
Measurement and Use of Antioxidants in Human Medicine

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Introduction

In previous publications (Medi-Sphere n^{os} 55 and 62, 1997) we have shown that oxygen, an element indispensable to life, can under certain circumstances become a lethal danger for the organism. This happens when highly reactive activated oxygen species are formed (free radicals⁴, hydrogen peroxide, singlet oxygen, hypochlorous acid). Once formed, activated oxygen species (AOS) can induce breaks in DNA, inactivate proteins, or induce lipid peroxidation (attack of polyunsaturated fatty acids) leading to a loss of cell membrane permeability.

Although AOS can, in some cases, have a physiological role (white blood cells destroy bacteria via intracellular AOS production), they nevertheless induce irreversible cell damage (oxidative stress) when produced in excess (as in inflammation, where endotoxin-activated white blood cells secrete AOS into the extracellular medium, where they attack healthy tissues).

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⁴ As mentioned in prior publications, a free radical is a molecule characterised by the presence of a free (unpaired) electron in its electron structure. This type of molecule is chemically unstable, and this makes it highly reactive towards a wide variety of biological substrates. The superoxide anion $O_2^{\bullet-}$ and the hydroxyl radical OH^{\bullet} (the dot indicates the presence of the free electron) are two examples of free-radical products of oxygen metabolism.

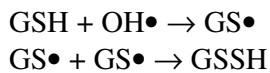
Our organism produces AOS continuously. It has been calculated, for instance, that the DNA of a single cell undergoes 10,000 free-radical attacks each day. To control or limit the harmful effects of AOS, our organism has developed a battery of antioxidant defences. These are what enabled living organisms in the first place to adapt to living in an oxygen-rich environment.

An effective antioxidant defence system

Generally speaking, an antioxidant can be defined as a substance which, when present at low concentration as compared to a substrate, can significantly delay or inhibit the substrate's oxidation. Antioxidant defences include:

- a primary defence system consisting of enzymes and antioxidant compounds. The function of these enzymes and compounds is to prevent the initiation or propagation of free-radical reactions. They include:
 - a) superoxide dismutase (SOD), which reduces the half-life of the superoxide anion $O_2^{\bullet-}$. There exist three types of SOD: one containing copper and zinc (CuZnSOD), located in the cytosol of eukaryotic cells and in red blood cells; one containing manganese (Mn), located in the mitochondria, and a high-molecular-weight factor displaying SOD activity (EC-SOD), located in human plasma and lungs;

- b) catalase, which converts hydrogen peroxide (H_2O_2) to a simple water molecule. It is present mainly in the peroxisomes of various cells, in platelets, and in the stroma of red blood cells;
- c) glutathione peroxidase (GPx) destroys both hydrogen peroxide and all lipid peroxides ($ROOH$, where R represents and unsaturated fatty acid). It is a seleno-enzyme (SE-GPx) using reduced glutathione (GSH) as a coenzyme and located in the cytosol and mitochondria of cells;
- d) the reader is reminded that iron in its free form plays, as a catalyst, a preponderant role in the initiation and maintenance of free-radical reactions. The iron storage proteins transferrin and ferritin keep this metal in an inactive form;
- e) the destruction of AOS (particularly free radicals) is usually carried out by antioxidant molecules or scavengers that react directly with free radicals to form oxidised derivatives. Thus glutathione (GSH) reacts with the hydroxyl radical $OH\bullet$ according to the reaction:

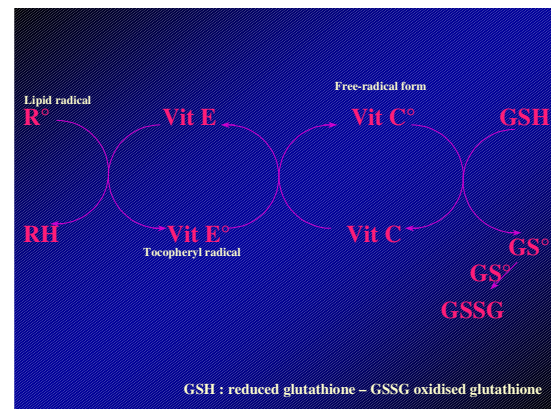


Other scavengers include uric acid, bilirubin, glucose, proteins bearing a thiol group (SH), provitamin A (β -carotene) and vitamins C (ascorbic acid), E (α -tocopherol), and Q (coenzyme Q-10 or ubiquinone). Because it is hydrophobic, vitamin E can insert into biological membranes where it acts to prevent the propagation of free-radical reactions involving lipids. In addition to its antioxidant properties, coenzyme Q-10, present at particularly high concentration in mitochondria, is an indispensable element of a cell's energy production chain.

Remarkably, these antioxidants can combat free radicals synergistically, as shown in Figure 1.

- a secondary defence system consisting of lytic enzymes whose role is to prevent intracellular accumulation of oxidised proteins or DNA and to degrade their toxic fragments. Phospholipases, DNA endonucleases and ligase, and macroxyproteinases are among the enzymes taking part in this last line of defence against AOS.

Figure 1: upon interacting with a lipid radical $R\bullet$, vitamin E (Vit E) is converted to the tocopheryl radical (Vit E \bullet). Vit E is regenerated through the action of vitamin C (Vit C) which, in turn, converts to a free-radical form (Vit C \bullet). Reduced glutathione (GSH) promotes Vit C regeneration through conversion to a thiyl radical (GS \bullet). The latter, upon reacting with a molecule of like kind, yields oxidised glutathione (GSSG).

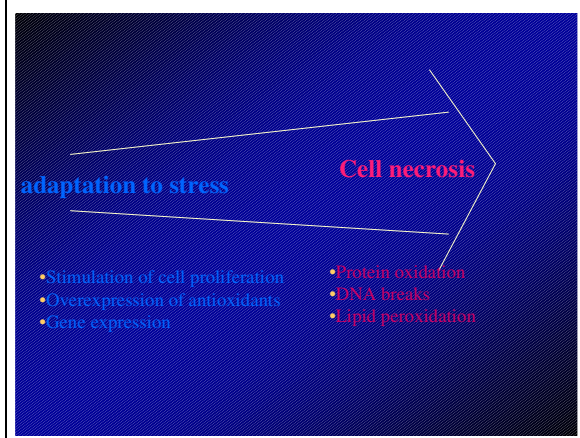


How a cell responds to AOS production

When produced in low amounts, AOS trigger in the cell an adaptive stress response: increased cell growth, overexpression of antioxidant enzymes (superoxide dismutase), and expression of genes coding for a variety of proteins.

When overproduced, AOS attack all the above-mentioned biological substrates, destroy the cell's antioxidant defences, and, through their toxic action, cause often-irreversible cell damage leading to tissue necrosis (Figure 2).

Figure 2: Intensity of oxidative stress



Antioxidant defences and disease

Over a hundred pathologies have been linked to overproduction of AOS leading to major alterations of antioxidant defences. An evaluation of the latter may thus be useful in diagnosis (Table 1), in the search for adequate therapies, and in monitoring the evolution of patients.

Enzyme antioxidants are usually assayed in red blood cells, whilst scavengers are analysed in the plasma of patients. Several firms have recently developed quick-assay kits for SOD and GPx in red blood cells. It is noteworthy that because GPx is a selenium-dependent enzyme, its level accurately reflects the level of this oligoelement. It is possible to determine vitamin C, uric acid, thiol proteins, and bilirubin in plasma by means of a simple colorimetric test, whereas it takes a more sophisticated technique called liquid-phase column chromatography to measure levels of β -carotene, **vitamin E**^{*}, and ubiquinone. As a complement to these determinations, it is also possible to measure an individual's overall antioxidant status, using the following method (Figure 3).

The presence of antioxidants in the plasma enables it, *in vitro*, to inhibit free radical reactions involving the attack of various targets and production of oxidation products

^{*} To evaluate correctly the vitamin E concentration, it is necessary to express it with respect to the total lipid or cholesterol concentration.

that are readily measurable by spectrophotometry. Any decrease in this inhibitory power means that endogenous antioxidants have been consumed through their interaction with AOS produced *in vivo*.

Antioxidant therapy in human clinical practice

In issue no. 55 of Medi-Sphere, we overviewed the various antioxidants that can be used clinically in cases of various pathologies where natural defences are largely destroyed. These antioxidants fall into four categories: molecules interacting directly with AOS (antioxidant enzymes, β -carotene, vitamins C and E, ubiquinone, flavonoids, glutathione, probucol, lipoic acid, etc.), molecules or cofactors reinforcing antioxidant defences (N-acetylcysteine, selenium, copper), molecules maintaining iron in an inactive state (Desferal[®], hydroxypyridines), and inhibitors of enzymes responsible for the formation of AOS (allopurinol).

Here are a few illustrative examples taken from the literature. Daily administration of 40 mg/kg Desferal[®] delays significantly the progression of HIV infection to full-blown acquired immunodeficiency syndrome (AIDS). Continuous daily intake of 600 mg N-acetylcysteine improves the quality of life of patients suffering from chronic destructive lung disease. In kidney transplant patients, injection of a vitamin cocktail (500 mg Vit C, 1 mg Vit E, 5 mg tocopherol acetate, and 5.5 mg retinol palmitate) just before reperfusion of the organ improves significantly the immediate functions of the kidney. After a myocardial infarction, administration of 300 mg allopurinol reduces cell damage subsequent to reperfusion of the heart.

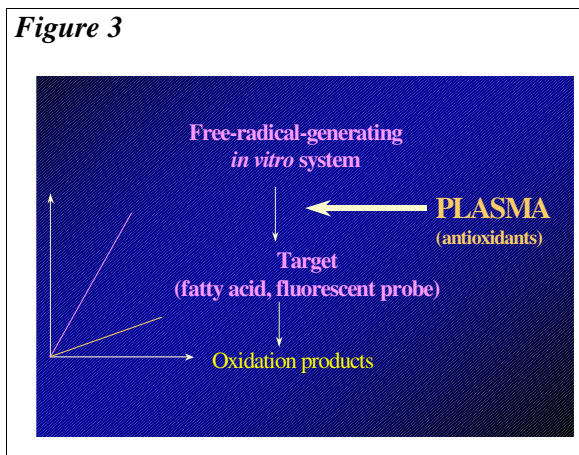
Antioxidants and diet

It is impossible to stop AOS formation because it is a normal process of our organism. In addition, our industrialised world imposes a lifestyle (prolonged exposure to sunlight, atmospheric pollution, ozone, work stress, radiation, pesticides, tobacco, alcohol, intake of various medicines) that triggers free-radical

Table 1: Examples of human pathologies associated with an altered antioxidant status

Evolution of the antioxidant	Pathological situation
Decreased vitamin C	Respiratory diseases (emphysema, chronic obstructive broncho-pulmonary disease, smokers) Acute pancreatitis Haemochromatosis
Decreased vitamin E	Respiratory distress syndrome Organ transplants Coronary bypass Septic shock Head trauma Haemochromatosis
Decreased glutathione, SH-proteins	Acquired immunodeficiency syndrome Respiratory distress syndrome
Decreased ubiquinone	Hyperlipidaemia
Increased uric acid	Ischaemia-reperfusion phenomena
Decreased total antioxidant capacity	Respiratory diseases (asthma, chronic obstructive broncho-pulmonary disease, smokers) Liver disease Premature birth
Decreased GPx, selenium deficiency	Cancer Alcoholism Mucoviscidosis Keshan's disease Cardiovascular diseases Sterility Crohn's disease Rheumatoid arthritis Cataract (lens)
Increased SOD	Leukaemia Hepatitis Diabetes Duchenne's muscular dystrophy Respiratory distress syndrome Down's syndrome
Decreased SOD	Rheumatoid arthritis Fanconi's anaemia Hypocuprosis Immunodeficiency syndromes

Figure 3



A healthy, varied diet (vegetables, fish, soy oil, fruit), which normally should supply all the antioxidants we need, is thus no longer sufficient to counter the harmful effects of AOS. This situation is all the more alarming because changes in cultivated soils and increasingly poor eating habits have dire consequences: the usual diet in Northern Europe contains fewer and fewer vitamins and oligoelements. In England, for instance, the average daily intake of selenium, cofactor of glutathione peroxidase, has dropped from 60 µg to 30 µg in less than 25 years, whereas the World Health Organisation recommends a daily intake of 50 to 200 µg. In 1985, a Finnish study of 12,000 subjects demonstrated a higher cancer risk among individuals deficient in selenium and vitamin E than in individuals displaying normal levels of these antioxidants.

In the light of this information, it is conceivable that the intake of antioxidants independently of the diet might be beneficial to our organism. Currently the French SUVIMAX study (SUPplémentation en VITamines et MINéraux AntioXidants - 15,000 subjects) is testing the hypothesis that daily ingestion of β-carotene (6 mg), vitamins C (120 mg) and E (15 mg), zinc (20 mg), and selenium (100 mg) might prevent the appearance of certain heart diseases and cancer. The final results of this study won't be known until 2002.

One should bear in mind, however, that the massive use of antioxidants can have harmful effects. Whatever the antioxidant used, it can

reactions in our organism over and above what Nature has equipped us to deal with. For example, antioxidant levels (vitamins C and E, selenium) are distinctly lower in smokers than in non-smokers because a puff on a cigarette produces some 10^{19} free radical. become, at high concentration, a pro-oxidant capable of initiating lipid peroxidation (notably by interacting with iron). This is notably the case of vitamin C. Minerals (selenium, zinc) at high dosage are also toxic. It is thus advisable to avoid vitamin abuse, a practice that has become particularly noticeable in the United States. Aware that their diet is poor, Americans do not hesitate to swallow up to 60 vitamin complexes daily. This practice, akin to drug abuse, can be very detrimental to the organism, as stressed by Professor Herczberg, head of the SUVIMAX study.

Conclusions

Evaluation of a patient's antioxidant status should contribute to better diagnosis of certain diseases (Table 1) and enable doctors to correct specific antioxidant deficiencies. On the other hand, doctors might be led to recommend preventive antioxidant treatment in some cases, e.g. for patients about to undergo major surgery causing serious damage to antioxidant defences through massive AOS production. In patients having undergone organ transplantation or coronary bypass surgery requiring heart-and-lung bypass, we have shown that the blood level of vitamin E, a crucial antioxidant in the fight against AOS, decreases by half in the post-op period. This might justify appropriate antioxidant treatment for two weeks prior to the operation.

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