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REVIEW ARTICLE

# Redox- and non-redox-metal-induced formation of free radicals and their role in human disease

**Marian Valko[1,4] · Klaudia Jomova[2] · Christopher J. Rhodes[3] · Kamil Kuča[4,5] · Kamil Musílek[5,6]**

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**Abstract** Transition metal ions are key elements of various biological processes ranging from oxygen formation to hypoxia sensing, and therefore, their homeostasis is maintained within strict limits through tightly regulated mechanisms of uptake, storage and secretion. The breakdown of metal ion homeostasis can lead to an uncontrolled formation of reactive oxygen species, ROS (via the Fenton reaction, which produces hydroxyl radicals), and reactive nitrogen species, RNS, which may cause oxidative damage to biological macromolecules such as DNA, proteins and lipids. An imbalance between the formation of free radicals and their elimination by antioxidant defense systems is termed oxidative stress. Most vulnerable to free radical attack is the cell membrane which may undergo enhanced lipid peroxidation, finally producing mutagenic and carcinogenic malondialdehyde and 4-hydroxynonenal and other exocyclic DNA adducts. While redox-active iron (Fe) and copper (Cu) undergo redox-cycling reactions, for a second group of redox-inactive metals such as arsenic (As) and cadmium (Cd), the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins. While arsenic is known to bind directly to critical thiols, other mechanisms, involving formation of hydrogen peroxide under physiological conditions, have been proposed. Redox-inert zinc (Zn) is the most abundant metal in the brain and an essential component of numerous proteins involved in biological defense mechanisms against oxidative stress. The depletion of zinc may enhance DNA damage by impairing DNA repair mechanisms. Intoxication of an organism by arsenic and cadmium may lead to metabolic disturbances of redox-active copper and iron, with the occurrence of oxidative stress induced by the enhanced formation of ROS/RNS. Oxidative stress occurs when excessive formation of ROS overwhelms the antioxidant defense system, as is maintained by antioxidants such as ascorbic acid, alpha-tocopherol, glutathione (GSH), carotenoids, flavonoids and antioxidant enzymes which include SOD, catalase and glutathione peroxidase. This review summarizes current views regarding the role of redox-active/inactive metal-induced formation of ROS, and modifications to biomolecules in human disease such as cancer, cardiovascular disease, metabolic disease, Alzheimer's disease, Parkinson's disease, renal disease, blood disorders and other disease. The involvement of metals in DNA repair mechanisms, tumor suppressor functions and interference with signal transduction pathways are also discussed.

**Keywords** Metals · Toxicity · Oxidative stress · Human disease · Reactive oxygen species

✉ Marian Valko
marian.valko@stuba.sk

1 Faculty of Chemical and Food Technology, Slovak University of Technology, 812 37 Bratislava, Slovakia

2 Department of Chemistry, Faculty of Natural Sciences, Constantine the Philosopher University, 949 74 Nitra, Slovakia

3 Fresh Lands, P.O. Box 2074, Reading, Berkshire RG4 5ZQ, UK

4 The Center for Basic and Applied Research, University Hradec Kralove, Hradec Kralove, Czech Republic

5 Biomedical Research Center, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

6 Department of Chemistry, Faculty of Science, University Hradec Kralove, Hradec Kralove, Czech Republic

## Introduction

In comparison with the majority of organic molecules, metals or metal ions are rather small and simple species, and

consequently, an understanding of their interactions with large biological molecules might appear deceptively straightforward. However, the currently emerging picture is that the interactions of such species with bio-macromolecules and in signal transduction pathways are actually rather complex in nature. Some metal ions may be transformed by processes which include reduction/oxidation reactions, and lead to the formation of reactive oxygen species (ROS), or reactive nitrogen species (RNS) (Halliwell and Gutteridge 1990). As already noted, the cumulative formation of ROS and RNS is termed oxidative stress which may induce a cellular redox imbalance, and this may be linked with various disease states of an organism (Valko et al. 2007).

Metal toxicity is a rather complex phenomenon to evaluate, as is especially true for mechanisms of metal-induced carcinogenesis (Beyersmann and Hartwig 2008). Metal carcinogenicity occurs via complex mechanisms which involve oxidative damage, DNA repair, maintenance of redox homeostasis and disturbance of signal transduction pathways.

An intriguing feature of metals in biological systems is that some metals essential for health (such as copper and iron) may become toxic under certain circumstances. These metal ions may compete with essential ions for high-affinity metal-binding sites, so causing structural modifications and disturbances in metal homeostasis (Kozlowski et al. 2014; Valko et al. 2005).

In contaminated areas, humans may be exposed to redox-inert toxic elements such as arsenic and cadmium, which are known to be toxic at low concentrations (Hartwig 2013). For this group of metals, the primary mechanism for their toxicity and carcinogenicity is depletion of the powerful cellular antioxidant glutathione, by bonding to protein sulfhydryl groups and other modes of action.

The present article surveys all the aforementioned aspects of metals acting in living systems, including providing an overview of the role of redox-active copper and iron and non-redox toxic metals arsenic and cadmium. Redox-inert zinc is the most abundant trace metal in the brain and has various functions in health and in disease. Accordingly, special attention is paid to the anti-inflammatory role of zinc and its potential to suppress oxidative stress, while the mechanism by which zinc may act against oxidative stress and chronic inflammation is discussed.

## Biochemistry of metal-induced oxidative stress

The toxicity of redox-active transition metal ions in living systems is a very complex process. Transition metal ions are integral parts of protein active sites and may undergo redox-cycling reactions. Metal ions are present in biological systems in a variety of oxidation states and may interact with physiologically relevant metabolites derived from oxygen (molecular oxygen, superoxide anion radical, hydroxyl radical, etc.), low molecular weight antioxidants (ascorbic acid, tocopherols, etc.) and other components (Stohs and Bagchi 1995).

In addition to kinetic criteria, the outcome of chemical reactions between various substances is governed by thermodynamic principles. The most important parameter to characterize the course of a chemical reaction is the half-cell reduction potential. The overall redox potential for two redox pairs is given by the electromotive force ($\Delta E$) and is calculated according to the equation (Jomova and Valko 2011a, b)

$$\Delta E = E_2\,(\text{electron-acceptor}) - E_1\,(\text{electron-donor}) \quad (1)$$

where $E_1$ and $E_2$ are the reduction potentials for both half-cell reactions.

Disruption of the homeostasis of redox-active metals such as iron, copper, cobalt, chromium, nickel and other metals renders them available as catalysts to form excess of ROS and RNS. In addition, redox-active metals can bind to phospholipids, so changing the integrity of the lipid bilayer and enhancing its susceptibility to lipid peroxidation. Conversely, redox-inactive metals (cadmium, arsenic, lead and other metals) mediate their oxidative stress effects via bonding to sulfhydryl groups of proteins, which results in depletion of glutathione (Koedrith and Seo 2011). Zinc has a special role among metals in living organisms, since it is a non-redox metal and can impact both on the immune system and on various important neurological processes.

To avoid the excessive formation of ROS/RNS by redox metal imbalance, and consequent oxidative stress to an organism, redox homeostasis is tightly regulated (Forman et al. 2010). Indeed, many disease states are a result of disrupted homeostasis of metals (most frequently iron and copper) which catalyze and participate in the formation of damaging free radicals.

### Radicals derived from oxygen

Mitochondria are known to play a key role in maintaining the bioenergetic status of multiple basic cellular processes. The formation of superoxide anion radical by mitochondria is dependent on the reaction between potential electron donors and molecular oxygen. The formation of the superoxide radical anion within the mitochondrial matrix depends on the proton-motive force ($\Delta p$), the NADH/$NAD^+$ and $CoQH_2$/CoQ (coenzyme Q) ratios and the local $O_2$ concentration (Murphy 2009)

$$O_2 + e^- \rightarrow O_2^{\cdot -} \quad (2)$$

The superoxide radical anion further reduces Fe(III) or Cu(II) ions (or another metal ions) according to reaction

$$\mathrm{Fe(III)/Cu(II) + O_2^{\cdot -} \rightarrow Fe(II)/Cu(I) + O_2} \quad (3)$$

Metal ions in reduced oxidation states are able to enter catalytic decomposition of hydrogen peroxide ($\Delta E^{\ominus} = +0.307$ V)

$$\mathrm{Fe(II)/Cu(I) + H_2O_2 \rightarrow Fe(III)/Cu(II) + HO^{\cdot} + OH^-} \quad \text{(Fenton reaction)} \quad (4)$$

The superoxide radical anion is not only produced as a by-product of mitochondrial respiration, but is formed in large quantities in phagocytes by NADPH oxidase, for the purpose of killing invading pathogens. Hence, the immune system defends its host against microorganisms by producing superoxide. Superoxide is also produced by a number of other enzymes, for example, xanthine oxidase (Valko et al. 2004).

Hydrogen peroxide is produced in specific cellular compartments and acts as a second messenger molecule in regulating various important biological processes. Metals in low oxidation states can catalyze decomposition of hydrogen peroxide via the Fenton reaction (Eq. 4) which yields hydroxyl radicals (Lloyd et al. 1997). Hydroxyl radicals can subsequently react at diffusion-limited rates with various biomolecules, including lipids, proteins and DNA and cause serious damage to them. Following reaction (2), the resulting superoxide radical anion can reduce hydrogen peroxide via the so-called Haber–Weiss reaction which can be described according to reaction (5) (Liochev and Fridovich 2002):

$$\mathrm{O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^{\cdot} + OH^-} \quad (5)$$

SOD enzymes can scavenge superoxide radical anions by either the removal or addition of an electron, so resulting in the less damaging species, molecular dioxygen or hydrogen peroxide

$$\mathrm{M(II)\text{-}SOD + O_2^{\cdot -} \rightarrow M(I)\text{-}SOD + O_2} \quad (6)$$

$$\mathrm{M(I)\text{-}SOD + O_2^{\cdot -} + 2H^+ \rightarrow M(II)\text{-}SOD + H_2O_2} \quad (7)$$

where M is copper, manganese or iron.

The biologically acceptable level of hydrogen peroxide is controlled by the enzymes catalase and glutathione peroxidase, both of which convert it to molecular dioxygen.

$$\mathrm{2H_2O_2 \rightarrow 2H_2O + O_2} \quad (8)$$

The following reactions involving hydrogen peroxide may also take place:

$$\mathrm{^{\cdot}OH + H_2O_2 \rightarrow H_2O + HO_2^{\cdot}} \quad (9)$$

$$\mathrm{^{\cdot}OH + Fe(II) \rightarrow Fe(III) + OH^-} \quad (10)$$

$$\mathrm{HO_2^{\cdot} + HO_2^{\cdot} \rightarrow H_2O_2 + O_2} \quad (11)$$

$$\mathrm{Fe(III)/Cu(II) + HO_2^{\cdot} + Fe(II)/Cu(I) + O_2 + H^+} \quad (12)$$

Redox metals, mainly iron and copper, can mediate lipid peroxidation by the reductive cleavage of hydroperoxides (ROOH) derived from membrane phospholipids

$$\mathrm{ROOH + Fe(II)/Cu(I) \rightarrow RO^{\cdot} + OH^- + Fe(III)/Cu(II)} \quad (13)$$

$$\mathrm{ROOH + Fe(III)/Cu(II) \rightarrow RO_2^{\cdot} + H^+ + Fe(II)/Cu(I)} \quad (14)$$

resulting in the formation of alkoxyl ($\mathrm{RO^{\cdot}}$) and peroxyl ($\mathrm{RO_2^{\cdot}}$) radicals. The mechanism of reactions (13) and (14) involves formation of Fe(II)–Fe(III) or Fe(II)–$\mathrm{O_2}$–Fe(III) complexes. Fe(II) oxidation is stimulated at increased pH and, by the addition of Fe(III), reaching a maximum at a Fe(II)/Fe(III) ratio approaching 1:1 (Tadolini and Hakim 1996). At low concentrations of hydrogen peroxide, Fe(II) induces decomposition of lipid peroxides forming alkoxyl and peroxyl radicals.

Cu(I) ions are effective catalysts for the Fenton reaction, while both Cu(II) and Cu(I) ions are effective species for decomposing organic hydroperoxides to form peroxyl and alkoxyl radicals according to reactions (15–17) (Li et al. 2000a, b).

$$\mathrm{Cu(I) + H_2O_2 \rightarrow [Cu(I){-}H_2O_2] \rightarrow Cu(II) + HO^{\cdot} + OH^-} \quad (15)$$

$$\mathrm{Cu(I) + ROOH \rightarrow RO^{\cdot} + OH^{\cdot} + Cu(II)} \quad (16)$$

$$\mathrm{Cu(II) + ROOH \rightarrow RO_2^{\cdot} + H^+ + Cu(I)} \quad (17)$$

Based on the above discussion and various in vitro studies, it has been shown that the mechanism of lipid peroxidation is a radical-dependent process with physiological relevance. Lipid peroxidation results in the formation of a wide variety of oxidation products, the most common of which are aldehydes. Among the most abundantly produced aldehydes are malondialdehyde (MDA), propanal, hexanal and 4-hydroxynonenal (4-HNE) (Marnett 2000). We may note that MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic (Esterbauer et al. 1991).

The initiation reaction is mediated by formation of hydroxyl radicals via the Fenton reaction, or by ferryl intermediates which are similarly powerful oxidants. Both species are able to abstract hydrogen atoms from unsaturated fatty acids forming alkyl radicals ($\mathrm{R^{\cdot}}$):

$$\mathrm{HO^{\cdot} + RH \rightarrow H_2O + R^{\cdot}} \quad (18)$$

Studies of the lipid peroxidation process in brain tissue revealed that hydrogen abstraction occurs at the allylic carbons in the ninth and tenth positions of the oleic acid chain (Hall et al. 2015). Secondary reactions then proceed

by hydrogen abstraction by $RO^{\cdot}$ and $ROO^{\cdot}$ at the tertiary carbon atoms:

$$RO^{\cdot} + RH \rightarrow ROH + R^{\cdot} \quad (19)$$

$$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot} \quad (20)$$

$$RO_2^{\cdot} + RH \rightarrow ROOH + R^{\cdot} \quad (21)$$

The radicals $R^{\cdot}$ and $ROO^{\cdot}$ are key intermediates in the ROS-mediated mechanism of lipid peroxidation. Thus, iron and copper ions promote peroxidation of lipids in phospholipid liposomes by homolytic cleavage of hydrogen peroxide via formation of hydroxyl radicals and by scission of ROOH to form alkoxyl and peroxyl radicals, in addition to the effect of altering the surface properties of liposomes.

Nickel and cobalt ions have been shown to catalyze metal-induced peroxidation; however, the mechanism is not fully understood. Nickel exists in a variety of oxidation states ranging from −1 to +4 of which +2 is the most prevalent form (Valko et al. 2005). Nickel(II) ions interfere with DNA repair and promote peroxidation of lipids, in addition to the formation of protein carbonyl moieties. Ni(II) does not catalyze the lipid peroxidation process when hydroperoxides are present alone, but it does so when included in the Fenton system (Fe(II)/hydrogen peroxide) (Salnikow and Kasprzak 2005). Cobalt(II) ions are directly not involved in the peroxidation of lipids and exhibit only a negligible effect when incorporated into the Fenton system.

### Radicals derived from nitrogen

Nitric oxide ($NO^{\cdot}$) contains one unpaired electron on the antibonding $2\pi_y$ orbital and is, therefore, a radical. Nitric oxide is formed in biological tissues by specific nitric oxide synthases (NOSs). NOSs are a family of enzymes catalyzing formation of $NO^{\cdot}$ from L-arginine. Nitric oxide is an important molecule playing significant roles in a variety of physiological processes, such as blood pressure regulation, neurotransmission, defense mechanisms, smooth muscle relaxation and immune regulation. In an aqueous environment, nitric oxide has a half-life of only several seconds (Matsubara et al. 2015). In analogy with the definition of oxidative stress, overproduction of radicals derived from nitrogen (RNS) is termed nitrosative stress. During the inflammatory processes, the immune system produces both the superoxide anion radical and nitric oxide. Under these conditions, these two radical species may react together to from significant amounts of peroxynitrite anion $ONOO^-$ ($NO^{\cdot} + O_2^{\cdot -} \rightarrow ONOO^-$), which is a very potent oxidant capable of participating in several oxidative reactions causing DNA fragmentation and oxidation of lipids (Prolo et al. 2015). The reaction of peroxynitrite formation has one of the highest rate constants (~$10^{10}$ $M^{-1}$ $s^{-1}$). Thus, the toxicity of nitric oxide is a consequence of its reactivity with superoxide radical anion to form peroxynitrite.

## Iron

The electronic structure of an iron atom predetermines its ability to carry out one-electron reactions, such that it is a key element in the formation and metabolism of ROS. In biological systems, iron is most frequently present in ferrous [Fe(II)] and ferric [Fe(III)] oxidation states and to a lesser extent in the highly oxidizing ferryl [Fe(IV)] form.

As discussed above, iron is a redox-active metal which can be involved in the various redox reactions that catalyze formation of ROS (Jungwirth et al. 2011). The standard redox potentials of Fe(III) complex + $e^-$ → Fe(II) complex may vary in the range of −1 to +1 V depending on the structure, strength of coordination and nature of ligands bound to the iron atom. Thus, variation of the ligand coordinated to iron allows a fine tuning of the various electron transfer reactions that are possible in a given system (Valko et al. 2005).

Of the order of 65 % of iron in living organisms is bound to the carrier protein hemoglobin, about 25 % is bound to the iron-storage proteins ferritin and hemosiderin, and about 10 % is an integral part of oxygen carrier proteins, myoglobin and cytochromes (Winter et al. 2014). Only a very small amount of iron is present in the form of a redox-active iron pool, sometimes termed as the labile iron pool (LIP), which represents iron bound to low-affinity intracellular low molecular weight ligands. It has been estimated that less than 5 % of the total cell iron is present in the redox-active form (50–100 μM) (Kakhlon and Cabantchik 2002; Wang and Pantopoulos 2011). The presence of a free redox-active iron pool in a cell may serve as a catalyst for the formation of free radicals via the Fenton reaction (4) which may cause damage to cellular components.

Since iron availability is essential for the synthesis of many proteins, including oxygen carriers, electron transport proteins and cytochromes, the iron balance has to be tightly regulated. In summary, iron is released into the plasma from intestinal enterocytes or macrophages, where it is further scavenged by transferrin which maintains $Fe^{3+}$ in a redox-conserved state. Scavenged iron is then delivered to and absorbed by tissues. Received iron is transported into mitochondria for the synthesis of iron–sulfur or hem cores which represent the active sites of various metalloproteins. Excess of iron is stored and detoxified in cytosolic ferritin.

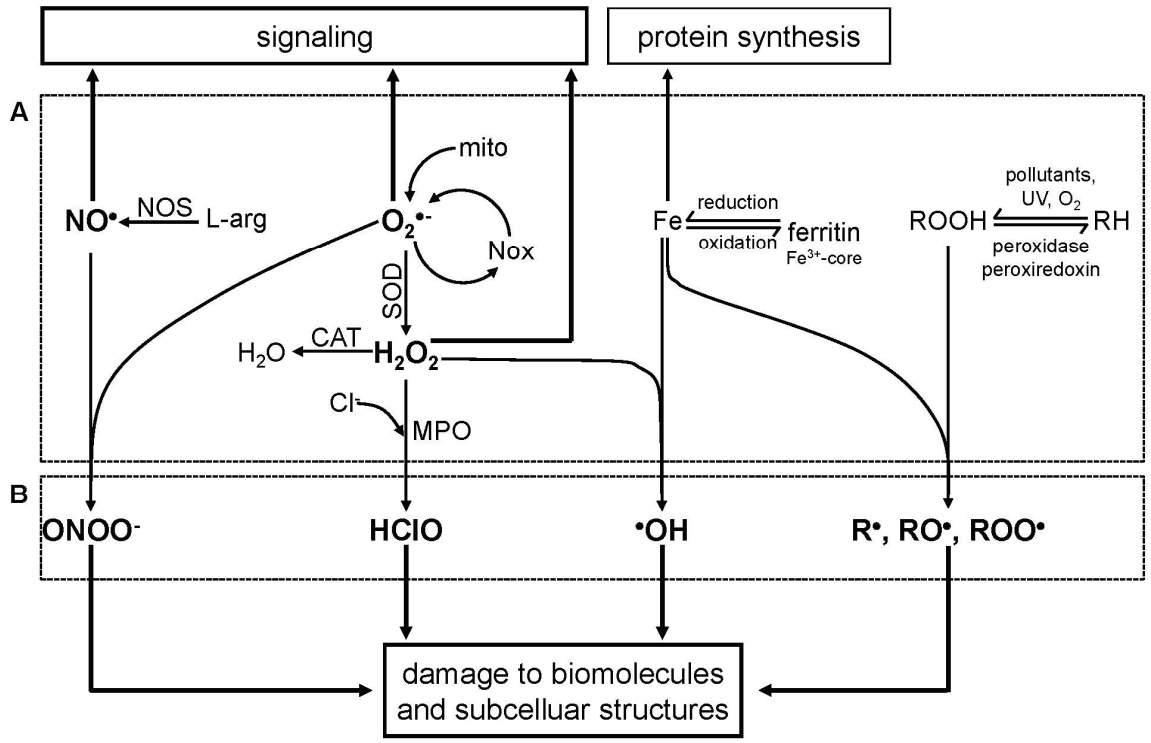


**Figure 1.** Scheme illustrating physiological and pathophysiological reactions of different reactive species. A, primary reactive species ($NO^{\bullet}$, $O_2^{\bullet-}$, Fe, ROOH) and the products of the interaction of two identical reactive species (dismutation of $O_2$ to $H_2O_2$) and transition metals (reactive oxygen, nitrogen and metal species = RONMS). B, secondary products of reactions between two different RONMS. Primary products predominantly contribute to physiological processes (e.g., signaling, protein synthesis); secondary products exert deleterious effects on diverse cell functions. Abbreviations: NO, nitric oxide; $O_2^{\bullet-}$, superoxide; Fe, iron; ROOH, lipid peroxide; $H_2O_2$ hydrogen peroxide; RH, non-oxidized lipid; $R^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, lipid radicals; NOS, nitric oxide synthase; L-arg, L-arginine; $ONOO^-$, peroxynitrite; NOX, NADPH oxidase; mito, mitochondria; SOD, superoxide dismutase; CAT, catalase; $H_2O$, water; $Cl^-$, chloride ion; MPO, myeloperoxidase; HClO, hypochlorous acid; $^{\bullet}OH$, hydroxyl radical; UV, ultraviolet radiation.