

Oxidative Stress in Human Pathology

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ABSTRACT

This review is aimed at presenting a general view on oxidative stress in biology and medicine. The nature of the mediators of oxidative stress, their source and their biological targets are explained in some detail, allowing the reader to understand the link between a defined environmental condition and its biological consequences involving a cascade of undesirable non-specific oxidations when the endogenous antioxidant defences are saturated. Although oxidative stress is associated with many pathological conditions, it is often difficult to evaluate its action as a cause and/or a consequence of a disease. We selected some diseases for which the involvement of oxidative stress has been extensively studied, to illustrate the influence of the matter explained from a theoretical point of view in a whole organism.

Keywords: ageing, antioxidants, cardiovascular diseases, free radicals, lipid peroxidation, lung diseases, neurodegenerative diseases, reactive oxygen species, skin diseases

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; COX, cyclooxygenase; GPX, glutathione peroxidase; PD, Parkinson's disease; ROS, reactive oxygen species; SOD, superoxide dismutase; UV, ultraviolet

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INTRODUCTION

All aerobic organisms require molecular oxygen as an electron acceptor to produce energy from organic fuels. However, oxygen is a strong oxidant, and secondary oxidations not involved in physiological metabolism are thus unavoidable. These random oxidations would have deleterious consequences if they were not neutralised by efficient endogenous antioxidant systems. Indeed, many pathological conditions are associated with the overproduction of reactive oxidant intermediates. Although it is often difficult to know whether the latter are the cause or the consequence of the disease, they always worsen the injuries to biological tissues by denaturing most functional biomolecules and neutralising endogenous antioxidants.

In this review, we tried to present oxidative stress in a practical manner, while being as close as possible to the physical reality of this abstract field.

Since it is impossible to deal with all aspects of oxidative stress in human pathology in a single article, we focused our review on common diseases that have been well documented as regards their association with oxidative stress, and we emphasized new developments in biochemical mechanisms involved in the production and signal transmission of reactive intermediates of oxidative stress.

WHAT IS OXIDATIVE STRESS?

From a classical point of view, oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation (Sies 1991). In other words, when the antioxidant capacity of the organism is saturated, and more and more oxidation reactions occur, there is an unstable situation that can evolve to the degradation of biomolecules, then to tissue injury and finally to the development of a pathological condition. However, we should bear in mind

that antioxidants are not only electron donors, i.e., reductants. Indeed, any chemical species is a reductant towards a more oxidant one, and also an oxidant towards a more reductant one. Thus, depending on the microenvironment, many "antioxidants" can donate electrons to other molecules at much higher concentrations to those at which they exert a biological activity. In particular, in mitochondria, where most of the free radical superoxide is generated under physiological circumstances, "antioxidants" (or "reductants") are involved in redox mechanisms that function as signal transducers. Such systems of signal transduction are very sensitive to the concentrations of oxidants and reductants, and the loss of their regulation can give rise to the overproduction of superoxide and other reactive intermediates. This redox equilibrium is tightly associated to cell signalling, and oxidative stress can be redefined as an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control (Jones 2006).

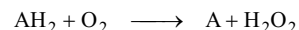
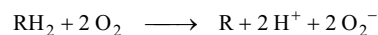
MEDIATORS AND SOURCES OF OXIDATIVE STRESS

The mediators of oxidative stress belong to two main families of molecules, the free radicals and reactive oxygen species (ROS). Some mediators belong to only one of these families, whereas others belong to both of them (Halliwell and Gutteridge 1999; Sorg 2004).

A free radical is an atom or a molecule that possesses at least one unpaired electron. In biology, most free radicals are the result of molecules that have lost one proton and one electron; they are symbolised by "R•". ROS are produced by reduction of molecular oxygen, giving unstable species that react easily with most of biomolecules (Table 1). Since molecular oxygen is a biradical, any reduction is necessarily sequential, giving rise to superoxide, although superoxide can be tightly bound to the reactive complex, before receiving one or more electrons; in these cases, hydrogen peroxide or water are released in the medium, instead of superoxide (Halliwell and Gutteridge 1999; Sorg 2004).

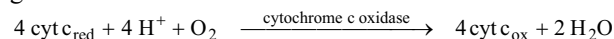
Several enzymatic reactions can generate ROS. Superoxide is usually produced during reactions catalysed by oxidases. These enzymes catalyse the transfer of two protons and two electrons from a substrate to molecular oxygen (or dioxygen). Depending on the enzymes and the microenvironment, superoxide or hydrogen peroxide are released in

the medium:

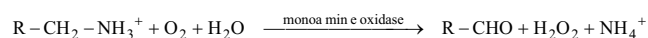
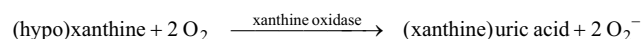
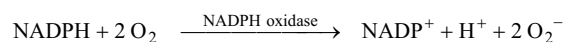
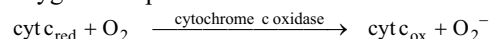


Examples of oxidases that account for a large part of superoxide are cytochrome c oxidase, NADPH oxidase, xanthine oxidase and monoamine oxidases.

Normally cytochrome c oxidase reduces molecular oxygen to water in a four-electron redox reaction:

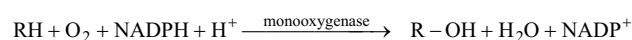


Sometimes cytochrome c oxidase reduces molecular oxygen to superoxide in a one-electron redox reaction:

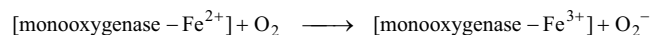


Monoxygenases (cytochromes P450) represent a large family of enzymes that catalyse the hydroxylation of lipophilic substrates, and contribute to the catabolism and elimination of xenobiotics as phase I enzymes. They introduce one of the two oxygen atoms of molecular oxygen to lipophilic substrates. The four electrons needed to complete this reaction come from the substrate and NADPH. Occasionally, dioxygen receives one electron from the enzyme, and is released as superoxide:

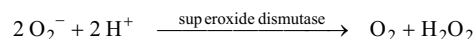
normally:



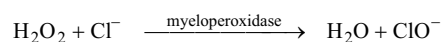
sometimes:



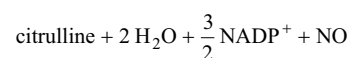
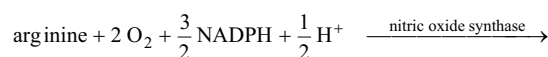
Superoxide dismutase, an antioxidant enzyme that scavenges superoxide, catalyses the dismutation of superoxide into oxygen and hydrogen peroxide:



Myeloperoxidase, an enzyme mostly found in activated neutrophils, produces hypochlorite from hydrogen peroxide and chloride:



Nitric oxide synthases, found as constitutive and inducible forms, produce the intercellular and vasoactive messenger nitric oxide in a complex reaction that oxidises arginine by oxygen (Halliwell and Gutteridge 1999; Sorg 2004):



The metabolism of arachidonate, released from membrane phospholipids following phospholipase A₂ activation, generates bioactive lipids such as prostaglandins, thromboxanes, leukotrienes and hydroperoxyeicosatetraenoates; this process requires the incorporation into arachidonate of several oxygen atoms from dioxygen, and is highly susceptible to release superoxide and hydrogen peroxide (Pompeia *et al.* 2003) (Fig. 1).

Table 1 Name and structure of ROS.

Name	Formula	Free radical	Structure
Superoxide	O_2^-	Yes	$\bullet\text{O}-\text{O}^-$
Hydroxyl radical	OH	Yes	$\bullet\text{OH}$
Alcoyl radical	RO	Yes	$\text{R}-\text{O}\bullet$
Peroxyl radical	ROO	Yes	$\text{R}-\text{O}-\text{O}\bullet$
Nitric oxide	NO	Yes	$\bullet\text{N}=\text{O}$
Hydrogen peroxide	H_2O_2	No	$\text{HO}-\text{OH}$
Singlet oxygen	$^1\text{O}_2$	No	$\text{O}=\text{O}$
Ozone	O_3	No	$\text{O}=\text{O}-\text{O}^{\delta-} \longleftrightarrow \text{O}^{\delta+}-\text{O}-\text{O}^{\delta-}$
Peroxynitrite	ONOO^-	No	$\text{O}=\text{N}-\text{O}-\text{O}^-$
Hypochlorite	ClO^-	No	$\text{Cl}-\text{O}^-$

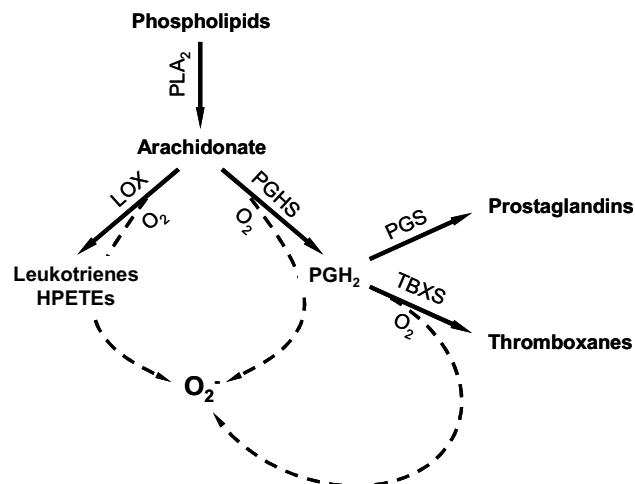
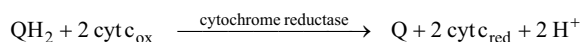


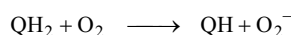
Fig. 1 Production of ROS during arachidonate metabolism. Abbreviations: HPETEs, hydroperoxyeicosatetraenoic acids; LOX, lipoxygenases; PGH₂, prostaglandin H₂; PGHS, prostaglandin H synthase; PGS, prostaglandin synthases (other than PGHS); PLA₂, phospholipase A₂; TBXS, thromboxane synthases.

ROS are produced during non-enzymatic reactions, too. Quinolic species such as ubiquinol (coenzyme Q) are susceptible to successive one-electron oxidations from dioxygen, and thus produce superoxide:

normally:



sometimes:

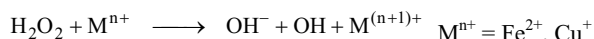


sometimes:

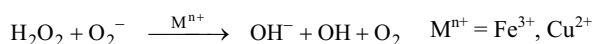


Partial reduction of hydrogen peroxide by reduced transition metals such as copper or iron (Fenton reaction) or by superoxide and catalysed by oxidised transition metals (Haber-Weiß reaction) can generate the strong oxidant hydroxyl radical:

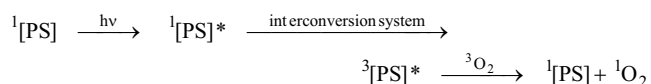
Fenton:



Haber-Weiß:



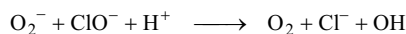
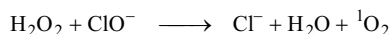
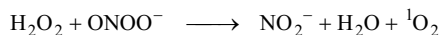
Singlet oxygen, the non-radical excited form of dioxygen, can be produced via the activation of photosensitizers (PS) such as retinoids, flavins or porphyrins: the photosensitizer (singlet state) absorbs UV or visible light and becomes excited, undergoes a spin inversion and becomes a biradical (triplet state), and then returns to its basal (singlet) level by reaction with triplet (biradical) oxygen to generate singlet (non-radical, excited) oxygen (Halliwell and Gutteridge 1999; Sorg 2004):



Two primary ROS can react together to generate secondary ROS. In particular, the reaction between the two radicals nitric oxide and superoxide gives rise to the strong oxidant peroxynitrite (also called oxoperoxonitrate (1-)):



On the other hand, the reaction between hydrogen peroxide and peroxynitrite or hypochlorite produces singlet oxygen, whereas the reaction between superoxide and hydrogen peroxide (Haber-Weiß) or hypochlorite can generate the hydroxyl radical (Khan 1995; Halliwell and Gutteridge 1999; Sorg 2004):



Pathology and oxidative stress

Many pathologies are associated with oxidative stress. The latter can be a cause or an aggravating consequence of the disease. In the case of inflammation, if an oxidative stress can induce inflammation, it is well known that inflammatory processes generate ROS, which participate in the defence against invading pathogens. The cornerstone of these processes is the activation of neutrophils, which produce several ROS following the activation of inducible nitric oxide synthase, NADPH oxidase and myeloperoxidase. The primary ROS produced by these enzymes (nitric oxide, superoxide and hypochlorite) can then react together to generate secondary ROS such as peroxynitrite, singlet oxygen and hydroxyl radical.

Various xenobiotics can generate ROS during their metabolism. For instance, the toxicity of carbon tetrachloride is due to its metabolism by the liver into trichloromethyl radical, which can by itself induce the peroxidation of lipids or can be further metabolised to oxidant species such as hydroxylamines (Recknagel *et al.* 1989). Paracetamol (acetaminophen) is metabolically activated by cytochrome P450 enzymes to N-acetyl-*p*-benzoquinone imine. This species is normally detoxified by glutathione, but following a toxic dose of paracetamol (>10 g), glutathione is depleted and ROS are produced (Hinson *et al.* 2004). Quinolic species such as the aglycones divicine and isouramil from the glycosides vicine and convicine found in fava beans are oxidised by oxygen to their quinone derivatives, thus producing superoxide and depleting erythrocyte glutathione in people (men) having a glucose-6-phosphate dehydrogenase deficiency, followed by haemolytic anaemia (favism) (Chevion *et al.* 1982).

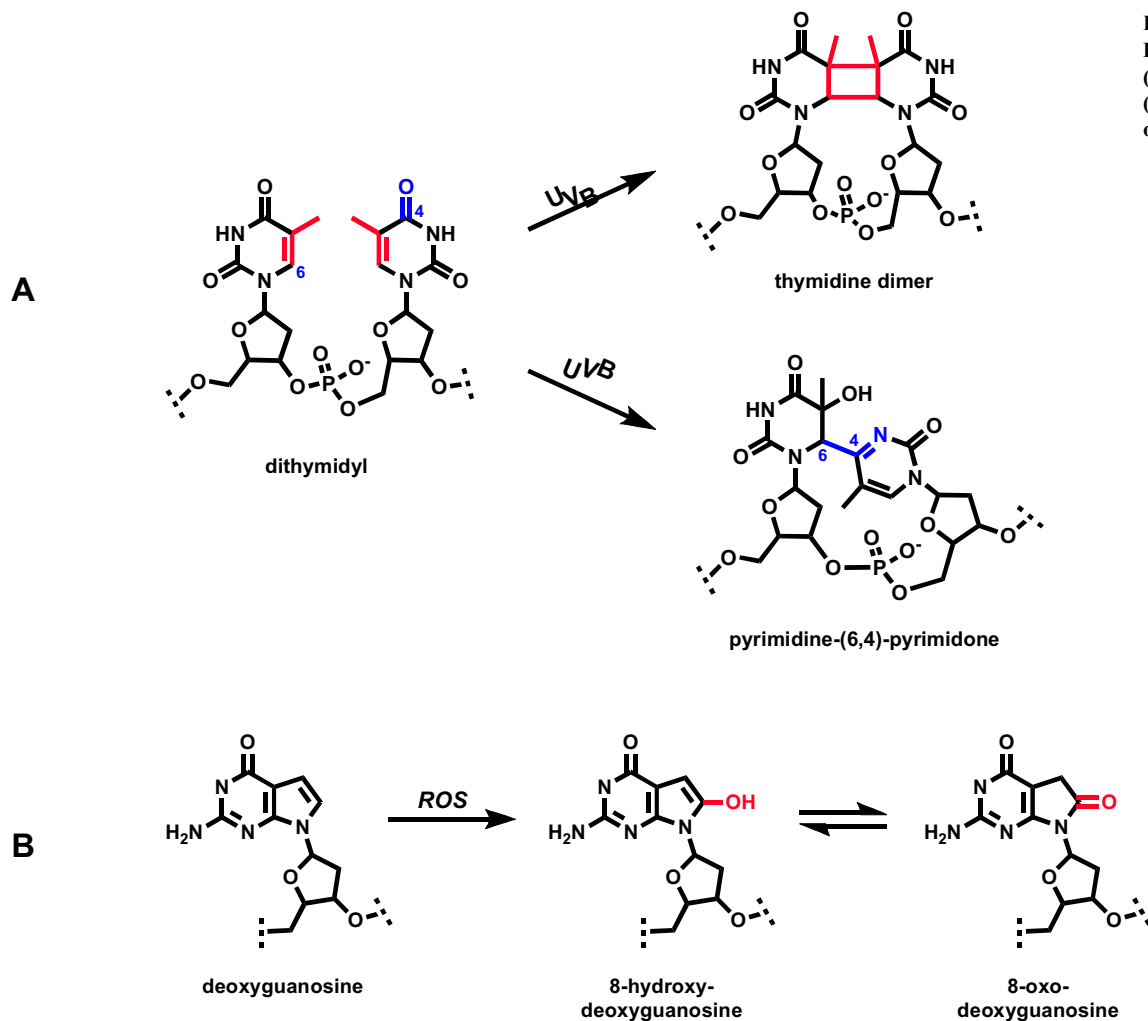
Ionizing radiations such as X rays and radioisotope radiations have enough energy to remove electrons from atoms and molecules, producing free radicals. On the other hand, the energy of visible and ultraviolet light received at the Earth's surface from the sun are too low to produce free radicals directly, but can generate ROS via activation of cellular chromophores (Schaich 1980).

TARGET MOLECULES OF OXIDATIVE STRESS

DNA

DNA, which absorbs electromagnetic radiations with a maximum at 260 nm, is a primary target of UVB light (280-320 nm) and a secondary target of UVA light (320-400 nm). Following UVB light exposure, two main photoproducts are observed at DNA sites with two adjacent pyrimidines: the pyrimidine dimers and pyrimidine-(6,4)-pyrimidone ((6,4)-photoproducts) (Fig. 2A). UVA light can induce the oxidation of guanine bases at the 8-position (Fig. 2B). These DNA damages can be repaired, but if the degree of damage is too high, complete reparation is impossible, leading to DNA mutations; in this case the cell becomes apoptotic or can escape apoptosis and keeps its DNA mutation, a situation potentially deleterious for the organism (Toyokuni 1999; Cadet *et al.* 2001).

Fig. 2 Generation of DNA photoinduced (A) and oxidative (B) products during oxidative stress.



Proteins

The alpha carbon of aminoacids is susceptible to hydroxylation and peroxidation by ROS, in particular the OH radical (Fig. 3). Cysteine and selenocysteine residues (the latter being found at the active site of the antioxidant enzymes glutathione peroxidases and thioredoxin reductases, for instance) are susceptible to oxidation by ROS, leading to the corresponding (seleno)sulphenic, (seleno)sulphinic and (seleno)sulphonic acids. The other sulphur containing amino-acid, methionine, is easily oxidised into methionine sulfoxide. Some aminoacids can be oxidised into peroxides, other are susceptible to carbonylation. The aromatic ring of phenylalanine, tyrosine and tryptophan can be hydroxylated

at various positions. Tryptophan can give its natural metabolites kynurenine and N-formylkynurenine by non-enzymatic reactions with ROS (Berlett and Stadtman 1997; Dalle-Donne *et al.* 2003, 2006) (Table 2).

Lipids

Lipids, especially unsaturated ones, are highly susceptible to peroxidation following a radical process initiated by free radicals such as hydroxyl or lipid radicals. The primary lipid radical can then react easily with dioxygen, which is a bi-radical, giving a peroxy radical. The process continues itself as long as there is oxygen in the system. The end products can be lipid hydroperoxides, malondialdehyde or con-

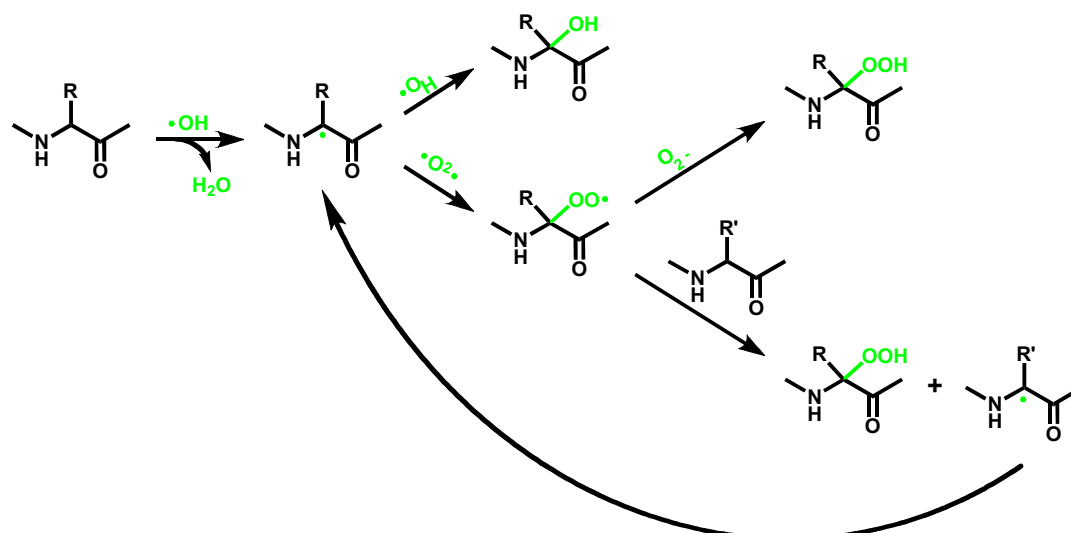


Fig. 3 Mechanism of protein oxidation by ROS.

Table 2 Oxidation of amino acids by ROS.

Amino acid	Oxidation products
Cys-SH	Cys-SOH, Cys-SO ₂ H, Cys-SO ₃ H
Cys-SeH	Cys-SeOH, Cys-SeO ₂ H, Cys-SeO ₃ H
Met-S-CH ₃	Met-S(O)-CH ₃
Trp	Kynurenine, <i>N</i> -formylkynurenine
Trp	Trp(OH) _n
Phe	Phe(OH) _n
Tyr	Tyr(OH) _n
Glu, Leu, Lys, Ile, Pro, Tyr, Val	Hydroperoxides (R-OOH)
Arg, Cys, His, Lys, Pro, Thr	Carbonyls (R-CO)

jugated dienes, depending on the microenvironmental conditions (Halliwell and Gutteridge 1999; Sorg 2004; Hwang and Kim 2007) (Fig. 4).

Cellular signalling pathways

A general mechanism of disruption of redox-dependent cell signalling is ROS-mediated membrane depolarisation. ROS released during oxidative stress oxidise protein components of ion channels, leading to activation of non-specific ion channels, disruption of ionic gradients, and membrane depolarisation, as well as massive calcium ion influx. Indeed, extracellular Ca²⁺ concentration (≈2.5 mM) is about 25,000 times higher than intracellular Ca²⁺ concentration (≈100 nM); this means that any condition inducing the activation of cationic channel leads to a massive influx of Ca²⁺ ions. Several deleterious processes can be initiated by a dramatic increase of cytosolic Ca²⁺ concentration: calcium-dependent proteases (calpains), lipases and endonucleases lead to the degradation of proteins, lipids and nucleic acids, other calcium-dependent enzymes such as phospholipase A₂ and caspases induce inflammation/oxidative stress and apoptosis, respectively, whereas mitochondrial Ca²⁺ influx, following increase of cytosolic Ca²⁺ concentration, inhibits ATP synthesis by depolarisation of the inner mitochondrial membrane (Mattson 2002; Chinopoulos and Adam-Vizi 2006).

The primary oxidation products of ROS on biomolecules can in turn lead to secondary oxidation products or interfere with cell signalling pathways. In particular 4-hydroxy-2-nonenal, a by-product of linoleate peroxidation, can potentiate a primary oxidative stress or modulate various biochemical pathways, such as inflammation via COX-2

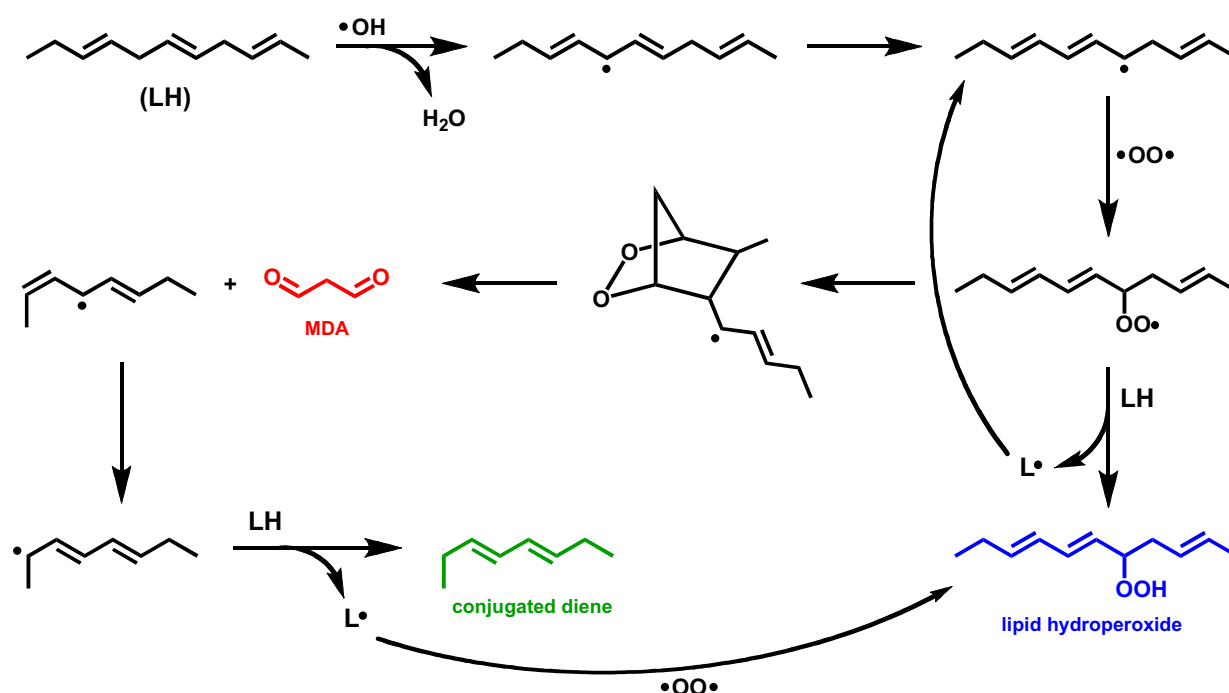
activation, cell signalling via the activation of the kinase cascade, or cell growth and apoptosis (Uchida 2003; Forman and Dickinson 2004).

ROS can induce inflammation by activating intracellular networks involved in cytokine and Toll-like receptor signalling. For instance, membrane lipid peroxides generated by ROS are able to activate a kinase complex that phosphorylates an inactive complex constituted by the transcription factor NF-κB and its inhibitor IκB; once phosphorylated, the latter is released from the complex, and then degraded in proteasomes; the active dimeric NF-κB (i.e. p50-p65) thus released is then translocated into the nucleus, where it binds to a DNA consensus sequence; this induces the expression of genes coding for inflammatory cytokines such as IL-1, IL-6, TNF-α, or ICAM (Schulze-Osthoff *et al.* 1997; Turpaev 2002) (Fig. 5).

INVOLVEMENT OF OXIDATIVE STRESS IN HUMAN PATHOLOGY

Ageing

Ageing is a complex, general and ineluctable process during which the characteristics acquired during development are progressively lost, leading to a degradation of the general condition and finally to death. As a natural and ineluctable process, ageing cannot be prevented, but it can be accelerated by environmental conditions. The general theories of accumulation of cellular damages explain ageing by the accumulation during the life of alterations of proteins, lipids, glucides, DNA and other biomolecules that the organism cannot repair or eliminate completely. Among these theories, that due to free radicals was introduced in 1956 by Denham Harman. According to this theory, ageing can be considered as a progressive, inevitable process characterised by the accumulation of oxidative damages into biomolecules due to an imbalance between prooxidants and antioxidants in favour of the former (Harman 1956; Harman 1993; Harman 2001; Biesalski 2002). Many studies confirmed the involvement of oxidative stress in ageing. Long-living animal species have more efficient antioxidant systems and higher liver superoxide dismutase I activity than shorter-living species (Cutler 1991). Diseases that frequently accompany ageing have oxidative stress as a major determinant (Ames *et al.* 1993; Wick 2000). Healthy old people have higher plasma/tissue levels of antioxidants compared to disabled ones

**Fig. 4** Mechanism of lipid peroxidation by ROS.

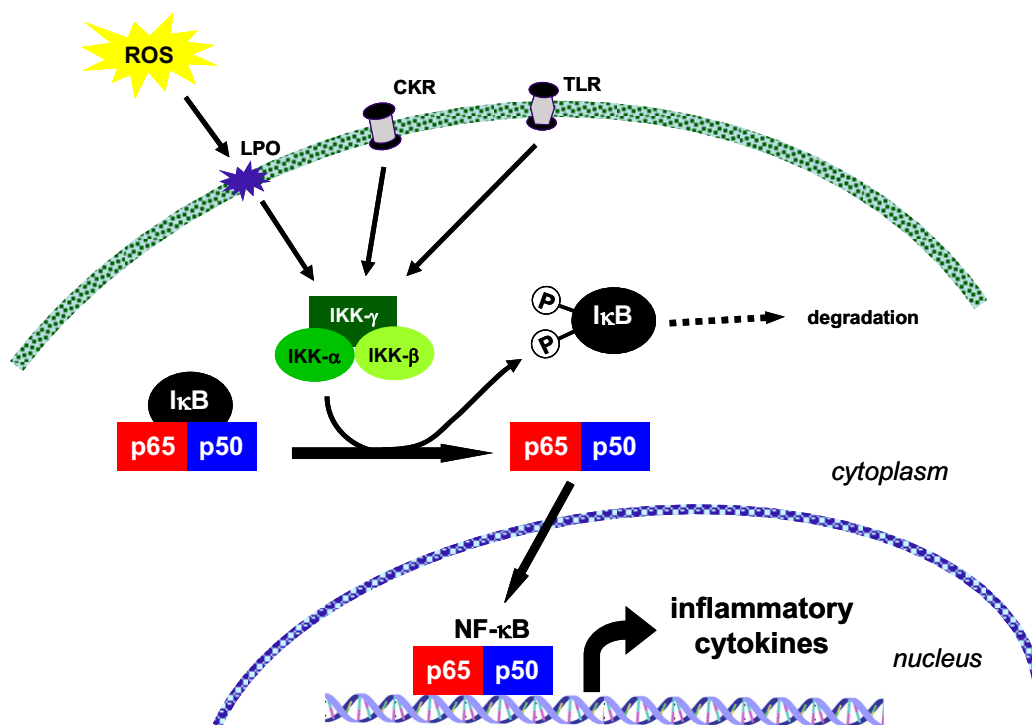


Fig. 5 Induction of inflammatory cytokine secretion by ROS. Abbreviations: CKR, cytokine receptor; IκB, inhibitor of NF-κB; IKK, IκB kinase; NF-κB, nuclear factor κB; LPO, lipid peroxide; TLR, Toll-like receptor.

(Paolisso *et al.* 1998; Mecocci *et al.* 2000; Junqueira *et al.* 2004; Gil *et al.* 2006). Decreased rate of protein degradation by proteasomes and repair of oxidised proteins has been associated with an increased susceptibility to oxidative stress and ageing (Friguet 2006). Similarly, other random processes produce alterations of tissues that accumulate with time: DNA crosslinks during mitoses and other DNA damages (Hamilton *et al.* 2001), protein misfolding, protein glycation (Mooradian and Thurman 1999), and unavoidable membrane damages lead to organic dysfunction (Halliwell and Gutteridge 1999). Generally-speaking, natural metabolic activity produces "metabolic waste" that the organism cannot always eliminate properly. In other words, the organism adapts to any situation by preventing undesirable reactions and repairing damaged molecules and tissues, but with a success that never reaches 100%. With time, these oxidised catabolites become oxidants for biomolecules if they are not eliminated. For this reason, the very few undesirable reactions that escape the prevention and repair systems accumulate little by little, and will irremediably end up becoming deleterious after a long period of time (Kuroo 2001; Tahara *et al.* 2001; Sander *et al.* 2002). Thus the free radical theory of ageing rejoins the general theory of accumulation of cellular damages, which accounts for the observations made in aged individuals (Sorg *et al.* 2004).

Cardiovascular diseases

Myocardial infarctions and localised cerebral ischaemia are often the consequence of atherosclerosis, a disease of arteries characterised by a local thickening of the inner coat of the vessels. Oxidative stress has been shown to play an important role in the initiation and the development of atherosclerosis, thus being both a cause and a consequence of the disease. Many genetic and environmental conditions such as hypercholesterolaemia, diabetes, hypertension, smoking or ageing can lead to the peroxidation of LDL lipids. These oxidised LDL bind to LDL scavenger receptors expressed by macrophages; the latter become then foam cells, i.e. lipid-laden distorted cells from macrophage or vascular origin that contribute to the formation of atheroma plaques. Activated enzymes such as xanthine oxidase, myeloperoxidase, NADPH oxidase and nitric oxide synthase then produce more ROS, leading to the formation of fibrous plaques; the latter consist of fibrous cap, composed

mostly of smooth muscle cells and dense connective tissue surrounding by macrophages, T lymphocytes and other smooth muscle cells (Dhalla *et al.* 2000; Ceconi *et al.* 2003; Harrison *et al.* 2003; Hamilton *et al.* 2004).

Paraoxonase, an esterase initially shown to hydrolyse non-endogenous organophosphates, and bound to HDL, seems to be the predominant antioxidant for HDL and LDL by removing oxidised lipids from these lipoproteins. Low serum paraoxonase activity has been related to diabetes, hypercholesterolaemia, lipid peroxidation and cardiovascular diseases such as atherosclerosis, whereas high paraoxonase activity decreases the biosynthesis of cholesterol by macrophages (Durrington *et al.* 2001; Aviram and Rosenblat 2004). This confirms the role played by oxidative stress and lipid peroxidation in particular in the development of atherosclerosis, and makes paraoxonase and hydrolysis of oxidised lipids in general putative targets for the design of new pharmacological treatments of atherosclerosis.

Ischaemia-reperfusion injury is a multifactorial, deleterious process that develops during the reperfusion of an organ that has been transiently subjected to reduced blood supply (Sorg 2004). It has been extensively studied in the heart (Ceconi *et al.* 2003), but can take place in other organs. During ischaemia, blood glucose falls, glycolysis and oxidative phosphorylation rates decrease, and subsequently ATP stores are converted to ADP. Two molecules of ADP can give ATP and AMP. Thus, if ATP is no longer regenerated by new blood substrates, ATP consumption leads to an accumulation of AMP, as well as a fall of the activity of Na^+/K^+ -ATPase, the ATP-dependent pump that maintains the physiological gradients of sodium and potassium ions between both sides of cellular membranes (Vander Heide *et al.* 1996). AMP is then metabolised to hypoxanthine. On the other hand, the decrease of the activity of ATP-dependent ionic pumps induces a depolarisation of the plasmic membrane, promoting a massive influx of calcium ions within cells (Katsura *et al.* 1993). High cytoplasmic Ca^{2+} has deleterious consequences for the cells: in particular, it induces the production of ROS in mitochondria due to the disruption of the mitochondrial proton gradient, which also impairs ATP production, and it activates calcium-dependent proteases such as calpains (Budd 1998). Calpains and ROS can convert xanthine dehydrogenase to xanthine oxidase, due to limited proteolysis and cysteine oxidation (Cheng *et al.* 1994). The consequence is the transfer of electrons from substrates to oxygen, instead of NAD^+ , which leads to the

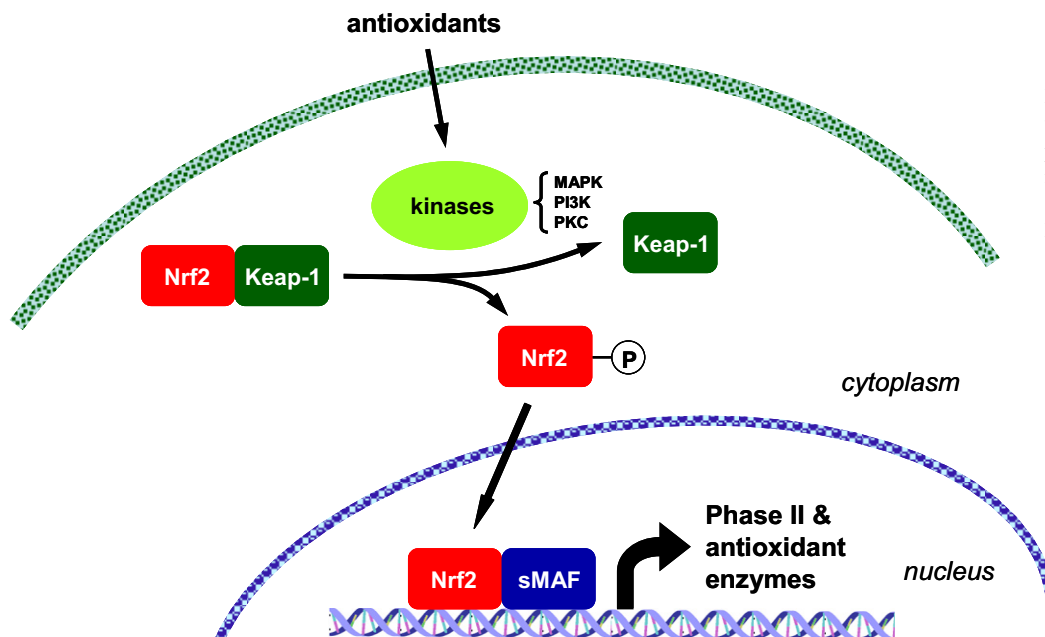


Fig. 6 Induction of detoxifying enzyme synthesis by antioxidants. Abbreviations: Keap-1, kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; Nrf2, NF-E2-related factor-2; PI3K, Phosphoinositide-3 kinase; PKC, protein kinase C; sMAF, small musculoaponeurotic fibrosarcoma.

formation of superoxide and hydrogen peroxide. Furthermore, due to hypoxanthine accumulation in response to ischaemia, xanthine oxidase is activated and gives rise to a dramatic production of superoxide ions as soon as oxygen is reperfused (Nishino 1994; Rees *et al.* 1994; Nishino *et al.* 1997). Finally, the activity of the main antioxidant enzymes, i.e., catalase and superoxide dismutase (SOD), are decreased following ischaemia (Homi *et al.* 2002). In other words, during ischaemia, xanthine dehydrogenase is converted to its oxidase form, which is activated by the accumulation of its substrate hypoxanthine, giving rise to ROS; because of the repression of antioxidant enzymes following ischaemia, this burst of ROS generates an oxidative stress.

Cancer and metastasis

As discussed above, DNA, proteins and cell membrane lipids are the main targets of oxidative stress. DNA oxidation can lead to mutations affecting genes involved in growth control and apoptosis, a condition that predisposes to cancer development. In particular, the oxidation product of the nucleotide 2'-deoxyguanosine, 8-hydroxy-2'-deoxyguanosine, has been demonstrated to be a reliable marker of DNA oxidation associated to various cancers (Wu *et al.* 2004; Hwang and Kim 2007). As discussed above, membrane lipids are targets of oxidative stress mediating intermediates. Lipid peroxides induce a loss of cell membrane integrity and biological functions, and exert a direct biological activity on various intracellular pathways related to cell growth, apoptosis and inflammatory processes; a dysregulation of these vital cellular processes leads to cell death or carcinogenesis (Dianzani 1993; Cejas *et al.* 2004). On the other hand, several chemotherapeutic agents activate the Nrf2 pathway: this transcription factor has been implicated as the central protein that interacts with the antioxidant response element, a *cis*-acting enhancer sequence that mediates transcriptional activation of genes in response to oxidative stress. The products of these genes are mostly antioxidant and detoxifying proteins such as glutathione peroxidase, catalase, superoxide dismutase and phase II enzymes (i.e., UDP-glucuronosyltransferase, sulphotransferases, *N*-acetyltransferase, glutathione *S*-transferases, etc.) (Kwak *et al.* 2002; Nguyen *et al.* 2003; Wang *et al.* 2006) (Fig. 6).

Cellular effects of ROS are thought to play a role in invasiveness and metastasis (Storz 2005). One potential mechanism of the action of ROS in tumour invasiveness and metastasis is the modulation of expression of adhesion molecules such as integrins; another is the suppression of apop-

toxis. In addition, ROS may also activate the matrix metalloproteinases which degrade the extracellular matrix (Halliwell 2007). Human metastatic prostate cancer cells produce more ROS than their primary malignant counterparts (Oberley 2002). The levels of lysyl oxidase, an H_2O_2 -producing enzyme, is increased in metastatic human breast cancer tissues when compared with those of primary cancer (Payne *et al.* 2005). Another possible mechanism is the stimulation of angiogenesis by ROS via increased cellular production of vascular endothelial growth factor (Huang and Zheng 2006).

Neurodegenerative diseases

From a theoretical point of view, the nervous system should be particularly vulnerable to oxidative stress. Indeed, the brain contains high concentrations of polyunsaturated fatty acids that are highly susceptible to lipid peroxidation; it utilizes the highest amount of oxygen per mass unit to produce energy, compared to the other organs; the biosynthesis and the catabolism of the neurotransmitters catecholamines generate significant amounts of ROS, as is the case following the release of glutamate and nitric oxide during neuronal activity (Rao and Balachandran 2002; Kedar 2003; Mariani *et al.* 2005). Paradoxically, the brain seems relatively deficient in antioxidant systems such as catalase and glutathione peroxidase, as well as in dietary low molecular weight antioxidants. For these reasons, oxidative stress plays a pivotal role in neurodegenerative disorders, and this has been well documented in Alzheimer's and Parkinson's diseases. ROS have been shown to decrease the uptake by astrocytes of extracellular glutamate following neuronal activity, a situation leading to excessive neuronal depolarisation, high calcium influx, activation of calcium-dependent enzymes, and finally neuronal death by the so-called excitotoxic process (Sorg 2004) (Fig. 7).

Alzheimer's disease (AD), one of several disorders that cause the gradual loss of brain cells, is the leading cause of dementia. Its neuropathological hallmarks are neurofibrillary tangles and senile plaques, two forms of protein aggregation, i.e., amyloid β -protein – a product of mutated amyloid protein precursor – and hyperphosphorylated tau, respectively. The involvement of oxidative stress as a cause of the disease is still a matter of debate, although mitochondrial dysfunction probably plays a role at an early stage of the disease. Many studies have clearly demonstrated that amyloid β -protein, hyperphosphorylation of tau, as well as mutated presenilins and apolipoprotein E, produced oxidative alterations in proteins, lipids, carbohydrates, RNA and

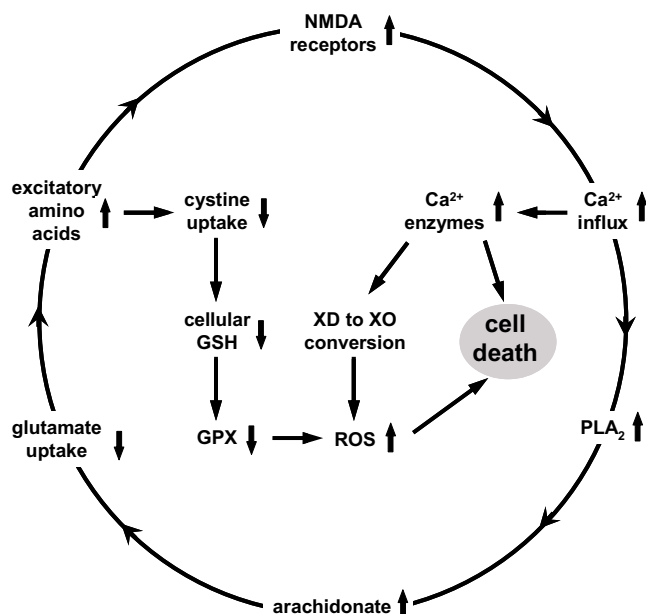


Fig. 7 Mechanism of excitotoxicity. Abbreviations: GSH, glutathione; GPX, glutathione peroxidase; PLA₂, phospholipase A₂; XD, xanthine dehydrogenase; XO, xanthine oxidase.

DNA in brain of AD patients and animal models (Chan *et al.* 2002; Zhu *et al.* 2004; Forero *et al.* 2006; Keller 2006). Moreover, mitochondria and various proteases were shown to contribute at various degrees to the increase of oxidative stress at a later stage of AD (Chauhan and Chauhan 2006). Thus oxidative stress is at least a consequence of early stage of AD, and greatly contributes to its development and the worsening of its symptoms (Moreira *et al.* 2005).

Amyotrophic lateral sclerosis (ALS) is caused by the degeneration of both upper and lower motor neurons, resulting in skeletal muscle atrophy and weakness, and culminating in respiratory insufficiency. Despite extensive research, the cause of ALS is still unknown; however several known mechanisms are implicated in the pathogenesis of motor neuron degeneration, including excitotoxicity, immune activation, mitochondrial dysfunction, protein aggregation, altered proteosomal function, and finally apoptosis (Abele 2002; Simpson *et al.* 2003; Strong and Rosenfeld 2003; Barber *et al.* 2006). These mechanisms are not mutually exclusive, but rather act in synergy to worsen the resulting cytotoxicity. Ten to fifteen percent of ALS cases are inherited, among them 25-30% are due to mutations of the antioxidant enzyme SOD. These mutations seem to be associated with a toxic gain of function rather than a loss of function; these abnormal forms of SOD are thus believed to play a role in the generation of an oxidative stress by a mechanism that remains to be elucidated (Brown 1995; Radunovic and Leigh 1996; Valentine *et al.* 1999). On the other hand, markers for protein, lipid and DNA oxidation were localised in motor neurons, reactive astrocytes and microglia in ALS patients, whereas they were absent in control spinal cords, confirming the role of oxidative stress in the pathology of ALS, although it is not clear whether it participates as an initiator or an amplifier of the symptoms, or both (Barber *et al.* 2006).

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. It is due to the degeneration of dopaminergic neurones in *substantia nigra*; the first symptoms appear after approximately 80% of these neurones have been lost. Autooxidation of dopamine, as well as its catabolism by monoamine oxidase, produce superoxide and hydrogen peroxide (Haavik *et al.* 1997; Galzigna *et al.* 2000). This led to the concept that the metabolism of dopamine might be responsible for the high basal levels of oxidative stress in *substantia nigra* (Jenner 2003). On the other hand, the parkinsonism-inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been studied

in animal models and was shown to be taken up by astrocytes, and then oxidised by monoamine oxidase in another compound, 1-methyl-4-phenyl-pyridinium ion (MPP⁺), which cannot diffuse through cellular membranes, but is specifically taken up by dopaminergic neurones and produces symptoms very similar to those of PD; once in the cytoplasm, MPP⁺ kills the cells by interfering with the mitochondrial respiratory chain by a mechanism that increases superoxide production (Singer *et al.* 1987; Speciale 2002). More recently, inflammatory processes involving microglia, as well as the cytokines ICAM-1, LFA-1, TNF α and IL-6 have been demonstrated in PD (Sawada *et al.* 2006). Thus oxidative stress, excitotoxicity, inflammation and mitochondrial dysfunction form a synergistic cascade of interrelated events that lead to death of dopaminergic neurones by way of apoptosis (Jenner and Olanow 2006).

Pulmonary diseases

Asthma is characterised by reversible airflow obstruction, airway hyperresponsiveness/hyperreactivity, and chronic inflammation. It has been shown that increased oxidative stress cause airway inflammation in asthmatic patients (Kirkham and Rahman 2006). Inflammatory cells in the airways, such as macrophages, neutrophils, and eosinophils, release increased amounts of ROS in asthmatic patients (Sedgwick *et al.* 1990). ROS can result in lung injury via direct oxidative damage to epithelial cells (Hulsmann *et al.* 1994). ROS have also been shown to induce bronchial hyperreactivity (Sadeghi-Hashjin *et al.* 1996) and to stimulate histamine release from mast cells and mucus secretion from airway epithelial cells (Krishna *et al.* 1998).

Chronic obstructive pulmonary disease (COPD) is a slowly progressive and largely irreversible disease characterized by airflow limitation. Cigarette smoking is the major etiological factor for COPD (Kirkham and Rahman 2006). The oxidative damage in the lungs of smokers is mediated by ROS released from macrophages and neutrophils (Rahman and MacNee 1996). Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce ROS and to release a series of mediators which attract inflammatory cells into the lungs. Both neutrophils and macrophages, which migrate in increased numbers into the lungs of COPD patients, can generate ROS via the NADPH oxidase system (Saetta *et al.* 2001). Increased amounts of myeloperoxidase found in neutrophils correlate with the degree of pulmonary dysfunction (Fiorini *et al.* 2000).

Skin diseases

Oxidative stress has been proposed to be involved in the pathogenesis of psoriasis and acne (Okayama 2005). Melanocytes in patients with vitiligo are thought to be more susceptible to oxidative stress (Boissy and Manga 2004). ROS may also participate in the pathogenesis of allergic reactions in the skin (Bickers and Athar 2006). Patients allergic to nickel show increased tissue iron and an elevated oxidized/reduced GSH ratio, which characterise an oxidative stress in skin mediated by the Fenton reaction (Kaur *et al.* 2001). Skin exposure to a number of irritants or proinflammatory agents such as UVA light and UVB light generates ROS in infiltrating leukocytes at the site of inflammation (Black 2004). Accumulation of ROS owing to catalase attenuation results in skin ageing and photoageing in human skin *in vivo* (Fisher *et al.* 2002). Leg ulcers due to venous insufficiency are characterised by chronic inflammation in which haeme and iron are deposited in the tissue. In drug-induced skin photosensitisation, the inflammatory response involves the generation of ROS (Briganti and Picardo 2003). It has been shown that spontaneous conversion of papillomas to carcinomas can be increased by treating papilloma-bearing mice with free radical-generating compounds (Athar *et al.* 1989). Pro-oxidant compounds can be metabolically converted into free radicals that induce skin malignancy (Bickers and Athar 2006). Various oxidants and free

Table 3 Endogenous antioxidants.

Antioxidant	Phase	Action
Superoxide dismutases	Hydrophilic	Dismutation of O_2^- into H_2O_2 and O_2
Catalase	Hydrophilic	Dismutation of H_2O_2 into H_2O and O_2
GPX	Hydrophilic or lipophilic	Reduction of R-OOH into R-OH
Glutathione reductase	Hydrophilic	Reduction of oxidised glutathione
GST	Hydrophilic	Conjugation of R-OOH to GSH to form GS-OR
Metallothioneins	Hydrophilic	Chelation of transition metals
Peroxiredoxin/thioredoxin	Hydrophilic	Reduction of R-S-S-R into R-SH and R-O-O-R into R-OH
GSH	Hydrophilic	Reduction of R-S-S-R into R-SH
		Free radical scavenger
		Cofactor for GPX and GST
Ubiquinol	Lipophilic	Free radical scavenger (prevents LPO)
Dihydrolipoic acid	Amphiphilic	ROS scavenger
		Induces antioxidant and phase II enzymes
		Recycles vitamins C and E
Ascorbic acid (vitamin C)	Hydrophilic	Free radical scavenger
		Recycles tocopherols (vitamin E)
		Maintains enzymes in their reduced state
Retinoids and carotenoids	Lipophilic	Free radical scavengers
		Singlet oxygen (1O_2) quenchers
Tocopherols (vitamin E)	Lipophilic	Free radical scavengers (prevent LPO)
		Increase selenium absorption
Selenium	Amphiphilic	Constituent of GPX and thioredoxin reductase

Abbreviations: GPX, glutathione peroxidase; GSH, glutathione; GST, glutathione *S*-transferase; LPO, lipid peroxidation.

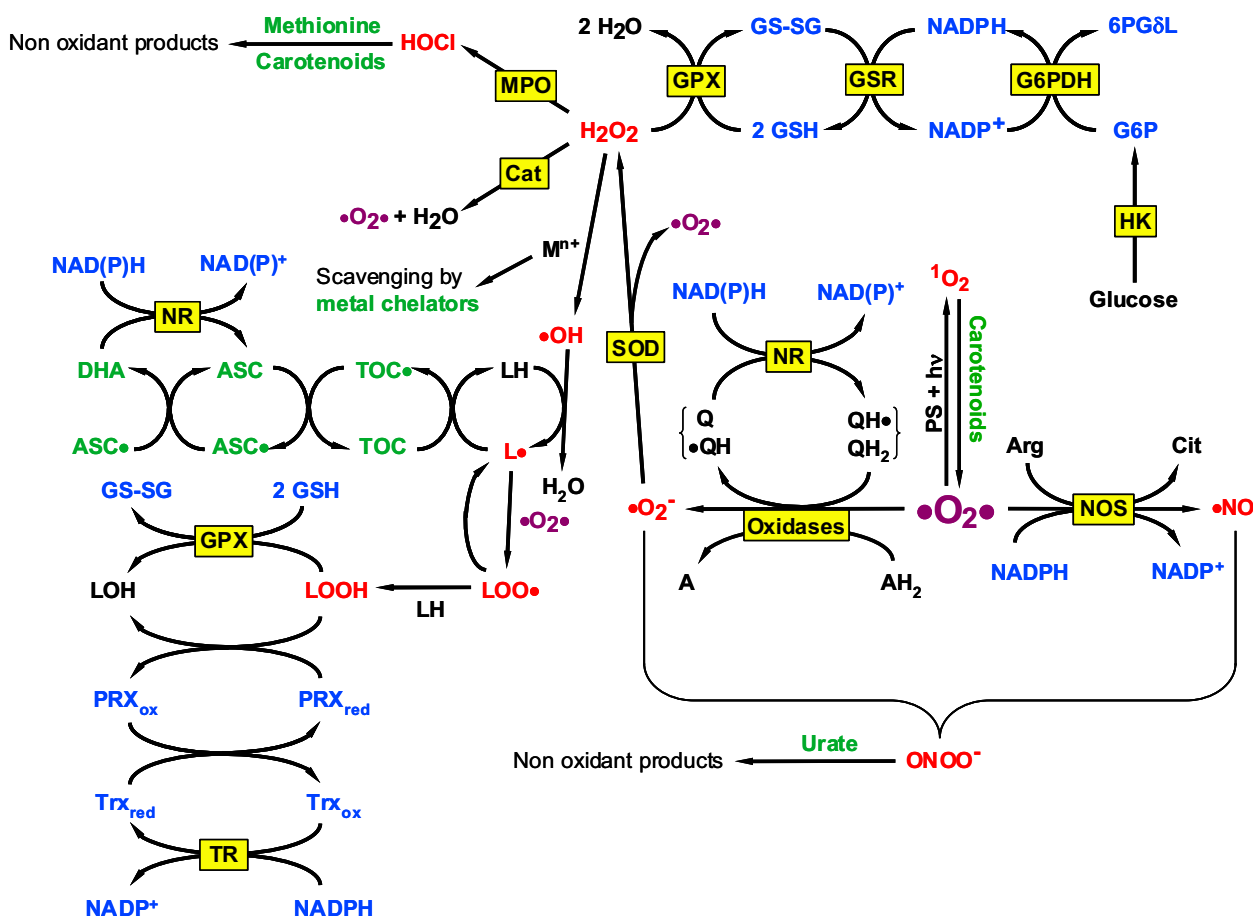


Fig. 8 Production and degradation of ROS. Abbreviations: 6PG δ L, 6-phosphoglucono- δ -lactone; ASC, ascorbate; Cat, catalase; DHA, dehydroascorbate; G6P, glucose 6-phosphate; G6PDH, glucose 6-phosphate dehydrogenase; GSH, glutathione; GPX, glutathione peroxidase; HK, hexokinase; MPO, myeloperoxidase; NOS, nitric oxide synthase; NR, NADPH:quinone reductase; PRX, peroxiredoxin; SOD, superoxide dismutase; TOC, tocopherol; Trx, thioredoxin.

radical-generating chemicals induce ornithine decarboxylase activity in murine skin and increase conversion of benign papillomas to squamous cell carcinomas (Sander *et al.* 2004).

ENDOGENOUS DEFENCES

All organisms that live in the presence of oxygen possess an efficient battery of antioxidant defences able to trap reactive intermediates before they have time to oxidise biomolecules or reduce those which have been oxidised (**Table 3; Fig. 8**) (Steenvoorden and van Henegouwen 1997; Blokhina *et al.*

2003; Pinnell 2003; Sorg 2004). Due to the great variety of reactive intermediates that must be neutralised, the variety of oxidised biomolecules which must be reduced, as well as the different physical phases in which undesirable oxidations may occur, several kinds of antioxidant systems play together to neutralise the deleterious effects of oxidative stress (Steenvoorden and van Henegouwen 1997; Halliwell and Gutteridge 1999; Thiele *et al.* 2001; Blokhina *et al.* 2003; Pinnell 2003; Wood *et al.* 2003; Sorg 2004).

The most efficient antioxidants are enzymes that catalyse the reduction of ROS: SOD catalyses the dismutation of superoxide into hydrogen peroxide and oxygen, catalase catalyses hydrogen peroxide dismutation into water and oxygen, glutathione peroxidases (GPX) reduce both hydrogen and organic hydroperoxides. Oxidised glutathione is then reduced by glutathione reductase. Various GPX isoforms exist, which are specific for hydrophilic or lipophilic phases. Metallothioneins are small proteins with several cysteine residues which bind transition metal ions efficiently: this can both detoxify metals and avoid them catalysing the Haber-Weiß and Fenton reactions which lead to the production of the hydroxyl radical. Peroxiredoxins are efficient in reducing organic hydroperoxides and disulphur bridges into the corresponding alcohols and thiols, respectively, as well as reducing thyl radicals; the whole system involves thioredoxin and the selenoprotein thioredoxin reductase to recycle reduced peroxiredoxin (Rhee *et al.* 2005; Jeong *et al.* 2006). Besides being an antioxidant by itself, glutathione is also the cofactor for GPX and glutathione reductase. Ubiquinol plays an essential role in the mitochondrial electron-transport chain in creating a proton gradient between both sides of the inner mitochondrial membrane. Due to its two-step oxidation via a radical intermediate, ubiquinol is both a promoter of superoxide formation and an efficient free radical scavenger in lipid phase. Dihydrolipoic acid, a component of pyruvate dehydrogenase complex, is a metal chelator and a ROS scavenger; it also induces the expression of antioxidant and phase II enzymes and recycles vitamins C and E (Biewenga *et al.* 1997; Lynch 2001; Chidlow *et al.* 2002). L-Ascorbic acid (vitamin C) is a water-soluble low molecular weight antioxidant required for collagen synthesis, iron absorption and maintenance of the redox status of cells (Englard and Seifter 1986). It recycles vitamin E, the predominant membrane antioxidant, as well as many other oxidised biomolecules, and scavenges free radicals (Chan 1993). Carotenoids are free radical scavengers, and most importantly, singlet oxygen quenchers. This is particularly important for the retina, where singlet oxygen is produced following interactions between visible light, oxygen (triplet) and various photosensitizers (Iyama *et al.* 1996; Böhm *et al.* 1997; Handelman 2001; Rosen 2003). Tocopherols (vitamin E) are the main antioxidants in the lipophilic phase; once oxidised, they become radicals, then they are converted to their functional reduced state by ascorbic acid (Chan 1993; Pehr and Forsey 1993; Steenvoorden and van Henegouwen 1997; Blokhina *et al.* 2003; Pinnell 2003). Selenium is a trace element present in the food as selenites, selenates or selenomethionine, which are precursors for selenocysteine.

CONCLUSION

In spite of the number of publications reporting the involvement of ROS and free radicals in biological processes, many physicians and biologists retain their scepticism towards oxidative stress and its aptitude to improve our knowledge in human physiology and pathology. This is probably due in part to the biophysical nature of the processes, leading to the feeling that oxidative stress might be a theoretical concept, rather than a concrete phenomenon that take part in biology. In this review, we present the production of ROS and secondary oxidations as an ineluctable process in any aerobic organism. The nature of the ROS and free radicals is well defined, and superoxide anion appears as the cornerstone in the formation of other ROS, be-

cause it represents the required intermediate in any reduction of molecular oxygen. We should bear in mind that molecular oxygen is a biradical: this explains its high reactivity in the presence of free radicals, compared to most (non-radical) biomolecules. The detrimental role of ROS in frequent pathologies such as ischaemia, atherosclerosis, ageing and neurodegenerative disorders has been extensively studied, and the knowledge of the biochemical mechanisms involving ROS should help to develop new strategies to prevent and care these diseases. A main point regarding the formation of ROS and free radicals is that these molecules are generated during any physiological process in aerobic organisms, and that they play a role in many biochemical mechanisms involved in biosynthesis, metabolism and cell signalling. The notion of "stress" appears only when the concentration of these molecules exceeds the capacity of endogenous antioxidants to maintain them at their physiological concentrations, a condition that leads to a degradation of biomolecules and a disruption of cell signalling, as illustrated here by selected pathologies for which a link with oxidative stress is well defined.

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