress, as a pathologic determinant, is a widely accepted concept. Unsaturated fatty acids in membranes, thiol groups in proteins, and nucleic acids are important targets in oxidative stress [51].

Established risk factors such as hypertension, smoking, environmental related diseases, etc., are all associated with increased oxidative stress due to excess ROS

activity [19]. When interest in oxidative damage in cardiology started, unsaturated fatty acids appeared to be the likely initial oxidative target and lipid peroxidation appeared to be a reliable explanation for the dramatic structural changes of cellular membranes and the loss of ion homeostasis occurring upon post-ischemic reperfusion [52,53].

As a consequence of the convincing evidences obtained from experimental research and pathophysiologic studies conducted in humans, it was thought that manipulation of oxidative stress should reflect an effective improvement in the treatment of heart disease patients. Unfortunately, not a single pathophysiologic achievement in the field of oxidative stress has had a significant impact on the cardiologic therapy so far.

Recent primary prevention clinical studies have investigated the role of antioxidants, mainly vitamin E, on patients at risk of cardiovascular diseases. Large prevention studies with an overall combined total population of 20,865 patients failed to indicate any benefit from treatment with randomized vitamin E (HOPE, GISSI) against death from all causes and/or on cardiovascular outcomes [54,55].

On the basis of these data, antioxidants, such as vitamin E, are not, at the moment, indicated for the treatment or prevention of coronary artery disease. The American Heart Association makes no specific recommendation regarding supplements of vitamin E or β-carotene for the general population in primary prevention or as nutritional advice [American Heart Association Eating Plan for Healthy Americans, 2000].

What went wrong with oxidative stress in the clinical practice?

Evidence for a role of oxidative damage in the pathophysiology of many diseases is clear and indisputable: the inconsistency with clinical results does not reduce its importance. It is critical therefore to understand the reasons for the lack of correspondence between experimental and human studies. Could the reason be on our understanding of the important determinants of oxidative stress?

In our view, this inconsistency may arise from several reasons that can be summarized in the problems with: (i) the concept, (ii) the methodology, and (iii) the mechanisms of oxidative stress in humans.

Concerning the concept, it is common knowledge that ROS are strong oxidants and are very toxic in view of their high reactivity. However, this is not entirely true: superoxide anion, for instance, is not a strong oxidant, but becomes a weak reductant in aqueous solutions at neutral pH. The availability of a proton to neutralize the charge restricts its ability to act as an oxidant. As a reductant it participates in nucleophilic reactions and competes with dismutation. Under this condition, superoxide does not appear to have the necessary reactivity to act as a deleterious or cytotoxic species. On the other hand, superoxide anion can readily react with ferric ion and with non-heme-iron–sulfur compounds and other forms of chelated iron complexes leading to release of ferrous ion, which can undergo the Fenton reaction to form highly reactive radical species. Nevertheless, whether, how, and when these chemical species occur in the progression of cardiovascular disease needs indeed to be demonstrated.

Concerning the methodology, oxidative stress, as seen before, involves disarrangement of membranes, MDA formation and lipid peroxidation, protein strand scission, protein cross-linking, and lipid–protein cross-linking. The methodology used in basic research laboratories has so far been applied directly to the clinical setting and to complex systems in vivo, with no modification of the techniques and perhaps with false results. The best example, from our point of view, is the MDA determination. MDA is a by-product of lipid peroxidation that has been assayed for a long time by the thiobarbituric acid (TBA) test, a reaction at high temperature between unstable lipid intermediates and a dye. This test is not easily applicable and reliable in complex biological material and should be applied in human studies with great care. For instance, we have demonstrated that, in the whole heart, TBA-reactive material—measured by the commonly used TBA test—does not correspond to MDA measured by high performance liquid chromatography (HPLC) [56]. During ischemia and reperfusion, TBA reactive material, chemically unidentified and unlikely to represent by-products of lipid peroxidation, accumulates. Its increase does not correspond to true MDA, which is actually lower in ischemia as a consequence of the low tension of oxygen. Accordingly, it can well be that artifactual results would have been obtained if MDA measurement is carried out from application of the TBA test under non-optimal conditions [51].

Concerning mechanisms, lipid peroxidation, experimentally demonstrated and target for antioxidants in primary prevention trials (vitamin E), may not represent the right target to address in clinical studies. That is to say that lipid peroxidation is likely to be the ultimate step of oxidative stress, usually preceded by many other steps which can just be functional and not structural, but important as targets for antioxidative tools.

Recently, a crucial role of cellular redox state as a possible early target of oxidative damage has been brought into focus. Cellular glutathione and thiol status are reduced during myocardial ischemia with no accumulation of oxidized glutathione, but with a concomitant decreased enzymatic activity of Mn-SOD. With reperfusion and readmission of oxygen also oxidized glutathione is accumulated and released [29,42].

Ischemia and reperfusion damage leads to abnormalities of intermediate metabolism which may alter the ultimate effect of oxidative stress. Among the many biochemical lesions occurring during myocardial ischemia and reperfusion, an inadequate synthesis of NADPH with effects on all redox states of thiols has been demonstrated. These effects influence other important equilibrium reactions. The decrease, in NADPH during ischemia may therefore contribute to explain the depletion of reduced glutathione and of total-SH, that was difficult to justify after observation of proton and reducing equivalent accumulation. Fig. 1 shows some important equilibrium reactions that regulate the interplay of reducing equivalents in the cell, and therefore determine modification of the equilibrium after alteration of one of these substances following oxidative stress. Oxidation of glucose-6-phosphate through the hexose monophosphate shunt provides, under physiologic conditions, the reducing equivalents (NADPH) for equilibrium activity of glutathione reductase and for major reactions of intermediate metabolism including those for the reduction of thioredoxin by thioredoxin reductase. NADPH is also a source of electrons for many biosynthetic reactions. Under ischemic conditions, a reduction of NADPH supply directly affects glutathione status and leads to accumulation of oxidized glutathione that cannot be reduced by glutathione reductase. At this point, other sources of SH groups, i.e., proteins, may become important as cellular defenses to reconstitute the reduced glutathione via glutathione *S*-transferase, leading to mixed sulfide formation [57,58].

Involvement of thiol oxidation and/or modification of redox state in proteins and channels is likely to alter ion homeostasis, or enzymatic activity of proteins involved in cardiac metabolism, such as glucose-6-phosphate dehydrogenase, pyruvate kinase, and malate dehydrogenase. Thiol oxidation or disulfide formation in proteins may lead to functional alteration such as the increase in diastolic pressure. ROS effects, involving ion channels, ATPases, and exchangers, produce alterations in intracellular calcium homeostasis, with transient intracellular calcium overload, decreased sensitivity of myofilaments to calcium, excitation-contraction uncoupling, and changes in sarcolemmal ion movements, such as activation of sodium/calcium exchange secondary to the activation of sodium/hydrogen exchange or leukocyte activation [59–66]. Also, a role of thiol oxidation in the initiation of the apoptotic process has been hypothesized [67].

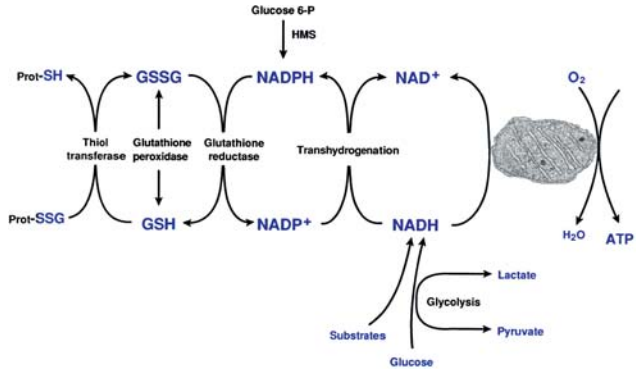

Fig. 1. Schematic representation of the interdependence of intracellular thiol pools and pyridine nucleotide redox couples.

Conclusive remarks

It is unthinkable not to consider that large clinical studies have given unexpected negative results on the protective effect of antioxidants. On the other hand, oxidative stress is the cause of many progressive cardiac diseases, as amply demonstrated in animal and human studies. This points out a dichotomy between strong pathophysiologic evidence and the myth of a drug useful for patients.

A second thought needs to be given to the reasons why this led to the discrepancy between the impressive bulk of evidence from experimental studies and the failure of medical treatment in clinical trials. Promising perspectives are, in our view, opened by the concept that very early phases of oxidative damage are only functional changes of the cellular redox metabolism. Indeed, further studies are needed to elucidate the relevance of these observations in the clinical setting.

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