Oxidative Stress: How To Detect It, Cope With And Treat Based On Evidence

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Abstract

INTRODUCTION

We have reasonable evidence that reactive oxygen species (ROS) play a key role in several pathological conditions. Monitoring oxidative stress in humans is achieved by assaying products of oxidative damage or by investigating the potential of an organism, tissue or body fluid to withstand further oxidation. Unfortunately there is little consensus about the selection of parameters of oxidative stress or antioxidant state in defined patients or diseases. This is not only due to the uncertainty whether or not a certain parameter is playing a causative role. Moreover, the methods of determination described in the literature represent very different levels of analytical practicability, costs and quality. Generally, accepted reference ranges and interpretations of pathological situations are lacking as well as control materials. Nowadays the situation is changing dramatically and sophisticated methods like High Performance Liquid Chromatography (HPLC) and immunochemical determinations have become more and more common standard. Test kits for photometric determinations applicable to small laboratories or Point of Care laboratories are increasingly available. In the screening of such simplified procedures, we found the Fort (Free Oxygen Radicals Testing) and Ford (Free Oxygen Radicals Defence) methods very suitable in clinical practice, because of their reliability and ease of use. Fort is a colorimetric test based on the properties of an amine derivative employed as chromogen, i.e. $CHNH_2$ (4-Amino-N-ethyl-N-isopropylaniline hydrochloride), aiming at producing a fairly long-lived radical cation. When the sample is added to a $CHNH_2$ solution, the coloured radical cation of the chromogen is formed and the absorbance at 505 nm, which is proportional to the concentration of hydroperoxyl molecules, is associated to the oxidative status of the sample.

The visible spectrum of the $CHNH_2$ radical cation shows two peaks of absorbance at 505 and 550 nm. The overall spectral intensity increases with time. The reactions occurring in the Fort test conditions are based on the capacity of transition metals to catalyse the breakdown of hydroperoxides (ROOH) into derivative radicals, according to the Fenton's reaction. Once they are formed, ROOH maintain their chemical reactivity and oxidative capacity to produce proportional amounts of alkoxy ($RO^{\cdot}$) and peroxy ($ROO^{\cdot}$) radicals. These derivative radicals are then preferentially trapped by a suitably buffered Fort chromogen and develop (in a linear kinetic based reaction at 37°C) a coloured fairly long-lived radical cation which is photometrically detectable. The intensity of the colour correlates directly with the quantity of radical compounds, according to the Lambert-Beer's law and it can be related to the oxidative status of the sample.

$$R\text{-}OOH + Fe^{2+} \rightarrow R\text{-}O^{\cdot} + OH^{-} + Fe^{3+}$$

$$R\text{-}OOH + Fe^{3+} \rightarrow R\text{-}OO^{\cdot} + H^{+} + Fe^{2+}$$

$$RO^{\cdot} + ROO^{\cdot} + 2CrNH_2 \rightarrow RO^{-} + ROO^{-} + [Cr\text{-}NH_2{}^{+\cdot}]$$

Ford is a colorimetric test based on the ability of plasma antioxidants to reduce a preformed radical cation. The principle of the assay is that, at an acidic pH and in the presence of a suitable oxidant solution ($FeCl_3$), 4-amino-N,N-diethylaniline, the FORD chromogen, can form a stable and colored radical cation.

Antioxidant molecules (AOH) present in the sample which are able to transfer a hydrogen atom to the Ford chromogen radical cation, reduce it quenching the colour and producing a decolouration of the solution which is proportional to their concentration in the sample. Preliminary experiments

showed that the choice of oxidant solution and the ratio between the concentration of the chromogen substance and the concentration of the oxidative compound are essential for the effectiveness of the method.

$$\text{Chromogen}_{\text{(uncoloured)}} + \text{oxidant } (Fe^{3+})\ H^{+} \rightarrow \text{Chromogen}^{\cdot+}_{\text{(purple)}}$$

$$\text{Chromogen}^{\cdot+}_{\text{(purple)}} + AOH \rightarrow \text{Chromogen}^{+}_{\text{(uncoloured)}} + AO$$

The UV-visible spectrum of the Ford chromogen radical cation shows maximum absorbance at approximately 330 nm, 510 nm and 550 nm.

ROS AND AGEING

The ageing process is very complex. It is determined by a genetic component but also lifestyle and environmental factors. Fifty years ago, Harman (1) suggested that the accumulation of oxidants could explain the alteration of physical and cognitive functions of ageing. Oxygen metabolism leads to reactive species production, including free radicals, which tend to oxidize the surrounding molecules such as DNA, proteins and lipids. Oxidative stress is an adaptive process which is triggered upon oxidant accumulation and which comprises the induction of protective and survival functions. Experimental evidence shows that the ageing organism is in a state of oxidative stress, which supports the free radical theory.

ROS AND CARDIO-CEREBROVASCULAR DISEASES

Common vascular risk factors, including hyperlipidaemia, hypertension, cigarette smoking, diabetes, obesity, physical inactivity, age, gender and familiar predisposition, only partially explain the excessive risk of developing cerebrovascular and coronary heart disease (CHD) and many studies support the role of oxidative stress in their pathogenesis. Atherosclerosis is a chronic pathology involving the deposition of plasma lipoproteins and the proliferation of cellular components in the artery wall that provide a barrier to arterial blood flow. Consistent evidence has supported the theory that free radical-mediated oxidative processes and specific related products play a key role in atherogenesis (2). At the base of this hypothesis are low-density lipoproteins (LDL) which occasionally enter the subendothelial space of arteries. Here LDL are oxidized. The oxidized form of LDL (oxLDL) is able to start mechanism leading to the formation of atherosclerotic plaques as it is taken up by macrophages and induces the release of factors that recruit other cells and stimulate smooth muscle cell proliferation. oxLDL may also up-regulate expression of cellular adhesion molecules that facilitate leukocyte binding. High levels of oxLDL can also down-regulate the expression of endothelial nitric oxide synthase (eNOS), an enzyme synthetizing most of vascular nitric oxide. Stroke is the main cause of disability and mortality in Western countries. Particularly the condition of ischemia and reperfusion occurring after stroke has been shown to be associated with free radical-mediated reactions probably leading to cell death (3). Even if ischemic and haemorrhagic stroke have different risk factors and pathophysiological mechanisms, there is evidence of an increased production of free radicals and other reactive oxygen species in both conditions, leading to oxidative stress.

ROS, METABOLIC SYNDROME AND OBESITY

Associations between obesity and markers of oxidative stress and the lipid sensitivity leading to oxidative modification have been observed in humans (4, 5). The processes that underlie observed associations between obesity and oxidative stress are unclear, even if several theories have been proposed. For example, it has been suggested that oxidative stress in obesity may result, partly, from the accumulation of intracellular triglycerides (6). In particular, intracellular triglycerides are supposed to elevate superoxide radical generation within the electron transport chain by inhibiting the mitochondrial adenosine nucleotide transporter. The inhibition of this transporter leads to a diminution in intra-mitochondrial adenosine diphosphate (ADP) that, in turn, reduces proton flux. It has been suggested that in diabetes the production of oxidative stress may be mostly due to hyperglycaemia (7, 8). The generation of free radicals correlated to chronic hyperglycaemia may result from non-enzymatic glycation (9) and glucose autooxidation (10). It has been hypothesized that hyperinsulinaemia may directly induce intracellular generation of free radicals (11). The effects of an acute increase in glycaemia on plasma antioxidant defences have also been investigated (12). Some studies report that the use of antioxidants can neutralize some effects acutely induced by hyperglycaemia, such as vasoconstriction (8, 13), activation of coagulation and the increase in intercellular adhesion molecule-1 (ICAM-1) plasma level (11).

ROS AND NEURODEGENERATIVE DISORDERS

Oxidative stress has been implicated as a common pathogenetic mechanism in various neurodegenerative diseases. Central nervous system is particularly exposed to free radical injury, given its high metal content, which can catalyze the formation of ROS, and the relatively low

content of antioxidant defences. Oxidative stress damage is also intimately correlated to glutamate neurotoxicity, known as “excitotoxicity”. An excessive concentration of extracellular glutamate over activates glutamate receptors, leading to very high intracellular calcium levels and a cascade of events resulting in neural cell death (14).

Alzheimer’s disease (AD) represents the most prominent cause of dementia in the elderly and is clinically characterised by memory dysfunction, loss of lexical access, spatial and temporal disorientation and impairment of judgement. Histopathologically, AD is characterised by synaptic and nerve cell loss, extracellular deposition of -amyloid protein forming senile plaques and intracellular precipitation of tau protein. The exact biochemical mechanism of the pathogenesis of AD is still unknown, but much attention is given to the role of the massive loss of the neurotransmitter acetylcholine (necessary for cognition and memory) and to the possible involvement of oxidative stress in its development (15). AD is essentially an acceleration of the ageing mechanism in affected brain regions that become progressively more damaged by free radicals.

Parkinson’s disease (PD) is clinically characterised by bradykinesia, postural inability, gait difficulty and tremor. It is the result of neurodegeneration occurring in specific brain areas (substantia nigra and striatum) and of dopamine depletion. The processes of cell death in PD have not yet been fully elucidated, but increased oxidative stress, abnormal mitochondrial function and excitotoxicity are perhaps among the most relevant initiators or mediators of neuronal damage. Amyotrophic lateral sclerosis (ALS) or Lou Gehrig’s disease is a progressive, fatal neurodegenerative disease characterized by gradual degeneration of motor neurons in the cortex, brainstem and spinal cord. Motor neurons are responsible for supplying electrical stimulation to the muscles necessary for the movement of body parts. The cause of sporadic ALS is unknown; however, in about 10% of all ALS cases, the disease is familial (FALS). About 20% of FALS cases are associated with mutations and lowered activity of Cu/Zn superoxide dismutase (SOD1). SOD1 catalyses the formation of hydrogen peroxide through the dismutation of superoxide radical anions playing a relevant role in regulating oxidative damage to cells.

ROS AND LUNG

The lung function is to exchange gases between the body tissues and the outside environment. In normal conditions (when an individual is in good health and resides in a relatively clean environment) the lung represents a unique tissue for oxidative stress, continually exposed to relatively-high O_2 tension, pollutants and the metabolic products derived from them. Inhalation of airborne irritants and pollutants, including cigarette smoke, ozone, carcinogens (e.g. diesel exhaust), other chemicals and dust particles, will produce excess ROS and reactive nitrogen species (RNS) in the lung. Additionally, such exposures lead to depletion of endogenous antioxidants (16).

ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Asthma is a chronic relapsing inflammatory disease of the airways. As inflammatory cells produce and release ROS, asthmatic airways are responsible for oxidative stress. Convincing evidence confirms that acute asthma in an adult is accompanied by oxidative and nitrosative stress (17). Exhaled air of patients with asthma contains high levels of some markers of oxidative stress; many studies have shown that inflammatory cells from peripheral blood and bronchoalveolar lavage fluid of asthmatic subjects produce more superoxide anion radicals than the ones from controls. Further evidence for an oxidant-antioxidant imbalance is provided by the finding of a decreased antioxidant capacity in plasma and bronchoalveolar lavage fluid of patients with asthma (18). Chronic obstructive pulmonary disease (COPD) is characterised by the progressive decline of lung function and the presence of airflow obstruction as a result of chronic bronchitis or emphysema. The single most important aetiological factor in the pathogenesis of this disease is cigarette smoke. It is estimated that 90% of all patients with COPD are, or have been, smokers but only about 20% of cigarette smokers develop the condition. As oxidants play a relevant role in cigarette smoke-induced lung damage, pulmonary antioxidant status defence mechanisms have a great importance. There is evidence that plasma antioxidant capacity is decreased in smokers and in association with exacerbations of COPD, supporting evidence of a depletion of vitamin C, E and other serum antioxidants. Various therapies lead to free-radical-induced tissue damage, i.e. O_2 therapy for the treatment of prematurely-born neonates, acute respiratory distress syndrome (ARDS) and chemotherapy and radiation therapy for cancer patients.

ROS AND LIVER DISEASE

Steatohepatitis can cause fibrosis and cirrhosis and ultimately lead to liver failure and hepatocellular carcinoma in a minority of patients (19, 20, 21, 22). The pathogenesis

of steatohepatitis remains unclear and the factors, which cause the progression from bland steatosis to steatohepatitis, often termed the “second hit”, remain poorly understood (23, 24). Oxidative stress is one of the potential biochemical mechanisms involved in the pathogenesis of steatohepatitis (25, 26).

ROS AND GASTRODUODENAL DISEASES

Helicobacter pylori is an important agent in the pathogenesis of active chronic gastritis, peptic ulcer, low-grade gastric MALT lymphoma and gastric carcinogenesis (27). Generation of cytotoxins, urease, ammonia, T-cell mediated damage and a mainly humoral reaction are among the events which involve mucosal integrity following Helicobacter pylori infection (28). Moreover, reactive oxygen metabolites have been found to play a relevant role in gastroduodenal inflammatory damage (29). Particularly, the acute mucosal inflammatory infiltrate (e.g. polymorphonuclear cells), which characterizes Helicobacter pylori-related chronic active gastritis, could be an important source of free radicals (30), as both in vivo an in vitro studies have reported a positive relation between Helicobacter pylori infection and reactive oxygen metabolites generation (29, 31)

ROS AND VIRUS INFECTIONS

Acquired Immune Deficiency Syndrome (AIDS) is the terminal phase of Human Immunodeficiency Virus (HIV) infection when it runs its natural course. Oxidative stress plays a relevant role in HIV infections. HIV-infected patients are in oxidative imbalance early in the disease (serum and tissue antioxidant levels are low and peroxidation products elevated). High plasma levels of malondialdehyde (MDA), reduced plasma glutathione (GSH), decreased glutathione peroxidase (GPx) and superoxide dismutase activities are normally found. HIV infection also results in considerably reduced vitamin E and C concentrations and very low plasma Zn and Se levels. Particularly, Se deficiency is related to the occurrence, virulence and disease progression of some virus infections including HIV progression to AIDS.

ROS, ARTHRITIS AND RHEUMATOID ARTHRITIS

ROS and RNS can directly or indirectly be responsible for joint damage and lead to the clinical expression of inflammatory arthritis. Several factors are involved in the development of oxidative stress in the joints of RA patients. ROS generation from locally activated leukocytes is followed by a pressure increase in the synovial cavity (32), a capillary density reduction, vascular changes and an increased metabolic rate of synovial tissue.

ROS AND HEARING LOSS

ROS have been implicated in hearing loss associated with ageing and noise exposure. Superoxide dismutases form a first line of defence against damage mediated by the superoxide anion, the most common ROS. Absence of SOD1 has been shown to potentiate hearing loss related to noise exposure and age. Conversely, overexpression of SOD1 may be hypothesized to afford a protection from age- and noise-related hearing loss. This evidence has been investigated by using a transgenic mouse model carrying the human SOD1 gene (33).

ROS AND EYE DISEASES

Oxidative stress mechanisms in ocular tissues have been hypothesized to play a role in diseases such as glaucoma, cataract, uveitis, retrolental fibroplasias, age-related macular degeneration and various forms of retinopathy providing an opportunity for new approaches to their prevention and treatment. The possible sources of increased oxidative stress might include increased generation of free radicals or impaired antioxidant defence system. Dietary supplementation with antioxidants in animal models of diabetic retinopathy inhibits retinal metabolic abnormalities and retinal histopathology, suggesting that oxidative stress is associated with the development of retinopathy. The mechanism by which antioxidants inhibit retinopathy in diabetes warrants further investigation, but animal studies show that therapy with many antioxidants provides significantly more protection if compared to treatment with any single antioxidant. Thus, supplementation with antioxidants represents an achievable adjunct therapy to help preserve vision in diabetic patients (34).

ROS AND CRITICALLY ILL SURGICAL PATIENTS

Nathens et al. (35) determined the effectiveness of early, routine antioxidant supplementation using alpha-tocopherol and ascorbic acid to reduce the rate of pulmonary morbidity and organ dysfunction in critically ill surgical patients. Oxidative stress has been associated with the development of ARDS and organ failure through direct tissue injury and activation of genes integral to the inflammatory response.

ROS AND PREGNANCY DISORDERS, ENDOMETRIOSIS

Oxidative processes exert a fundamental regulatory function

during pregnancy. It depends on the influence of oxygen, nitric oxide, ROS and RNS metabolic pathways upon the vascular changes in the maternal organism, as well as on the regulation of uterine and cervical tone throughout gestation and delivery. Endometriosis is associated with a general inflammatory response in the peritoneal cavity and oxidative stress has been proposed as a potential factor involved in the pathophysiology of the disease. Endothelial nitric oxide synthase, the enzyme that produces nitric oxide, is also overexpressed in endometriosis and adenomyosis. The endometrium shows altered expression of enzymes such as superoxide dismutase and GPx involved in defence against oxidative stress. Also vitamin E levels are significantly lower in the peritoneal fluid of women with endometriosis in the presence of redox-active metal ions while estrogens are established oxidants. This mechanism might be due to estrogen pro-inflammatory effect. In fact hormone therapy results in an increased amount of C-reactive protein, a marker of inflammation (36). It is important to underline that estrogens and their metabolites may exert have both pro-oxidant and antioxidant properties depending on the availability of metal ions and/or their formulation.

ROS AND MENOPAUSE

Lack of estrogens in menopause leads to a redox status imbalance and a change in the lipid profile. The symptoms accompanying the menopause (e.g. hot flushes) suggest that when they are manifest there is an increased metabolic activity (37) which, together with their repetitive character, could cause a redox status imbalance toward oxidative processes. ROS react with many biologic substrates, especially polyunsatured fatty acids leading to lipid peroxidation (increased lipid peroxides in plasma) and membrane destruction. Other components of the biologic response to the presence of reactive oxygen intermediates is the decrease in antioxidants, including reduced sulfhydryl groups.

ROS AND HUMAN INFERTILITY

The correlation between varicocele and male infertility is still obscure. Several factors seem to be involved in sperm modifications: backflow of noxious substances from the kidney or adrenal glands, testicular temperature increased, tissue hypoxia induced by venous stasis, hypothalamic or pituitary dysfunctions (38). Oxidative damage because of the testicular venous backflow may represent one of the causes of gonad injury and seems to precede the histological alteration (39).

ROS AND KIDNEY DISEASES

There is a relevant evidence suggesting that ROS are implicated in the pathogenesis of ischemic, toxic and immunologically-mediated renal injury (40, 41).

CONCLUSIONS

There is now reasonable evidence that ROS play a major role in several diseases, and in the ageing process itself with specific morphopathological traits, through cytokine-mediated inflammation leading to necrosis and final fibrosis. Biochemical investigations, aiming to promptly detect the danger of an oxidative imbalance and restoring antioxidant reservoir before triggering the irreversible damage cascade, are thus mandatory. In the future, they should be performed as routine exams, during standard blood evaluation, and repeated along the treatment plan to monitor the efficacy of antioxidant compounds administered as primary unique therapy or as complementary therapy intervention (like antibiotics in severe infections and sepsis). The improvement of symptoms as well as prevention of inflammatory damage, degenerative diseases or cancer should be the goals of antioxidant therapy with step by step confirmation of the serum red-ox balance restoration. In the future we cannot exclude that an oxidative status check-up, followed by immediate targeted treatment, will help prevent illnesses.

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