What Is Oxidative Stress?

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Abstract: Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. Therefore, a more useful definition of oxidative stress may be "a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them." The biomarkers that can be used to assess oxidative stress *in vivo* have been attracting interest because the accurate measurement of such stress is necessary for investigation of its role in lifestyle diseases as well as to evaluate the efficacy of treatment. Many markers of oxidative stress have been proposed, including lipid hydroperoxides, 4-hydroxynonenal, isoprostan, 8-hydroxyguanine, and ubiquinol-10. To prevent the development of lifestyle diseases, advice on how to lead a healthy life should be given to individuals based on the levels of oxidant and antioxidant activity assessed by pertinent biomarkers. Individual genetic information should also be taken into consideration.

Key words: Oxidative stress; Free radicals; Active oxygen; Biomarkers

Introduction

The close association between oxidative stress and lifestyle-related diseases has become well known. Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs.

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Table 1 Major Active Oxygen Species

$O_2^{\bullet -}$	Superoxide radical
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
$^1O_2$	Singlet oxygen
$HOO^{\bullet}$	Hydroperoxyl radical
LOOH	Alkylhydroperoxide
$LOO^{\bullet}$	Alkylperoxyl radical
$LO^{\bullet}$	Alkoxyl radical
CIO^-	Hypochlorite ion
$Fe^{4+}O$	Ferryl ion
$Fe^{5+}O$	Periferryl ion
$NO^{\bullet}$	Nitric oxide

Free Radicals, Active Oxygen Species, and Oxidative Stress

Usually, an atom is composed of a central nucleus with pairs of electrons orbiting around it. However, some atoms and molecules have unpaired electrons and these are called free radicals. Free radicals are usually unstable and highly reactive because the unpaired electrons tend to form pairs with other electrons. An oxygen molecule (O_2) undergoes four-electron reduction when it is metabolized *in vivo*. During this process, reactive oxygen metabolites are generated by the excitation of electrons secondary to addition of energy or interaction with transition elements. The reactive oxygen metabolites thus produced are more highly reactive than the original oxygen molecule and are called active oxygen species. Superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen are active oxygen species in the narrow sense. Active oxygen species in a broad sense are listed in Table 1. Only active oxygen species having an unpaired electron, indicated with a dot above and to the right of the chemical formula in the table, are free radicals.

For aerobic organisms, a mechanism to remove these highly reactive active oxygen species is essential to sustain life. Therefore, various antioxidant defense mechanisms have developed in the process of evolution. It is also true that the high reactivity of these oxygen metabolites is utilized to control various biological phenomena.

From a biological viewpoint, various oxygen-derived free radicals have been attracting attention for the following reasons: Various active oxygen species are generated in the body during the process of utilizing of oxygen. Because the body is furnished with elaborate mechanisms to remove active oxygen species and free radicals, these by-products of oxygen metabolism are not necessarily a threat to the body under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress. Consequently, active oxygen species and free radicals can attack molecules in biological membranes and tissues, thus inducing various diseases. In other words, oxidative stress is defined as a "state harmful to the body, which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost."

However, oxidative stress is actually useful in some instances. For example, oxidative stress induces apoptosis to prepare the birth canal for delivery. Also, biological defense mechanisms are strengthened by oxidative stress during appropriate physical exercise and ischemia. Therefore, a more useful definition of oxidative stress may be a "state where oxidation exceeds the antioxidant systems because the balance between them has been lost."

Biomarkers of Oxidative Stress

The biomarkers that can be used to assess oxidative stress have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions, as well as to evaluate the efficacy of drugs. Assessment of the extent of oxidative stress using biomarkers is interesting from a clinical standpoint. The markers found

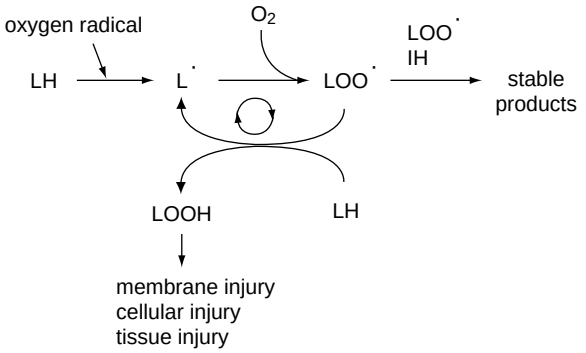


Fig. 1 The chain reaction causing lipid peroxidation

in blood, urine, and other biological fluids may provide information of diagnostic value, but it would be ideal if organs and tissues suffering from oxidative stress could be imaged in a manner similar to CT scanning and MR imaging. In recent years, attempts have been made to use electron spin resonance techniques for this purpose, but it will take time before such methods can be applied to humans.

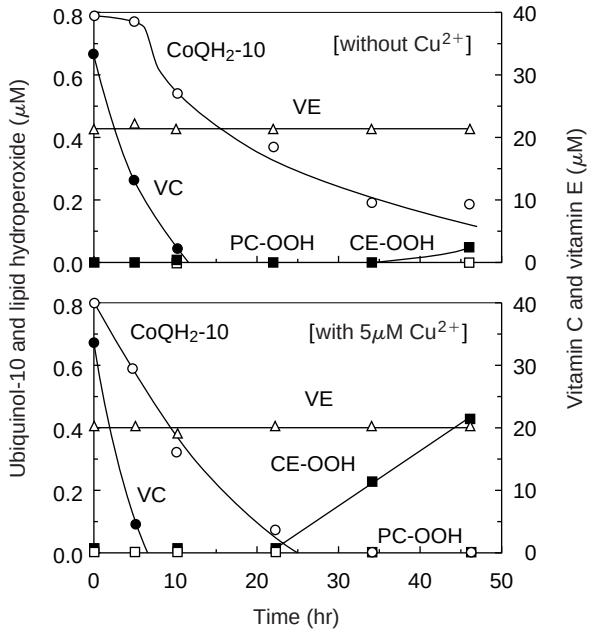
Because the body is not necessarily fully protected against oxidative damage, some of its constituents may be injured by free radicals, and the resultant oxidative products have usually been used as markers. Many markers have been proposed, including lipid peroxides, malondialdehyde, and 4-hydroxynonenal as markers for oxidative damage to lipids; isoprostan as a product of the free radical oxidation of arachidonic acid; 8-oxoguanine (8-hydroxyguanine) and thymineglycol as indicators of oxidative damage to DNA; and various products of the oxidation of protein and amino acids including carbonyl protein, hydroxyleucine, hydrovaline, and nitrotyrosine. Lipid peroxide was assessed in clinical samples even in relatively early studies, and the analytical methods for this substance have improved.

The famous method of Yagi, which measures substances that react with thiobarbituric acid, has been widely used in both clinical and experimental studies. Such substances have been the most frequently used marker of oxidative stress partly because lipid peroxidation (Fig. 1) is a very important mechanism of cell membrane destruction. Lipid peroxidation is a chain reaction by which unsaturated fatty acids (cell membrane components) are oxidized in various pathological conditions.

When a hydrogen atom is removed from a fatty acid molecule for some reason, the free radical chain reaction proceeds as shown in Fig. 1. Thus, radicals that can be involved in the extraction of hydrogen atoms from lipids include the hydroxyl radical ($HO^{\bullet}$), the hydroperoxyl radical ($HOO^{\bullet}$), the lipid peroxyl radical ($LOO^{\bullet}$), and the alkoxyl radical ($LO^{\bullet}$). Metal-oxygen complexes, particularly iron-oxygen complexes, are also important *in vivo*. The peroxidation chain reaction propagates itself once it has started. The process by which lipid radicals ($L^{\bullet}$) are generated from lipids (LH) is called the chain initiation reaction. Lipid radicals ($L^{\bullet}$) thus generated react immediately with oxygen, resulting in the formation of $LOO^{\bullet}$, which attacks another lipid and removes a hydrogen atom from it, resulting in the formation of lipid hydroperoxide (lipid peroxide; LOOH) and another $L^{\bullet}$. This new $L^{\bullet}$ also reacts with oxygen and forms $LOO^{\bullet}$, which attacks another lipid to generate lipid peroxide, so lipid peroxide accumulates as the chain reaction proceeds.

Gastric mucosal injury occurs in patients with extensive burns. Before the development of mucosal lesions, the blood level of skin-derived substances that react with thiobarbituric acid shows an increase. Then these substances also increase in the gastric mucosa, leading to the development of mucosal lesions. The free radical peroxidation of lipids is an important factor in local injury to cell membranes and impairment of the activity of enzymes and receptors bound to the membrane, and the lipid peroxide thus produced can affect even remote organs.

Among the agents that protect the body from lipid peroxidation, vitamin E is consid-



$CoQH_2$-10; coenzymeQ_{10}
VC; vitamin C, VE; vitamin E
PC-OOH; phosphatidylcholine hydroperoxide
CE-OOH; cholesterylester hydroperoxide

Fig. 2 Changes of antioxidants and generation of lipid peroxides during incubation of human plasma at 37°C in air

Source: Yamamoto, Y. *et al.*: *Oxidative Damage and Repair*. ed. Davies, K.J.A., Pergamon Press, 1991; pp. 287–291.

ered to be the most important. This vitamin has attracted attention as an antioxidant because it can scavenge lipid peroxyl radicals and hence stop the propagation of the free radical chain reaction. The lipid peroxyl radical removes a hydrogen atom from the phenyl group of vitamin E and the molecule that has accepted the hydrogen atom is stabilized. In turn, vitamin E is converted into a radical, which is also stable and less reactive. Consequently, this vitamin E-derived radical is unlikely to attack lipids and perpetuate the chain reaction. Instead, it is thought to react with another peroxyl radical and thus become stable. This antioxidant reaction protects biological membranes from injury caused by free radicals and lipid peroxides.

However, lipid peroxides are still generated in the plasma despite the presence of an adequate concentration of vitamin E. Consequently, plasma vitamin E levels seem unlikely to be a useful biomarker of oxidative stress. In addition, vitamin E is lipid soluble, so its blood level varies depending on the lipid content.

When human plasma is incubated at 37°C in air, the concentrations of antioxidants and lipid peroxides change as shown in Fig. 2. Of the three antioxidants, vitamin C decreases first, followed by reduced coenzyme Q-10 (ubiquinol-10). This suggests that vitamin C and ubiquinol-10 are the antioxidants that are most sensitive to oxidative stress. Vitamin E may be protected by vitamin C and ubiquinol-10 because it is an important antioxidant. Vitamin C and ubiquinol-10 levels were measured to assess oxidative stress in patients with various liver diseases. In patients with chronic hepatitis, liver chirrhosis, and liver cancer, the vitamin C and ubiquinone-10 (oxidized coenzyme Q-10) levels were significantly decreased and increased, respectively, when compared with those in the control group, with a significant percent increase of oxidized coenzyme Q-10. In contrast, there was no significant difference of the vitamin E level.

Oxidative Stress as a Biological Modulator and as a Signal (Fig. 3)

Oxidative stress not only has a cytotoxic effect, but also plays an important role in the modulation of messengers that regulate essential cell membrane functions, which are vital for survival. It affects the intracellular redox status, leading to the activation of protein kinases, including a series of receptor and non-receptor tyrosine kinases, protein kinase C, and the MAP kinase cascade, and hence induces various cellular responses. These protein kinases play an important role in cellular responses such as activation, proliferation, and differentiation, as well as various other functions. Accordingly, the protein kinases have attracted the most attention in the investigation of the association between oxidative stress and disease.

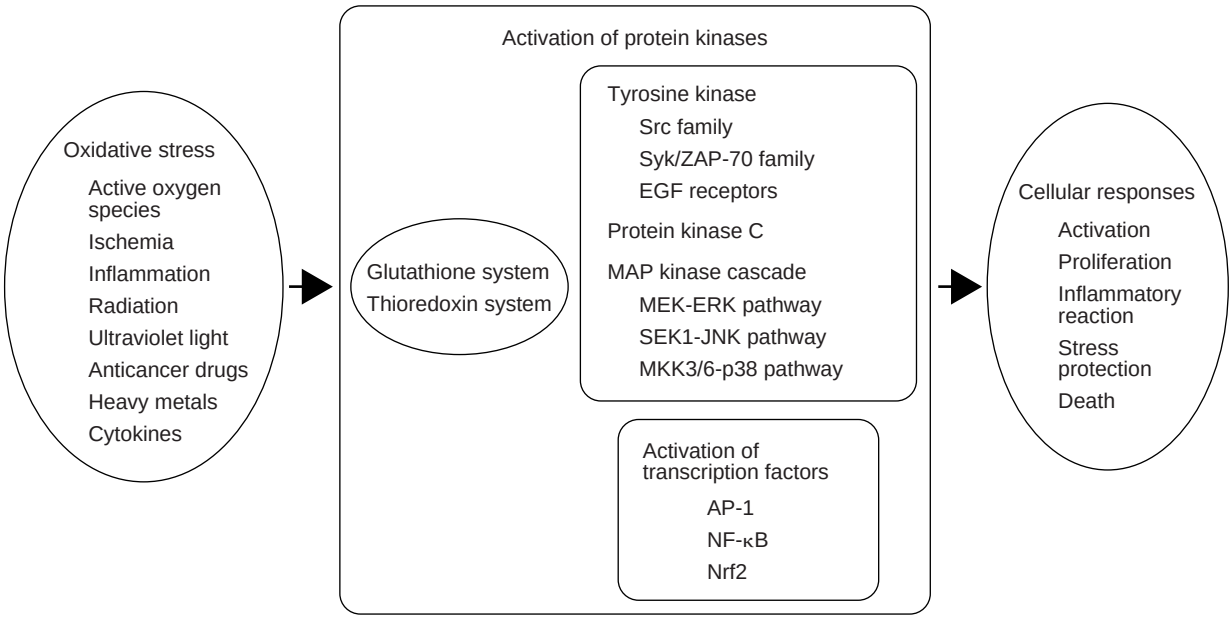


Fig. 3 Oxidative stress and cellular responses

Oxidative stress can influence many biological processes such as apoptosis, viral proliferation, and inflammatory reactions. In these processes, gene transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) act as oxidative stress sensors through their own oxidation and reduction cycling. This type of chemical modification of proteins by oxidation and reduction is called reduction-oxidation (redox) regulation.

The transcription factor NF-κB undergoes translocation from the cytoplasm to the nucleus in response to an extracellular signal. This translocation induces its ability to bind to DNA, leading to transcriptional up-regulation of the expression of many genes related to inflammation and immunity. Thus, NF-κB seems to be involved in development and aggravation of many diseases. Recently, it was also suggested that this factor may be involved in the process of carcinogenesis because it is located upstream to a series of transcription regulation factors and because it possesses the ability to suppress apoptosis.

With respect to the role that oxidative stress plays in the activation of NF-κB, many new findings have been obtained recently. Stimulation with tumor necrosis factor (TNF)-α, phorbol myristate acetate (PMA), interleukin (IL)-1, lipopolysaccharide, viral infection, and ultraviolet light leads to the generation of active oxygen species, which function as a second messenger in the activation of NF-κB. The mitochondrial respiratory chain is considered to be the major source of active oxygen species. In cells lacking mitochondria, damage caused by TNF-α and NF-κB dependent IL-6 production is suppressed. It has also been shown that antimycin A, an inhibitor of mitochondrial electron transport, increases the intracellular generation of active oxygen species and enhances the activation of NF-κB. In resting cells, NF-κB is bound to IκB and remains in the cytoplasm. An extracellular signal causes the dissociation of these two molecules and IκB decomposes, whereupon NF-κB migrates to the nucleus and activates transcription.

The phosphorylation cascade that produces the NF-κB/IκB complex has been shown to depend on the interaction between proteins

derived from activation of IL-1 and TNF receptors. The activation of NB-κB requires a signal derived from active oxygen species. The possible involvement of active oxygen species in the release of NF-κB is partly suggested because IκB undergoes phosphorylation via a group of kinases involved in a phosphorylation cascade. Induction of the expression of thioredoxin by active oxygen species is also involved in the activation of NF-κB, since thioredoxin gives NF-κB the ability to bind to DNA in a process that is regulated by redox reactions.

NF-κB seems to be the key transcription factor for elucidating the relationship of oxidative stress to lifestyle diseases and identification of the precise mechanisms involved may lead to the development of new therapies for such diseases.

Conclusion

The causes of lifestyle diseases can be divided into three major categories, which are genetic, habitual, and environmental. Many of the genes that are associated with biological oxidative stress have been identified, with the genes for NO synthetase (NOS) and heme oxygenase (HO) being considered as candidates for such diseases. However, lifestyle diseases are often multifactorial, so it is difficult to identify the causative factors. Recent progress in the field of molecular biology has made it possible to store massive amounts of genetic information on DNA microchips and has provided various efficient computer programs for analysis, thus promising rapid progress in this field.

Many daily habits are closely associated with oxidative stress, which is augmented by smoking, drinking, and an irregular diet. In Japan, dietary habits have undergone a marked change over the years. When the energy intake related to major nutrients is calculated, lipids provide over 25%, reflecting this change. Many environmental factors can generate active oxygen species and DNA damage caused by such oxygen radicals is extremely serious because it may be related to carcinogenesis. To prevent the development of lifestyle diseases, instructions on how to lead a healthy life should be given individually depending on the level of antioxidant activity assessed by pertinent biomarkers. Individual genetic information should also be taken into consideration when giving such instructions. Such health issues may become central to medical care in the 21st century.

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