Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Metal induced Oxidative Damage

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Abstract: Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. Redox-active metals, such as iron, copper and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury and others deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes. Either redox-active or redox-inactive metals may cause an increase in production of reactive oxygen species (ROS) such as hydroxyl radical ($HO^{\cdot}$), superoxide radical ($O_2^{\cdot -}$) or hydrogen peroxide (H_2O_2). Enhanced generation of ROS can overwhelm cells' intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. Consequently, it is suggested that metal-induced oxidative stress in cells can be partially responsible for the toxic effects of heavy metals. Several studies are underway to determine the effect of antioxidant supplementation following heavy metal exposure. Data suggest that antioxidants may play an important role in abating some hazards of heavy metals. In order to prove the importance of using antioxidants in heavy metal poisoning, pertinent biochemical mechanisms for metal-induced oxidative stress should be reviewed.

INTRODUCTION

Increasing evidence indicates that multifactorial mechanisms might be involved in metal-induced toxicity and it is suggested that one of the well-known mechanisms is a metal-induced reactive oxygen species. Fenton-like reactions, Fig. (**1**), appear to play a major role in the oxidative stress observed in redox-active metal toxicity [1]. Depletion of a cell's major sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress that is induced by redox-inactive metals [2]. Mechanisms for this stress are not as clear and as easily understood as are the mechanisms for redox-active metal-induced oxidative stress, which has been studied by many scientists [2-10]. Therefore, this review will focus on redox-inactive toxic metals, including lead, cadmium, arsenic and mercury. Lead-induced oxidative stress and the role of antioxidants were reviewed in detail in one of our recent publications [11]. We strongly suggest that readers interested in lead-induced oxidative stress read that review article even though a summary of it is presented here.

$$M^{(n)} + O_2^{\cdot -} \longrightarrow M^{(n-1)} + O_2$$

$$2\,O_2^{\cdot -} + 2\,H^{+} \longrightarrow H_2O_2 + O_2$$

$$M^{(n-1)} + H_2O_2 \longrightarrow M^{(n)} + HO^{\cdot} + OH^{-}$$

Fig. (1). Fenton like reactions of metals.

The heavy metals, lead, mercury and cadmium, all have electron-sharing affinities that can result in formation of covalent attachments [8]. These attachments are mainly formed between heavy metals and sulfhydryl groups of proteins [12]. The tripeptide, glutathione (GSH), is found in mammalian tissues at millimolar concentrations and, therefore, accounts for more than 90% of the total non-protein sulfur [13]. Its physiological and pathological roles in metabolic regulation have been reviewed extensively [13-15]. Interaction of toxic metals with GSH metabolism is an essential part of the toxic response of many metals [10].

When GSH is depleted by any metal, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle. GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic metal exposure [2,10,12]. Several enzymes in antioxidant defense systems may protect this imbalance. Unfortunately, most of these enzymes become inactive also due to direct binding of the metal to the enzymes' active sites, if the sites contain sulfhydryl groups [12]. Furthermore, zinc, which usually serves as a cofactor of many enzymes, could be replaced by heavy metals, thereby making the enzymes inactive [16].

Possible mechanisms involved in metal-induced oxidative stress will be summarized in this review, Fig. (**2**). Lead, cadmium, arsenic and mercury are included because these metals are commonly found in the environment and have been suggested as being very toxic and carcinogenic. The proposed mechanisms for metal-induced oxidative stress will be reviewed by addressing their role in the generation of ROS plus their effect on the antioxidant defense system. If

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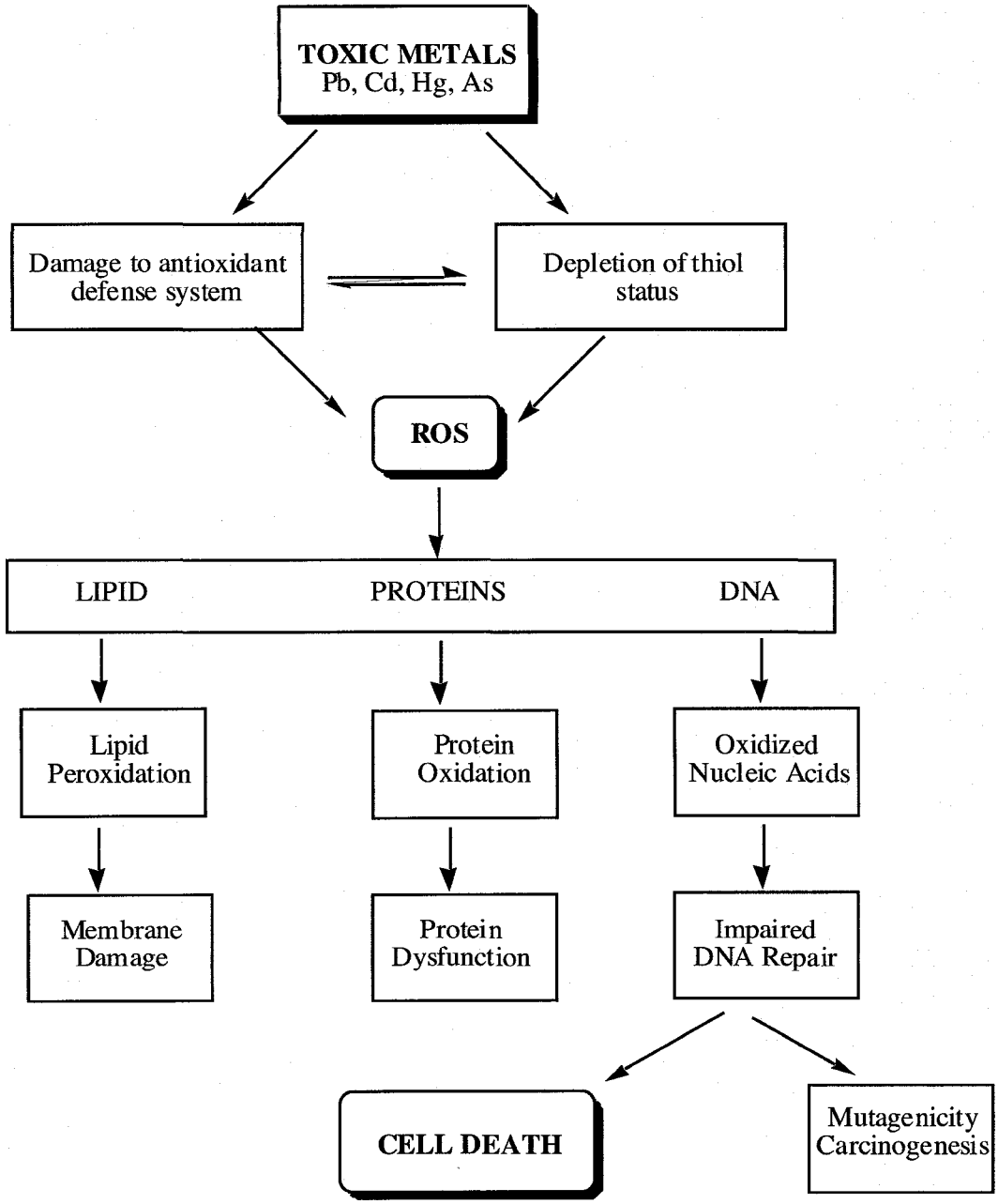

Fig. (2). Possible mechanisms for metal-induced oxidative stress.

oxidative stress is scientifically proven to be an important mechanism for heavy metal toxicity, then inclusion of antioxidants in the treatment of metal-induced toxicity deserves further consideration.

THE MECHANISMS FOR METAL-INDUCED OXIDATIVE DAMAGE

Lead

Because lead (Pb) cannot readily undergo valance changes, the mechanisms that enable lead to induce oxidative stress are not clear [11]. The earliest paper regarding lead-induced oxidative stress was published in 1965 [17]. In this study, several metals were shown to increase the rate of essential fatty acid oxidation. Lead, however, was found to be ineffective. Many years later, lipid peroxidation was assessed by malondialdehyde (MDA) analysis and was found to be increased by lead [18]. This has also been proven by other investigators [19-23]. Lipid peroxidation in the brains of lead-exposed rats was observed by several researchers [19-21], and a direct correlation was observed between lead concentration and lipid peroxidation [20]. A similar effect in the liver of lead-exposed rats was reported [22].

Several mechanisms are proposed for lead-induced oxidative stress: 1) Direct effect of lead on cell membranes, 2) Lead-hemoglobin interactions, 3) δ-aminolevulinic acid (δ-ALA)-induced generation of reactive oxygen species, and 4) Effect of lead on the antioxidant defense systems of cells.

1) Direct Effect of Lead on Cell Membranes

In order to study lead's effect on cell membranes, red blood cell (RBC) membranes were intensively analyzed. Osmotic and mechanic susceptibilities of RBCs were reported to be increased in lead toxicity [24]. It is not clear whether oxidative stress is the cause or the consequence of these reported toxic effects of lead, but lead exposure probably may further increase the susceptibility of membranes by altering their integrity via causing deterioration of their components [11].

Several studies investigated the toxic effects of lead on membrane components and found a direct correlation between these effects and lead-induced oxidative damage. When lead was incubated with various polyunsaturated fatty acids, a marked enhancement in MDA concentration was observed. MDA concentration was found to increase as the number of double bonds of fatty acids increased [18]. Arachidonic acid and the arachidonate/linoleate ratio in the livers, serum and RBC membranes of lead-exposed chicks were increased [25,26]. Since fatty acid chain length and unsaturation are important determinants of membrane susceptibility to peroxidation, it was suggested that arachidonic acid augmentation might be responsible for the enhanced lipid peroxidation in cellular membranes [26]. Lead is also shown to strongly bind to phosphatidylcholine membranes *in vitro*, thereby decreasing the levels of phosphatidylcholine [27]. Furthermore, the same group determined lead, phospholipid and lipid peroxidation levels in various regions of the brains of lead-exposed rats [20]. An inverse correlation was observed between the rate of lipid peroxidation and the phospholipid level in lead-exposed brain samples [20]. In summary, these data suggest that altered lipid composition of cellular membranes may alter membrane integrity, permeability and function, thereby increasing susceptibility to lipid peroxidation.

2) Lead-hemoglobin Interactions

Some heavy metals, including Ag^+, Hg^{2+}, Cu^{2+}, and Pb^{2+}, cause hemolysis and lipid peroxidation [28]. Lead-induced lipid peroxidation was suggested to be the possible mechanism for hemolysis [29]. However, because Pb^{2+} cannot initiate lipid peroxidation on the membrane lipids directly, indirect mechanisms were investigated [17,30,31]. The interaction of heavy metals with oxyhemoglobin (oxyHb) has been suggested as an important source of superoxide radical formation in RBCs [32]. As a result, Ribarov *et al.* found that Pb^{2+} substantially increased the auto-oxidation of hemoglobin (Hb) in an *in vitro* liposome model [30]. Hb auto-oxidation by lead was inhibited by antioxidant enzymes, suggesting that $O_2^{\cdot-}$ and H_2O_2 were involved in this process. Therefore, it was speculated that lead might induce oxidative stress by interacting with oxyhemoglobin, leading to peroxidative hemolysis in RBC membranes [30,31].

3) δ -Aminolevulinic Acid (ALA)-Induced Generation of Reactive Oxygen Species

Effects of lead on the hematological system have been known for many years. Anemia is a well-known symptom of lead poisoning. It is seen in plumbism (an old term for lead poisoning), because lead inhibits Hb synthesis and changes RBC morphology and survival [11]. In heme synthesis, there are several pathways and enzymes catalyzing these pathways. Activities of δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase, which are involved in heme synthesis, are inhibited by lead. ALAD is a sulfhydryl-containing enzyme. Lead binds to the -SH group of this enzyme, making it inactive. Condensation of two molecules of ALA, which is catalyzed by ALAD, is then blocked and levels of ALA become very high in blood [33,34]. Many studies, particularly that of Bechara et al., focused on the effects of ALA on ROS generation [35] and showed that ALA undergoes enolization at physiological pH. The enolised ALA then autoxidizes and generates superoxide anion, as evidenced by the parallel reduction of ferricytochrome c and also by electron spin resonance spin-trapping experiments [36,37]. Monterio *et al.* suggested that ALA/oxyHb coupled oxidation also results in ROS [37]. Proposed mechanisms for ALA oxidation and generation of free radicals are shown in Fig. (**3**). As shown in that figure, HO· radicals, which are the most reactive free radicals, are generated as a result of both ALA and ALA/oxyHb coupled autoxidation. Inhibition of ALA/oxyHb coupled oxidation by antioxidants supports the hypothesis that free radicals are involved in the process [37]. Douki *et al.* demonstrated that the final oxidation product of ALA, 4,5-dioxovaleric acid, is an effective alkylating agent (of the guanine moieties within both nucleoside and isolated DNA). Rats, chronically treated with ALA, have high levels of DNA oxidation products such 8-oxo-7, 8-dihydro-2'-deoxyguanosine and 5-hydroxy-2'-deoxycytidine [38]. These studies suggest that ALA accumulation in lead poisoning might be partially responsible for ROS generation.

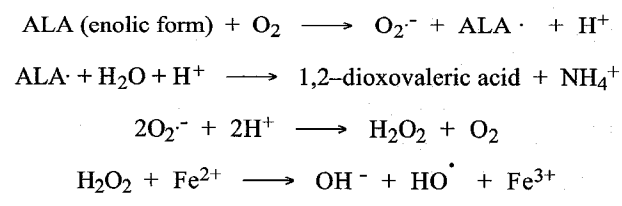

Fig. (3). Enolization of ALA followed by aerobic oxidation which induces the generation of superoxide and peroxide radicals.

4) Effect of Lead on the Antioxidant Defense Systems of Cells

Cells have developed various antioxidant defense systems against free radical attacks. GSH, γ-glutamyl-cysteinyl-glycine, plays a major role in protecting cells against oxidative stress [39]. GSH has carboxylic acid groups, an amino group, a sulfhydryl group and two peptide linkages as sites for the reaction of metals. Its functional group, -SH,

plays an important role in metal binding. Several groups have demonstrated that GSH is decreased in the brains, livers, and eye-lens of rats exposed to lead [11,39-49]. Glutathione reductase (GR) reduces glutathione disulfide (GSSG) to GSH, thereby supporting the antioxidant defense system. GR has a disulfide bond in its active site, but lead interferes with the disulfide bond and inhibits the enzyme. This inhibition prevents the reduction of GSSG, making cells more susceptible to oxidative damage [22,50,51].

Other antioxidant enzymes, which remove peroxides, and superoxide radicals including glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), are also potential targets for lead. Because GPx requires selenium for its activity, when lead forms a complex with selenium, GPx activity decreases [52,53]. Lead is known to inhibit heme synthesis, and since CAT is a heme-containing enzyme, it causes CAT activity to decrease [54]. SOD dismutates $O_2^{\cdot-}$ and requires copper and zinc for its activity. Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme [55]. Several studies showed decreased RBC SOD activity in lead-exposed rats [52,56-59]. In summary, lead's inhibitory effects on these antioxidant enzymes appear to impair the antioxidant defenses of cells and to render them more susceptible to oxidative attacks.

Cadmium

Cadmium (Cd) is a nonessential, group II-B metal. It is found in foods (vegetables, grains and cereals), water and tobacco leaves, and is also produced as a by-product of zinc and lead mining and smelting [60]. Because of Cd's widespread nature, it can be ingested or inhaled but, since Cd is not a redox-active metal like Pb, its oxidant role is not clear. It has been shown that Cd is mainly stored in soft tissues, especially in the liver and kidneys, and induces lipid peroxidation in the liver, kidneys, brains, lungs, heart and testes of rats. Unlike other heavy metals, Cd has a long biological half-life (10-30 years) and is excreted very slowly from the body [61].

The mechanisms responsible for Cd-induced toxicity may be multifactorial. Cd has been officially listed as a pulmonary carcinogen for rats and human by the International Agency for Research on Cancer [62]. Proposed mechanisms for Cd-induced oxidative stress can be studied in three groups: 1) Adverse effects of cadmium on cellular defense systems and thiol status, 2) Enhancement of lipid peroxidation by cadmium, 3) Deleterious effects of cadmium on cellular enzymes.

1) Adverse Effects of Cadmium on Cellular Defense Systems and Thiol Status

Cd remains in the lungs and induces a cysteine (Cys) rich protein called metallothionein (MT). There are several isoforms of MTs, which are known to protect cells from oxidative stress. Since MTs are comparatively cysteine-rich (20%-30% of the protein is Cys), and metals have a high affinity for thiols, MTs are known to sequester metals [63]. Therefore, metals (particularly Cd) are stored as a Cd-MT complex in the liver. This Cd-MT is transferred from the liver to the kidneys over time, and is then filtered and reabsorbed by the renal proximal tubules. Cd-MT is metabolized in lysosomes to liberate Cd ions. These liberated Cd ions again bind to preexisting or newly made MT. If MT synthesis cannot keep up with the demand and the non-MT bound Cd overwhelms other defense systems, Cd toxicity ensues [64,65].

Several studies indicated that Cd alters GSH levels. For example, Cd administered orally for 30 days was found to increase tissue GSH levels in rats [66]. In another study, GSH was also increased in the livers and kidneys of rats given 0.228 mg Cd/kg, 3 days/week for 1-year [67]. GSH is known to protect cells against oxidative stress and any alteration in GSH levels (either a decrease or an increase) indicates a disturbed oxidant status. When cells are oxidatively challenged, GSH synthesis increases. As oxidative stress continues, GSH synthesis cannot efficiently supply the demand; therefore, GSH depletion occurs. Alterations in GSH levels have been observed in Cd toxicity, and most studies reported an increase in GSH levels after Cd exposure [66-69]. A few studies reported GSH depletion in tissues [70,71]. In order to support the hypothesis that Cd toxicity changes GSH levels, a GSH-depleting agent, buthionine sulfoximine (BSO), was given to animals (BSO inhibits γ-glutamylcysteine synthetase). Depletion of GSH caused nephrotoxicity within 6 hours after a Cd injection as indicated by a several-fold increase in urinary lactate dehydrogenase (LDH) activity. Acute hepatotoxicity was not observed in animals treated with BSO and Cd [65]. Moreover, co-treatment with N-acetylcysteine (NAC), a GSH replenishing agent and a free radical scavenger, protected against chronic hepatotoxicity and nephrotoxicity as well. These studies indicate that Cd toxicity causes oxidative stress by challenging the thiol status of cells.

2) Enhancement of Lipid Peroxidation by Cadmium

Lipid peroxidation has also been observed in Cd toxicity. Malondialdehyde (MDA) is a well-known lipid peroxidation indicator and has been found to increase in the liver and kidneys after Cd exposure [69]. Reasons for lipid peroxidation after Cd exposure are not completely known, but we believe that disturbances in GSH and MT levels may allow free radicals to be "free" such that $HO\cdot$ and $O_2^{\cdot-}$ radicals can attack double bonds in membrane lipids and result in an increase in lipid peroxidation. Moreover, mitochondrial respiration as the major source of ROS, is promoted by lipid peroxidation and therefore enhances oxidative stress induced Cd toxicity [70]. In another study, Yiin *et al.* demonstrated that administration of Cd in various doses significantly increased thiobarbituric acid-reactive substances (TBARS), a well-known indicator of lipid peroxidation, in rat adrenal glands that structurally contain large amounts of polyunsaturated lipids [72]. This same research group also showed the presence of lipid peroxidation of arachidonic acid catalyzed by cadmium chloride in vitro. In this particular study, it was indicated that when Cd concentration, exposure time, and temperature of incubation were increased, the production of lipid peroxidation was also elevated [73].

3) Deleterious Effects of Cadmium on Cellular Enzymes

Metal-induced alterations in antioxidant enzyme activities have been extensively studied over the years. It has been reported that especially CAT and SOD, two major antioxidant enzymes, are affected by Cd. SOD activity was found to be inhibited by administration of cadmium acetate to liver and kidneys *in vitro*, as well as *in vivo*. High levels of lipid peroxidation were found in both tissues [74]. Mateo *et al.* examined CAT activity in erythrocytes. Administration of 0.2 mM Cd inhibited CAT activity in colon cancer and showed less malignancy (when compared to colon cancer) at the same concentration, whereas it increased CAT activity in gastric neoplasia [69]. Aminotriazole (AT) is a CAT inhibitor. Shaikh *et al.* studied the effects of AT on Cd nephrotoxicity. A single dose of AT was given to rats, prior to the last Cd injection. A significant increase in urinary LDH activity was seen indicating nephrotoxicity [75].

More support for Cd-induced oxidative stress following Cd exposure comes from studies on the levels of heme-oxygenase-1 (HO) and GST-Ya, one of the isoforms of glutathione-S-transferase(GST). In the Gong *et al.* study, exposure of lung epithelial cells to Cd resulted in time and dose-dependent increases in mRNA levels for MT, GST-Ya and HO. Northern hybridization results also indicated that, at low Cd concentrations (5 μM), MT gene expression is more sensitive than that of GST-Ya or HO. On the other hand, higher concentrations of Cd (10-20 μM) mostly induced the HO gene. Moreover, GSH depletion via BSO treatment enhanced the Cd-induced expression of MT, GST-Ya and HO genes with administration of 10μM $CdCl_2$. In the same study, epithelial cells were pretreated for 2-30 hours with 10 mM NAC before $CdCl_2$ exposure in order to determine the effect of thiol supplementation. It was observed that, after 8 hours of NAC treatment, Cd-induced expression of all three genes was suppressed. MT mRNA levels, in particular, were significantly lower when compared to basal MT mRNA levels of non-pretreated cells [65].

Arsenic

Arsenic (As) is associated with several diseases, including diabetes [76], hypertension [77], and tumors of the skin, bladder, liver and lung [78]. The mechanisms by which arsenic may induce cancer have not been fully understood [79]. Besides various mechanisms that have been proposed, oxidative stress is a relatively new theory for arsenic-induced carcinogenesis. As-induced oxidative stress has been previously reviewed by Kitchin *et al.* [80] and Bernstam *et al.* [81].

1) Direct Evidence for Arsenic-Induced Free Radical Formation

Arsenic-induced free radical formation was first indicated by Yamanaka *et al.* [82,83]. It is suggested that dimethylarsine (a trivalent arsenic form and a minor in vivo metabolite of dimethylarsinic acid) reacts with molecular oxygen to form a dimethylarsenic radical and superoxide anion. The addition of another molecule of molecular oxygen onto the dimethylarsenic radical results in a dimethylarsenic peroxyl radical. It was also suggested that a hydroxyl radical generates during these reactions via involvement with cellular iron and other transition metals [83]. Recently Liu *et al.* provided direct evidence of an inorganic arsenite-induced free radical formation. Mice were given either 100μmol/kg of arsenite or 500μmol/kg of arsenate subcutaneously, as well as a spin trap agent. Thirty minutes later, the livers were removed and analyzed with the electron spin resonance technique. Both arsenite and arsenate gave positive results where stronger signals were detected from arsenite-exposed mice [84].

The source of the ROS stimulated by arsenite has not been defined. Barchowsky *et al.* reported that there may be both mitochondrial and extra-mitochondrial sources of oxygen consumption and radical production [85].

2) Indirect Evidence for Arsenic-Induced Oxidative Stress

The radical scavenging enzymes, CAT and SOD, were shown to suppress the frequency of arsenic-induced sister chromatid exchanges in human lymphocytes [86,87]. H_2O_2-resistent Chinese hamster ovary (CHO) cells were shown to be cross-resistant to arsenite. On the other hand, CAT-deficient CHO cells are reported to be hypersensitive to the toxic effects of arsenite [88]. The addition of CAT to those cells was found to completely inhibit sodium arsenite-provoked micronuclei induction [88]. Recently, using single cell alkaline electrophoresis, Liu *et al.* detected DNA strand breaks (DSBs) in bovine aortic endothelial cells by using a 4-hour treatment with sublethal concentrations of arsenic. Since nitric oxide synthase inhibitors could decrease As-induced DSBs, nitric oxide (NO) was suggested as being involved in As-induced DSBs. Moreover, the results showing that As-induced DSBs could be decreased by superoxide and peroxynitrite scavengers further indicated that As-induced NO may react with superoxide to produce peroxynitrite and cause DNA damage [89]. Results were reported confirming arsenite-induced NO production in several cell lines [90,91]. On the other hand, it has also been reported that arsenite does not increase NO production in rat aortic muscle cells [92], hepatocytes, and human liver cells [93]. According to their reported results Liu and Jan concluded that the effect of arsenite on NO production could be cell type specific [89].

Biomarkers of oxidative stress were reported with arsenite treatment over the last decade. 8-hydroxy-2-deoxyguanosine (8-OHdG) is a major biomarker for ROS-induced DNA damage. Yamanaka *et al.* reported increased 8-OHdG in urine samples of mice gavaged with 720 mg/kg of dimethylarsinic acid [94]. In a long-term carcinogenesis study of rats, hepatic 8-OHdG levels were increased in dimethylarsinic acid-treated rats, suggesting an increased rate of reactive oxygen species attack on DNA [95].

3) Effects of Arsenic on Cellular Antioxidant Defense Systems

Several studies have shown the effect of arsenic compounds on cellular antioxidant defense activities. Lee *et al.* reported that treatment of human fibroblasts with sodium

arsenite resulted in elevated HO activity and ferritin levels [96]. The increased HO synthesis was inhibited by co-treatment with the antixodants, sodium azide and dimethyl sulfoxide [96]. Furthermore, sodium arsenite treatment significantly increased GSH levels and SOD activity, slightly decreased GPx activity and significantly decreased CAT activity. The addition of CAT to the culture medium decreased the sodium arsenite toxicity, which indicated a possible role of H_2O_2 in the toxicity [96]. Maiti and Chatterjee [97] reported that the liver and kidneys have different adaptive cellular protective mechanisms against arsenic exposure. The kidneys, in general, were observed to be more vulnerable to arsenic treatment in male Wistar rats that had been exposed to sodium arsenite (3.33mg/kg b.w. per day) for 14 days. Significantly increased lipid peroxidation and decreased SOD and CAT activities were identified in the rats' kidneys. On the other hand, lipid peroxidation and SOD activity in their livers remained unchanged following arsenite treatment. The livers showed significantly increased GSH levels, and activities of GR and GST were reported to be protected from arsenite-induced oxidative damage by some antioxidant components, such as GSH, GST, and glucose 6-phosphate dehydrogenase (G6PD).

Mercury

The primary sources of chronic, low-level mercury (Hg) exposure are dental amalgams and fish. Hg enters water as a natural process of off-gassing from the earth's crust and as a result of industrial pollution. Algae and bacteria methylate Hg entering the waterways. Methylmercury (MeHg) makes its way through the food chain into fish and shellfish and ultimately into humans [98].

The two major, highly absorbed subspecies of Hg are elemental mercury (Hg^0) and MeHg. So-called "silver dental amalgams" contain over 50% Hg^0, which is the only metal with a melting point below room temperature. Hg^0 vapor is highly lipophilic and is efficiently absorbed through the lungs and oral mucosa. After entering the blood, it rapidly passes the cell membranes, including the blood-brain barrier and placental barrier. Once inside a cell, Hg^0 is oxidized by CAT and becomes highly reactive Hg^{2+}. On the other hand, MeHg, derived from fish, is readily absorbed in the gastrointestinal tract (more than 95% absorption from food). Because of its lipid soluble nature, MeHg can also easily cross both the blood-brain and placental barriers [98,99].

Once absorbed, Hg has a low excretion rate. A major proportion of absorbed Hg accumulates in the kidneys, neurological tissue and the liver. All forms of mercury exhibit toxic effects, including neurotoxicity, nephrotoxicity and gastrointestinal toxicity.

Among the various mechanisms suggested as explaining the neurotoxic effects of Hg, no single mechanism explains all of the neuropathological outcomes. The chemical reactivity and some biological features of the metal suggest that oxidative stress might be involved in Hg-induced toxicity. Since MeHg is the most common and most toxic environmental form of Hg, the majority of experimental studies have focused on MeHg. Because 3% to 6% of the organic Hg accumulated in a brain is converted to Hg^{2+}, mercury-induced oxidative damage is also worth considering.

1) Effects of Mercury on the Cellular Thiol Pool

One of the most important mechanisms for Hg-induced oxidative damage is its known sulfhydryl reactivity. Once absorbed in the cell, both Hg^{2+} and MeHg form covalent bonds with GSH and the cysteine residues of proteins. GSH, the primary intracellular antioxidant and the conjugating agent, was shown to be depleted and to have impaired function in Hg toxicity. A single Hg ion can bind to and cause irreversible excretion of up to two GSH molecules [12]. In fact, GSH serves as a primary line of cellular defense against Hg compounds. Releasing the Hg ions from complexes with GSH and cysteine results in greater activity of the free Hg ions disturbing GSH metabolism and damaging cells [10]. However, interaction of Hg compounds and GSH suggested the production of oxidative damage via accumulation of reactive oxygen species, which had normally been eliminated by GSH [100].

2) Inhibition of Mitochondrial Oxidative Phosphorylation by Mercury

In eucaryotic organisms, mitochondria are the primary sites for production of $O_2^{\cdot-}$ and H_2O_2, converting 1-5% of cellular O_2 to $O_2^{\cdot-}$ during normal metabolism [101]. Agents that block electron transport in any step are shown to stimulate $O_2^{\cdot-}$ production [102]. Inorganic Hg is suggested to increase H_2O_2 production by impairing the efficiency of oxidative phosphorylation and electron transport at the ubiquinone-cytochrome b5 step [103-105]. This mechanism is highly plausible for the nephrotoxic effects of Hg, which is known to accumulate in kidney at high levels. On the other hand, MeHg is known to be an uncoupling agent, stimulating state IV respiration [106]. By accelerating electron transfer rates in the electron transport chain in mitochondria, premature shedding of electrons to molecular oxygen increases and generates $O_2^{\cdot-}$ and H_2O_2 .

3) Effects of Mercury on Calcium Homeostasis

The third molecular mechanism for Hg-induced oxidative stress appears to be its effect on calcium homeostasis. The role of calcium in the activation of hydrolytic enzymes, such as proteases, endonucleases and phospholipases, is well-known. Activation of phospholipase A_2 results in increased generation of arachidonic acid, which rapidly influences lipoxygenase and cyclooxygenase and results in production of $O_2^{\cdot-}$. Therefore, activation of phospholipase A_2 is related to the elevation of ROS [107-109]. Furthermore, arachidonic acid, an unsaturated fatty acid with four double bonds, is known to be an important target of ROS [55]. Another consequence of increased calcium is the conversion of xanthine dehydrogenase to xanthine oxidase, which then catalyzes reactions with $O_2^{\cdot-}$ and H_2O_2 forming as by-products. Both organic and inorganic Hg alter calcium homeostasis, but by different mechanisms [110]. MeHg is believed to increase intracellular calcium via accelerating the influx of calcium from the extracellular medium and by

mobilizing intracellular calcium stores. However, it was suggested that Hg^{2+} increases intracellular calcium only by increasing the influx from the extracellular medium [110]. Although a direct relation between increased calcium levels and Hg-induced oxidative damage was not reported by the authors [110], elevated levels of cytoplasmic calcium could possibly increase oxidative damage [100].

It is also suggested that Hg^{+2} displaces iron and copper from their intracellular binding sites, thereforeaccelerating Fenton-mediated ROS formation [100,111].

4) Mercury-Induced Lipid Peroxidation

Increased lipid peroxidation was reported in animals [111-113] and plants [114] that were exposed to various Hg compounds. Huang *et al.* reported increased concentrations of MDA in the livers, kidneys, lungs and testes of $HgCl_2$-treated rats [111], which correlated with the severity of hepatotoxicity and nephrotoxicity. Furthermore, $HgCl_2$–associated lipid peroxidation was significantly reduced by an antioxidant, selenium dioxide pretreatment. Selenium can achieve a protective effect, either by directly binding to Hg or serving as a cofactor for GPx, and thereby facilitating its ROS scavenging activity. Another well-known antioxidant, vitamin E, was shown to protect against $HgCl_2$–induced hepatic lipid peroxidation in high concentration [115]. However, excess dietary β-carotene [115], sodium ascorbate and promethazine [111] did not protect against $HgCl_2$–associated lipid peroxidation.

5) Mercury's Effects on Other Antioxidant Enzymes

In addition to inducing lipid peroxidation (by means of MDA levels) and altering GSH concentrations, $HgCl_2$ was also reported to affect other antioxidant enzyme activities of cells. Ariza *et al.* showed that Hg^{+2} induces H_2O_2 formation and stimulates the activities of copper-zinc SOD and xanthine oxidase in AS52 cells. Hg^{2+} did not affect other antioxidant enzymes, such as CAT, GPx, and GR [57]. On the other hand, Hussain *et al.* reported increased CAT, and GPx activity, and GSH content in the livers, kidneys and brains of mice exposed to 1 mg/kg/day $HgCl_2$ for 14 days. The activated antioxidant defense system of cells was reported to be a compensatory mechanism for $HgCl_2$ –induced oxidative stress [116]. Similarly, Woods *et al.* reported that increased levels of GSH and the activities of GR and GPx in rats exposed to MeHg in drinking water were an adaptive response to Hg exposure in renal epithelial cells [117]. Because neurons did not appear to have a similar adaptive capacity [118], this was the explanation given for Hg being relatively more neurotoxic then nephrotoxic [12].

CONCLUSION

Fenton-like production of ROS seems to be involved with redox-active metals, including iron, copper, chromium and vanadium. However, for redox-inactive toxic metals (such as lead, cadmium, mercury and arsenic) depletion of cells' major antioxidant reserves, GSH, and protein-bound sulfhydryls may play a pivotal role in the overall toxic manifestations. Mounting evidence indicates that multiple mechanisms may be responsible for the production of ROS in toxic metal exposure. Among them, alterations in thiol status, increased lipid peroxidation, production of ROS, and damage to a cell's antioxidant defense systems are well-known for all redox active and inactive metals. Although, toxic metal exposure has been strictly controlled and is rather uncommon in the USA, it is seen quite often in developing countries. Moreover, once it occurs, treatment is rather ineffective. Metal chelators are given to increase the excretion of metals but unfortunately, their side effects are numerous. New treatments are essential, since the total elimination of metals from the environment is not feasible. If oxidative stress proves to play a major role in manifestations of metal-induced toxicities, then inclusion of antioxidants in treatment of metal-toxicity should be seriously considered.

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ABBREVIATIONS

8-OHdG = 8-Hydroxy-2-deoxyguanosine

ALA = δ-Aminolevulinic acid

ALAD = δ-Aminolevulinic acid dehydratase

AT = Aminotriazole

BSO = Buthionine sulfoximine

CAT = Catalase

CHO = Chinese hamster ovary

Cys = Cysteine

DSBs = DNA strand breaks

G6PD = Glucose 6-phosphate dehydrogenase

GPx = Glutathione peroxidase

GR = Glutathione disulfide reductase

GSH = Glutathione (reduced form)

GSSG = Glutathione disulfide (oxidized form)

GST = Glutathione-S-transferase

Hb = Hemoglobin

HO = Heme-oxygenase-1

LDH = Lactate dehydrogenase

MDA = Malondialdehyde

MeHg = Methylmercury

MT = Metallothionein

NAC = N-acetylcysteine

NO = Nitric oxide

oxyHb = Oxyhemoglobin

RBC = Red blood cells

ROS = Reactive oxygen species

-SH = - thiol

SOD = Superoxide dismutase

TBARS = Thiobarbituric acid-reactive substances

REFERENCES

[1] Liochev, S.I. The mechanism of "Fenton-like reactions and their importance for biological systems. A biologist's view. In *Metal Ions in Biological Systems*; Sigel, A.; Sigel, H.; Eds.; Marcel Dekker, Inc: New York, **1999**, pp 1-39.

[2] Stohs, S.J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* **1993**, *18*, 321-336.

[3] Li, N.C.; Manning, R.A. Some metal complexes of sulfur-containing amino acids. *J. Am. Chem. Soc.* **1955**, *77*, 5225-5228.

[4] Jocelyn, P.C. Metal derivatives of thiols. In *Biochemistry of the SH group*. Academic Press, London, **1972**, pp 77-116.

[5] Vallee, B.L.; Ulmer, D.D. Biochemical effects of mercury, cadmium, and lead. *Annu. Rev. Biochem.* **1972**, *41*, 91-128.

[6] Cooper, A.J.L. Biochemistry of sulfur-containing amino acids. *Annu. Rev. Biochem.* **1983**, *52*, 187-222.

[7] Hultberg, B.; Andersson, A.; Isaksson, A. Thiol and redox reactive agents exerts different effects on glutathione metabolism in HeLa cell cultures. *Clin. Chim. Acta* **1999**, *283*, 21-32.

[8] Bondy, S.C. Oxygen generation as a basis for neurotoxicity by metals. In: *Toxicology of Metals*. Chang, L.W.; Eds,; CRC Press, Baco Raton, **1996**, pp 699-706.

[9] Cuypers, A.; Vangronsveld, J.; Clijsters, H. The chemical behavior of heavy metals play a prominent role in the induction of oxidative stress. *Free Rad. Res.* **1999**, *31*, 39-43.

[10] Hultberg, B.; Andersson, A.; Isaksson, A. Interaction of metals and thiols in cell damage and glutathione distribution: potentiation of mercury toxicity by dithiothreitol. *Tox.* **2001**, *156*, 93-100.

[11] Gurer, H.; Ercal, N. Can antioxidants be beneficial in the treatment of lead poisoning. *Free Radic. Biol. Med.* **2000**, *29*, 927-945.

[12] Quig, D. Cysteine metabolism and metal toxicity. *Alter. Med. Rev.* **1998**, *3*, 262-270.

[13] Meister, A. Glutathione metabolism and its selective modification. *J. Biol. Chem.* **1988**, *263*, 17205-17208.

[14] Meister, A. Glutathione, ascorbate, and cellular protection. *Cancer Res.* **1994**, *54*(suppl), 1969s-1975s.

[15] Meister, A.; Anderson, M.E. Glutathione. *Annu. Rev. Biochem.* **1983**, *52*, 711-760.

[16] Donaldson, W.E. Interaction of dietary lead with fish oil and antioxidant in chicks. *Biol. Trace Elem. Res.* **1991**, *31*, 215-222.

[17] Willis E.D. Mechanisms of lipid peroxide formation in tissues. Role of metals and haematin proteins in the catalysis of the oxidation of unsaturated fatty acids. *Biochem. Biophys. Acta* **1965**, *98*, 238-251.

[18] Yiin, S. J.; Lin, T.H. Lead-catalyzed peroxidation of essential unsaturated fatty acid. *Biol. Trace Elem. Res.* **1995**, *50*, 167-172.

[19] Gerber, G.B.; Maes, J.; Gilliavod, N.; Casale, G. Brain biochemistry of infants and rats exposed to lead. *Tox. Lett.* **1978**, *2*, 51-63.

[20] Shafiq-ur-Rehman Lead-induced regional lipid peroxidation in brain *Tox. Lett.* **1984**, *21*, 333-337.

[21] Shafiq-ur-Rehman; Rehman, S.; Chandra, O.; Abdulla, M. Evaluation of malondialdehyde as an index of lead damage in rat brain homogenates. *Biometals* **1995**, *8*, 275-279.

[22] Sandhir, R.; Gill K.D. Effect of lead on lipid peroxidation in liver of rats. *Biol.Trace Elem. Res.* **1995**, *48*, 91-97.

[23] Somashekaraiah, B.; Padmaja, K.; Prasad, A.R.K. Lead-induced lipid peroxidation and antioxidant defense components of developing chick embryos. *Free Radic. Biol. Med.* **1992**, *13*, 107-114.

[24] Waldron, H.A. The anemia of lead poisoning: A review. *Brit. J. Indust. Med.* **1966**, *23*, 83-100.

[25] Knowles, S.O.; Donaldson, W.E. Dietary modification of lead toxicity: effects on fatty acid and eicosanoid metabolism in chicks. *Comp. Biochem. Physiol.* **1990**, *95C*, 99-104.

[26] Lawton, L.J.; Donaldson, W.E. Lead-induced tissue fatty acid alterations and lipid peroxidation. *Biol. Trace Elem. Res.* **1991**, *28*, 83-97.

[27] Shafiq-ur-Rehman; Abdulla, M. Impacted lead on phospholipid metabolism in human erythrocyte membranes. *Bul. J. Tox. Occup. Environ. Health* **1993**, *2*, 35-47 .

[28] Ribarov, S.R.; Benov, L.C. Relationship between the hemolytic action of heavy metals and lipid peroxidation. *Biochim. Biophys. Acta* **1981**, *640*, 721-726.

[29] Levander, O.A.; Morris, V.C.; Ferretti, R.J. Filterability of erythrocytes from vitamin E-deficient lead-poisoned rats. *J. Nutr.* **1977**, *107*, 363-372.

[30] Ribarov, S.R.; Benov, L.C.; Benchev, I.C. The effect of lead on hemoglobin- catalyzed lipid peroxidation. *Biochim. Biophys. Acta* **1981**, *664*, 453-459.

[31] Ribarov, S.R.; Bochev, P.G. Lead-hemoglobin interaction as a possible source of reactive oxygen species- A chemiluminescent study. *Arch. Biochem. Biophys.* **1982**, *213*, 288-292.

[32] Carrell, R.W.; Winterbourn, C.C.; Rachmillewitz, E.A. Activated oxygen and haemolysis. *Br. J. Haematol.* **1975**, *30*, 259-264.

[33] Klaassen, C.D.; Amdur, M.O.; Doull, J. Eds.; *Casarett and Doull's Toxicology*. McGraw-Hill, New York; **1996**.

[34] Ratcliffe, J.M., Ed.; *Lead in Man and The Environment*. Ellis Horwood Ltd.: Chichester, **1981**, pp 34-47.

[35] Bechara, E.J.H. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. *Brazilian J. Med. Biol. Res.* **1996**, *29*, 841-851.

[36] Monteiro, H.P.; Abdalla, D.S.P.; Augusto, O.; Bechara, E.J.H. Free radical generation during δ-aminolevulinic acid autoxidation: induction by hemoglobin and connections with porphyrinopathies. *Arch. Biochem. Biophys.* **1989**, *271*, 206-216.

[37] Monteiro, H.P. Abdalla, D.S.P.; Faljoni-Alario, A.; Bechara, E.J.H. Generation of active oxygen species during coupled autoxidation of oxyhemoglobin and δ-aminolevulinic acid. *Biochim. Biophys. Acta* **1986**, *881*, 100-106.

[38] Douki, T.; Onuki, J.; Medeiros, M.H.; Bechara, E.J.; Cadet, J.; Di Mascio, P. DNA alkylation by 4,5-dioxovaleric acid, the final oxidation product of 5-aminolevulinic acid. *Chem. Res. Toxicol.* **1998**, *11*, 150-157.

[39] Christie N.T.; Costa M. In vitro assesment of the toxicity of metal compounds. IV. Disposition of metals in cells: interaction with membranes, glutathione, metallothionein, and DNA. *Biol. Trace Elem. Res.* **1984**, *6*, 139-158.

[40] Monteiro, H.P.; Abdalla, D.S.P.; Arcuri, A.S.; Bechara, E.J.H. Oxygen toxicity related to exposure to lead. *Clin. Chem.* **1985**, *31*, 1673-1676.

[41] Ito, Y.; Niiya, Y.; Kurita, H.; Shima, S.; Sarai, S. Serum lipid peroxide level and blood superoxide dismutase activity in workers with occupational exposure to lead. *Int. Arch. Occup. Environ. Health* **1985**, *56*, 119-127.

[42] Sugawara, E.; Nakamura, K.; Miyake, T.; Fukumura, A.; Seki, Y. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. *Brit. J. Indust. Med.* **1991**, *48*, 239-242.

[43] Chiba, M.; Shinohara, A.; Matsushita, K.; Watanabe, H.; Inaba, Y. Indices of lead-exposure in blood and urine of lead-exposed workers and concentrations of major and trace elements and activities of SOD, GSH-Px and catalase in their blood. *Tohoku J. Exper. Med.* **1996**, *178*, 49-62.

[44] Solliway, B.M.; Schaffer, A.; Pratt, H.; Yannai, S. Effects of exposure to lead on selected biochemical and hematological variables. *Pharmacol. Tox.* **1996**, *78*, 18-22.

[45] Valle, B.L.; Ulmer, D.D. Biochemical effects of mercury, cadmium and lead. *Ann. Rev. Biochem.* **1972**, *41*, 91-128.

[46] Bernard, A.; Lauweys, R. Metal-induced alterations of δ-aminolevulinic acid dehydratase. *Ann. New York Acad. Sci.* **1987**, *514*, 41-47.

[47] Korsrud, G.O.; Meldrum, J.B. Effect of diet on the response in rats to lead acetate given orally or in the drinking water. *Biol. Trace Elem. Res.* **1988**, *17*, 167-173.

[48] Fahey, R.C.; Sundquist, A.R. Evolution of glutathione metabolism. *Adv. Enzymol. Rel. Areas Molec. Biol.* **1991**, *64*, 1-53.

[49] Sandhir, R.; Julka, D.; Gill, K.D. Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. *Pharmacol. Tox.* **1994**, *74*, 66-71.

[50] Schrauzer, G.N. Effects of selenium antagonists on cancer susceptibility: new aspects of chronic heavy metal toxicity. *Sangyo Ika Daigaku Zasshi* **1987**, *9* Suppl, 208-215.

[51] Otthman, A. I.; El Missiry, M.A. Role of selenium against lead toxicity in male rats. *J. Biochem. Mol. Tox.* **1998**, *12*, 345-349.

[52] Howard, J.K. Human erythrocyte glutathione reductase and glucose 6-phosphate dehydrogenase activities in normal subjects and in persons exposed to lead. *Clin. Sci. Molec. Med.* **1974**, *47*, 515-520.

[53] Whanger, P.D. Selenium in the treatment of heavy metals poisoning and chemical carcinogenesis. *J. Trace Elem. Electrolytes. Health Dis.* **1992**, *6*, 209-221.

[54] Mylroie, A.A.; Umbles, C.;Kyle, J. Effects of dietary copper supplementation on erythrocyte superoxide dismutase activity, ceruloplasmin and related parameters in rats ingesting lead acetate. In: *Trace Substances in Environmental Health.* Hemphill Ed.; Vol 18 Univ. of Missouri Press: Columbia, **1984**, pp 497-504.

[55] Halliwell, B.; Gutteridge, J.M.C. eds. *Free Radicals in Biology and Medicine.* 2nd edition. Oxford: Clarendon Press; **1989**.

[56] Adler, A.J.; Barth, R.H.; Berlyne, G.M. Effect of lead on oxygen free radical metabolism: Inhibition of superoxide dismutase activity. *Trace Elem. Med.* **1993**, *10*, 93-96.

[57] Ariza, M.E.; Bijur, G.N.; Williams, M.V. Lead and mercury metagenesis: Role of H_2O_2, superoxide dismutase, and xanthine oxidase. *Environ. Mol. Mutagen* **1998**, *31*, 352-361.

[58] Treatment guidelines for lead exposure in children. American Academy of Pediatrics Committee on Drugs. *Pediatrics* **1995**, *96*, 155-160.

[59] Porru, S.; Alessio, L. The use of chelating agents in occupational lead poisoning. *Occup. Med.* **1996**, *46*, 41-48.

[60] Stohs, S.J.; Bagchi, D.; Hassoun, E.; Bagchi, M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Tox. Oncol.* **2000**, *19*, 201-213.

[61] Jones, M.M.; Cherian, G.M. The search for chelate antagonists for chronic cadmium intoxication. *Tox.* **1990**, *62*, 1-25.

[62] IARC. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. *IARC Monogr. Eval. Carcinogen. Risk Hum.* **1993**, *58*, 119-237.

[63] Simpkins, C.O. Metallothionein in human disease. *Cell. Mol. Biol.* **2000**, *46*, 465-488.

[64] Klaassen, C.D.; Liu J. Role of metallothionein in cadmium-induced hepatotoxicity and nephrotoxicity. *Drug Met. Rev.* **1997**, *29*, 79-102.

[65] Gong, Q.; Hart, B.A. Effect of thiols on cadmium-induced expression of metallothionein and other oxidant stress genes in rat lung epithelial cells. *Tox.* **1997**, *119*, 179-191.

[66] Rana, S.V.; Boora, P.R. Antiperoxidative mechanisms offered by selenium against liver injury caused by cadmium and mercury in rat. *Bull. Environ. Contam. Tox.* **1992**, *48*, 120-124.

[67] Kamiyama, T.; Miyakawa, H.; Li, J.P.; Akiba, t.; Liu, J.H.; Liu, J; Marumo, F.; Sato, C. Effects of one-year cadmium exposure on livers and kidneys and their regulation to glutathione levels. *Res. Commun. Mol. Path. Pharmacol.* **1995**, *88*, 177-186.

[68] Rana, S.V.; Verma, S. Protective effects of GSH, vitamin E, and selenium on lipid peroxidation in cadmium-fed rats. *Biol. Trace Elem. Res.* **1996**, *51*, 161-168.

[69] Shaikh, Z.A.; Vu, T.T.; Zaman, K. Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. *Tox. Appl. Pharmacol.* **1999**, 154, 256-263.

[70] Karmakar, R.; Banik, S; Bandyopadhyay, S; Chatterjee, M. Cadmium-induced alterations of hepatic lipid peroxidation, glutathione S-transferase activity and reduced glutathione level and their possible correlation with chromosomal aberration in mice: a time course study. *Mut. Res.* **1998**, *397*, 183-190.

[71] Shibasaki, T.; Matsumoto, H.; Gomi, H.; Ohno, I.; Ishimoto, F.; Sakai, O. Effects of a Hepatoprotective agent and a hepato-secreting chelator on cadmium-induced nephrotoxicity in Syrian hamsters. *Biol. Trace Elem. Res.* **1996**, *52*, 1-9.

[72] Yiin, S.J.; Sheu, J.Y.; Lin, T.H. Lipid peroxidation in rat adrenal glands after administration cadmium and role of essential metals. *J. Tox. Environ. Health. Part A* **2001**, *62*, 47-56.

[73] Yiin, S.J.; Sheu, J.Y. Effects of metallic antioxidants on cadmium-catalyzed peroxidation of arachidonic acid. *Ann. Clin. Lab. Sci.* **1998**, *28*, 43-50.

[74] Hussain, T.; Shukla, G.S.; Chandra, S.F. Effects of cadmium on superoxide dismutase and lipid peoxidation in liver and kidney of growing rats: In vivo and in vitro studies. *Pharmacol. Tox.* **1987**, *60*, 355-358.

[75] Mateo, M.C.M.; Martin, B.; Beneit, M.S.; Rabadan, J. Catalase activity in erythrocytes from colon and gastric cancer patients. Influence of nickel, lead, mercury, and cadmium. *Biol. Trace Elem. Res.* **1997**, *57*, 79-90.

[76] Lai, M.S.; Hsueh, Y.M.; Chen, C.J.; Shyu, M.P.; Chen, S.Y.; Kuo, T.L.; Wu, M.M.; Tai, T.Y. Injested inoganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol.* **1994**,*139*, 484-492.

[77] Chen, C.J.; Hsueh, Y.M.; Lai, M.S.; Shyu, M.P.; Chen, S.Y.; Wu, M.M.; Kuo, T.L.; Tai, T.Y. Increased prevalence of hypertension and long term arsenic exposure. *Hypertension* **1995**, *25*, 53-60.

[78] Chen, J.C.; Wang, C.J. Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. *Cancer Res.* **1990**, *50*, 5470-5474.

[79] National Academy of Sciences Arsenic in drinking water. Washington D.C.: National Academy of Sciences Press; **1999**.

[80] Kitchin, K.T. Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites. *Tox. Appl. Pharmacol.* **2001**, *172*, 249-261.

[81] Bernstam, L.; Nriagu, J. Molecular aspects of arsenic stress. *J. Tox. Environ. Health B* **2000**, *3*, 293-322.

[82] Yamanaka, K.; Hasegawa, A.; Sawamura, R.; Okada, S. Dimethyl arsenics induce DNA strand breaks in lung via the production of active oxygen in mice. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 43-50.

[83] Yamanaka, K.; Hoshino, M.; Okanato, M.; Sawamura, R.; Hasegawa, A.; Okada, S. Induction of DNA damage by dimethylarsine, a metabolite of inorganic arsenics, is for the major part likely due to its peroxyl radical. *Biochem. Biophys. Res. Commun.* **1990**, *168*, 58-64.

[84] Liu, J.; Kadiiska, M.; Liu, Y.; Qu, W.; Mason, R.P.; Waalkes, M.P. Acute arsenic-induced free radical production and oxidative stress-related gene expression in mice. *Toxicologist* **2000**, *54*, 280-281.

[85] Barchowsky, A,; Klei, L.R.; Dudek, E.J.; Swartz, H.M.; James, P.E. Stimulation of reactive oxygen, but not reactive nitrogen species, in vascular endothelial cells exposed to low levels of arsenite. *Free Radic. Biol. Med.* **1999**, *27*, 1405-1412.

[86] Guzzo, A.; Karatzios, C.; Diorio, C.; DuBow, M.S. Metallothionein-II and ferritin H mRNA levels are increased in arsenite-exposed HeLa cells. *Biochem. Biophys. Res. Commun.* **1994**, *205*, 590-595.

[87] Nordenson, I.; Beckman, L. Is genotoxic effect of arsenic mediated by oxygen free radicals? *Hum. Hered.* **1991**, *41*, 71-73.

[88] Wang, T.S.; Huang, H. Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. *Mutagenesis* **1994**, *9*, 253-257.

[89] Liu, F.; Jan, K.Y. DNA damage in arsenite- and cadmium-treated bovine aortic endothelial cells. *Free Radic. Biol. Med.* **2000**, *28*, 55-63.

[90] Lynn, S.; Lai, H.T.; Gurr, J.R.; Jan, K.Y. Arsenite retards DNA break rejoining by inhibiting DNA ligation. *Mutagenesis* **1997**, *12*, 353-358.

[91] Mordan, L.J.; Burnett, T.S.; Zhang, L.X.; Tom, J.; Cooney, R.V. Inhibitors of endogenous nitrogen oxide formation block the promotion of neoplastic transformation in C3H 10T1/2 fibroblasts. *Carcinogenesis* **1993**, *14*, 1555-1559.

[92] Christodoulides, N.; Durante, W.; Kroll, M.H.; Schafer, A.I. Vascular smooth muscle cell heme oxygenases generate guanylyl cyclase-stimulatory carbon monoxide. *Circulation* **1995**, *91*, 2306-2309.

[93] De Vera, M.E.; Kim, Y.M.; Wong, H.R.; Wang, Q.; Billiar, T.R.; Geller, D.A. Heat shock response inhibits cytokine-inducible nitric oxide synthase expression in rat hepatocytes. *Hepatology* **1996**, *24*, 1238-1245.

[94] Yamanaka, K.; Mizoi, M.; Kato, K.; Hasegawa, A.; Nakano, M.; Okada, S. Oral administration of dimethylarsinic acid, a main metabolite of inorganic arsenic, in mice promotes skin tumorigenesis initiated by dimethylbenz(a)anthracene with or without ultraviolet B as a promoter in mice. *Biol. Pharm. Bull.* **2001**, *24*, 510-514.

[95] Wanibuchi, H.; Hori, T.; Meenakshi, V.; Ichihara, T.; Yamamoto, S.; Yano, Y.; Otani, S.; Nakae, D.; Konishi, Y.; Fukushima, S. Promotion of rat hepatocarcinogenesis by dimethylarsinic acid as assessed in rat in vivo models. A review. *Mutat. Res.* **1997**, *386*, 353-361.

[96] Lee, T.C.; Ho, I.C. Modulation of cellular antioxidant defense activities by sodium arsenite in human fibroblasts. *Arch. Tox.* **1995**, *69*, 498-504.

[97] Maiti, S.; Chatterjee, A.K. Differential response of cellular antioxidant mechanism of liver and kidney to arsenic exposure and its relation to dietary protein deficiency. Environ. *Tox. Pharmacol.* **2000**, *8*, 227-235.

[98] Crinnion, W.J. Environmental Medicine, part three: Long-term effects of chronic low dose mercury exposure. *Altern. Rev. Med.* **2000**, *5*, 209-223.

[99] Zalups, R.K.; Lash, L.H. Interactions between glutathione and mercury in the kidney, liver and blood. In: *Toxicology of Metals*; Chang, L.W.; Eds.; CRC Press, Boca Raton, **1996**, pp 145-163.

[100] Sarafian, T.A. Methylmercury-induced generation of free radicals: biological implications. *Met. Ions Biol. Syst.* **1999**, *36*, 415-444.

[101] Boveris, A.; Oshino, N.; Chance, B. The cellular production of hydrogen peroxide. *Biochem. J.* **1972**, *128*, 617-630.

[102] Schulze-Osthoff, K.; Bakker, A.C.; Vanhaesebroeck, R.; Beyaert, R.; Jacob, W.A.; Fiers, W. J. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J. Biol. Chem.* **1992**, *267*, 5317-5322.

[103] Lund, B.O.; Miler, D.M.; Woods, J.S. Mercury-induced H_2O_2 formation and lipid peroxidation in vitro in rat kidney mitochondria. *Biochem. Pharmacol.* **1991**, *42*, S181-S187.

[104] Chavez, E.; Holguin, J.A. Mitochondrial calcium release as induced by Hg^{2+}. *J. Biol Chem.* **1988**, *263*, 3582-3587.

[105] Nath, K.A.; Croatt, A.J.; Likely, S.; Behrens, T.W.; Warden, D. Renal oxidant injury and oxidant response induced by mercury. *Kidney Int.* **1996**, *50*, 1032-1043.

[106] Verity, M.A.; Brown, W.J.; Cheung, M. J. Organic mercurical encephalopathy: in vivo and in vitro effects of methylmercury on synaptosomal respiration. *Neurochem.* **1975**, *25*, 759-766.

[107] Bazan, N.G. Arachidonic acid in the modulation of excitable membrane function and at the onset of brain damage. *Ann. NY Acad Sci.* **1989**, *559*, 1-16.

[108] Siesjo, B.K.; Bengtsson, F.; Grampp, W.; Theander, S. Calcium, excitotoxins, and neuronal death in the brain. *Ann. NY Acad. Sci.* **1989**, *568*, 234-251.

[109] Keyser, D.O.; Alger, B.E. Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals. *Neuron* **1990**, *5*, 545-553.

[110] Tan, X.; Tang, C.; Castoldi, A.F.; Manzo, L.; Costa, L.G. Effects of inorganic and organic mercury on intracellular calcium levels in rat T lymphoctes. *J. Tox. Environ. Health* **1993**, *38*, 159-170.

[111] Huang, Y.L.; Cheng, S.L.; Lin, T.H. Lipid peroxidation in rats administrated with mercuric chloride. *Biol. Trace Elem. Res.* **1996**, *52*, 193-206.

[112] Fukino, H.; Hirai, M.; Hsueh, Y.M.; Moriyasu, S.; Yamani, Y. Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. *Tox. Appl. Pharmacol.* **1984**, *73*, 395-401.

[113] Lund, B.O.; Miler, D.M.; Woods, J.S. Studies on Hg(II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochem. Pharmacol.* **1993**, *45*, 2017-2024.

[114] Ali, M.B.; Vajpayee, P.; Tripathi, R.D.; Rai, U.N.; Kumar, A.; Singh, N.; Behl, H.M.; Singh, S.P. Mercury bioaccumulation induced oxidative stress and toxicity to submerged macrophyte Potamogeton crispus L. *Bull. Environ. Tox.* **2000**, *65*, 573-582.

[115] Andersen, H.R.; Andersen, O. Effects of dietary α-tocopherol and β-carotene on lipid peroxidation induced by methyl mercuric chloride in mice. *Pharmacol. Tox.* **1993**, *73*, 192-201.

[116] Hussain, S.; Atkinson, A.; Thompson, S.J.; Khan, A.T. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. *J. Environ. Sci. Health, B* **1999**, *34*, 645-660.

[117] Woods, J.S.; Ellis, M.E. Up-regulation of glutathione synthesis in rat kidney by methylmercury. Realtionship to mercury-induced oxidative stress. *Biochem. Pharmacol.* **1995**, *50*, 1719-1724.

[118] Sarafian, T.A.; Bredesen, D.E.; Verity, M.A. Cellular resistance to methylmercury. *Neurotox.* **1996**, *17*, 27-36.