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Review

Advances in metal-induced oxidative stress and human disease

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ABSTRACT

Detailed studies in the past two decades have shown that redox active metals like iron (Fe), copper (Cu), chromium (Cr), cobalt (Co) and other metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders (Alzheimer's disease, Parkinson's disease), chronic inflammation and others. The underlying mechanism of action for all these metals involves formation of the superoxide radical, hydroxyl radical (mainly via Fenton reaction) and other ROS, finally producing mutagenic and carcinogenic malondialdehyde (MDA), 4-hydroxynonenal (HNE) and other exocyclic DNA adducts. On the other hand, the redox inactive metals, such as cadmium (Cd), arsenic (As) and lead (Pb) show their toxic effects via bonding to sulphydryl groups of proteins and depletion of glutathione. Interestingly, for arsenic an alternative mechanism of action based on the formation of hydrogen peroxide under physiological conditions has been proposed. A special position among metals is occupied by the redox inert metal zinc (Zn). Zn is an essential component of numerous proteins involved in the defense against oxidative stress. It has been shown, that depletion of Zn may enhance DNA damage via impairments of DNA repair mechanisms. In addition, Zn has an impact on the immune system and possesses neuroprotective properties. The mechanism of metal-induced formation of free radicals is tightly influenced by the action of cellular antioxidants. Many low-molecular weight antioxidants (ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants) are capable of chelating metal ions reducing thus their catalytic acitivity to form ROS. A novel therapeutic approach to supress oxidative stress is based on the development of dual function antioxidants comprising not only chelating, but also scavenging components. Parodoxically, two major antioxidant enzymes, superoxide dismutase (SOD) and catalase contain as an integral part of their active sites metal ions to battle against toxic effects of metal-induced free radicals. The aim of this review is to provide an overview of redox and non-redox metal-induced formation of free radicals and the role of oxidative stress in toxic action of metals.

Abbreviations: NF-κB, nuclear factor kappa B; ATF, activating transcription factor; AP-1, activating protein; NFAT, nuclear factor of activated T-cells; IRPs, iron regulatory proteins; c-acon, aconitase; SOD, superoxide dismutase; HO-1, heme oxygenase; LIP, labile iron pool; 8-OH-G, 8-hydroxyguanine; ROO•, peroxyl radicals; MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; NGAL, neutrophil gelatinase-associated lipocalin; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; APP, amyloid precursor protein; NFT, neurofibrillary tangle; hCtr1, human copper transporter protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TBARS, thiobarbituric acid-reactive substances; $ONOO^-$, peroxynitrite anion; STAT3, signal transducer and activator of transcription 3; IFN, interferon; IL, interleukin; MNC, mononuclear cells; TNF-alpha, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule-1; ROS, reactive oxygen species; RNS, reactive nitrogen species; VEGF, vascular endothelial growth factor; GR, glutathione reductase; ALA, delta-aminolevulinic acid; DMSA, meso-2,3-dimercaptosuccinic acid; ALAD, deltaaminolevulinic acid dehydrogenase; DFB, deferoxamine; CQ, 5-chloro-7-iodoquinolin-8-ol; BAL, dimercaprol.

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Contents

1. Introduction

Metals play important roles in a wide variety of biological processes of living systems. Homeostasis of metal ions, maintained through tightly regulated mechanisms of uptake, storage and secretion is therefore critical for life and is maintained within strict limits (Bertini and Cavallaro, 2008). Metal ion transporters participate in maintaining the required levels of the various metal ions in the cellular compartments (Rolfs and Hediger, 1999).

Breakdown of metal-ion homeostasis can lead to the metal binding to protein sites different to those designed for that purpose or replacement of other metals from their natural binding sites (Nelson, 1999). The results have provided evidence that toxic metals can interact with DNA and proteins causing oxidative deterioration of biological macromolecules. Thus the process of breakdown of metal-ion homeostasis has been involved in a plethora of diseases (Halliwell and Gutteridge, 1990, 2007; Stohs and Bagchi, 1995; Valko et al., 2005; Matés, 2000; Matés et al., 1999). For example, iron is critical for cell growth, oxygen utilization, various enzymatic activities and responses of immune systems. Despite iron is an abundant trace metal in food, more than 2 billion people worldwide suffer from anemia (Stoltzfus, 2001). Iron deficiency results in impaired production of iron-containing proteins, the most prominent of which is hemoglobin. Cellular iron deficiency inhibits cell growth, and subsequently leads to cell death. Conversely, abnormal iron uptake has been related to the most common hereditary disease hemochromatosis, leading to tissue damage derived from free radical toxicity (Toyokuni, 1996). In addition, disruption of iron (and copper) homeostasis has been found to play a key role in the etiology of neurological disorders such as Alzheimer's disease and Parkinson's disease (Bush and Curtain, 2008).

Metals are known to modulate gene expression by interfering with signal transduction pathways that play important roles in cell growth and development (Valko et al., 2006). Deregulation of cell growth and differentiation is a typical characteristic of the cancer phenotype. Actions of metals interfere with deregulation of cell proliferation by activating various transcription factors, controlling cell cycle progression and apoptosis (Evan and Vousden, 2001). The most important involve the nuclear factors NF-κB, AP-1, NFAT and the tumour suppressor protein p53.

Since the generation of free radicals in living systems is closely linked with the participation of redox-active metals such as iron, copper, chromium and cobalt, the redox state of the cell is maintained within strict physiological limits (Rahman, 2007; Matés et al., 2002, 2008). Redox active metals may undergo cycling reactions participating in the transfer of electrons between metals and substrates and therefore may play an important role in the maintenance of redox homeostasis, a phenomenon tightly linked with metal homeostasis (Lindeque et al., 2010). Disruption of metal homeostasis may lead uncontrolled metal-mediated formation of deleterious free radicals participating in the modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulphydryl homeostasis (Gutteridge, 1995; Valko et al., 2007).

Humans may be exposed to redox-inert elements such as cadmium and arsenic which have no known biological function and are even known to be toxic at low concentrations. In contaminated areas, exposure to these elements arises from a variety of natural sources, including air, drinking water and food. While redox active metals undergo redox-cycling reactions, for the group of redox-inert elements, the primary route for their toxicity and carcinogenicity is depletion of glutathione, bonding to sulphydryl groups of proteins and other mechansisms of action (Speisky et al., 2008; Sinicropi et al., 2010; Peralta-Videa et al., 2009).

All these aspects of metals acting in biological systems make the purpose of this paper to provide an overview of the current state of knowledge of the following: the role of redox-active metals, namely iron, copper, chromium, cobalt and redox-inert metals cadmium and arsenic in the formation of reactive oxygen and nitrogen species and their involvement in the development of human disease and ageing. A special attention is paid to the anti-inflammatory role of the redox-inert metal zinc.

2. Iron

Iron occurs in the oxidation states +II and +III. The ferrous ions are soluble in biological fluids and generate in the presence of oxygen damaging hydroxyl radicals. The ferrous ions are unstable in aqueous media and tend to react with molecular oxygen to form ferric ions and superoxide anion radical. The oxidized form of iron is insoluble in water at neutral pH and precipitates in the form of ferric hydroxide (Jones-Lee and Lee, 2005). Paradoxically, despite the fact that both iron ions, ferrous and ferric are so inaccessible, iron is the key catalytic site of many of the enzymes and oxygen-transporting proteins in cells.

2.1. Iron metabolism

Although iron is vital for life, it can be toxic when it is present in excess (Lee et al., 2006a). Iron homeostasis is a complex process, as there are many different proteins that respond not only to the total body burden of iron, but also to stimuli such as hypoxia, anemia and inflammation.

About 65% of iron is bound to hemoglobin, 10% is a constituent of myoglobin, cytochromes, and iron-containing enzymes, and 25% is bound to the iron storage proteins, ferritin and hemosiderin (Cheng and Li, 2007). About 0.1% of body iron circulates in the plasma as an exchangeable pool, essentially all bound to transferrin. The process of chelation not only facilitates the transport of iron into cells, but also prevents iron-mediated free radical toxicity.

The process of cellular iron uptake and storage is regulated by iron regulatory proteins (IRPs) (Eisenstein and Blemings, 1998). Several studies have demonstrated, that dysregulation of IRP expression can be deleterious and even lethal. IRPs are cytosolic trans regulators able to bind to specific RNA stem-loop structures called iron-responsive elements (IREs). Both IRPs have similar affinity for natural IREs, but in most mammalian cells IRP1 is far more abundant than IRP2. IRP2 is homologous to IRP1and does not sense iron. IRP1 is a bifunctional protein which also exhibits aconitase activity in the cytosol. There are two binding mechanisms by which excess iron inactivates IRP1 RNA (Deck et al., 2009). The first mechanism is the so-called iron–sulphur switch, represented by a [4Fe–4S] cluster converting IRP1 to the cytosolic isoform of aconitase (c-acon) (Clarke et al., 2006). A second mechanism depends on iron-mediated degradation of the IRP1 apoprotein. The key role in this process plays phosphorylation of Ser138 which makes the [4Fe–4S] cluster highly sensitive to both cluster perturbants and iron concentration. Electron Paramagnetic Resonance (EPR) spectroscopy has shown that phosphorylation of Ser138 is linked to cluster cycling (between $[4Fe–4S]^{2+}$ and $[3Fe–4S]^{0}$ forms) which regulates iron availability (Deck et al., 2009). IRP2 responds to iron in different ways and does not form a [Fe–S] cluster. It has been revealed that degradation of IRP2 is triggered by iron which regulates the level of the ubiquitin ligase that is responsible for IRP2 degradation (Takahashi-Makise et al., 2009).

2.2. Oxidative stress and iron

The redox state of the cell is predominantly dependent on an iron (and copper) redox couple and is maintained within strict physiological limits (Park et al., 2009). Homeostatic mechanisms prevent excessive iron absorption in the proximal intestine and regulate the rate of iron release involved in recycling. Cellular iron that is not used by other ferroproteins accumulates in ferritin, however its iron-binding capacity is limited (Ganz, 2003). Iron overload is a condition typical for patients suffering from hemochromatosis that causes widespread organ damage. The toxic effects of free iron are substantiated by its ability to catalyze via Fenton reaction the generation of damaging reactive free radicals (Ganz, 2003).

Many studies documented that mutations in superoxide dismutase enzymes (Deng et al., 1993) and iron-uptake regulator (Iolascon et al., 2009) may lead to excess levels of superoxide anion radicals and iron overload. Such a condition leads to the possibility of redox active iron to participate in organic and inorganic oxygen radical reactions, such as stimulating lipid peroxidation and catalyzing the formation of damaging hydroxyl radicals with subsequent tissue damage.

Superoxide radicals are normally produced by the enzyme NADPH oxidase in order to activate the defense mechanisms against invading pathogens (Halliwell and Gutteridge, 2007). Superoxide is produced by the electron transport chain from oxygen occupying the final position and acting as the terminal electron acceptor. Some electrons can randomly "leak" from the electron transport chain (Campian et al., 2004) and interact with oxygen to produce superoxide radicals. Thus under physiological conditions, about 1–3% of the oxygen molecules in the mitochondria are converted into superoxide radicals.

Superoxide radical is normally present mainly in the form of an anion radical and is removed by a dismutation reaction (Liochev and Fridovich, 2000):

$$2O_2^{-}\cdot + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2 \quad (1)$$

While without SOD this reaction proceeds very slowly ($k \sim 0.2\ M^{-1}\ s^{-1}$), the reaction becomes biologically relevant when it is catalyzed by the SOD. The kinetic constant of the SOD-catalyzed superoxide depletion dismutation reaction has been estimated to be $2.5 \times 10^{9}\ M^{-1}\ s^{-1}$ (Liochev and Fridovich, 2003).

A mutual link between superoxide radicals and iron shows, that under in vivo stress conditions, an excess of superoxide releases "free iron" from iron-containing molecules (e.g. ferritin). The release of iron by superoxide has also been demonstrated for the [4Fe–4S] cluster-containing enzymes. Inactivation of these enzymes by $O_2^{-\bullet}$ is a rapid process that leads to oxidation of the iron-sulphur cluster. The native clusters contain two Fe(II) and two Fe(III) ions, and the oxidation [one Fe(II) is oxidized to Fe(III)] may be denoted as follows (Liochev and Fridovich, 1994):

$$[2Fe(II)\ \ 2Fe(III)–4S]^{2+} + O_2^{-\bullet} + 2H^{+} \rightarrow [Fe(II)\ \ 3Fe(III)–4S]^{3+} + H_2O_2 \quad (2)$$

The rate constant for reaction (2) has been estimated in the range of 10^{8} to $10^{9}\ M^{-1}\ s^{-1}$. Since the oxidized protein binds the Fe(III)

more firmly, Fe(II) ions are released from protein according to the following reaction:

$$[\mathrm{Fe(II)}\quad 3\mathrm{Fe(III)}\text{–}4\mathrm{S}]^{3+} \rightarrow [3\mathrm{Fe(III)}\text{–}4\mathrm{S}]^{+} + \mathrm{Fe(II)} \quad (3)$$

The released Fe(II) can participate in the Fenton reaction, generating highly reactive hydroxyl radicals ($^{\bullet}$OH) (Prousek, 2007)

$$\mathrm{Fe(II)} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe(III)} + {}^{\bullet}\mathrm{OH} + \mathrm{OH^-} \quad \text{(Fentonreaction)} \quad (4)$$

The Fenton reaction has its in vivo significance mainly under state of an organisms overloaded by iron (as in the conditions of hemochromatosis, b-thalassemia, hemodialysis). Thus high amounts of "free available iron" can have deleterious effects (Kakhlon and Cabantchik, 2002). The superoxide radical participates in the Haber–Weiss reaction (Liochev and Fridovich, 2002):

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

which is a combination of Fenton reaction and the reduction of Fe(III) by superoxide:

$$\mathrm{Fe(III)} + \mathrm{O_2}^{-\bullet} \rightarrow \mathrm{Fe(II)} + \mathrm{O_2} \quad (6)$$

The hydroxyl radical is highly reactive with a half-life in aqueous solution of less than 1 ns (Pastor et al., 2000). When produced in vivo it reacts close to its site of formation. Production of $^{\bullet}$OH close to DNA could lead to this radical reacting with DNA bases or the deoxyribose backbone of DNA to produce damaged bases or strand breaks.

It is assumed that the most abundant in vivo production of hydroxyl radical according to the Fenton reaction occurs when M^{n+} is iron and copper. However, the Fenton reaction has also been observed for chromium, cobalt and certain other metals (Lloyd et al., 1997). Although Fenton chemistry is known to occur in vitro, its significance under physiological conditions is not fully understood. Due to the effective sequestration of iron by the various metal-binding proteins, the cells contain only the negligible amounts of "free catalytic iron". To avoid harmful effect of free iron, its proper chelation is of key importance (Kell, 2009).

The peptide hormone hepcidin is a 25-amino acid polypeptide regulator of iron proteins and plays a central role in iron homeostasis (Ganz, 2003; Kemna et al., 2008). Hepcidine is expressed in the liver and regulated by iron, hypoxia, and inflammation. Hypoxia is known to enhance formation of superoxide radicals and suppressed formation of hepcidin leading to more iron being absorbed from the intestine and effluxed in the circulation (Donovan et al., 2005). Thus there is a complex interplay between positive and negative regulation and distribution of iron within the organism caused by changes in the level of hepcidin (Nemeth et al., 2004). P53 is known to activate the formation of hepcidin that plays a role in the degradation of atherosclerotic plaques (Weizer-Stern et al., 2007).

If iron is not appropriately chelated it can participate in the formation of harmful free radicals including the hydroxyl radical. Low molecular weight chelators occurring in cytoplasm can bind iron and thus contribute to the formation of a labile iron pool (LIP) consisting of both Fe(II) and Fe(III) ions chelated by citrate, carboxylates, nucleotides and other ligands (Kakhlon and Cabantchik, 2002). LIP represents a steady state exchangeable, and readily chelatable iron that rapidly passes through the cell (Ponka and Lok, 1999). The quantification of cellular LIP represents only a minor fraction (<5%) of the total cell iron (50–100 μM) (Kakhlon and Cabantchik, 2002; Doulias et al., 2008; Inoue and Kawanishi, 1987), however, there still exists serious methodological problems associated with the estimation of LIP concentrations ranging 0.2–230 μM obtained for the same types of cells and tissues.

2.3. Iron and human disease

Permanent modification of genetic material resulting from free radical attacks represents the initial step involved in mutagenesis, carcinogenesis and ageing (Durackova, 2010). In fact, as it has been well documented, in various cancer tissues free radical-mediated DNA damage has occurred (Marnett, 2000). The hydroxyl radical produced via the catalytic action of iron(II) (Fenton reaction) is able to add to double bonds of DNA bases. To date, more than 100 oxidative products of DNA have been identified, the best known being of 8-hydroxyguanine (8-OH-G) (Dizdaroglu et al., 2002; England et al., 1998) (see Fig. 1). The presence of 8-OH-G in human urine was first reported by Ames and co-workers (Shigenaga et al., 1989). The oxidized DNA products are mutagenic and carcinogenic and represent a good biomarker of oxidative stress of an organism and carcinogenesis.

The process of lipid peroxidation is catalyzed by the iron and results in the formation of peroxyl radicals (ROO$^{\bullet}$). Once formed, peroxyl radicals can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (MDA) (Fig. 2). The major aldehyde product of lipid peroxidation other than malondialdehyde is 4-hydroxy-2-nonenal (HNE).

MDA can react with DNA bases guanine, adenine, and cytosine to form M_1G, M_1A and M_1C adducts, respectively (Marnett, 1999). M_1G adducts were detected significantly elevated in human breast tissues and rodent tissues (Wang et al., 1996). The role of free radicals in the etiology of breast cancer via hydroxyl radical-induced DNA damage has been well established (Malins et al., 1996).

It has been proposed that intestinal exposure to ingested iron and iron-induced oxidative stress may be key determinants of human colorectal cancer in highly developed, meat-eating countries (Nelson, 1992). DNA analysis from colon and rectum biopsies revealed also a significantly increased level of 8-OH-G, 2-hydroxyadenine and 8-hydroxy-adenine adducts (Skrzydlewska et al., 2005). These lesions caused by hydroxyl radical attack could signify the increase in DNA damage and/or decrease in their repair. Interestingly, long-term use of anti-inflammatory drugs such as aspirin lowers by 40% the incidence of colon cancer, thus the development of cancer may be linked with an inflammatory component. We have proposed an alternative mechanism in which the bile acids (deoxycholic acid), the K vitamins, iron(II) complexes and oxygen interact to induce an oncogenic effect in the colon by the generation of free radicals (Valko et al., 2001).

The iron carrier NGAL (21 kDa, also known as lipocalin-2 or siderocalin), a small siderophore-binding protein involved in the maintenance of iron equilibrium has been found to be expressed in various tumours (Bolignano et al., 2009). Significant changes in NGAL expression have also been observed, for instance, during kinase-mediated signalling (Cowland et al., 2003), in cardiovascular disease (Elneihoum et al., 1996) and in cancer (Stoesz et al., 1998).

Occupational exposure of workers to asbestos (and related fibers) containing approximately 30% (weight) of iron has been related to an increased risk of mutagenesis and carcinogenesis (Stayner et al., 1996). The mutagenic and carcinogenic properties of asbestos were related to the ability of iron to catalyze the formation of hydroxyl radicals. Thus in this context, iron chelation may be a useful strategy for cancer prevention.

Iron metabolism has been found to be significantly disturbed in type 2 diabetes and interferes with glucose metabolism (Lee et al., 2006b). Lowering iron pools generally improves insulin sensitivity. In addition, iron has been strongly implicated in nonalcoholic steatohepatitis, considered an early marker of insulin resistance (Machado and Cortez-Pinto, 2006).

Elevated iron levels can predispose to coronary disease and myocardial infarction. Hypertension is believed to be a common

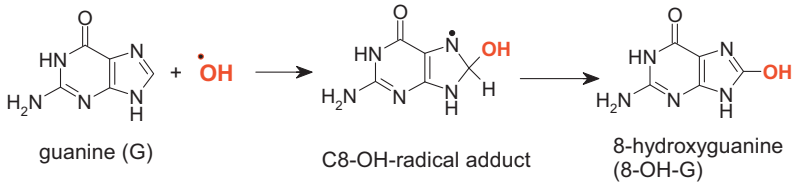

Fig. 1. Reaction of guanine base with hydroxyl radical.

risk factor of cardiovascular disease, related to metabolic syndrome and obesity, mediated mainly by elevated levels of ROS in which iron plays a key role (LaMarca et al., 2008). Positive effects of iron depletion in women due to menstruation have been associated with the lowering risk of cardiovascular-disease that disappears in post-menopause.

Cardiovascular disease is a multifactorial disorder in which lipid metabolism, life style (smoking, stress), coronary artery disease and others play their concerted roles (Touyz and Schiffrin, 2004). It has been communicated that iron mediated formation of superoxide radical and hydroxyl radical during development of heart disease, mainly during reperfusion injury, can be inhibited by iron chelators.

Anemia is a potential risk factor and has been associated with heart failure (Mozaffarian et al., 2003; Bolger et al., 2006), pointing to a role for dysregulation of iron metabolism. This points to the necessity of our understanding that exact speciation of iron in chronic anemias is linked to inflammatory diseases (Weiss and Goodnough, 2005).

Atherosclerosis is an inflammatory condition accompanied by the accumulation of iron and oxidized lipids and fibrous elements in arteries as plaques. There is a correlation between iron status and atherosclerosis; free or poorly ligated iron can participate in lipid peroxidation and protein peroxidation. The iron levels found in plagues correlated with the amount of oxidized pro-

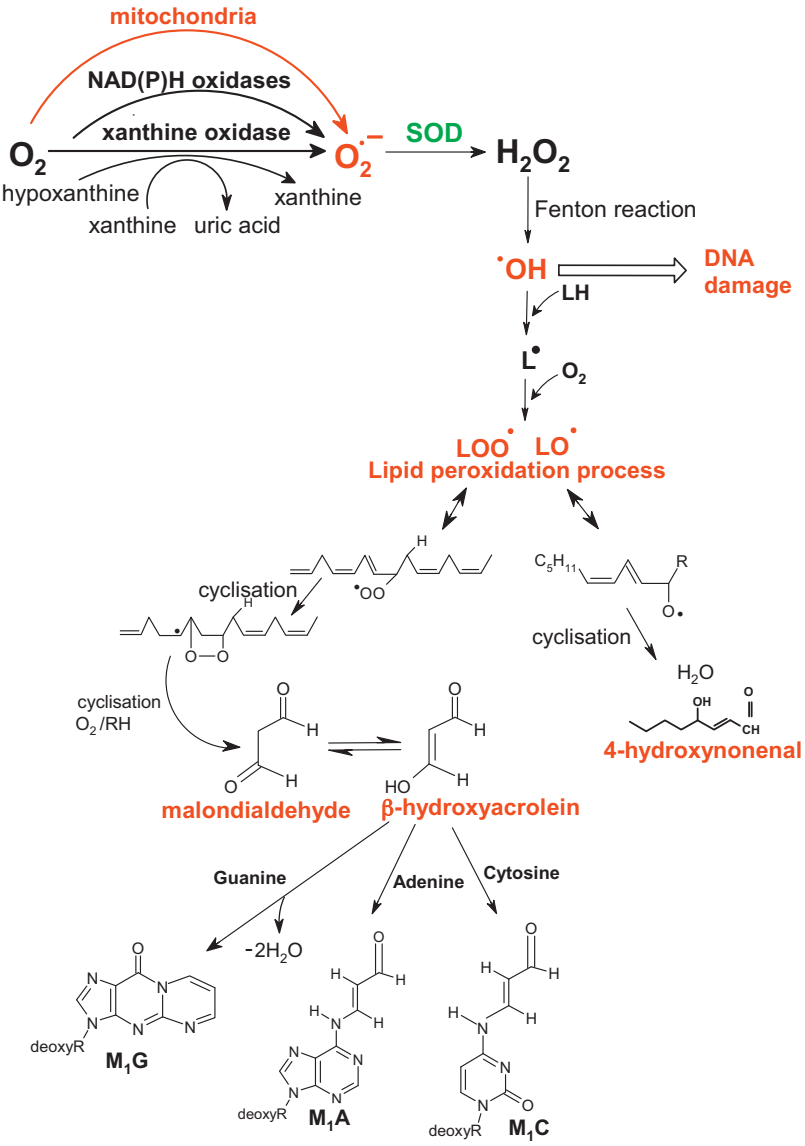

Fig. 2. ROS formation and the lipid peroxidation process.

teins. Electron Paramagnetic Resonance (EPR) has been employed to demonstrate that atherosclerotic tissue contained 17 times more iron (EPR detectable ferric) than equivalent healthy tissue (Stadler et al., 2004).

Transition metal ions have been implicated in etiology of neurodegenerative disorders (Bush, 2003). Dysregulation of brain iron (and also copper, see below) homeostasis is a key factor to early neuropathological events in Alzheimer's disease (AD), including oxidative stress, inflammatory processes, amyloid β deposition, tau phosphorylation, and neuronal cell cycle regulatory failure, leading to apoptosis (Bush and Curtain, 2008). Very recently, combining the three ion beam techniques of transmission ion microscopy, scattering spectrometry and particle induced X-ray emission in conjunction with a high energy proton microprobe, it has been proved that there is an increase in the metal concentrations within the amyloid plaques compared with the surrounding tissue as follows: iron (85 ppm compared with 42 ppm), copper (16 ppm compared to 6 ppm), and zinc (87 ppm compared to 34 ppm) (Rajendran et al., 2009). Iron is capable of stimulating free radical formation, increased protein and DNA oxidation in the Alzheimer's brain, enhanced lipid peroxidation, decreased level of cytochrome c oxidase and advanced glycation end products, carbonyls, malondialdehyde (MDA), peroxynitrite and HO-1 (Dröge, 2002). Excess of iron in brain tissue may activate the iron-dependent HIF-1 prolyl-4-hydroxylase, resulting in the proteasomal-mediated degradation of HIF. Iron-chelating drugs have been shown to stabilize HIF-1, which, in turn, would transactivate the expression of established protective genes, including vascular endothelial growth factor (VEGF), erythropoietin, aldolase and p21. In conclusion, considering the multiple iron-operating sites in Alzheimer's disease, iron chelators, possessing several active neuroprotective moieties can suppress the wide spectrum of oxidative stress-associated neuropathologies, as well as amyloid precursor protein (APP) translation, Aβ generation, and amyloid plaque and neurofibrillary tangle (NFT) formation (Amit et al., 2008).

Rheumatoid arthritis is another disorder linked with the effect of ROS (Dröge, 2002). This disorder is characterized by an overall low level of body iron (anemia), however elevated iron is found in the synovial fluid of arthritic joints (Gutteridge, 1987). This suggests a marked disorder in iron metabolism and points to a mechanism in which elevated superoxide radical liberates free (catalytic) iron from ferritin in synovial fluid catalysing thus the formation of damaging hydroxyl radicals via the Fenton reaction. Some studies evidenced that effective iron chelators can improve symptoms of rheumatoid arthritis.

3. Copper

The most oxidation numbers of copper in living organisms are Cu(II) and Cu(I). The essential trace element copper is a cofactor of many enzymes involved in redox reactions, such as cytochrome c oxidase, ascorbate oxidase, or superoxide dismutase. In addition to its enzymatic roles, copper is used in biological systems for electron transport (Valko et al., 2005). The blue copper proteins that participate in electron transport include azurin and plastocyanin.

3.1. Copper metabolism

Copper is readily absorbed from the diet across the small intestine (~2 mg/day) and stored in the liver. The major excretory route of copper stored in liver is via the biliary pathway (~80%) (Linder and Hazegh-Azam, 1996). Copper is bound to either serum albumin or histidine and trafficked through the bloodstream for delivery to tissues or storage in the liver. Copper is imported into the hepatocytes via the high-affinity human copper transporter, hCtr1 (Zhou and Gitschier, 1997), localized on the plasma membrane. hCtr1 also participates in the intracellular compartmentalization of this metal. Once inside the cell, copper is escorted to (i) metallothionein pool, or (ii) transported to the mitochondria for cytochrome c oxidase incorporation, or (iii) for delivery to emerging Cu, Zn-SOD or (iv) transported to the Wilson disease P-type ATP-ase in the trans-golgi network for subsequent incorportation to the ceruloplasmin (Shim and Harris, 2003). Ceruloplasmin contains about 95% of the copper found in serum.

Copper can catalyze ROS formation via Fenton and Haber–Weiss chemistry and therefore under physiological conditions, free copper very rarely exists inside cells. In the process of the investigation of copper chaperone for SOD, Rae et al. (1999) explored that the upper limit of so-called "free pools of copper" was far less than a single atom per cell. This finding is of great importance, especially when considering other physiologically important trace metal ions.

3.2. Oxidative stress and copper

Copper can induce oxidative stress by two mechanisms. First, it can directly catalyze the formation of ROS via a Fenton-like reaction (Prousek, 2007; Liochev and Fridovich, 2002). Second, exposure to elevated levels of copper significantly decreases glutathione levels (Speisky et al., 2009).

Cupric and cuprous ions can act in oxidation and reduction reactions. The cupric ion (Cu(II)), in the presence of superoxide anion radical or biological reductants such as ascorbic acid or GSH, can be reduced to cuprous ion (Cu(I)) which is capable of catalyzing the formation of reactive hydroxyl radicals through the decomposition of hydrogen peroxide via the Fenton reaction (Aruoma et al., 1991; Prousek, 1995; Barbusinski, 2009):

$$Cu(II) + O_2^{-\bullet} \rightarrow Cu(I) + O_2 \quad (7)$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + {}^{\bullet}OH + OH^- \quad \text{(Fentonreaction)} \quad (8)$$

The hydroxyl radical is extremely reactive and can further react with practically any biological molecules in the near vicinity, for example via hydrogen abstraction leaving behind a carbon-centered radical, e.g. form a lipid radical from unsaturated fatty acids. Copper is also capable of causing DNA strand breaks and oxidation of bases via ROS. Copper in both oxidation states (cupric or cuprous) was more active that iron in enhancing DNA breakage induced by the genotoxic benzene metabolite 1,2,4-benzenetriol. DNA damage occurred mainly by a site-specific Fenton reaction (Moriwaki et al., 2008).

Glutathione is a substrate for several enzymes that removes ROS and is also a powerful cellular antioxidant present in the cells in millimolar concentration. It has multiple functions in intracellular copper metabolism and detoxification. Glutathione can suppress copper toxicity by directly chelating the metal (Mattie and Freedman, 2004) and maintaining it in a reduced state making it unavailable for redox cycling. Disruption of copper homeostasis resulting in elevated pools of copper may contribute to a shift in redox balance towards more oxidizing environment by depleting glutathione levels (Linder, 1991). The depletion of glutathione may enhance the cytotoxic effect of ROS and allow the metal to be more catalytically active, thus producing higher levels of ROS. The large increase in copper toxicity following GSH depletion clearly demonstrates that GSH is an important cellular antioxidant acting against copper toxicity (Steinebach and Wolterbeek, 1994).

The effect of copper on oxidation of low-density lipoprotein (LDL) has been studied (Harris, 1992). Such studies have various clinical consequences involving promotion of atherogenesis and prothrombotic properties. In vitro studies clearly demonstrated LDL oxidation induced by copper. In addition to copper ions, ceruloplasmin, containing seven copper atoms per molecule may serve

as a source of free Cu and thus be involved in LDL oxidation (Witting et al., 1995).

It has also been reported that high-density lipoprotein (HDL) is susceptible to oxidation. Oxidation of HDL may significantly affect their cardioprotective properties since HDL is more sensitive to oxidation by copper than LDL. Dose-dependent oxidative damage to HDL and protective effect of vitamin E against oxidation of HDL was observed in the studies of copper incubated with HDL. Experimental results demonstrate that vitamin C also inhibits lipid oxidation in HDL and preserves the antioxidant activity associated with this lipoprotein fraction (Hillstrom et al., 2003).

Homocysteine is an atherogenic amino acid and is known to promote copper and iron-dependent oxidation of LDL (Hillstrom et al., 2003). Investigation whether ascorbate could protect LDL from homocysteine-mediated oxidation has shown, that ascorbate (concentrations ~50–100 μM) protected LDL from oxidation as evidenced by an increased lag time preceding lipid diene formation, decreased thiobarbituric acid-reactive substances (TBARS) accumulation and decreased lipoprotein anodic electrophoretic mobility. Partial protection was observed even at lower concentrations of ascorbate (5–10 μM).

Spin traping EPR spectroscopy has been employed to study the combined effect of selenium and vitamin E on copper-induced oxidation of LDL (Kadiiska and Mason, 2002). Observation of increased concentration of lipid-derived radicals has confirmed copper-mediated formation of free radicals in vitamin E and selenium deficient rats. These findings support the proposal that dietary selenium and vitamin E can protect against lipid peroxidation and copper toxicity (Gaetke and Chow, 2003).

The effect of smoking on copper plasma level and lipid peroxidation process has been studied (Lapenna et al., 1995). The results have shown, that concentration of copper was higher in smokers that in non-smokers. As expected, the damage products of lipid peroxidation evaluated by fluorescence spectroscopy were also increased in smokers. This indicates that cigarette smoke is at least partly responsible for enhanced prooxidant action of copper.

As described above, superoxide dismutases normally protect cells from oxidative damage, therefore the role of SOD in DNA damage was also investigated. Of interest were experimental studies exploring the effect of copper deficiency on oxidative DNA damage in Jurkat T-lymphocytes (Pan and Loo, 2000). The results have shown that copper deficiency increased cellular susceptibility to oxidative damage. Copper depletion leads to decreased capability of cells to produce SOD, thus increasing their propensity to oxidative damage.

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion ($ONOO^-$) (Carr et al., 2000):

$$NO^{\bullet} + O_2{}^{-\bullet} \rightarrow ONOO^- \quad (9)$$

Peroxynitrite anion is a potent cytotoxic oxidising agent capable of attacking proteins and causing DNA fragmentation and lipid oxidation. Peroxynitrite has been shown to destroy the transport protein ceruloplasmin and release Cu ions that may induce formation of a copper–lipoprotein complex, which stimulates lipid peroxidation.

The combination of ascorbate, copper (or iron), and hydrogen peroxide is an efficient hydroxyl radical generating system called "the Udenfriend system" (Udenfriend et al., 1954). Prooxidant behaviour of ascorbate under in vitro conditions in this system has been well documented. To see whether ascorbate acts as a pro-oxidant in the presence of copper (or iron) under physiological conditions, an experimental study using human plasma has been conducted. The results have shown that even in the presence of redox-active iron or copper and hydrogen peroxide, ascorbate acts as an antioxidant preventing lipid peroxidation and protein oxidation in human plasma (Suh et al., 2003).

Exposure to metals has been shown to activate components of MAPK signalling cascades (Mattie and Freedman, 2004). Transcriptional activation by copper involves MAPK pathways and changes in cellular glutathione status. Results from various studies suggested that copper is capable of activating transcription through both metal- and oxidative stress-mediated mechanisms. However, the molecular mechanisms exploring gene expression induced by copper have still not been adequately described. The role of ROS in mediating the ability of copper to activate MAPK signalling pathways was previously demonstrated using PKC, p38, ERK, and JNK inhibitors. The recent results obtained by fine-tuning of the level of intracellular-copper-induced oxidative stress have shown altered levels of protein binding to AP-1 and ARE. This clearly demonstrates a role for the copper-induced and ROS-mediated activation of MAPK signalling pathways (Mattie et al., 2008).

Copper-induced formation of ROS can cause peroxidation of lipids, subsequently leading to an increase in the levels of a signalling molecule HNE (Mattie et al., 2008). HNE acts as a second messenger and may increase the levels of phosphorylation and activation of the c-Jun N-terminal kinase/stress-activated protein kinase and p38 pathways. HNE production correlates with an increase in AP-1 activity and the expression of several genes, including collagen type I, transforming growth factor β1 and γ-glutamylcysteine synthetase. HNE is also capable of increasing c-Jun expression and of activating PKC and JNK/SAPK.

3.3. Copper and human disease

3.3.1. Cancer

Literature to date has shown that both serum and tumour tissue copper levels in cancer patients are significantly elevated compared to healthy subjects. In addition to copper, the majority of these studies have focused on determining the concentrations of zinc, iron and selenium. Interestingly, while the zinc, iron and selenium concentrations were significantly lowered in cancer patients, the copper concentrations were almost always found to be either elevated or significantly elevated compared to healthy subjects.

The most elevated levels of copper have been documented in cancer patients suffering from breast, cervical, ovarian, lung, prostate, stomach cancer and leukemia. Furthermore, it has been also shown that the Cu:(Zn, Se, Fe) ratios are very frequently higher in cancer patients compared to normal subjects (Gupte and Mumper, 2009).

Since copper is known to promote oxidative stress and inflammation, these data document that it is likely that under non-physiological conditions of increased copper levels, it could play a role in the development of various cancers. Increased markers of oxidative stress have been documented in a variety of tumours, possibly due to the combination of factors such as elevated active metabolism, mitochondrial mutation, cytokines, and inflammation (Roberts et al., 2010). Elevated copper levels have been shown to be directly linked to cancer progression (Gupte and Mumper, 2009).

Copper is important also for angiogenesis, a process of the growth of any tumour beyond a few millimeters. In the process of angiogenesis, newblood supplies that feed the malignant cells are formed (Folkman, 1995). Angiogenesis is a multi-step process, involving degradation of the endothelial cell basement membrane, endothelial cell migration to the perivascular stroma and capillary sprouting. To stop the growth of tumour in the early stage, the concept of anti-angiogenic therapy has gained enormous interest. Such therapy uses findings in the description of endogenous angiogenesis stimulators including growth factors (e.g. VEGF, EGF,

angiogenin, basic Fibroblast Growth factors and others), cytokines (e.g. Interleukin (IL-1)) and transition metal elements, such as copper. In fact, copper has been shown to stimulate angiogenesis in chick embryo chorioallantoic models. In addition, the expressions of various angiogenic cytokines/growth factors such as IL-1, 6 and, b-FGF, TNF-α and VEGF are suppressed following copper elimination. In this respect, several anti-angiogenic agents, based on copper chelators have been designed and tested (Brem et al., 1990).

3.3.2. Neurological disorders

The majority of papers link the origin of Alzheimer's disease (AD), and to a lesser extent also to Parkinson's disease (PD), with direct evidence supporting increased oxidative stress of the brain (Bush and Curtain, 2008; Jomova et al., 2010).

The "null hypothesis" in studies of Alzheimer's disease has been centered on Amyloid-β (Aβ) (Cuajungco et al., 2000). The central tenet of Aβ toxicity is linked with the presence of redox metals, mainly copper and iron. Direct evidence of increased metal concentrations within amyloid plaques is based on physical measurements that proved that there is an increase in the metal concentrations within the amyloid plaques (see above) (Rajendran et al., 2009).

Copper is known to bind to Aβ via histidine (His13, His14, His6) and tyrosine (Tyr10) residues (Hung et al., 2010). Besides Cu(II), Aβ also binds Zn(II) and Fe(III). Cu(II) interaction with Aβ promotes its neurotoxicity which correlates with the metal reduction [Cu(II) → Cu(I)] and the generation of hydrogen peroxide which in turn can be catalytically decomposed forming hydroxyl radical. Cu(II) promotes the neurotoxicity of Aβ with the greatest effect for Aβ (1–42) > Aβ (1–40), corresponding to the capacity to reduce Cu(II) to Cu(I), respectively and form hydrogen peroxide (Cuajungco et al., 2000). The copper complex of Aβ(1–42) has a highly positive reduction potential, characteristic of strongly reducing cupro-proteins.

EPR spectroscopy has been employed to show, that the *N*-terminal residues of His13, His14, His6 and Tyr10 are involved in the complexation of Cu in Aβ (Cerpa et al., 2004; Butterfield et al., 2001). It has recently been proposed that *N*-terminally complexed Cu(II) is reduced by electrons originating from the C-terminal methionine (Met35) residues according to the reaction:

$$\mathrm{MetS} + \mathrm{A\beta\text{-}Cu(II)} \leftrightarrow \mathrm{MetS^{+\bullet}} + \mathrm{A\beta\text{-}Cu(I)} \quad (10)$$

forming the sulphide radical of Met35 ($\mathrm{MetS^{+\bullet}}$) and reducing Cu(II). Based on the thermodynamic calculations the above reaction is rather unfavourable. However, the rate of electron transfer between MetS and Aβ-Cu(II) may be enhanced by the subsequent exergonic reaction of deprotonation of $\mathrm{MetS^{+\bullet}}$, leaving behind the 4-methylbenzyl radical, thus making the reaction (16) viable in vivo (Valko et al., 2005). The sulphide radical $\mathrm{MetS^{+\bullet}}$ may react for example with superoxide anion radical:

$$\mathrm{MetS^{+\bullet}} + \mathrm{O_2^{-\bullet}} \rightarrow 2\mathrm{MetO} \quad (11)$$

forming Met-sulphoxide (MetO) which has been isolated from AD senile plaques.

Amyloid-β has neurotoxic properties and has been proved to stimulate copper-mediated oxidation of ascorbate (Dikalov et al., 2004):

$$\mathrm{A\beta\text{-}Cu(II)} + \mathrm{AscH^-} \leftrightarrow \mathrm{A\beta\text{-}Cu(I)} + \mathrm{Asc^-} + \mathrm{H^+} \quad (12)$$

$$\mathrm{A\beta\text{-}Cu(II)} + \mathrm{Asc^-} \leftrightarrow \mathrm{A\beta\text{-}Cu(I)} + \mathrm{Asc} \quad (13)$$

$$\mathrm{A\beta\text{-}Cu(I)} + \mathrm{H_2O_2} \rightarrow \mathrm{A\beta\text{-}Cu(II)} + {}^{\bullet}\mathrm{OH} + \mathrm{OH^-} \quad \text{(Fenton)} \quad (14)$$

$$\mathrm{A\beta\text{-}Cu(I)} + \mathrm{O2} \leftrightarrow \mathrm{A\beta\text{-}Cu(II)} + \mathrm{O_2^{-\bullet}} \quad (15)$$

Cu(I) may catalyze free radical oxidation of the peptide via the formation of free radicals by the Fenton reaction. It should be noted, that copper plays an important role in other neurodegenerative disorders, such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (Lou Gehrig's disease), and prion diseases such as Jacob-Creutzfeldt disease. All these conditions are characterized by the copper-dependent formation of misfolded proteins forming inclusion bodies.

3.3.3. Chronic disease: diabetes, cardiovascular disease and atherosclerosis

Ceruloplasmin has been noted to be increased in both type 1 and type 2 diabetic humans with respect to healthy subjects (Uriu-Adams and Keen, 2005). In addition, some studies reported increased concentration of copper in plasma of diabetic patients with complications, such as hypertension and retinopathy (Kang et al., 2000). Altered copper metabolism interfering with increased glycated proteins may contribute to the progression of diabetes-related pathologies. Glycated proteins exhibit increased affinity for transition metal ions, including copper. Despite copper being bound to proteins it can catalytically participate in the formation of free radicals and thus provide stable active sites for producing free radicals that in turn can contribute to increased oxidative stress in diabetes (Yim et al., 2001). In fact, increased markers of oxidative damage, including damaged proteins, lipid peroxidation and DNA damage, have been observed and implicated in the pathogenesis of diabetic complications (Aydin et al., 2001; Dinçer et al., 2002; Flores et al., 2004).

The serum level of ceruloplasmin plays an important role also in cardiovascular disease. Epidemiological studies have shown, that an elevated level of ceruloplasmin is an independent risk factor for cardiovascular disease (Cunningham et al., 1995). Increased concentration of copper in serum has also been associated with mortality from coronary disease. HDL, a normally anti-inflammatory molecule changes during acute phase response to one that is pro-inflammatory. When ceruloplasmin was a constituent of HDL and was added to aortic endothelial cell/smooth muscle cell cultures, HDL had a suppressed capability to inhibit LDL oxidation, and increased the expression of a chemotactic factor, MCP-1, which induced monocyte migration (Van Lenten et al., 1995).

Copper is also linked with atherosclerosis (Haidari et al., 2001). The most profound evidence for the involvement of copper in atherosclerosis is probably the interaction of copper and homocysteine generating free radicals and thus oxidising LDL, which has been found in the atherosclerotic plaques. Elevated homocysteine levels are a known risk factor for atherosclerosis (as well as AD), and it may be this toxic interaction with copper that makes it a risk factor.

In addition, an association between elevated copper and ceruloplasmin levels with atherosclerotic disease has been noted (Burkitt, 2001). Ceruloplasmin belongs to the multi-copper oxidase family of enzymes and contains the trinuclear copper center. It has been found to present in human atherosclerotic tissues (Swain and Gutteridge, 1995), suggesting that effects of ceruloplasmin at the level of the atherosclerotic lesion may be involved in disease pathology. One of the copper ions of ceruloplasmin can interact with LDL which is a component of atherosclerotic plaques. Similarly to copper, iron has been found to play a positive role in the development of atherosclerosis and supports the concept of a positive role for copper in the etiology of this disease.

Animal models have been adopted to reveal the association between abnormal copper metabolism and diabetes. A rat model of diabetes with heart failure revealed improved progress after treatment with anticopper chelating agent trientine used for treatment of Wilson's disease (WD). WD is a rare inherited autosomal recessive disorder of copper metabolism, resulting in copper toxicity. Studies using animal models have shown that copper interacts with

glycated proteins and produces neuropathy, one of the complications of diabetes in humans (Eaton and Qian, 2002).

It has been recently characterized that hyperglycemic complications contributing to cardiovascular disease are linked with disturbed copper homeostasis. Chelatable copper level was found to be increased in the diabetic hearts and elevated extracellular copper might be implicated in the mechanism of cardiovascular damage in diabetes (Cooper et al., 2004). Heart disease in diabetes is accompanied by left ventricular hypertrophy, cardiomyopathy and increased incidence of heart failure. Copper balance in type 2 diabetes can be improved by treatment with copper(II)-selective chelator trientine (Cooper et al., 2009). It has been hypothesised that hyperglycemia-induced impairment of tissue copper balance is an important mechanism of left-ventricular hypertrophy in diabetes and that effective copper(II) chelation can be used as a new way of treatment for cardiac disease in diabetes.

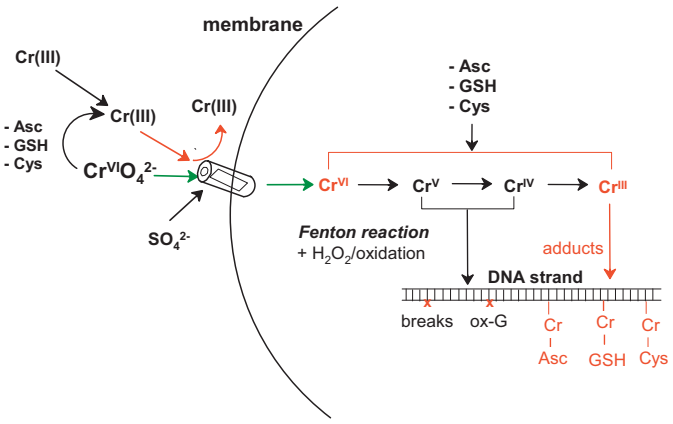

Fig. 3. Uptake and DNA damage by Cr(VI).

4. Chromium

Chromium, one of the most common elements in the earth's exists in several oxidation states (Cieslak-Golonka, 1996). The most important stable states are 0 (elemental metal), +III (trivalent), and +VI (hexavalent). The health effects and toxicity/carcinogenicity of chromium are primarily related to the oxidation state of the metal at the time of exposure. Trivalent (Cr[III]) and hexavalent (Cr[VI]) compounds are thought to be the most biologically significant (US Department of Health, 1993).

4.1. Oxidative stress and chromium

Cr(III) is an essential dietary mineral in low doses, found in most fresh foods, including breads, meats and vegetables and drinking water (Vincent, 2010). It is required to potentiate insulin and for normal glucose metabolism. Solubilities of Cr(VI) compounds greatly vary from those that are readily soluble to those which are practically insoluble in water (Proctor et al., 2002). All Cr(VI) compounds, regardless of their degree of solubility in water, are considered occupational carcinogens. Cr(VI) compounds are carcinogenic in higher doses, generally considered much more toxic than Cr(III). Carcinogenicity of Cr(VI) is site specific, targeted mainly to the lung and requires massive exposures (Singh et al., 1998).

Trivalent chromium Cr(III) is is not a substrate of the cellular anion transport system and therefore is unable to cross the cell membrane (Costa, 1997; Salnikow and Zhitkovich, 2008). However, it has been proposed that small amounts of Cr(III) enter the cell through the energy intensive process of pinocytosis. Carcinogenic Cr(VI) is commonly present in tetrahedral coordination and thus emulates biological phosphates and sulphates. Therefore it can be readily taken up through channels for the transfer of the isoelectric and isostructural anions into cells.

Following oral administration of Cr(VI), it is efficiently detoxified upon reduction by saliva and gastric juice, and sequestration by intestinal bacteria (De Flora, 2000). Chromium(VI) absorbed by the intestine is effectively reduced in the blood and then in the liver. This is in agreement with rather low genotoxicity and carcinogenicity of Cr(VI), with the exception of long-term exposed individuals to high doses of this carcinogenic metal (De Flora et al., 1990).

In the lungs (and also in the liver) Cr(VI) is efficiently reduced probably by the glutathione (Izzotti et al., 1998). Thus the risk of lung cancer increases only when Cr(VI) doses overwhelm the cellular defense mechanisms.

The process of intracellular reduction of Cr(VI) by chelators reduces pools of this potentially carcinogenic metal ion (Fig. 3). Enhanced diffusion of Cr(VI) from plasma to erythrocytes represents a mechanism of depletion of Cr(VI) from blood plasma. In the erythrocytes, in the course of detoxification of Cr(VI), it is reduced to lower oxidation states and forms chromium protein complexes (Kerger et al., 1997; Petrilli and De Flora, 1978). Complexed chromium with various ligands, cannot leave the cell and move back into the plasma (Zhitkovich, 2005; De Flora et al., 1995). It has been estimated, that that the rate of uptake of Cr(VI) by red blood cells is synchronised with the reduction capacity of Cr(VI) to Cr(III) species.

The process of reduction of Cr(VI) to Cr(III) by chelation is not absolutely safe, because during this process various free radicals are generated, which will result either in activation or in detoxification depending on the site of the intracellular reduction and its proximity to DNA.

The results have shown that ascorbate is the most efficient biological reductant of Cr(VI) in cells under in vivo conditions and plays a dual role in Cr(VI) toxicity: protective-antioxidant outside and prooxidative inside the cell. In fact, reactions utilizing ascorbate in the reduction of chromium(VI) inside the cells generate high levels of chromium–DNA adducts and produce mutation-inducing DNA damage (Fig. 3) (Quievryn et al., 2003, 2002; O'Brien et al., 2002).

In addition to primary reduced Cr(VI) by ascorbate, it can be accomplished through non-enzymatic reactions with cysteine and glutathione; however, in the target tissues of chromate toxicity, such as lung, ascorbate is the primary reducer of Cr(VI). In mitochondria, the primary reductant of Cr(VI) appears to be NAD(P)H, leading to the formation of stable Cr(III) which binds DNA more effectively than Cr(VI) (De Flora and Wetterhahn, 1989). Intermediate oxidation states of chromium, i.e. Cr(V) and Cr(IV), are also proposed to play a role in chromium genotoxicity and carcinogenicity, either directly or through reaction (e.g. via the Fenton reaction) with other cellular components, resulting in the generation of reactive oxygen species (see Fig. 4). It has been demonstrated that Cr(III) can be reduced to Cr(II) by the biological reductants, for example by L-cysteine and NAD(P)H, which in turn reacts with hydrogen peroxide via the Fenton reaction to produce hydroxyl radicals, detected by both Electron Paramagnetic Resonance spectroscopy and HPLC (Shi et al., 1993a,b). Cr(III) species have been found to be capable of producing reactive oxygen species from both hydrogen peroxide and lipid peroxides. The formation of intermediate oxidation states of chromium, Cr(V) and Cr(IV) in both in vitro studies and in vivo animal studies administered Cr(VI) have been directly detected using EPR spectroscopy (Shi et al., 1993a,b). In the course of the Cr(VI) reduction, many reactive oxygen species, including free radicals, such as the hydroxyl radical, singlet oxygen, superoxide anion are formed. Generated hydroxyl radicals are able to react with DNA bases, e.g. guanine producing a variety of radical adducts, the best described is 8-hydroxyguanosine (8-OH-dG), a good marker of oxidative damage of an organism. Several types of DNA dam-

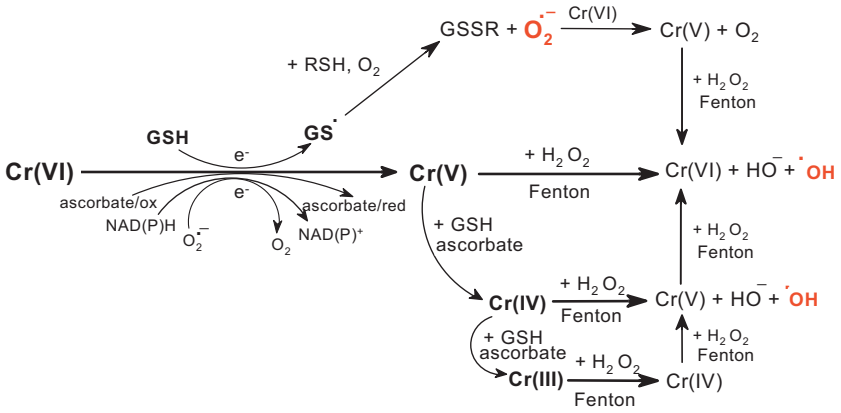

Fig. 4. Biological reductants of Cr(VI) and its reactions.

age occur in chromium(VI)-exposed cells, including single-strand breaks, DNA–DNA interstrand crosslinks, DNA–protein crosslinks, chromium–DNA adducts, oxidative nucleotide changes and chromosomal aberrations (De Flora and Wetterhahn, 1989; Singh et al., 1998).

Chromium is known to activate the MAP kinase signal transduction pathway. NF-κB, ATF-2 and p53 participate in regulation of critical cellular processes, including apoptosis. Cr(VI)-induced oxidative stress triggers the hypoxia signalling pathways, leading to increase in HIF-1α and VEGF protein levels. Chromium(III) deficiency in humans has been associated with cardiovascular disease, metabolic disease (e.g. diabetes) and infertility (see below).

4.2. Chromium and human disease

Chromium(VI) at high doses is considered to be the greatest health risk (Keegan et al., 2008). Cr(VI) enters the body by all three of routes of exposure: inhalation, ingestion or absorption through the skin. For occupational exposure, the airways and skin are the primary routes of uptake (De Flora et al., 1995). Breathing high levels of chromium(VI) can cause irritation to the nasal cavity, breathing difficulty (asthma and cough). Skin contact with certain chromium(VI) compounds can cause skin ulcers. Allergic symptoms such as redness and swelling of the skin have been reported following contacts with chromium compounds.

According to the International Agency for Research on Cancer (IARC), carcinogenicity of Cr(VI) compounds in the lung and in the nasal cavity has been confirmed. Hexavalent chromium is recognized as a human carcinogen via inhalation and known to cause lung cancer in humans (Quievryn et al., 2002). Welders are heavily exposed to chromium and therefore are at particular risk. For example, workers exposed to hexavalent chromium in workplace air had significantly increased incidence of lung cancer than workers in control group. However, lung cancer can only be induced when Cr(VI) doses overwhelm these defense mechanisms. Incidences of cancers of nasal cavity have also significantly increased over the past decade (Sunderman, 2001). In conclusion, Cr(VI) compounds are carcinogenic to humans, but epidemiological studies provide evidence that its carcinogenicity is strictly site-specific.

Various case reports of occupational and nonoccupational Cr(VI) ingestion have been reviewed (Barceloux, 1999a,b). Adverse health effects seen in these cases include gastrointestinal symptoms, hypotension, and hepatic and renal failure. An increase rate in stomach tumours was observed in humans and animals exposed to chromium(VI) in drinking water. Sperm damage and damage to the male reproductive system have also been seen in laboratory animals exposed to chromium(VI).

The Occupational Safety and Health Administration (OSHA) announced limits of occupational exposure to Cr(VI) (Occup. Safety, 2006). Safe environment represents less than 5 μg of Cr(VI) per cubic meter of air. Very recent studies using cells cultures revealed a much greater potential for Cr(VI) to cause chromosomal damage and mutations (Reynolds et al., 2007) than was previously expected.

The metal, Cr(0), is less common and does not occur naturally. Cr(0) is not currently believed to cause a serious health risk.

The US National Academy of Science has established a safe daily intake for chromium in adults of 50–200 μg per day (Institute of Medicine, 2001). Chromium(III) is an essential mineral which has a beneficial role in the regulation of insulin, metabolic syndrome and cardiovascular disease. Chromium potentiates insulin and therefore plays a role in the normal glucose metabolism. Decreased levels of chromium in human tissues have been found which correlated with the incidence of diabetes 2.

Deficiency of chromium has been associated with disturbed glucose tolerance, fasting hyperglycemia, glucosuria, increased body fat, dyslipidemia and impaired fertility (De Flora et al., 1995).

There is growing evidence that chromium may facilitate insulin signalling and chromium supplementation therefore may improve systemic insulin sensitivity (Hummel et al., 2007).

Chromium metabolism has signs of disturbance in humans with cardiovascular disorders. Picolinate is a byproduct of the amino acid tryptophan and chromium picolinate (200 μg per day) has been shown to reduce insulin resistance and to help reduce the risk of cardiovascular disease and type 2 diabetes (Bagchi et al., 2002). In order to explore whether chromium intake in the form of dietary supplements would be effective in cardiovascular disease prevention, clinical trials are necessary to conduct (Alissa et al., 2009).

5. Cobalt

Cobalt forms a number of organic and inorganic salts with the most stable oxidation numbers being +3 [Co(III)], and +2 [Co(II)]. Cobalt is an element that occurs naturally in many different chemical forms throughout our environment (Lison et al., 2001).

5.1. Cobalt and oxidative stress

Vitamin B12 contains 4% cobalt which confirms that this element is essential to man (Kim et al., 2008). Experimental studies confirmed that cobalt can not only interfere with DNA repair processes but can also cause direct induction of DNA damage, DNA-protein crosslinking, and sister-chromatid exchange. It is

well-established that cobalt-mediated free radical generation contributes to the toxicity and carcinogenicity of cobalt.

Cobalt particles in suspension [Co(0)] do not react with hydrogen peroxide via the Fenton reaction. EPR spin trapping experiments in the presence of oxygen indicated the generation of the radical intermediate Co(I)-OO$^{\bullet}$ species described by the reaction (Leonard et al., 1998; Valko et al., 2005):

$$Co + O_2 \rightarrow Co(I) + O_2^{-\bullet} \rightarrow Co(I)\text{-}OO^{\bullet} \quad (16)$$

In the presence of SOD, the enzyme catalyzes the decomposition of Co(I)-OO$^{\bullet}$ species to H_2O_2 and Co(I):

$$Co(I) - OO \cdot \xrightarrow{SOD} H_2O_2 + Co(I) \quad (17)$$

where H_2O_2 is produced from $O_2^{-\bullet}$ via a dismutation reaction and $O_2^{-\bullet}$ by one-electron reduction of molecular dioxygen catalyzed by Co. EPR spectroscopy revealed the Fenton reaction for Co(I) as well as for Co(II) (Leonard et al., 1998):

$$Co(I) + H_2O_2 \rightarrow Co(II) + {}^{\bullet}OH + OH^{-} \quad \text{(Fenton)} \quad (18)$$

$$[Co(II)\text{-chelate}] + H_2O_2 \rightarrow [Co(III)\text{-chelate}] + {}^{\bullet}OH + OH^{-} \quad \text{(Fenton)} \quad (19)$$

The catalytic activity of cobalt ions depends on the applied chelators. Cobalt(II) complexed with GSH or cysteine has been found to generate under physiological conditions hydroxyl radicals and other oxygen- and carbon-centered radicals from model lipid peroxides (Shi et al., 1993a,b). NADH, GSH and anserine (beta-alanyl-N-methylhistidine) render Co(II) reactive with hydrogen peroxide to produce hydroxyl radicals (Mao et al., 1996).

Co(II) plus hydrogen peroxide was found to induced DNA cleavage at all bases with a preference for G > T, C ≫ A. Spin trapping EPR experiments showed that Co(II) reacts with hydrogen peroxide forming not only $^{\bullet}$OH, but also singlet oxygen (using TEMPOL) especially in the presence of chelators (Kawanishi et al., 1989). The cobalt-mediated formation of free radicals according to the reactions outline above suggests the involvement of Co(II) in oxidative stress mediated toxicity and carcinogenicity, as proved in the studies of hepatocytes (Pourahmad et al., 2003).

Cobalt(II) exposure is known to deplete intracellular ascorbate (Salnikow et al., 2004). To understand the molecular mechanism of this process, both uptake and efflux of ^{14}C-labeled ascorbate in the presence of Co(II) have been investigated. Interestingly, while the influx of ascorbate is blocked by cobalt, the efflux is metal-independent process. Compared with that in the control cells, the initial rapid uptake of ascorbate in cobalt(II)-exposed cells has stopped 2–4 h after the addition of the cobalt to the cell culture medium. Then, within the next 16–18 h, the cellular [^{14}C] ascorbate decreased gradually to barely detectable levels. This time course could be the result of a relatively slow interaction of the metals (or metal complexes) with critical target molecules (ligands) in the medium and/or cells, including ascorbic acid. Exposure of cells to cobalt(II) causes activation of the HIF-1 transcription factor and up-regulates many of the hypoxia-inducible genes (Yuan et al., 2003). However, the exact mechanism of HIF-1 activation by cobalt (and also other metals) is not known.

Very recently it has been shown that HIF-1alpha stabilization in human lung epithelial cells occurred following exposure to various metal ions, including those that cannot substitute for iron in the hydroxylases. In each case addition of the reducing agent (ascorbic acid) abolished HIF-1alpha protein stabilization. To better understand the role of iron oxidation in hydroxylase inhibition and to define the role of ascorbic acid in the enzyme recovery, applied molecular modeling techniques were adopted. The results indicate that the energy required for iron substitution by divalent metal ions in the enzyme is high and unlikely to be achieved in a biological system (Kaczmarek et al., 2009).

5.2. Cobalt and human disease

As described above, cobalt is a potent inducer of oxidative stress causing free radical generation, which in turn induce DNA damage, inhibit DNA repair mechanisms and the exchange of DNA between sister-chromatids and aneuploidy (Galanis et al., 2009).

The toxicity of cobalt is relatively low compared to many other metals (Gal et al., 2008). Its toxic effect in higher concentrations affects mainly the lungs, leading to asthma, pneumonia and wheezing. Overdosing of cobalt (>5 mg/day) may lead to abnormal thyroid functions, polycythemia and overproduction of red blood cells (erythropoiesis), with increased production of the hormone erythropoietin. There is also a risk of pulmonary edema, peripheral vascular thrombosis, optic nerve atrophy. Intranasal use of vitamin B12 includes symptoms such as headache, sore throat and rhinitis.

Inhalation of Co alone can cause asthma (Barceloux, 1999a,b) and simultaneous inhalation of cobalt and tungsten carbide (WC) particles induce the development of hard metal lung disease via ROS mechanisms. The International Agency for Research on Cancer (IARC) recently classified the mixture Co/WC as “probably carcinogenic to humans”. Cobalt alone was only classified as “possibly carcinogenic to humans”. Several studies reported that metallic cobalt acquires a higher genotoxicity when associated to WC or to other carbides.

Investigators have demonstrated greater generation of ROS for Co in the presence of WC relative to ROS generation among the individual constituents (Co or WC) (Fenoglio et al., 2008). Thus, available data suggest that WC particles in association with Co particles, rather than WC or Co particles alone, should be considered a specific toxic combination in development of hard metal lung disease. The free radical formation has possible consequences of oxidative damage, as detected in the murine RAW 264.7 cell line using EPR spectroscopy.

Particle size-dependent differences in ROS generation were observed for all study powders [tungsten (W), tungsten carbide (WC, W_2C), cobalt (Co) and admixture (WC, W_2C and Co)] except Co alone, which did not generate radicals in the cellular model (Stefaniak et al., 2010). When the dose of powders was normalized to surface area (expressed as m^2/g), the formation of hydroxyl radicals was independent of particle size, suggesting that particle surface chemistry may be an important exposure factor.

Inhaled particles interact primarily with the lung surface made up by surfactants and antioxidants (Fenoglio et al., 2008). GSH acts as a ROS scavenger, thus constituting one of the first lines of defense against lung injury due to the over-production of ROS. Both ascorbic acid and GSH are able to scavenge superoxide and hydroxyl radicals. In addition, GSH and cysteine residues in proteins also have an important role in redox regulation. The concentration of GSH and Cys is significantly reduced in the presence of the Co/WC mixture, while the single components alone do not react or react to a much lesser extent with GSH and Cys. The extent of the reduction of the thiols concentration correlates to the amount of dust and, consequently, with the surface area exposed.

The reactivity of Co/WC mixture with cysteine and thiols (GSH) is quite significant. Cysteine alone reacts with Co/WC more extensively than the cysteinyl fragment in the tripeptide GSH. The results are consistent with the oxidation occurring at the surface containing mainly cysteine S–H groups involved in the generation of sulphur-centered radicals. Such a reaction, will enhance the level of oxidative stress caused by particles and cell-generated free radicals (Stefaniak et al., 2009). A detailed experiment on particle surface chemistry elucidated the importance of close contacts of metals

with biologically active surface area in the formation of free radicals by particle mixtures.

Interestingly, a reversed effect of cobalt on free radical generation has been reported (Shukla et al., 2009). Hypobaric hypoxia is accompanied by increased formation of free radicals and suppressed activities of antioxidant enzymes. Exposure of rats to hypobaric hypoxia revealed increased oxidation of lipids and proteins and decreased reduced oxidized glutathione (GSH/GSSG) ratio and increase in SOD, GPx, and GST levels. In addition, increase in heme oxygenase 1 (HO-1) and heat shock protein 70 (HSP70) was also recorded. Intake of cobalt significantly suppressed free radical formation, oxidation of lipids and proteins. In addition, the GSH/GSSH ratio was similar to that of control cells activated by HO-1. These results look promising in view of the prospective pharmacological benefits of cobalt in preventing hypoxia-induced oxidative stress.

6. Cadmium

Cadmium is a heavy metal and the most common oxidation number of cadmium is +2. Food is the main source of cadmium for the non-smoking population (Cuypers et al., 2010). Estimates of dietary cadmium intake worldwide range from 10–40 μg/day in nonpolluted areas to several hundred micrograms in cadmium-polluted regions.

6.1. Cadmium, oxidative stress and human disease

The routes of cadmium intake involve the lungs, intestines and skin. Cadmium in the body is predominantly bound to metallothioneins (Hamer, 1986). The cadmium–metallothionein complex is distributed to various tissues and organs and is ultimately reabsorbed in kidney tubuli (Ohta and Cherian, 1991). There is no mechanism for the excretion of cadmium in humans, thus cadmium accumulates in tissues. The half-life of cadmium in kidney cortex is 20–35 years. In humans, the largest amount of cadmium is deposited in the kidneys, liver, pancreas and lungs.

Cadmium itself is unable to generate free radicals directly, however, indirect formation of ROS and RNS involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Waisberg et al., 2003). Some experiments also confirmed the generation of non-radical hydrogen peroxide which itself in turn may be a significant source of radicals via Fenton chemistry (Elinder et al., 1976). Cadmium can activate cellular protein kinases (protein kinase C) which result in enhanced phosphorylation of various transcription factors which in turn lead to activation of target gene expression.

An interesting mechanism explaining the indirect role of cadmium in free radical generation was presented, in which it was proposed that cadmium can replace iron and copper in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), thus increasing the amount of unbound free or poorly chelated copper and iron ions participating in oxidative stress via Fenton reactions (Price and Joshi, 1983). These results are supported by recent findings by Watjen and Beyersmann (2004). Displacement of copper and iron by cadmium can explain the enhanced cadmium-induced toxicity, because copper, displaced from its binding site, is able to catalyze breakdown of hydrogen peroxide via the Fenton reaction.

The toxic mechanisms of cadmium are not well understood, but it is known to act intracellularly, mainly via free radical-induced damage, particularly to the lungs, kidneys, bone, central nervous system, reproductive organs and heart (Waalkes, 2000).

The effect of cadmium exposure in drinking water on markers of oxidative stress in rat cardiac tissue has shown significantly increased lipoperoxides, MDA and decreased activities of SOD and glutathione peroxidase (GPx) (Novelli et al., 2000). No alterations were observed in catalase activity. In addition, decreased glucose levels and increased total lipid content in cardiac tissue of rats following cadmium exposure were observed. The decreased activities of alanine transaminase and aspartate transaminase reflected decreased metabolic protein degradation and increased lactate dehydrogenase activity. Since the metabolic pathways were altered by cadmium exposure, it can be concluded that Cd^{2+}-induced formation of ROS initiates a series of events that occur in the heart which in turn resulted in alterations of metabolic pathways.

The testis is a good marker of cadmium exposure. Cadmium-induced testicular damage and testicular necrosis have been documented by many reporters (see for example Dalton et al., 2005). Various studies have been performed on the cadmium-induced testicular toxicity in rat models. A significantly increased content of malondialdehyde and glutathione peroxidase (GSH-Px) in exposed groups has been observed (Yang et al., 2003). Glutathione was found to scavenge intracellular oxygen radicals either directly or via the GSH peroxidase/GSH system. The activity of superoxide dismutase in the tested animals was lowered. This study also revealed that the number of cells with DNA single strand breaks and the levels of cellular DNA damage were significantly higher in exposed groups than in controls.

Cadmium is a potent human carcinogen causing preferentially prostate, lung and gastro-intestinal (kidney and pancreas) cancers. Smoking synergistically increases the carcinogenic effect of cadmium (Flora et al., 2008; Flora and Pachauri, 2010). The effect of environmental exposure to cadmium on cancer incidence (particularly that of the lung) in the environmentally contaminated north-east Belgium (the neighbourhood of zinc smelters) has been extensively investigated (Sartor et al., 1992). The results have shown an association between risk of cancer and cadmium exposure as shown by 24-h urinary excretion – a finding that remained consistent after adjustment for sex, age and smoking.

New findings in the explanation of cadmium-induced carcinogenicity with respect to cell adhesion have recently been published. E-cadherin, a transmembrane Ca(II)-binding glycoprotein playing an important role in cell–cell adhesion, can bind cadmium to Ca(II)-binding regions, changing the glycoprotein conformation (Pearson and Prozialeck, 2001). Thus the disruption of cell–cell adhesion induced by cadmium could play an important role in tumour induction and promotion.

6.2. Cadmium and antioxidants

Intoxication with cadmium led to significantly increased concentration of lipid peroxides in rats and altered activity of antioxidant enzymes such as Cu, Zn-SOD, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase (Ognjanovic et al., 2003). Pretreatment with vitamin E revealed a protective role against the toxic effects of cadmium as substantiated by the hematological values of lipid peroxides. Supplementation with vitamin C and/or vitamin E has been shown to reduce the level of ROS-initiated testicular damage. The combined effect of vitamins restored normal testicular function in Cd-exposed rats (Sen Gupta et al., 2004). The effect of dietary vitamin E intake on lipid peroxidation as measured by the production of thiobarbituric acid reactive substances (TBARS) was assessed. It appears that reduction in the increase in TBARS due to Cd-induced toxicity may be an important factor in the action of vitamin E (Beytut et al., 2003).

The protective role of melatonin, an effective antioxidant and free radical scavenger, against cadmium was also studied (Karbownik et al., 2001). Melatonin slightly reduced lipid peroxidation in the testes induced by cadmium.

7. Arsenic

The most common oxidation numbers of arsenic are +5, +3, and −3, in which the element is able to form both inorganic and organic compounds in the environment and within the human body (Hei and Filipic, 2004). In combination with other elements such as oxygen, sulphur and chlorine the element is referred to as inorganic arsenic and as combined with hydrogen and carbon as organic arsenic. Since most arsenic compounds are colourless and/or do not smell, the presence of arsenic in food, water or air, is a serious human health risk.

7.1. Arsenic, toxicity and free radicals

Inorganic arsenic includes arsenite (As(III)) and arsenate (As(V)) and can be either methylated to form monomethylarsonic acid (MMA) or dimethylated as in dimethylarsinic acid (DMA) (Arnold et al., 2006; Wang and Rossman, 1996). The metabolism of inorganic arsenic involves a two-electron reduction of pentavalent arsenic, mediated by GSH, followed by oxidative methylation to form pentavalent organic arsenic.

Arsenic trioxide (As_2O_3) is the most prevalent inorganic arsenical found in air, while a variety of inorganic arsenates (AsO_4^{3-}) or arsenites (AsO_2^-) occur in water, soil, or food (Ding et al., 2005). Gallium arsenide (GaAs) is used in electronics industry and has also negative impact on human health. Although gallium arsenide is poorly soluble, it undergoes slow dissolution and oxidation to form gallium trioxide and arsenite (Webb et al., 1986). The toxic effects of GaAs consist of liberated arsenic enhanced by the other effects of the gallium.

Arsenic is toxic to the majority of organ systems; inorganic arsenic being more toxic than methylated organic arsenic (Mandal and Suzuki, 2002). The trivalent forms are the most toxic and react with thiol groups of proteins. The pentavalent forms possess less toxicity, however uncouple oxidative phosphorylation.

Trivalent arsenic inhibits various cellular enzymes, including for example pyruvate dehydrogenase, resulting in a reduced conversion of pyruvate to acetyl coenzyme A (CoA) (Wang and Rossman, 1996). Enzyme inhibition occurs through binding to sulphydryl groups. Arsenic also inhibits the uptake of glucose into cells, gluconeogenesis, fatty acid oxidation, and further production of acetyl CoA. Most importantly, arsenic inhibits the synthesis of GSH, one of the most powerful cellular antioxidant.

The toxicity of pentavalent inorganic arsenic occurs via its reduction to trivalent arsenic (Ferrario et al., 2008). Pentavalent arsenic resembles to inorganic phosphate and substitutes for phosphate in glycolytic and cellular respiration pathways. Uncoupling of oxidative phosphorylation occurs because of the loss of the high-energy ATP phosphate bonds due to the preferential formation of ADP-arsenate.

As mentioned above, methylated organic arsenicals are usually viewed as being less toxic than the inorganics (Mandal and Suzuki, 2002). This is substantiated by the majority of studies supposing that the acute toxicity of inorganic arsenic was greater than organic arsenic. Thus, the methylation of inorganic arsenic was considered to be a detoxication process. However, the results presented in the past decade show that human cells are more sensitive to the cytotoxic effects of MMA^{III} than arsenite (Petrick et al., 2000; Styblo et al., 2001) and that DMA^{III} is at least as cytotoxic as arsenite in several human cell types (Styblo et al., 2000). Thus the process of methylation of arsenic does not have to be a detoxication mechanism. Further detailed studies dealing with the possible toxic effects of organic arsenic are awaited. Several organic arsenicals are found to accumulate in fish and shellfish. These include arsenobetaine and arsenocholine, both referred to as "fish arsenic" that have been found to be essentially nontoxic (Hindmarsh, 2000).

Many studies confirmed the generation of various types of ROS during arsenic metabolism in cells (reviewed in Valko et al., 2005). Oxidative stress has been linked with the development of arsenic related diseases including cancers. In addition to ROS, reactive nitrogen species (RNS) are also thought to be directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic. Many recent studies have provided experimental evidence that arsenic-induced generation of free radicals can cause cell damage and death through activation of oxidative sensitive signalling pathways (Roy et al., 2009).

Arsenic-mediates formation of the superoxide anion radical ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), the peroxyl radical ($ROO^\bullet$), nitric oxide ($NO^\bullet$), hydrogen peroxide (H_2O_2), dimethylarsinic peroxyl radicals ($[(CH_3)_2AsOO^\bullet]$) and also the dimethylarsinic radical $[(CH_3)_2As^\bullet]$ (Yamanaka and Okada, 1994). The exact mechanism responsible for the generation of all these reactive species is not yet clear, but some studies proposed the formation of intermediary arsine species.

Recent studies on the arsenite toxicity in the brain reported that some of its effects have been connected to the generation of the damaging hydroxyl radical (Mishra and Flora, 2008). The time-evolution of the formation of the hydroxyl radical in the striatum of both female and male rats who underwent a direct infusion of different concentrations of arsenite was investigated. The treatment with arsenite induced significant increase in hydroxyl radical formation. These results support the participation of hydroxyl radicals in arsenic-induced disturbances in the central nervous system. In this connection, an interesting route to produce H_2O_2 was explained by the oxidation of As(III) to As(V) which, under physiological conditions, results in the formation of H_2O_2 (a source of damaging hydroxyl radical):

$$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2$$
$$(\Delta_r G^{\Theta} = -40.82\ \text{kcal/mol}) \quad (20)$$

The above reaction is spontaneous and exergonic with an estimated standard reaction free energy change for H_2O_2 formation of −40.82 kcal/mol (−170.87 J/mol).

In addition to ROS, arsenic exposure can also initiate the generation of RNS. Several conflicting reports concerning arsenic-induced production of $NO^\bullet$ have been published (Shi et al., 2004). One report concluded that there was no cadmium-induced increase in $NO^\bullet$ generation in hepatocytes and human liver cells, which inhibited inducible NO synthase gene expression in cytokine-stimulated human liver cells and hepatocytes (Germolec et al., 1996). In another report, arsenite was found to inhibit inducible NO synthase gene expression in rat pulmonary artery smooth muscle cells (Kodavanti et al., 1996). Similarly, a third study with low levels of arsenite reported no change in intracellular concentration of Ca(II) as well as no $NO^\bullet$ generation as detected by EPR spectroscopy (Barchowsky et al., 1999).

GSH is a very effective cellular antioxidant and plays an important role in maintaining cellular redox status. In addition, GSH level is a good marker of oxidative stress of an organism (Halliwell and Gutteridge, 2007). Several papers have reported decreased levels of GSH after exposure to arsenic. It was reported that following oral intake of arsenic, the GSH concentration was significantly decreased in the liver of male Wistar rats (Maiti and Chatterjee, 2001). After 6 months exposure to arsenic, hepatic GSH and the enzymes glucose-6-phosphate dehydrogenase and GPx were significantly lowered in mice. Overall, from these studies follow that GSH possibly acts as an electron donor for the reduction of pentavalent to trivalent arsenicals and that arsenite has high affinity to GSH.

7.2. Arsenic and human disease

The exact molecular mechanism of arsenic toxicity and carcinogenesis is still not known. Current views of molecular mechanisms of arsenic toxicity involve genetic changes, the involvement of increased oxidative stress, enhanced cell proliferation and altered gene expression. Arsenic is known to induce hypoxia signalling pathways. For example in prostate cancer cells treated with arsenite induced HIF-1alpha expression in a concentration- and time-dependent manner, whereas the level of HIF-1beta remained unaffected (Posey et al., 2008). The VEGF protein level was also elevated. ROS formation was linked with the activation of the PI3K/Akt pathway and the subsequent induction of HIF-1alpha and VEGF.

Arsenic is a well-documented carcinogen in a number of studies (Waalkes et al., 2004). Chronic exposure to inorganic arsenic from contaminated water is responsible for various adverse health effects such as developing tumours of the lung, skin, liver, bladder and kidney. Skin lesions, peripheral neuropathy and anemia are hallmarks of chronic arsenic exposure. Arsenic is also a potential risk factor for atherosclerosis.

While cardiovascular disorders following oral exposure to arsenic are well documented, there is some evidence from epidemiological trials that also inhaled inorganic arsenic can affect the cardiovascular system (Das et al., 2010). A systematic review of the epidemiologic evidence on the association between arsenic and cardiovascular outcomes in Taiwan has been performed (Tseng, 2008). In addition, the estimation of relative risks for coronary disease, for stroke, and for peripheral arterial disease has been conducted. Methodological constraints, however, limited interpretation of the moderate-to-strong associations between high arsenic exposure and cardiovascular outcomes in Taiwan. Such studies of arsenic and cardiovascular outcomes should be a research priority.

An interesting association between intellectual deficiencies in children and exposure to arsenic has been found (Wang et al., 2007). Adolescents from various regions of Taiwan and China exposed to low (0.0017–0.0018 mg As/kg/day) levels of inorganic arsenic in the drinking water showed decreased performance in the switching attention task, while children in the high exposure group (0.0034–0.0042 mg As/kg/day) showed decreased performance in both the switching attention task and in tests of pattern memory, relative to unexposed controls.

Neurological effects have also been confirmed in animal studies. Changes in levels of neurotransmitters such as dopamine, norepinephrine, and 5-hydroxytryptamine were noted in rats exposed to sodium arsenite in drinking water over a period of 16 weeks (Kannan et al., 2001).

There is a positive health effect of arsenic trioxide used in treatment of acute promyelocytic leukemia (AML), the most common type of acute leukemia (Wang and Chen, 2008; Wetzler et al., 2007). AML is a fast-growing cancer in which the bone marrow produces abnormal myeloblasts, which would normally develop into white blood cells that fight infection. AML is the most malignant form of acute leukemia with a severe bleeding tendency and a fatal prognosis. For more than two and half decades therapeutic applications of arsenic in the treatment of this type of leukemia have been investigated. An effort is now made to characterize the underlying mechanisms of arsenic trioxide action and its interactions with different proteins to enhance its therapeutic potential (Ferrara, 2010).

8. Zinc

The most common and most stable oxidation number of zinc is +2 [Zn(II)]. Zinc is a ubiquitous trace element found in plants and animals. The adult human body contains approximately 1.5–2.5 g of zinc, present in all organs, tissues, fluids and secretions. The level of free intracellular Zn(II) is as low as 0.5 nM, as estimated from measurements of the zinc-specific ^{19}F-NMR signal of a fluorinated metal chelating probe (Benters et al., 1997). Zinc is an element present in more than 70 different enzymes that function in many aspects of cellular metabolism, involving metabolism of proteins, lipids and carbohydrates.

8.1. Zinc, metabolism and oxidative stress

The observations performed in 1961 on Iranian males have shown that zinc deficiency may cause growth retardation and hypogonadism in humans (Prasad et al., 1961). Following studies later showed that zinc was essential for humans and that zinc deficiency was prevalent in the Middle East (Prasad et al., 1963). Zinc deficiency is related to poor dietary zinc intake, excessive dietary phytate intake, chronic illness or over-supplementation with iron or copper. Zinc deficiency incidence in well-nourished humans is unknown due to difficulties in sufficiently diagnosing zinc deficiency and the diversity of its metabolic roles. Other symptoms of zinc deficiency include loss of appetite, dermatitis, reduced taste acuity, delayed wound healing, impaired reproduction and poor immune function. Zinc helps manage insulin action and blood glucose concentration and has an essential role in the development and maintenance of the body's immune system. Severe zinc deficiency is rare and usually caused by genetic or acquired conditions.

Zinc is a redox inert metal and does not participate in oxidation-reduction reactions. Zinc's function as an antioxidant involves two different mechanisms: (i) the protection of sulphydryl groups of proteins against free radical attack and (ii) reduction of free radical formation through the prevention mechanisms or in other words antagonism of redox-active transition metals, such as iron and copper (Bray and Bettger, 1990). Any of these models result in a decreased reactivity of sulphydryl groups.

The first model considers direct binding of zinc to the sulphydryl groups, the second model assumes binding of zinc to a binding site close to the sulphydryl groups and finally the third assumed binding of zinc to another site of the protein resulting in a conformational change of the protein. Zinc was found to protect various sulphydryl-containing proteins, for example dihydroorotase (Kelly et al., 1986), DNA zinc-binding proteins (zinc fingers) (Klug and Rhodes, 1987), protein farnesyltransferase (Fu et al., 1996) and others.

The process of protein oxidation is a site-specific reaction and oxidative modifications occur predominantly around the metal binding site. In the second mechanism outlined above, there are two potential processes that would antagonize/prevent the formation of hydroxyl radicals. The first process involves removal or "pull" of the metal from its binding site through the use of a high-affinity ligand-chelator. The second process consists of "pushing" the redox metal off of its binding site through replacement by an isostructurally similar redox-inactive metal (e.g. copper replacement by zinc) (Stadtman, 1990). The displaced redox metal can then leave the cell, reducing thus its ability to catalyze decomposition of Fenton reaction (hydroxyl radical formation). An example of the zinc antagonism mechanism is documented by iron-mediated xanthine/xanthine oxidase-induced peroxidation of erythrocyte membranes. Antagonism of radical formation by zinc was reported in copper–iron ascorbate-induced DNA strand breaks, superoxide and hydroxyl radical from xanthine oxidase and NADPH oxidase, Fe(III)-ascorbate-induced methemoglobin formation in red blood cells and other systems.

Zinc deficiency has been associated with increased levels of oxidative damage including increased lipid, protein and DNA oxidation (Prasad, 2009). Animal studies confirmed that chronic or long-term absence of zinc makes an organism more to oxidative stress-induced injury. Zinc deficiency effects, combined with ROS

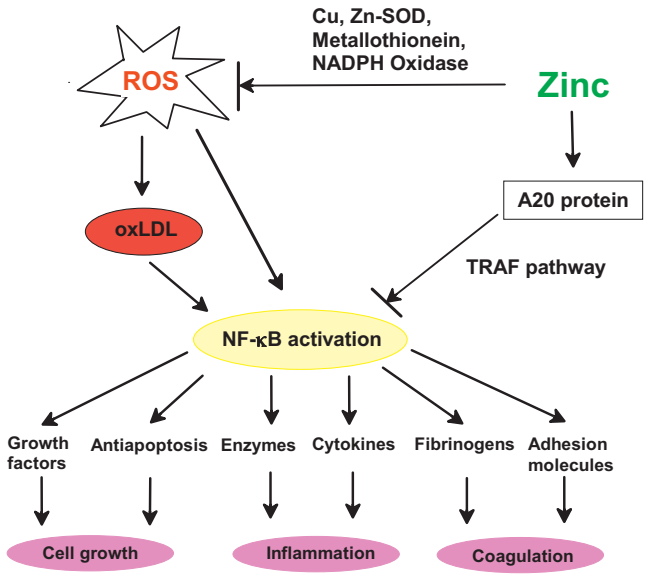

Fig. 5. Zinc as an antioxidant and anti-inflammatory agent.

formation has been documented by carbon centered free radical production and lipid peroxidation in lung damage, formation of conjugated dienes and malondialdehyde in liver microsomes and lipoprotein oxidation and galactosamine-induced hepatitis in rats (reviewed in Valko et al., 2005).

The metallothioneins are metal-binding proteins (6000–7000 kDa) containing 60–68 amino acid residues. The beneficial effects of long-term administration of zinc can be linked to the induction of some other species that serves as the ultimate antioxidants, among which one of the most effective seems to be metallothioneins (Powell, 2000). About 25–30% of all aminoacids in metallothioneins are cysteine, containing no aromatic amino acids or disulphide bonds and therefore can effectively bind 5–7 g zinc (mol/protein). Recent studies have reported that the metallothioneins represent a connection between cellular zinc and the redox state of the cell (Maret, 2008). Under conditions of high oxidative stress, changes in the cellular redox state result in release of zinc from metallothionein as a result of sulphide/disulphide exchange.

Zinc as an antioxidant, reduces formation of free radicals by several ways (Prasad, 2009) (Fig. 5). Zinc acts as an inhibitor of NADPH oxidase, inducer of metallothionein (effective scavenger of radicals) and is an integral metal of Cu, Zn-SOD. ROS are known to activate NF-kappaB which in turn activates growth factors, antiapoptotic molecules resulting in cell proliferation (cancer), inflammatory cytokines and adhesion molecules (Prasad, 2009). Zinc reduces inflammatory cytokine production by upregulation of a zinc-finger protein, A20, which inhibits NF-kB activation via TRAF pathway (Prasad, 2008). Thus zinc functions not only as an antioxidant but also as an anti-inflammatory agent.

8.2. Zinc and human disease

A beneficial effect of intake of the zinc on oxidative stress markers in elderly people has been reported (Prasad et al., 2007). Interleukin (IL-2) is a molecule of cytokine immune system responding to microbial infection. IFN-γ is a cytokine that is critical for adaptive immunity against bacterial and viral infections. Zinc deficiency in humans is characterized by a reduction of IL-2 and IFN-γ. A randomized double-blind, placebo-controlled trial of zinc supplementation was conducted in elderly people (Prasad et al., 2007). The zinc supplementation decreased incidence of infections and ex vivo generation of TNF-alpha and plasma oxidative stress markers than in the placebo group. Zinc supplementation was effective in decreasing incidences of infections in the elderly patients with sickle cell disease (Bao et al., 2008) and has beneficial effect on respiratory tract infections in children (Veverka et al., 2009).

Zinc may have a preventive role in some cancers such as colon and prostate and in atherosclerosis inasmuch as chronic inflammation has been implicated in the development of these disorders. Clinical trials have confirmed that the group taking zinc supplements had a shorter mean overall duration of cold and shorter duration of cough. The results of zinc supplementation in AIDS are contradictory (Bobat et al., 2005). It has been observed that only zinc deficient patients would respond to zinc supplementation and zinc sufficient patients may not have any beneficial effects. More studies are needed in this respect.

Zinc supplements intake together with IFN-alpha was more effective against chronic hepatitis C than therapy with IFN-alpha alone (Takagi et al., 2001). It is also possible that zinc has an antioxidant effect and this may have benefited a few cases of hepatitis. Zinc intake seems also promising to inhibit herpes simplex virus (Kumel et al., 1990) and rhinoviruses (Korant et al., 1974). While one study reported the beneficial effects of zinc supplementation with respect to joint swelling in patients with rheumatoid arthritis, two other studies did not confirm this observation (Overbeck et al., 2008).

Preventive effects of zinc supplemention in a group receiving zinc gluconate have shown significantly decreased incidence of infections and ex vivo generation of TNF-alpha and plasma oxidative stress markers with respect to a placebo group (Prasad et al., 2007).

The zinc-supplemented group of patients with sickle cell disease had decreased incidences of infection in comparison to the placebo group (Bao et al., 2008). After zinc supplementation, antioxidant power increased. In addition, plasma nitrite and nitrate (NOx), lipid peroxidation products, DNA oxidation products, and soluble vascular cell adhesion molecule-1 (VCAM-1) decreased compared to the placebo group.

Since oxidative stress and chronic inflammation may play important causative roles in many chronic diseases, including atherosclerosis, cancers, neurological disorders, and autoimmune diseases, more thorough studies exploring the status of zinc deficiency and supplementation are necessary.

9. Lead

Lead has atomic number 82 (symbol Pb) and is one of the heavy metals. Lead is a persistent metal and because of its unusual physical–chemical properties it is used in various industrial applications (Brannvall et al., 1999). Well known is its use as a radiation shield. Lead is a toxic metal to humans and animals and its persistency causes prolonged occurrence in the environment – in water, soil, dust and in manufactured products containing lead. Since young organisms bear the heaviest burden of sensitivity to lead exposure, lead-based paint covers represent a serious health threat to children worldwide (Kumar and Clark, 2009). Soil containing lead also represent a serious hazard for children. Gastrointestinal absorption of lead is higher in children (40–50%) than in adults (3–10%). Lead toxicity is most commonly diagnosed through elevated blood levels. Blood levels of 10 μg/dL (equivalent to 0.48 μmol/L) or higher are considered toxic and result in neurological disorders, cognitive impairments, hypertension and other disorders (Patrick, 2006a).

9.1. Lead and oxidative stress

Similar to other persistent toxic metals such as arsenic, cadmium and mercury, lead damages cellular components via elevated

levels of oxidative stress. The pathogenetic effect of lead is multifactorial since it directly interrupts the activity of enzymes, competitively inhibits absorption of important trace minerals and deactivates antioxidant sulphydryl pools (Patrick, 2006b).

Free radical-induced damage by lead is accomplished by two independent, although related mechanisms (Ercal et al., 2001). The first involves the direct formation of ROS including singlet oxygen, hydrogen peroxides and hydroperoxides and the second mechanism is achieved via depletion of the cellular antioxidant pool. Interrelations between these two mechanisms exist so that the increase in ROS on one side simultaneously leads to depletion of antioxidant pools on the other (Gurer and Ercal, 2000). Glutathione represents more than 90% of the non-tissue sulphur pool of human body and the major effect of lead is on glutathione metabolism (Hunaiti and Soud, 2000). In addition, glutathione is an important substrate acting in the metabolism of specific drugs and toxins via glutathione conjugation in the liver. The sulphydryl groups of glutathione bind effectively toxic metals such as arsenic and mercury. Therefore an organism exposed to lead has significantly lowered levels of glutathione, with respect to the control groups, which may in turn enhance the toxicity of other metals.

There are two specific enzymes, glutathione reductase (GR) and deltaaminolevulinic acid dehydrogenase (ALAD) that are both inhibited by lead (Hoffman et al., 2000). An epidemiological survey of lead exposure among children (lead concentration >10 μg/dL) in India has shown significantly suppressed levels of ALAD with respect to children with lead concentration (<7 μg/dL) (Ahamed et al., 2005). A direct correlation between blood lead levels, ALAD activity and erythrocyte levels of MDA has been observed among workers exposed to lead. Inhibition of ALAD by lead increases levels of the substrate delta-aminolevulinic acid (ALAD) which is known to stimulate the formation of ROS substantiated by the elevated levels of MDA.

GR is an enzyme responsible for recycling of oxidized glutathione (GSSG) to reduced glutathione (GSH) and lead has been shown to interfere with this cycle resulting in depressed GSH levels. Both trends, elevated and suppressed blood levels of catalase, SOD and glutathione peroxidase have been observed (Sugawara et al., 1991).

Studies using animal models and human populations have shown a causal relationship between low-level lead exposure and hypertension (Abadin et al., 2007). Since there are various factors such dietary intake of calcium, exposure to various environmental toxins, fat diet and intake of alcohol, it is difficult to separate unambiguously lead as a risk factor. However, hypertension is clearly linked with the enhanced levels of oxidative stress and exposure to low levels of lead has been shown to increase production of ROS. ROS-induced oxidative stress has been identified in lung, sperm, testes, liver and brain (Hsu and Guo, 2002). ROS formation following exposure to lead in animal studies has been linked with decreased sperm counts.

9.2. Lead toxicity and antioxidants

In addition to ROS, RNS has also been shown to play a significant role in incidence of hypertension following lead exposure in humans (Valko et al., 2007). Nitric oxide is known as an endothelium-derived relaxing factor. ROS formed as a consequence of lead exposure may oxidize nitric oxide in vascular endothelial cells by forming peroxynitrite ($ONOO^-$) which is a highly reactive ROS capable of damaging DNA and lipids. Depleted $NO^\bullet$ following lead exposure causes hypertension in animal models. Suppressed availability of $NO^\bullet$ can be recovered using antioxidants. In hypertensive rats with blocked glutathione production, the administration of vitamin E (5000 IU/kg) and vitamin C (3 mmol/L of drinking water) completely eliminated the hypertension. In addition the level of glutathione returned nearly to normal (Vaziri et al., 2000). In another animal model of lead-induced hypertension, a SOD-mimetic drug tempol (dimethylthiourea) was applied (Vaziri et al., 2001). Administration of tempol completely suppressed lead-induced hypertension via elimination of superoxide radical anion.

Methionine is known to react with ROS forming methionine sulphoxide (Jomova et al., 2010). Administration of methionine led to increases in thiol group containing molecules (mainly proteins with –SH groups) acting as antioxidants preventing lipid peroxidation processes in the kidneys and liver. N-acetylcysteine has also been shown to be effective not only in reducing but also reversing the oxidant effect of increased levels of aminolevulinic acid enhanced as a consequence of the lead effect.

Lead-exposed animals supplemented with zinc exhibited restored level of SOD and ALAD (Batra et al., 1998). It has been proposed that zinc acts as an antioxidant and possibly as a chelator agent in lead toxicity.

Selenium supplementation has been shown to have a protective effect when administrated to animals prior to lead exposure (Othman and El Missiry, 1998). Selenium increased levels of SOD, GSH and GPx in kidney and liver tissues. Selenium creates a stable lead–selenium complex which has been proposed to play a protective role against lead toxicity.

Alpha-lipoic acid is an effective antioxidant with chelating properties. In studies of lead-induced toxicity, alpha-lipoic acid suppressed the harmful effect of lead on liver and kidney glutathione and oxidative stress markers (Pande and Flora, 2002).

In vitro studies using cell cultures treated with lead have shown improved cell survival and decreased MDA levels following taurine treatment (Selvaraj et al., 2006). In these experiments taurine exhibited antioxidant and membrane-stabilizing properties.

There are several effective chelators of lead used in treatment of lead toxicity. The most effective chelators used in both pediatric and adult treatment of lead toxicity are meso-2,3-dimercaptosuccinic acid (DMSA) and calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) (Gurer et al., 1998; Flora et al., 2003). In addition, DMSA has been shown to have antioxidant properties lowering ROS level in erythrocytes.

10. Metal-chelation therapy in medicine

Chelation therapy is a medical treatment used to treat heavy metal poisoning and chelate redox active metals. The aim of chelation therapy is an attempt to prevent or reverse health problems of individuals exposed to high levels of metals.

10.1. Chelation of redox active iron and copper

As described above, the reactivity of iron significantly varies depending upon its ligand environment and damage caused by iron-mediated formation of hydroxyl radicals evoke the following question. Can suitable iron chelator inhibit production of hydroxyl radicals to desirable extent? (Kell, 2009; Andersen, 1999). Quantification of the effectiveness of a given chelate to inhibit formation of ROS is often rather difficult because some chelators can only suppress formation of ROS by chelating iron. However, other chelators can trap produced radicals or act by additional mechanisms.

Catalytic action of iron in the Fenton reaction involves the participation of its *d* orbitals. More saturated coordination sites of iron reflect the lower catalytic activity of metal (d'Hardemare et al., 2006). Generally, ligands containing oxygen atoms stabilize Fe(III) and ligands with nitrogen (and also sulphur) donor atoms prefer Fe(II) (Valko et al., 2005). Thus ligands bearing oxygen atoms promote the oxidation of ferrous to ferric ions and chelators containing nitrogen ligands such as phenantroline and bipyridine inhibit oxi-

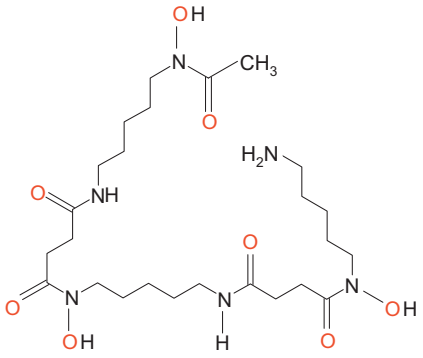

Fig. 6. The iron chelator deferoxamine.

dation of ferrous ions. The maximum coordination number of iron and copper is six. Thus hexadentate chelators can saturate the coordination environment around the iron atom and thus completely deactivate the "free iron". Iron complexes with bidentate or tridentate chelators have at least one or two coordination sites left free to maintain catalytic activity of iron and thereby promote free radical formation.

A proper iron chelator should fulfill certain requirements such as high affinity for Fe(III), oral activity, low toxicity and penetration ability through biological membranes. Deferoxamine (DFO, DFB, desferrioxamine B, known also as Desferal) (Fig. 6) is a bacterial siderophore produced by a gram-positive bacteria *Streptomyces* species (Henretig et al., 1983; Imbert et al., 1995). It is hexadentate and the most frequently used chelator proved to be very effective in the treatment of a number of diseases originating in excess body iron. Deferoxamine can bind iron both oxidation states (Kell, 2010). Ferriprox (deferiprone) is a bidentate chelator with a high affinity for iron acting at molecular, cellular, tissue and organ levels (Kell, 2009). Another effective chelator used in the treatment of neurological disorders is clioquinol (CQ, 5-chloro-7-iodoquinolin-8-ol) a hydroxyquinoline antibiotic containing the 8-hydroxy quinoline motif. CQ was found to be an effective high-affinity chelator of iron in blocking the formation of hydrogen peroxide by Amyloid beta (Bush, 2008).

Various copper chelators, such as D-Penicillamine (D-pen), dimercaprol, trientine, tetrathiomolybdate and clioquinol have been used in cancer treatment, especially in inhibiting angiogenesis both in vitro and in vivo (Brem et al., 1990; Gooneratne and Christensen, 1997; Pan et al., 2002). Brem et al. (1990) observed a reduced tumour growth following a low copper diet and D-pen treatment in glioma implanted intracerebrally in rabbits. D-pen and triente are chelators used to remove excess copper associated with Wilson's disease. Trientine is another copper chelator, acting primarily by enhancing urinary copper excretion. A decreased tumour growth and lowered production of IL-8 with trientine treatment in hepatocellular carcinoma has been observed (Moriguchi et al., 2002). Copper deficiency induced by tetrathiomolybdate resulted in impairment of tumour growth and angiogenesis in two animal models of breast cancer.

A number of clinical trials with copper chelators such as D-pen and tetrathiomolybdate to determine their anti-angiogenic activity have also been conducted (Brewer, 2005). A phase II trial of copper depletion and penicillamine as anti-angiogenesis therapy for glioblastoma reported an effective ceruloplasmin depletion after two months of combination therapy of penicillamine and a low copper diet. However, the achievement of hypocupremia was reported not to significantly increase survival in glioblastoma patients.

Polyphenolic compounds represent one of the most commonly occurring groups of plant metabolites (Melidou et al., 2005; Flora, 2009; Perron et al., 2010). Their structure consists of a diphenylpropane moiety containing aromatic rings linked through three carbon atoms that form an oxygenated heterocycle. Polyphenols may not only act as antioxidants terminating free radical chain reactions but they may act as effective chelators of redox-active metals capable of catalyzing lipid peroxidation.

Catechol (contains two hydroxyl groups) and gallol (contains three hydroxyl groups) and the many functionalized derivatives including the majority of polyphenol compounds are effective metal chelators (Perron and Brumaghim, 2009). They possess the key structural features responsible for the chelation of redox-active metals and thus prevent catalytic decomposition of hydrogen peroxide via Fenton chemistry. Polyphenols containing gallol or catechol groups are not only efficient redox-metal chelators, but they are effective antioxidants, primarily because of the large iron-binding stability constants for these compounds.

Several conflicting results in studies discriminating the effect of metal-chelation and antioxidant activity of flavonoids have been reported. One of the most effective flavonoids is quercetin which has been studied for discrimination between its antioxidant versus iron-chelating properties in the system containing tert-butylhydroperoxides. The results have shown that the prominent activity of quercetin resides in its efficiency to chelate redox active iron (Sestili et al., 1998). Thus the inhibitory effects of quercetin on DNA damage caused by the hydroperoxides were explained by an iron chelating mechanism. Conversly, another study (van Acker et al., 1998) reported that iron chelation by flavonoids does not play a significant role in the antioxidant activity in microsomal lipid peroxidation. From this study it follows, that only flavonoids with a low antioxidant activity may benefit from its metal-chelating ability.

10.2. Chelation of toxic metals

As described above, heavy metal toxicity is a serious condition and can cause a wide range of complications including severe injury to the body organs and the brain. Chelation therapy of toxic metals involves the use of chelates injected into the blood, muscle or taken orally to bind metals that are present in toxic concentrations so they can be excreted from the body, most frequently in urine (Rogan et al., 2001).

One of the most frequently used chelators applied in the treatment of heavy metal toxicity is dimercaprol ((RS)-2,3-disulphanylpropan-1-ol, BAL) (Blanusa et al., 2005). BAL is a compound containing two –SH groups and is used as a preferred agent for arsenic, mercury, cadmium and other metal toxicity. Dimercaprol competes with the thiol groups of enzymes for binding the arsenic or other metals to form a stable metal-chelate which is then excreted from the body in the urine. Dimercaprol is however, itself toxic with a tendency to accumulate arsenic in some organs and exhibits side effects including nephrotoxicity and hypertension.

Another effective chelator used in the treatment of lead toxicity mentioned above is $CaNa_2EDTA$ (Patrick, 2006b). Since this drug chelates only extracellular lead (not intracellular) it is frequently used in conjunction with BAL to increase its efficiency.

Progress in chelation therapy for cadmium removal is rather difficult since this metal is tightly bound to metallothionein in the liver and kidneys. There is a lack of evidence of beneficial effects of chelating agents on cadmium toxicity after prolonged exposure. Chelation therapy with $CaNa_2EDTA$ may be prescribed in the early period after acute cadmium exposure. Besides its beneficial effects, this chelating agent has several disadvantages. The most adverse effect of $CaNa_2EDTA$ administration is the redistribution of lead to

the brain. Its gastrointestinal absorption is rather limited and therefore must be given parenterally. $CaNa_2EDTA$ causes renal toxicity and can deplete the body of essential minerals (Aposhian et al., 1995).

Dimercaptosuccinic acid (DMSA) is an analogue of dimercaprol and is indicated for the treatment of lead or arsenic poisoning in children (Bradberry and Vale, 2009; Andersen and Aaseth, 2002). DMSA can cross the blood brain barrier of some laboratory animals, but not that of humans, limiting thus its use in the treatment of the central nervous system. One of the major disadvantages of DMSA applicability in clinical practice is its low efficiency to remove lead from the intracellular sites because of its lipophobic nature (Kalia and Flora, 2005).

Application of various chelating agents exhibited a range of side effects. A significant amount of patients treated with BAL experienced vomiting, fever, nausea and cardiological complications (Andersen and Aaseth, 2002). In the course of DMSA chelation therapy in patients with chronic lead intoxication, hemolytic anemia has been observed (Andersen and Aaseth, 2002). After termination of therapy, hematological values returned back to normal.

When antioxidants were combined with chelating agents, one trial clearly showed a synergism that improved chelating ability. A combination of DMSA with alpha-lipoic acid in lead-exposed animals was more effective in preventing oxidative damage as measured by alterations in erythrocyte membrane enzyme levels (Sivaprasad et al., 2004). A similar effect of improved chelating ability was observed for $CaNa_2EDTA$ administrated in conjunction with zinc (Batra et al., 1998). It appears that chelating agents used in conjunction with antioxidants can be a standard strategy in treatment of heavy metal toxicity.

A new trend in clinical practice is combined chelation therapy treatment. This includes the use of structurally different chelators in order to achieve a more effective removal of toxic metals (Kalia and Flora, 2005).

11. Concluding remarks

The current knowledge in the field of metallo-biochemistry of oxidative stress indicates that metal-induced and metal-enhanced formation of free radicals and other reactive species can be regarded as a common factor in determining metal-induced toxicity and carcinogenicity.

The above discussion provides an insight into the role of metals capable of direct or indirect generation of free radicals through various mechanisms. Among these mechanisms, Fenton– and Haber–Weiss type reactions are most common leading to generation of the superoxide and hydroxyl radicals (Fig. 7).

Through ROS-mediated reactions, metals cause “indirect” DNA damage, lipid peroxidation, and protein modification. Metal-induced formation of free radicals has most significantly been evidenced for iron and copper then for chromium and partly for cobalt. The “direct” damage by metals may involve conformational changes to biomolecules due to the coordinated metal.

Studies with cadmium revealed that the primary route for its toxicity is depletion of glutathione and bonding to sulphydryl groups of proteins. It has been described that arsenic also binds directly to critical thiols, however, an alternative mechanism leading to formation of hydrogen peroxide by oxidation of As(III) to As(V) under physiological conditions has been proposed. Nitric oxide seems to be involved in arsenite induced DNA damage and pyrimidine excision inhibition. Arsenic-induced formation of free radicals and depletion of antioxidant pools results in disruption of the antioxidant/prooxidant equilibrium of cells.

Metals interfere with cell signalling pathways and affect growth receptors, tyrosine and serine/threonine kinases, and nuclear transcription factors by ROS-dependent and ROS-independent

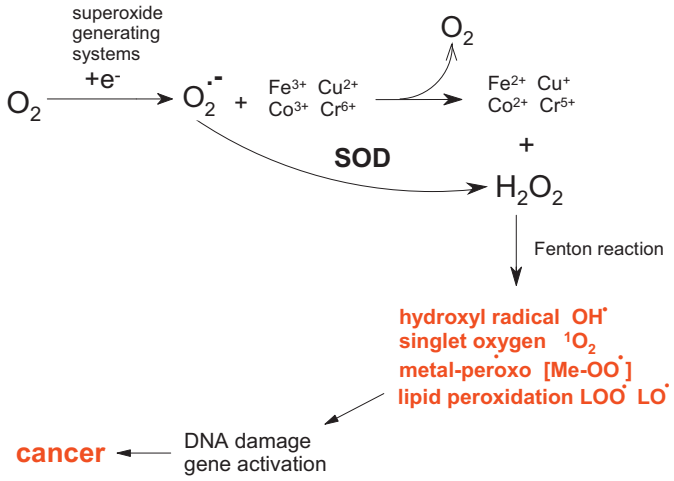

Fig. 7. Pathways of redox active metal-induced oxidative stress.

mechanisms. Many of the DNA base modifications caused by free radicals are pro-mutagenic, pointing to a strong link between oxidative damage and the carcinogenesis of metals. Various antioxidants (both enzymatic and non-enzymatic) provide protection against deleterious metal-mediated free radical attacks. Generally, antioxidants can protect against redox-metal (iron, copper) toxicity by (i) chelating ferrous ion and preventing the reaction with molecular oxygen or peroxides, (ii) chelating iron and maintaining it in a redox state that makes iron unable to reduce molecular oxygen and (iii) trapping any radicals formed. One of the most effective classes of antioxidants are thiol compounds, especially glutathione, which provide significant protection by trapping radicals, reduce peroxides and maintain the redox state of the cell. The non-enzymatic antioxidant vitamin E can prevent the majority of metal-mediated damage both in vitro systems and in metal-loaded animals.

As outlined above, metal-induced oxidative stress is linked with a number of diseases and results partly from declined antioxidant mechanisms. Thus design of dual functioning antioxidants, possessing both metal-chelating and ROS/RNS-scavenging properties is awaited.

Conflict of interest

None.

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