Oxidative Stress: Pathogenetic Mechanisms

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Key Words

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Summary

Radical and nonradical 'reactive oxygen species (ROS)' are normally occurring intermediates of aerobic cellular metabolism. Under physiologic conditions, they are kept from causing noticeable damage by antioxidant enzymes and small molecular weight compounds. Oxidative stress develops when antioxidants are overwhelmed by ROS. This may occur via overproduction of the latter and/or by deficiency of antioxidant defense. ROS in excess, especially the highly reactive hydroxyl radical (HO·), which is usually generated with the catalytic help of transition metals such as ferrous ions, can attack virtually all biological structures. Consequences may be functionally relevant when sensitive macromolecules are altered by ROS, in particular DNA (e.g. mutations, base modifications), proteins (formation of disulfide bridges etc.), lipids (lipid peroxidation), and polysaccharides (depolymerisation). Type and severity of the damage and cellular responses to it, including adaptive enzyme induction, determine the outcome in terms of cellular dysfunction or death. Many human disorders are thought to be associated with oxidative stress. ROS seem to play a predominant role, for instance, in radiation effects, damage by oxidant chemicals (many poisons and chemotherapeutic drugs), lung lesions due to hyperbaric oxygen, and chronic deficiencies of selenium and vitamin E. Oxidative stress is also thought to be important in other diseases and processes, e.g. in ischemia-reperfusion damage, central nervous system injury and stroke, severe chronic inflammatory disorders (for example rheumatoid arthritis), the development of arterial atherosclerosis, cancerogenesis, and ageing.

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Schlüsselwörter

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Krebs

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Zusammenfassung

Oxidativer Stress: pathogenetische Mechanismen

Radikale und nichtradikale «reaktive Sauerstoff-Spezies (RSS)» sind natürlich vor-kommende Zwischenprodukte des aeroben Zellstoffwechsels. Unter physiologischen Bedingungen sorgen antioxidative Enzyme und niedermolekulare Substanzen dafür, dass von den RSS kein erheblicher Schaden ausgeht. Ein oxidativer Stress entwickelt sich, wenn die antioxidative Abwehr von den RSS überwunden wird. Dies kann bei Überproduktion der letzteren und/oder bei antioxidativer Abwehrschwäche geschehen. RSS im Überfluss, insbesondere das hochreaktive Hydroxylradikal (HO·), das meist im Beisein von Fe²⁺ entsteht, vermögen die

verschiedensten Zellstrukturen anzugreifen. Die Folgen eines solchen Geschehens dürften vor allem dann ins Gewicht fallen, wenn wichtige und empfindliche Makromoleküle davon betroffen sind. Im besonderen ist hier an die DNS (Mutationen, Basenmodifika-

tionen), Proteine (Bildung von Disulfidbrücken u.a.), Lipide (Lipidperoxidation) und Polysaccharide (Depolymerisation u. a.) zu denken. Art und Schweregrad der Schäden sowie die zelluläre Antwort darauf (z. B. durch adaptive Enzyminduktion) bestimmen den Ausgang dieser Prozesse im Sinn einer gestörten Funktion oder des Todes der Zelle. Man nimmt an, dass zahlreiche krankhafte Zustände beim Menschen mit oxidativem Stress einhergehen. Beispielsweise scheinen RSS eine führende Rolle bei Strahlenschäden, Vergiftungen durch gewisse Chemikalien (u. a. auch bestimmte Chemotherapeutika), Lungenveränderungen nach Beatmung mit hyperbarem Sauerstoff sowie chronischem Mangel an Vitamin E und/oder Selen zu spielen. Dem oxidativen Stress kommt aber sehr wahrscheinlich auch bei vielen anderen Krankheiten bzw. Prozessen eine erhebliche Bedeutung zu, etwa bei Ischämie-Reperfusionsschäden, zentralnervösen Traumen und Hirnschlag, schweren chronischen entzündlichen Prozessen (z.B. rheumatoide Arthritis), bei der Entwicklung der arteriellen Atherosklerose, bei der Krebsentstehung und beim Altern.

Introduction

The notion of possible oxygen-mediated noxious effects on living matter is as old as the discovery, in 1775, of O_2 by Joseph Priestly [1]. The first proposition that free-radical chemistry may be related to normal metabolism was advanced, however, only 25 years ago [2]. Today, it is common knowledge that oxygen-centered radicals participate – among other – in such vital processes as oxidative phosphorylation through electron transport chains that generate most of the ATP needed for cellular metabolism, oxidation of fatty acids, a major mechanism in the formation of acetylcoenzyme A, and oxidation of amino acids, another source of energy for cells [3].

Some Definitions

The term ‘free radical’ denotes any species, molecular or – of lesser importance – atomic, that are capable of independent existence and contain one or more unpaired electron(s) in the valence shell, i.e. electrons that are alone in the respective orbital. Free radicals are indicated by a point next to the chemical formula (e.g. $HO\cdot$). Oxidation occurs by either adding oxygen, removing hydrogen, or transferring electrons, the latter being the primary source of metabolic radical production. Normally, in aerobic cells, most of the oxygen ends up combined with hydrogen as water, and only 4–5% of the $\frac{3}{4}$ consumed will form superoxides as electrons escape from the respiratory chain [4]. ‘Radical scavengers’ are molecules that can react with radicals before these cause damage. ‘Reactive oxygen species (ROS)’ is a collective term that includes not only oxygen-centered free radicals but also some nonradical derivatives of $\frac{3}{4}$ with oxidant capacity. ‘Antioxidants’ comprise all compounds able to counteract ROS. An ‘oxidative stress’ develops when antioxidants are overwhelmed by ROS via overproduction of the latter and/or deficiency of anti-oxidant defenses [5]. In fact, this term is not very precise, since macromolecular damage can occur through the sequestering of electrons independent of oxygen. ‘Oxidation-reduction’ denotes a transfer of electrons from an electron donor (reducing agent or anti-oxidant) to an electron acceptor (oxidizing agent) [3].

Generation of ROS

The most important ROS and non-oxygen-centered radicals are listed in table 1. The half-life of the radicals, due to their high reactivity, is very short and ranges, e.g., from ns ($HO\cdot$) to 7s

(peroxyl radicals, ROO·). The lifetime of the radicals also determines to a great extent their diffusion range. The highly reactive, short-lived HO· [6] usually exerts its damaging effects before it meets an antioxidant [7]. ROS generation may originate from intrinsic and/or extrinsic sources (table 2). The formation of the superoxide anion radical ($O_2^{\cdot -}$) an early – or the earliest – event in the chains of oxygen-centered radical production, can be described as the acceptance of an electron by ground-state oxygen:

$O_2 + e^- \rightarrow O_2^{\cdot -}$

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$e^- + O_2 \rightarrow O_2^{\cdot -}$

This may occur, e.g., during mitochondrial respiration, especially when the electron flux is poor [8], or with the help of

Table 1. Reactive oxygen species (ROS)

Superoxide anion radical

$O_2^{\cdot -}$

Hydroperoxyl radical

$HO_2^{\cdot}$

Hydroxyl radical

$HO^{\cdot}$

Nitric oxide

$NO^{\cdot}$

Alkoxyl radical

$RO^{\cdot}$

Peroxyl radical

$ROO^{\cdot}$

Nitroxyl radical

$RNO^{\cdot}$

Nonradicals

Hydrogen peroxide

H_2O_2

Singlet oxygen

1O_2

Hydrochlorous acid

$HOCl$

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Table 2. Generation of ROS

Table 3. Antioxidant defense

Intrinsic sources Mitochondrial respiration Metabolism of peroxisomes NADPH oxidase system of phagocytes Enzymatic synthesis of nitric oxide Xanthine oxidase system

Extrinsic sources Radiation (uv, ionizing) Heat

Ultrasound

Xenobiotics (e.g. carbon tetrachloride, anthracycline drugs, etc.) Various redox cycling compounds Mineral dusts Ozone, nitrogen oxides Phytoalexins, plant stress metabolites

NADPH oxidase, a family of superoxide-forming enzymes of phagocytes [7]. Superoxide generates hydrogen peroxide as a secondary product by dismutation on a longer time scale, depending on the pH [9]:

The most important role of H_2O_2 in the pathogenesis of ROS-mediated damage probably lies in the relatively long lifetime and diffusion range of this intermediate. The further course of events largely depends on the presence of transition metals, especially iron. The so-called Fenton reaction [10, 11] can be summarized as follows:

It should be mentioned at this point that Fe^{3+} -reducing agents may stimulate Fenton reactions [12], e.g.:

This indicates that vitamin C may not only have beneficial effects. The prominent feature of the Fenton reaction lies in the production of the highly reactive hydroxyl radical which is a much more powerful oxidant than either $\text{O}_2^{\cdot -}$ or H_2O_2 . However, the latter two intermediates may interact in the so-called Haber-Weiss reaction [13] to generate more hydroxyl radicals:

There are several other pathways of oxyradical and nonradical ROS formation [14–16] that we will not discuss in detail.

Antioxidant Defense

This defense (table 3) may be directed at a) preventing the initial generation of ROS, b) trapping ROS once they are formed, or c) repairing the oxidant-induced damage [17]. One important protective principle consists in the topographic separation (compartmentalization and/or sequestration) of the

Enzymes Superoxide dismutase (SOD): $2\text{O}_2^{\cdot -} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$

Catalase: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$

Glutathione (GSH) peroxidase: $\text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{GSSG}$

Low molecular weight antioxidants Water soluble (hydrophilic)

Glutathione

Ascorbate

Sugars

Unsaturated fatty acids and amino acids

Pyruvate

Urate

Bilirubin Lipid soluble (lipophilic)

Carotenoids (β -carotene etc.)

Vitamin E (α -tocopherol etc.)

Ubiquinol

Polyphenols

Butylated food additives

Iron or copper ion (complex)- binding proteins Ferritin (Fe, intracellular) Transferrin (Fe, extracellular) Hemopexin (heme) Haptoglobin (heme proteins) Ceruloplasmin (Cu) Albumin (Cu and others)

cell organelles apt to give rise to ROS generation on the one hand and those containing sensitive structures on the other hand [9]. Major ROS-scavenging enzymes and the reactions they catalyze are the following [for review see 17]:

Superoxide dismutases (SOD): $2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$

Catalase: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$

Glutathione (GSH) peroxidase: $\text{ROOH} + 2\text{GSH} \rightarrow$

$\text{ROH} + \text{H}_2\text{O} + \text{GSSG}$

Peroxides may preferentially be removed by GSH. Trace elements, especially metals, are important components of free radical-scavenging enzymes, e.g. Cu/Zn-SOD in the cytosol and Mn-SOD in mitochondria. Selenium also plays an important role. The distinction of hydrophilic and lipophilic low molecular weight antioxidants is important because the latter exert their action most often in membranes and other lipidic structures (e.g. to interrupt lipid peroxidation), while the former function mainly in the cytosol, in the intercellular space, and in the circulating blood [3]. Adaptive responses to ROS, involving modulation of gene expression (coding, for instance, for transcription factors, anti-oxidant and repair enzymes, cytokines, adhesion molecules, and proteins involved in cell proliferation or deletion), play an essential role, particularly in conditions with repetitive oxidative stress [18–21]. Major contributors to antioxidative defense in the extracellular

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space and the circulating blood are body fluid proteins that bind iron (transferrin) or copper ions (ceruloplasmin and albumin), heme proteins (haptoglobin) or heme (hemopexin) [12]. Repair mechanisms are especially relevant in the case of DNA damage [e.g. by excision repair, see 17].

Effects of Oxidative Stress

Under physiological conditions, the effects of ROS are counteracted by antioxidants (see above), resulting in a balance without noticeable short-term damage. However, oxidative stress, injury, and disease may ensue in case of excessive ROS production and/or deficient antioxidant defenses [4, 5, 12]. In such situations, ROS may interact with virtually all components of the cell, directly and indirectly [review 12], (table 4). Injury can be aggravated by ROS-mediated depression of antioxidant defense [22, 23]. As mentioned before, damage will be particularly severe if certain transition metals, such as Fe^{2+} , become available and the extremely reactive $\text{HO}^\cdot$ may be formed [24]. In addition to enzymes, other protein molecules can suffer ROS-mediated damage, usually by oxidation of critical thiol groups, for instance microfilaments [25], adhesion molecules, receptors, translational factors, and others that may become deactivated [17, 26].

Another important effect of oxidative stress is lipid peroxidation [reviews 4, 12]. In the initiation phase of this process, in which $\text{HO}_2^\cdot$ may be more important than $\text{HO}^\cdot$, the lipid molecule [usually a polyunsaturated fatty acid (L)] becomes an organic radical, e.g.:

$\text{HO}_2^\cdot + \text{LH} \rightarrow \text{L}^\cdot + \text{H}_2\text{O}_2$

A chain reaction is thus generated that alters membrane functions. It also leads to the release of toxic products that are able to diffuse away and cause further damage, e.g. malondialdehyde, which is a potentially mutagenic and carcinogenic compound [27].

Oxyradicals and their reactive derivatives are able to attack DNA directly and indirectly and form DNA adducts, result in DNA base modifications and/or induce strand breaks. $\text{HO}^\cdot$ seems to be the most aggressive in this respect; it may be generated – among other – with the help of iron complexed to DNA [28]:

$\text{DNA (Fe}^{II}) + \text{H}_2\text{O}_2 \rightarrow \text{DNA (Fe}^{III}) + \text{HO}^\cdot + \text{OFT}$.

As will be discussed later, ROS-induced mutations appear to play a major role in cancerogenesis. Oxidative stress is apt to cause a variety of other cellular changes that should be considered as secondary (indirect), e.g. rises of the intracellular Ca^{2+} concentration, with consecutive

activation of proteases and nucleases that may attack the cell's own structures, induction of the monophosphate shunt of glucose metabolism [29], activation of K⁺ channels in the cell membrane [30], and many others.

Table 4. Effects of ROS

Direct DNA (strand breaks, DNA base modifications, etc.) Proteins (enzymes, structural proteins, receptors, translational factors and others, often by oxidation of critical thiol groups) Lipids (membrane and other lipids, usually by lipid peroxidation) Polysaccharides (e.g. glycosaminoglycans, often by depolymerisation)

Indirect Activation of membrane K⁺ channels Rise in intracellular Ca²⁺ concentration (activation of proteases and nucleases etc.)

Altered hexose metabolism (e.g. induction of the hexose monophosphate shunt) Adoptive cellular responses (enzyme induction etc.)

In considering all these effects of oxidative stress, it should be remembered that most of them were observed in vitro or in animal experimentation and that considerable species differences exist with respect to damage caused by and cellular or organic responses to the action of ROS [31]. Extrapolations of these findings to humans should thus be made with caution.

A Note on Detection and Measurement of Oxidative Stress

Methodologic problems go beyond the scope of this short overview. The readers are referred to excellent reviews of recent developments in this field of research [12,17]. The direct, quantitative control of oxyradical generation has proved to be difficult or – as regards the very short-lived ROS – almost impossible even in vitro. Reliable detection and measurements of ROS effects in vivo pose even greater problems, however, several techniques are now available that allow for a reasonably accurate estimate of oxidative stress in the organism [32–37].

Overview of Human Disorders Thought to be Associated with Oxidative Stress

A selective list of endogenous and exogenous causes of oxidative stress is presented in table 5. The key question to be asked is what relative role such processes play in the various disorders. Four categories of pathologic conditions may be distinguished [38]:

a) Some disorders may actually be caused or largely be mediated by oxidative stress, in particular, untoward effects of uv and ionizing radiation or many chemicals (e.g. certain air pollutants, toxins and chemotherapeutic drugs), chronic dietary deficiency of selenium (Keshar disease) or α -toco-pherol (severe defects of fat absorption in the gut), Wilson's disease, and hemochromatosis.

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Table 5. Human disorders thought to be associated with oxidative stress

Disease/process	Role of ROS
Predominant radiation effects	
damage by oxidant xenobiotics	
lung damage due to hyperbaric oxygen	
chronic dietary deficiencies of, e. g.	
selenium (Keshar disease)	
α -tocopherol (severe fat absorption defect)	
Important	arterial atherosclerosis
ischemia-reperfusion damage	
central nervous trauma and stroke	

severe chronic inflammatory disorders, e. g.
chronic rheumatoid arthritis
colitis chronica

cancer

ageing

Unimportant smaller wounds

minor infections many others

In other diseases, excessive ROS generation, although secondary, most probably makes an important contribution to their pathogenesis, for instance in ischemia-reperfusion damage [39–41], myocardial stunning [42], central nervous trauma or stroke [43], inflammatory bowel disease [44], and chronic rheumatoid arthritis [45].

Three important long-term conditions are thought to be closely associated with ROS-mediated injury, be it by chronic accumulation of damage (arterial atherosclerosis and ageing), be it by genomic mutations or other changes of gene expression (cancerogenesis). A more detailed discussion of these three processes will follow below.

In still other diseases, oxidative stress may occur but not be a clinically significant mechanisms of tissue injury [6].

Pathogenetic pathways involving oxidative stress differ considerably in the various disorders.

Inflammatory reactions, especially those associated with bacterial infection and phagocytosis of microorganisms by neutrophils and macrophages, go along with an activation of the membrane-bound NADPH oxidase which results in an oxidative burst and the generation of ROS [18]. This process is most often accompanied by the release of various proinflammatory cytokines and other mediators and – if it gets out of control – may end up in infectious-toxic shock [46].

Furthermore, inflammation-induced reduction of NO⁺ production by endothelial cells can promote the adhesion of neutrophils in the microvasculature and thus aggravate oxidative stress [47].

Particular mechanisms of ROS overproduction and effects have been observed in viral infections [5, 48–51]. HIV infection, for instance, may reduce the cellular antioxidant capacity [48].

Oxidative stress is also apt to disrupt intercellular contacts by damaging adhesion molecules and thus contribute, e.g., to the development of intestinal barrier failure [46] or acute renal insufficiency [52].

With the advent of therapeutic thrombolysis and bypassing or reopening arterial stenoses (e.g. in coronary atherosclerosis), ischemia-reperfusion damage has gained increasing importance in clinical medicine [53]. In the rat jejunum closed-loop perfusion system, perfusion damage was achieved with peroxy- and hydroxyl radicals, but not with O₂^{•-} [54]. Presence of transition metals and inhibition of mitochondrial functions appear to play a major role by increasing the oxyradical production in such situations [39].

The complexity of ROS-induced lung injury has well been recognized in the context of exposure to hyperbaric oxygen and of infectious-toxic shock [46]. Oxyradicals, cytokines and other mediators appear to be involved in the generation of a proinflammatory cascade, in which various sources of ROS participate, e. g. respiratory burst of phagocytes and activated xanthine oxidase [55].

Special emphasis should be placed on ROS generation in the presence of excess transition metals, in particular iron and copper ions, as is the case in hemochromatosis and Wilson's disease [56].

Various halogenated hydrocarbons, for instance bromobenzene and carbon tetrachloride, exert at least some of their toxic effects by inducing lipid peroxidation in vivo [12]. The same pertains to many chemotherapeutic agents [57]. Elucidation of these events is complicated by considering adaptive responses which may render cells more resistant to the attack by oxyradicals and their derivatives.

Arterial Atherosclerosis

The predominant role of arterial atherosclerosis in causing human disease and death justifies a short separate discussion of the possible role played by ROS in the pathogenesis of this vascular change [review 17, 38]. Most recent reports indicate that lipid peroxidation, especially that achieved via the production of ROS by activated monocytes/macrophages adhering to the arterial endothelium [58], makes an early and significant contribution to the development of atherosclerotic plaques [12]. This latter lesion is now better understood since it was shown that oxidatively altered LDL is avidly taken up by macrophages, while nonoxidized LDL is not [59]. The presence of Fe²⁺ in plaques (e.g. following entry of blood through plaque fissures and consecutive local hemolysis) enhances the oxidation of LDL and thus promotes the accumulation of foam cells. In addition, toxic products of lipid peroxidation favor local necrosis, which may, in concert with other factors, initiate an inflammatory process. Furthermore, oxidative modifications of LDL can, in conjunction with cytokines like IL-1 and TNF- α , enhance the expression of genes coding for adhesion molecules (e.g. VCAM-1) and thus promote the attachment of even more monocytes to the endothelium. The notion that oxidative stress is an important part of atherogenesis is supported by results of animal experimentation [17]. Cigarette smoke,

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which contains many prooxidative aldehydes, is thought to favor oxidative stress in the inner layers of the arterial wall.

Cancerogenesis

In the past decade, much effort has been directed at elucidating the relationship between ROS and the development of malignant neoplasias, mainly in the hope that dietary antioxidants – or combinations thereof – might be identified that help to reduce cancer morbidity and lethality. Some present notions in this field of research may be summarized as follows [reviews: 17, 18]: If one adopts the three-stage model of carcinogenesis (initiation, promotion, and progression), the most important phase of ROS-mediated induction of malignant neoplasia is thought to be initiation. Oncogenic mutations may be direct, e.g. interactions of oxyradicals (especially HO in the presence of Fe²⁺) with DNA to cause strand breaks and/or DNA base modifications. Oxidative DNA damage can also be indirect, caused, e.g., via the action of peroxy radicals freed by endogenous lipid peroxidation or derived from the metabolism of classical chemical carcinogens [benz(a)pyrenes, aromatic amines, N-nitroso compounds and others]. Oxidative injury to DNA appears to occur at high rates already under normal conditions but is most often successfully repaired [60]. If mutations occur, they are most likely to initiate malignant neoplasia if they result in overexpression of oncogenes coding for growth factors and/or in the deletion of tumor suppressor genes [61]. The promotion and progression phases of cancerogenesis also seem to be subject to ROS influence and depend, among other, on a delicate balance between the various intracellular antioxidants, in particular enzymes [62]. Results of in vitro studies and animal experimentation seem to indicate that α -

tocopherol [63], carotenoids (especially β -carotene), and selenium may have an anticarcinogenetic effect, while inconsistent findings were made with supplementation of vitamin C [17].

Ageing

Lipofuscin, a peroxidized lipoproteidic complex, accumulates with age in various long-lived cell types, for instance in the liver, and has long been known among pathologists as 'age pigment'. This and many other observations have led to the concept that accumulation of damage to biosystems by ROS may be an important component of the ageing process [64]. It has also been found that the activities, e.g., of catalase, glutathione peroxidase, and D-glucose-6-phosphate dehydrogenase (all three H₂O₂ scavenging enzymes) in various cell types decline as a function of age. However, more quantitative evidence must be awaited, and effects of dietary antioxidants [65, 66] should carefully be evaluated, before part of the ageing process can be linked to ROS effects in more detail.

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