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Review Article

OXIDATIVE MECHANISMS IN THE TOXICITY OF METAL IONS

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Abstract—The role of reactive oxygen species, with the subsequent oxidative deterioration of biological macromolecules in the toxicities associated with transition metal ions, is reviewed. Recent studies have shown that metals, including iron, copper, chromium, and vanadium undergo redox cycling, while cadmium, mercury, and nickel, as well as lead, deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species as superoxide ion, hydrogen peroxide, and hydroxyl radical. As a consequence, enhanced lipid peroxidation, DNA damage, and altered calcium and sulfhydryl homeostasis occur. Fenton-like reactions may be commonly associated with most membranous fractions including mitochondria, microsomes, and peroxisomes. Phagocytic cells may be another important source of reactive oxygen species in response to metal ions. Furthermore, various studies have suggested that the ability to generate reactive oxygen species by redox cycling quinones and related compounds may require metal ions. Recent studies have suggested that metal ions may enhance the production of tumor necrosis factor alpha (TNFα) and activate protein kinase C, as well as induce the production of stress proteins. Thus, some mechanisms associated with the toxicities of metal ions are very similar to the effects produced by many organic xenobiotics. Specific differences in the toxicities of metal ions may be related to differences in solubilities, absorbability, transport, chemical reactivity, and the complexes that are formed within the body. This review summarizes current studies that have been conducted with transition metal ions as well as lead, regarding the production of reactive oxygen species and oxidative tissue damage.

Keywords—Iron, Copper, Cadmium, Chromium, Mercury, Nickel, Vanadium, Lead, Zinc, Free radicals, Oxidative stress, Redox cycling, Glutathione depletion, Lipid peroxidation, DNA damage, Stress proteins

INTRODUCTION

A growing body of evidence indicates that transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due at least in part to oxidative tissue damage. Recent studies have shown that metals such as iron, copper, cadmium, chromium, lead, mercury, nickel, and vanadium exhibit the ability to produce reactive oxygen species, resulting in lipid peroxidation, DNA damage, depletion of sulfhydryls, and altered calcium homeostasis.

The toxicities produced by the transition metals generally involve neurotoxicity, hepatotoxicity, and nephrotoxicity. Specific differences in the toxicities of metal ions may be related to differences in solubilities, absorbability, transport, chemical reactivity, and the complexes that are formed within the body. In

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spite of these factors, the basic mechanisms involving production of reactive oxygen species are the same for these transition metal ions. Furthermore, the basic mechanism of reactive oxygen species production and ultimate toxicity produced by metal ions may involve mechanisms that are common to redox cycling organic xenobiotics, as, for example, the quinones. Furthermore, various studies have suggested that the ability to generate reactive oxygen species by redox cycling quinones and related compounds may require metal ions such as iron or copper. Thus, common molecular mechanisms may be involved in the production of reactive oxygen species and the toxicities of numerous xenobiotics. In the current review, recent studies involving the production of reactive oxygen species and oxidative tissue damage by metal ions will be summarized.

METAL IONS AND REACTIVE OXYGEN SPECIES

Iron

The two most commonly studied transition metals are the cations iron and copper. A variety of studies have demonstrated the ability of iron chelates or complexes to catalyze the formation of reactive oxygen species and stimulate lipid peroxidation. Aust[1] has reviewed the relationship between metal ions, oxygen radicals, and tissue damage. The role of iron in the initiation of lipid peroxidation has also been reviewed by Minotti and Aust[2] and Alleman et al.[3] These investigators have presented evidence that lipid peroxidation requires both Fe(III) and Fe(II), probably as a dioxygen–iron complex. Iron is capable of catalyzing redox reactions between oxygen and biological macromolecules that would not occur if catalytically active iron were not present. Iron complexed with adenosine 5′-diphosphate (ADP), histidine, ethylenediaminetetraacetic acid (EDTA), citrate, and other chelators has been shown to facilitate the formation of reactive oxygen species and enhance production of lipid peroxidation.[1,4]

Evidence indicates that chelated iron acts as a catalyst for the Fenton reaction, facilitating the conversion of superoxide anion and hydrogen peroxide to hydroxyl radical, a species frequently proposed to initiate lipid peroxidation.[5,6]

Fenton Reaction

$$Fe(III) + O_2^{\cdot -} \rightarrow FE(II) + O_2$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + OH^-.$$

However, criticisms have been levied against the involvement of Fenton reactions in vivo, and these have been reviewed in depth by Halliwell and Gutteridge.[7] The major criticisms are that the rate constant for the Fenton reaction is too low, the reactive oxygen species produced is not the hydroxyl radical, and there are no metal catalysts available in vivo. In response to these criticisms, calculations based on rate constants suggest that hydroxyl radicals may be generated at the rate of approximately 50 per cell per s, which could have enormous biological consequences. Several studies have suggested that ferryl, an iron-oxygen complex, is the reactive species formed, although the vast majority of evidence supports hydroxyl ion as the reactive oxygen species produced by the Fenton reaction.[7] In complex biological systems it is difficult to discriminate between hydroxyl radicals and ferryl species as the initiators of peroxidative reactions. Ample evidence exists that metal ions are required for hydroxyl radical formation, and these ions may be bound at specific sites that are not readily accessible to some scavengers and chelators.[7]

In hereditary hemochromatosis and various forms of secondary hemochromatosis, a pathologic expansion of body iron stores occurs primarily due to an increase in the absorption of dietary iron. The major pathologic manifestations associated with chronic iron overload include fibrosis and ultimately cirrhosis. The mechanisms associated with liver injury in chronic iron overload are believed to include increased lysosomal membrane fragility mediated by iron-induced lipid peroxidation, and peroxidative damage of organelles as microsomes and mitochondria.[8]

Upon ingestion of iron, iron is either oxidized and stored in the iron storage protein ferritin or associates with the iron transport protein transferrin in the blood stream. For iron to facilitate the formation of reactive oxygen species via the Fenton reaction, the iron must be in a free or catalytically active form. Most iron is complexed, and little free iron actually exists in nature. A variety of xenobiotics has been shown to facilitate the release of iron from ferritin, including paraquat, diquat, nitrofurantoin, adriamycin, daunomycin, and diaziquone.[4] Thus, a variety of xenobiotics may enhance the formation of reactive oxygen species not only by undergoing redox cycling, but may also facilitate the release of iron, which catalyzes the formation of reactive oxygen species.

Studies using iron nitrilotriacetate have shown that this complex exhibits a proxidant activity, resulting in enhanced lipid peroxidation of hepatocyte cultures with the leakage of lactate dehydrogenase and transaminase enzymes, as well as a substantial increase in Trypan blue staining, which indicates membrane damage.[9,10] However, in these studies, the protein and non-

protein thiol content of the cells was not affected, although a dramatic increase in the conjugated diene content of mitochondria of iron nitrilotriacetate-treated hepatocytes was observed.[10] Lipid peroxidation was shown to occur in both inner membranes and plasma membrane. Free radical scavengers including superoxide dismutase, vitamin E, and mannitol reduced lipid peroxidation intracellularly in response to iron nitrilotriacetate, while catalase and thiourea seemed to protect plasma membranes as evidenced by a decrease in enzyme leakage.[9] The mechanism whereby superoxide dismutase decreased intracellular lipid peroxidation is unclear because this enzyme cannot penetrate the cell membrane.

Studies on the interaction of paraquat with microsomes and ferric complexes have demonstrated an increase in oxygen radical generation.[11] Ferric ion complexed with citrate, ATP, EDTA, and diethylenetriamine pentaacetic acid (DETAPAC) increased the catalytic effectiveness of paraquat in promoting microsomal generation of oxygen radicals. Thus, the interaction of iron complexes with paraquat may contribute to the oxidative stress and toxicity produced by paraquat in biological systems. Burkitt et al.[12] have developed a radical-trapping technique that has allowed them to demonstrate that the administration of both iron and paraquat results in the generation of greater levels of hydroxyl radical than in the absence of either paraquat or iron. Redox cycling of adriamycin has been shown to result in a parallel reductive release of membrane-bound nonheme iron.[13] Lipid peroxidation was shown to occur in the presence of low levels of adriamycin, which favored only partial Fe(II) autooxidation and a Fe(II)/Fe(III) of approximately 1:1, providing further evidence that both forms of iron are required for the initiation of lipid peroxidation.

Miller et al.[14] have examined the roles of hydrogen peroxide and superoxide anion produced by the enzyme xanthine oxidase on iron-catalyzed lipid peroxidation. These studies concluded that hydrogen peroxide and superoxide anion produced by xanthine oxidase support iron-catalyzed lipid peroxidation through their participation in redox reactions of iron by facilitating Fe(II) oxidation or Fe(III) reduction. In another model of iron-initiated lipid peroxidation, studies by Linseman et al.[15] demonstrated that endogenous vitamin E in cellular membranes was depleted before membrane lipids were peroxidized and membrane-bound protein thiols were oxidized. In the in vitro system that was used involving whole rat brain homogenate, depletion of the endogenous antioxidant vitamin E preceded evidence of oxidative tissue injury, providing evidence for the antioxidant role of vitamin E in membranes.

Iron has also been shown to play a role in the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD). Microsomal lipid peroxidation induced by TCDD requires iron.[16] TCDD markedly alters the distribution of iron as well as copper, zinc, and magnesium.[17] Endrin also alters iron distribution in hepatic mitochondria and microsomes of rats in a manner similar to the results observed for TCDD.[18] Wahba et al.[19] have shown that TCDD administration to rats results in an increase in the amount of catalytically active iron associated with microsomes. Endrin may produce a similar effect with respect to the amount of catalytically active or available iron. The ability of iron to act as a synergist for hepatocellular carcinoma induced by polychlorinated biphenyls (PCBs) in Ah-responsive mice has been demonstrated by Smith et al.[20] The precise role of iron in potentiating carcinomas in mice is not clear. However, because iron facilitates the formation of reactive oxygen species, and reactive oxygen species are believed to play a central role in tumor formation,[21] the iron may act to facilitate carcinogenesis in this manner.

Evidence indicates that patients with iron overload are more susceptible to hepatic damage from alcohol than individuals with normal iron levels. Therefore, Stal and Hultcrantz[22] have examined the role of iron in ethanol-induced hepatocellular damage in rats. Both biochemical and morphological evidence of increased hepatocellular damage following the combination of iron and ethanol was obtained. The mechanism involved in iron-stimulated ethanol toxicity is not known. However, the oxidation of ethanol by alcohol dehydrogenase results in the enhanced production of the reducing agent nicotinamide adenine dinucleotide (NADH), which in turn is a cofactor in the iron-dependent generation of hydroxyl radicals. The NADH might also facilitate mobilization of iron from ferritin. Furthermore, ethanol is activated to a hydroxyethyl radical, and the formation of this radical may be iron dependent. Shaw and Jayatilleke[23] observed that lipid peroxidation may be initiated by an iron-dependent acetaldehyde–xanthine oxidase system during the metabolism of ethanol. Thus, a complex series of interactions involving iron may exist.

Desferrioxamine (DFX) is an excellent chelating agent for Fe(III), and has been used extensively in vitro to assess the role of iron in the toxicity of various xenobiotics. DFX can also bind other transaction metals, but with much lower stability constants. Wahba et al.[19] have shown that DFX can modulate the toxicity of TCDD. DFX blocks the depletion of hepatic glutathione and the formation of MDA by *tert*-butyl hydroperoxide in isolated perfused rat livers.[24] Furthermore, DFX inhibits *tert*-butyl hydroperoxide-induced glutathione depletion, lipid peroxidation, and hepatotoxicity

in rats. These results emphasize the importance of iron in *tert*-butyl hydroperoxide-induced lipid peroxidation and hepatotoxicity, as well as the important role of iron in propagation of lipid peroxidation reactions.[25]

Due to the short half-life of DFX, it cannot readily be used to prevent oxidative stress associated with chemical-induced toxicities. However, the use of hydroxyethyl starch conjugated DFX has been shown to modulate the neurologic injury that occurs during brain ischemia and reperfusion in rats.[26] This complex may also be useful in modulating the neurotoxicity and hepatotoxicity associated with a wide range of toxicants. In addition to its ability to act as an iron chelator, DFX is also a peroxyl radical scavenger independent of its iron chelating ability,[27] and has been shown to act as a lipid chain-breaking antioxidant.[28] In this process, DFX is enzymatically oxidized to a nitroxide free radical.[29] These investigators have also noted that as long as the concentrations of DFX are kept below 10^{-3} M, reactions of reactive oxygen species with DFX are minimal, and, as such, DFX can be used as the chelator of choice to inhibit the iron catalyzed Fenton reaction.[30]

Under conditions of oxidative stress in myocytes, DFX has been reported to reduce the ferryl form of myoglobin with the formation of the DFX nitroxide radical and the ferryl myoglobin radical.[31] These studies further indicated that the ferryl myoglobin radical was capable of inducing membrane lipid peroxidation.

The induction of DNA single strand breaks in macrophage by Fe(III) under cool-white fluorescent light in the presence and absence of low molecular weight chelators was investigated by Chao and Aust.[32] The results indicate that the photochemical reduction of Fe(III) to Fe(II) by chelators, including citrate, nitrilotriacetate, or EDTA results in the formation of DNA single strand breaks without the addition of hydrogen peroxide. The results further indicate that Fe(II) and hydroxyl radical or similarly reactive species are involved in the induction of the DNA damage.

Toyokuni and Sagripanti[33] have examined the in vitro production of both single and double strand breaks in DNA mediated by Fe(III) or the iron chelated with nitrilotriacetate. Supercoiled plasmid pZ189 DNA was used. The iron nitrilotriacetate complex effectively oxidized the DNA, producing both single and double strand breaks. Neither the iron nor the nitrilotriacetate alone broke DNA. These authors discussed the role of localized DNA damage in relation to the renal tubular carcinogenesis produced by nitrilotriacetate. Studies by Okada et al.[34] have shown that the ferric nitrilotriacetate that is filtered by the renal tubules is rapidly reduced, and the resulting Fe(II) initiates lipid peroxidation in the lumen. Peroxidation of polyunsaturated fatty acids occurs, followed by acute renal tubular necrosis. Thus, the evidence indicates that a relationship exists between the ability of this iron chelate to initiate lipid peroxidation and tissue damage as well as renal carcinogenesis.

Numerous studies have shown that the brains of patients with Alzheimer's disease contain more aluminum than age- and sex-matched controls. Various investigators have suggested that aluminum may in part induce neurotoxicity by influencing iron homeostasis and iron-mediated free radical-induced neural cell damage. Aluminum has a fixed oxidation number, and therefore cannot participate in redox reactions. However, aluminum may cause a rearrangement of membrane lipids, thus facilitating iron initiated lipid peroxidation. Aluminum has been shown to accelerate iron-stimulated peroxidation of membrane lipids.[35] Furthermore, aluminum may bind to ferritin in the brain and produce increased levels of catalytically active iron.[36] Thus, aluminum may enhance iron-dependent free radical-induced tissue damage via an indirect mechanism or mechanisms.

Copper

Copper is widely distributed in nature and is an essential element. Acute poisoning occurs most frequently from the ingestion of copper sulfate or other copper salts, and hepatic necrosis is characteristic of copper poisoning. The redox properties of copper and iron complexes of adriamycin, bleomycin, and thiosemicarbazones have been investigated by electron spin resonance spectroscopy (ESR).[37] A common property of these metal complexes is their ready ability to be reduced by thiol compounds and oxidized by iron or reduced species of iron to produce radicals. Copper is a common cofactor for many enzymes including oxidases and oxygenases.[38] Similar to iron, copper acts as a catalyst in the formation of reactive oxygen species and catalyzes peroxidation of membrane lipids.[39] Studies have shown that when cupric acetate is added with adriamycin in the Ames salmonella mutagenicity test, the presence of copper results in more than a 700% increase in the mutagenicity of adriamycin, supporting the contention that drug-metal ion-DNA associations might contribute to genotoxicity.[40]

$$Cu(II) + O_2^{\cdot -} \rightarrow Cu(I) + O_2$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + {}^{\cdot}OH + OH^-$$

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

The role of copper in the oxidation of hydroquinone

to benzoquinone as well as the cytotoxicity of hydroquinone has been examined.[41] Copper was shown to significantly accelerate the oxidation of hydroquinone to benzoquinone in a concentration-dependent manner. Furthermore, copper added to primary bone marrow stromal cell cultures significantly enhanced hydroquinone-induced cytotoxicity. Glutathione and dithiothreitol, through their actions as antioxidants and/or metal-chelating agents, completely prevented the enhanced cytotoxicity of hydroquinone by copper while catalase was unable to do so.

Further studies have demonstrated that copper markedly enhances the formation of DNA strand breaks in the presence of hydroquinone.[42] The presence of singlet oxygen scavengers but not hydroxyl radical scavengers provided partial protection, suggesting that singlet oxygen rather than hydroxyl radical may play a role in the induction of DNA strand breaks. However, these scavengers are lacking in absolute specificity,[7] and the involvement of singlet oxygen may be small because there is currently no known mechanism by which it can be formed from oxyradicals, although Steinbeck et al.[43] have reported that phagocytosing neutrophils can generate singlet oxygen intracellularly. Other metal ions including Fe(III), Mn(II), Cd(II), and Zn(II) did not significantly enhance oxidation of hydroquinone or the induction of DNA strand breaks.[42] Thus, copper may be an important factor in the generation of reactive oxygen species, the cytotoxicity, and the formation of DNA damage in target cells by hydroquinone.

Milne et al.[44] have examined the effects of glutathione and other chelating agents on copper-mediated DNA oxidation. Glutathione was shown to inhibit free radical formation by copper ions in the presence of hydrogen peroxide, ascorbate and DNA. The protective effect of the glutathione was attributed to its ability to stabilize copper in the Cu(I) oxidation state, preventing redox cycling and the generation of free radicals. Thus, glutathione in cell nuclei serves to prevent rather than promote copper-induced DNA damage. The effect of glutathione on copper-mediated DNA oxidation may be due to the "radical sink" effect described by Winterbourn.[45] Via this system, a variety of radicals, either directly or through reduced glutathione, can transfer their unpaired electron to oxygen to give superoxide. Superoxide dismutase is essential in this system to prevent oxidative stress.

Ozawa et al.[46] have used the conversion of closed circular double-stranded supercoiled DNA to the nicked circular and linear forms to assess DNA single and double strand breaks by the reaction of Cu(II) ethylene diamine with hydrogen peroxide. Using this simple system, a dose–response curve for DNA scission was observed, providing evidence that Cu(II) causes DNA damage in the presence of hydrogen peroxide. Previous studies by these investigators have shown that this Cu(II) complex reacts with hydrogen peroxide to yield high concentrations of hydroxyl radicals, which may be directly responsible for the DNA damage.[46]

Cadmium

Cadmium is an abundant, nonessential element that is generating concern due to its accumulation in the environment as a result of industrial practices. It is widely used in electroplating and galvanizing, as a color pigment in paints, and in batteries. It is a byproduct of zinc and lead mining, and smelting. Soluble cadmium salts accumulate and result in toxicity to liver, kidneys, brain, lungs, heart, testes, and the central nervous system. The mechanisms responsible for the toxicity of cadmium are not well understood. Cadmium does not appear to generate free radicals,[47] but does elevate lipid peroxidation in tissues soon after exposure.[48] Studies by Fariss[49] have shown that free radical scavengers and antioxidants are useful in protecting against cadmium toxicity.

Manca et al.[50] examined the susceptibility of liver, kidneys, brains, lungs, heart, and testes of rats given intraperitoneal doses of cadmium chloride. The animals received from 25–1250 μg cadmium/kg as cadmium chloride, and the animals were sacrificed 24 h posttreatment. Greatest increases in lipid peroxidation were demonstrated in lungs and brain as well as liver, based on the formation of thiobarbituric acid reactive substances (TBARS). These studies indicate that lipid peroxidation is an early and sensitive consequence of cadmium exposure.

Treatment of rats with a single carcinogenic dose of cadmium chloride (30 mμmol/kg) has been shown to cause severe hemorrhagic damage in testis within 12 h after administration of the metal. Furthermore, lipid peroxidation levels, iron content, and cellular production of hydrogen peroxide were markedly elevated in testicular Leydig cells, the target population for cadmium carcinogenesis.[51] In addition, glutathione peroxidase activity increased while glutathione reductase and catalase activities decreased. The increased iron levels may be due to iron displacement from binding sites with redistribution, resulting in enhanced iron-mediated lipid peroxidation. The results support the contention that oxygen species such as hydrogen peroxide may play an important role in the initiation of carcinogenesis within the target cell population.

The influence of ascorbic acid on lipid peroxidation in guinea pigs treated with cadmium (1 mg cadmium/

animal/day in drinking water) has been examined by Hudecova and Ginter.[52] Cadmium administration in conjunction with a low intake of ascorbic acid (2 mg/animal/day) increased lipid peroxidation (lipid peroxides) in kidney, liver, and serum. A high intake of ascorbic acid (100 mg/animal/day) decreased formation of malondialdehyde (MDA) in these same tissues. The antioxidant action of ascorbic acid is complex. The ascorbic acid may complex cadmium or redox metal ions displaced by cadmium and thus prevent lipid peroxidation. Furthermore, ascorbic acid may also involve regeneration and/or reduction of other antioxidants such as vitamin E or uric acid.

Hussain et al.[53] examined the in vitro and in vivo effects of cadmium on superoxide dismutase (SOD) and lipid peroxidation in liver and kidney of rats. Cadmium acetate administered in vivo inhibited the activity of SOD and increased the levels of lipid peroxides and lipid peroxidation in liver and kidneys. Addition of cadmium in vitro also inhibited SOD in both tissues. Furthermore, lipid peroxidation was markedly increased after addition of cadmium to fresh homogenates of both tissues. Thus, the results demonstrate, both in vitro and in vivo, the ability of cadmium to induce lipid peroxidation. However, whether the increase in lipid peroxidation occurs as a direct effect of cadmium, via cadmium-induced displacement of redox metal ions or a decrease in glutathione content, is not clear.

The detection of lipid peroxidation products in the urine provides a convenient, noninvasive method of assessing lipid metabolism and oxidative stress in response to various xenobiotics. Four major lipid metabolites have been identified in the urine of rats by high performance liquid chromatography (HPLC), and include acetaldehyde (ACT), formaldehyde (FA), MDA, and acetone (ACON).[54] Recent studies by Bagchi and Stohs (unpublished) have shown that the administration of a single oral dose of cadmium chloride (44 mg/kg) to Sprague–Dawley rats results in a sharp increase in the urinary excretion of these four lipid metabolites. Initial increases do not occur until at least 24 h postadministration of the cadmium chloride, and are maximal at approximately 48–60 h. At 60 h after cadmium administration, the urinary excretion of MDA, FA, ACT, and ACON were 2.6-, 2.6-, 3.4-, and 2.8-fold, respectively, higher than control values. Thus, the results clearly demonstrate enhanced lipid metabolism and an oxidative stress.

By manipulating the cadmium concentrations in the media of V79 Chinese hamster fibroblasts in culture, some of the cells developed cadmium resistance and cross-resistance to oxidative stress.[55] Most of the cross-resistance to oxidative stress in cadmium-challenged cells can be accounted for by a parallel increase in glutathione content, which can act both as an antioxidant and as a metal-chelating agent. Metallothionein content did not seem to exert a major effect against oxidative stress in cadmium challenged cells.

Chin and Templeton[56] have demonstrated that in cultured rat glomerular mesangial cells, cadmium produced dose- and time-dependent increases in intracellular glutathione concentrations. The results suggest that the cadmium-induced elevation of glutathione in these cells may serve as a protective mechanism, particularly at cadmium concentrations that arise during environmental or occupational exposure. However, at high cadmium concentrations, glutathione depletion occurs, presumably due to the production of reactive oxygen species at a rate that exceeds the ability to regenerate reduced glutathione.

Li et al.[57] have also examined the effects of cadmium on cellular glutathione levels. In addition, these authors have studied the effects of cadmium on cytoskeletal protein sulfhydryls, and the organization of the cytoskeletal elements, microtubules and microfilaments, in cultured 3T3 cells. Exposure of these cells to cadmium resulted in a dose-dependent increase in both cellular glutathione levels and cytoskeletal protein sulfhydryls. Following cadmium exposure, a biphasic response was observed with an initial decrease in the two sulfhydryl parameters. However, restoration of cytoskeletal protein sulfhydryls occurred after cellular recovery of glutathione, suggesting that cellular glutathione plays an important role in regulating cytoskeletal protein sulfhydryls.

Many chemicals and physical stressors enhance the synthesis of a group of proteins referred to as stress or heat shock proteins. These proteins may serve as biomarkers of exposure and cellular injury, and are believed to be a defense mechanism against the stressor or insult that initiated the synthesis. Goering et al.[58] have examined the synthesis of stress proteins in rats in response to cadmium. Following the administration of 2 mg Cd/kg to rats, enhanced de novo synthesis of hepatic 70-, 90-, and 110-kilodalton (kDa) proteins were detected 2 h after exposure, with maximum increases occurring at 2–4 h. Increase in the synthesis of these proteins occurred prior to overt hepatic injury based on two biochemical assays and histopathological evidence. Furthermore, stress protein synthesis appeared to occur only in target tissues such as liver, based on a lack of evidence of renal injury or increased stress protein synthesis.

Exposure of HeLa and HL60 cells to cadmium chloride or sodium arsenite leads to a marked increase in the synthesis of a stress protein.[59] Further studies demonstrated that this protein in these human cells is

heme oxygenase, suggesting that an oxidative stress is involved in the toxicity of cadmium. More recent studies by Bauman et al.[60] have shown that the incubation of rat hepatocytes with cadmium results in the induction of a 70 kDa stress protein. The cadmium in these studies also was an effective inducer of metallothionein. Cadmium was a better inducer of both 70 and 90 kDa proteins than nickel, mercury, or zinc.

Studies by Yamada and Koizumi[61] have shown that human peripheral lymphocytes synthesize a 70 kDa protein that is distinct from metallothionein. Cadmium as well as zinc induce both of these proteins, while copper, mercury, nickel, and silver are inducers of metallothionein but not the 70 kDa stress protein. Cobalt induced the synthesis of the stress protein but not metallothionein. The reason for these differences is not clear. The induction of the stress protein may be involved in the basic cellular defense mechanisms. However, the relationship between the induction of stress proteins and the production of free radicals is more complex than a direct cause and effect relationship, because known free radical generators (copper, mercury, and nickel) did not induce stress proteins, whereas zinc, which does not induce free radical production, did facilitate stress protein formation.

Selenium prevents the carcinogenicity of cadmium through undefined mechanisms, and numerous studies on the protective effects of selenium have been summarized by Wahba et al.[62] These authors have shown that selenium prevents acute cadmium toxicity through a mechanism that involves a markedly enhanced retention of cadmium but does not involve induction of metallothionein.

Chromium

Chromium is a widely used industrial chemical, finding uses in steel, alloy cast irons, chrome, paints, metal finishes, and wood treatment. Chromium is widely known to cause allergic dermatitis as well as toxic and carcinogenic effects in humans and animals. Von Burg and Liu[63] have published a toxicological update on chromium and chromium(VI). These authors have summarized the acute toxicity, chronic toxicity, neurotoxicity, reproductive toxicity, genotoxicity, carcinogenicity, and environmental toxicity of chromium.

The role of physiological antioxidants in chromium(VI)-induced cellular injury has been reviewed by Sugiyama.[64] This author has reviewed recent in vitro and in vivo effects of oxygen scavengers, glutathione, vitamin B2, vitamin E, and vitamin C on chromate-induced injuries including DNA damage, lipid peroxidation, enzyme inhibition, cytotoxicity, and mutagenesis. Vitamin E was shown to dramatically decrease chromate-induced cytotoxicity, lipid peroxidation, and DNA damage, while vitamin B2 did not exhibit these effects.[65] Chromium occurs in the workplace primarily in the valence forms Cr(VI) and Cr(III). The chromate ion $[CrO_4]^{-2}$, the dominant form of Cr(VI) in neutral aqueous solutions, can readily cross cellular membranes via nonspecific anion carriers.[66]

While it has been postulated that Cr(V) is the ultimate carcinogenic form of chromium compounds, Kawanishi et al.[67] have demonstrated that it is not Cr(V) itself that is carcinogenic, but rather oxygen free radicals such as superoxide anion, singlet oxygen, and hydroxyl radicals. These investigators examined the mechanism of DNA cleavage induced by Cr(VI) in the presence of hydrogen peroxide. Reactive oxygen species were produced by the decomposition of $Cr(V)(O_2)_4^{-3}$ ion, resulting in DNA damage. The generation of hydroxyl radical was detected by ESR.

Shi and Dalal[68] have also used ESR to demonstrate the formation of long-lived Cr(V) intermediates in the reduction of Cr(VI) by glutathione reductase in the presence of NADPH, and the generation of hydroxyl radical. Hydrogen peroxide suppresses Cr(V) and enhances the formation of hydroxyl radicals through a Cr(V) catalyzed Fenton-like reaction. Subsequent investigations with superoxide dismutase showed no significant participation of superoxide anion in the generation of hydroxyl radicals.[69] These results indicate that the Cr(V) complexes produced in the reduction of Cr(VI) by cellular reductants react with hydrogen peroxide to generate hydroxyl radicals, which may be the initiators of the primary events in Cr(VI) cytotoxicity. Related studies by Jones et al.[70] have provided evidence that suggests that hydroxyl radicals are generated from a Cr(V) intermediate that is responsible for causing DNA strand breaks.

$$Cr(III) + O_2^{\cdot -} \rightarrow Cr(II) + O_2$$

$$Cr(II) + H_2O_2 \rightarrow Cr(III) + {}^{\cdot}OH + OH^-$$

$$Cr(VI) + O_2^{\cdot -} \rightarrow Cr(V) + O_2$$

$$Cr(V) + H_2O_2 \rightarrow Cr(VI) + {}^{\cdot}OH + OH^-$$

Until recently, Cr(III) was thought to be relatively nontoxic. However, Ozawa et al.[71] demonstrated that Cr(III) can be reduced to Cr(II) by the biological reductants L-cysteine and NADH, and in turn, the newly formed Cr(II) reacts with hydrogen peroxide to produce hydroxyl radical, which can be detected by ESR and HPLC. The resulting hydroxyl radicals are presumably responsible for tissue damaging effects.

Shi et al.[72] investigated free radical generation from hydrogen peroxide and lipid hydroperoxides

in the presence of Cr(III) using ESR spin-trapping techniques. At physiological pH, incubation of Cr(III) with hydrogen peroxide resulted in the generation of hydroxyl radical. Diethylenetriamine pentaacetic acid (DTPA) significantly reduced hydroxyl radical yield, while L-cysteine, glutathione, and NADH exhibited no significant effect. In addition, incubation of Cr(III) with cumene hydroperoxide and *tert*-butyl hydroperoxide resulted in the generation of lipid peroxide-derived free radicals. The results indicate that Cr(III) is capable of producing free radicals from both hydrogen peroxide and lipid hydroperoxides, and the authors have concluded that the generation of these radicals may have significant implications regarding the mechanism of chromium-induced carcinogenesis, although no direct evidence to support this hypothesis has been provided.

Sugden et al.[73] have employed the Salmonella reversion assay to identify mutagenic Cr(III) complexes. Relaxation of supercoiled DNA was used to show in vitro interactions with plasmid DNA. These investigations demonstrated that mutagenic Cr(III) complexes display characteristics of reversibility and positive shifts of the Cr(III)/Cr(II) redox couple, consistent with the ability of these Cr(III) complexes to serve as cyclical electron donors in a Fenton-like reaction. These same mutagenic Cr(III) complexes relaxed supercoiled DNA, presumably by the induction of single-strand breaks. The results suggest that the mechanism involved in the potentiation of mutagenesis by chromium complexes involves an oxygen radical as an active intermediate. Furthermore, Cr(III) may be one of the most biologically active oxidation states of chromium.

The effects of Cr(III) and Cr(VI) on indices of oxidative stress including lipid peroxidation, chemiluminescence, iodonitrotetrazolium reduction, and excretion of urinary lipid metabolites were assessed by Bagchi et al.[74] Increases in lipid peroxidation of 1.8- and 2.2-fold occurred in rat hepatic mitochondria and microsomes, respectively, 48 h after the oral administration of 25 mg sodium dichromate (Cr(VI))/kg (0.50 LD_{50}), while increases of 1.2- and 1.4-fold, respectively, were observed after 895 mg chromium chloride hexahydrate (Cr (III))/kg (0.50 LD_{50}). The urinary excretion of malondialdehyde, formaldehyde, acetaldehyde, and acetone were determined at 0–96 h after Cr administration. Between 48 and 72 h posttreatment, maximum excretion of the four lipid metabolites was observed. In Cr(VI)-treated rats, the excretions of all four lipid metabolites were 1.7- to 3.0-fold greater than for Cr(III)-treated animals.[74] Peritoneal macrophages from Cr(VI)-treated animals at 48 h posttreatment resulted in 1.4- and 3.6-fold increases in chemiluminescence and iodonitrotetrazolium reduction, indicating enhanced production of superoxide anion, while macrophages from Cr(III)-treated animals showed negligible increases. Thus, Cr(VI) induced greater oxidative stress in rats as compared to Cr(III)-treated animals.

In summary, the literature indicates that both Cr(VI) and Cr(III) are biologically active oxidation states of chromium, although Cr(VI) is more toxic and produces a greater oxidative stress. Furthermore, both oxidation states of chromium are involved in redox cycling with the production of reactive oxygen species. Further studies are required to elucidate the mechanisms involved in the regulation of tissue damaging effects by chromium complexes.

Mercury

The toxicity of mercury and its ability to react with and deplete free sulfhydryl groups are well known.[75] Elemental, inorganic, and organic forms of mercury exhibit toxicologic characteristics including neurotoxicity, nephrotoxicity, and gastrointestinal toxicity with ulceration and hemorrhage. Primary exposure occurs through environmental contamination as the result of mining, smelting, and industrial discharge, and include ingestion via inhalation and the food chain.[75] The decrease in free sulfhydryl groups may lead to the formation of an oxidative stress, resulting in tissue-damaging effects. The SC administration of mercuric chloride to rats results in nephrotoxic acute renal failure.[76] Mercury causes a depletion of glutathione in the renal tubules, and also a reduction in the activities of SOD, catalase, and glutathione peroxidase, enzymes responsible for the protection of cells against the peroxidative action of superoxide anion and hydroperoxides. Thus, nephrotoxicity may be due to mercury-induced alterations in membrane integrity via the formation of reactive oxygen species and the perturbation of antioxidant defense mechanisms.

Lipid peroxidation occurs in rat kidney as early as 12 h after mercury administration to rats.[77] A decrease in vitamin C and vitamin E contents in the kidney are also observed 12 h postmercury administration. When rats are pretreated with zinc, a decrease in kidney lipid peroxidation occurs with a concomitant increase in the glutathione content. When rats are pretreated with the thiol antidotes 2,3-dimercapto-1-propanesulfonic acid (DMPS) and D-penicillamine (PA) prior to the administration of mercury, protection against mercury-induced lipid peroxidation is afforded by both thiols in the liver, while in the kidneys only PA exhibited a protective effect.[78] In in vitro experiments, these same investigators demonstrated that both antidotes can act as oxygen radical scavengers and inhibitors of lipid peroxidation. However, PA was significantly more ef-

fective. Thus, the antioxidant properties of these chelating agents may be beneficial in metal intoxications.

Exposure of catfish to mercuric chloride ($HgCl_2$, 0.20 mg/l) for up to 30 days resulted in significant increases in lipid peroxidation in liver, brain, and muscle.[79] When rat kidney mitochondria are incubated with mercuric ion in vitro, approximately a 4-fold increase in hydrogen peroxide formation occurs at the ubiquinone-cytochrome *b* (antimycin A inhibited) region, and a 2-fold increase occurs in the NADH dehydrogenase (rotenone inhibited) region.[80] Concomitantly, a 3.5-fold increase in iron-dependent lipid peroxidation occurs at the NADPH dehydrogenase region with a small increase at the ubiquinone-cytochrome *b* region. In addition, mitochondrial glutathione reductase concentrations decrease as a function of both mercury concentration and incubation time. Thus, at low concentrations (12–30 nmol/mg protein), mercury depletes mitochondrial glutathione and enhances hydrogen peroxide formation under conditions of impaired respiratory chain electron transport. The increased hydrogen peroxide may lead to oxidative tissue damage, including lipid peroxidation, resulting in mercury-induced nephrotoxicity.

Lund et al.[81] have also demonstrated that the administration of mercury as Hg(II) (1.5 or 2.25 mg $HgCl_2$/kg) to rats results in increased hydrogen peroxide formation, glutathione depletion, and lipid peroxidation in kidney mitochondria. Moreover, Hg(II) induces alterations in mitochondrial calcium homeostasis. The results indicate that Hg(II) effects hydrogen peroxide formation principally at the ubiquinone-cytochrome *b* region of the mitochondrial respiratory chain, supporting the previous in vitro studies.[80] The combination of these effects results in an oxidative stress that is believed to be responsible for the nephrotoxicity of mercury.

Tan et al.[82] have also shown that mercury alters calcium homeostasis. The role of calcium in the activation of hydrolytic enzymes including proteases, endonucleases, and phospholipases is well known.[83–85] Using rat T lymphocytes, these investigators have demonstrated that methyl mercury induces an increase in intracellular calcium due to both an influx of calcium from the extracellular medium as well as the mobilization of intracellular calcium stores. $HgCl_2$, however, was shown to increase intracellular calcium only through an influx of calcium from the extracellular medium. Thus, both organic and inorganic mercury alter calcium homeostasis, but appear to involve different mechanisms. Whether mercury-associated oxidative membrane damage and reactive oxygen species are associated with the influx and mobilization of calcium is not known, and was not determined by these investigators.

The exposure of mice to mercury, chromium, or silver results in enhanced production of MDA in liver and kidneys.[86] MDA levels remain elevated for approximately 60 min and return to normal in kidneys, whereas MDA levels in liver are elevated for over 48 h after exposure. Furthermore, synergistic lipid peroxidation and toxicity was observed between inorganic mercury and carbon tetrachloride. The depletion of protective sulfhydryl groups and increased production of hydrogen peroxide[81] in combination with the metabolic activation of carbon tetrachloride may account for the synergistic effects. Preexposure to chromium, organic mercury, or silver compounds, which do not rapidly deplete sulfhydryl groups and stimulate mitochondrial hydrogen peroxide production, did not enhance carbon tetrachloride toxicity as measured by organ content of MDA. The reasons for these differences are currently unclear.

As with other heavy metal-induced oxidative stress, high levels of dietary α-tocopherol protected against methyl mercury chloride-induced hepatic lipid peroxidation, and enhanced the activity of selenium-dependent glutathione peroxidase activity.[87] However, excess dietary β-carotene did not provide protection and had no effect on glutathione peroxidase activity. The differences in response to α-tocopherol and β-carotene may be related to distribution and site-specific effects of the methyl mercury chloride.

DNA damage has also been demonstrated in response to the in vivo administration of mercuric acetate.[88] These investigators observed that in cultured human KB cells, the inhibition of dUTPase and DNA polymerase alpha activities and the activation of uracil-DNA glycosylase activity correlated with the induction of DNA single strand breaks and the decrease in cell viability.

LeBel et al.[89] have examined the hypothesis that methyl mercury may exert its neurotoxicity by way of iron-mediated oxidative damage. The formation of reactive oxygen species in rat brain was estimated based on the rate of oxidation of fluorescent probes. A significant increase in the rate of formation of reactive oxygen species was observed 7 days after a single intraperitoneal injection of methyl mercury (5 mg/kg IP), while pretreatment with DFX (500 mg/kg) completely prevented this effect either by acting as a chelator or by serving as a radical scavenger.[27,43] These findings indicate that iron-catalyzed oxygen radical production plays a role in methyl mercury neurotoxicity, and provides support for the use of DFX in xenobiotic-induced oxidative damage. When cerebellar granule cells from rats are incubated with methyl mercury, time- and concentration-dependent

cell killing occurs.[90] Significant protection from methyl mercury-induced cell death was observed by the addition of the chelators ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetic acid (EGTA) DFX and potassium cyanide (KCN), while vitamin E and EDTA had no effect. The authors interpret the results to indicate that oxidative processes contribute to the cytotoxicity of methyl mercury in isolated cerebellar granular neurons. However, the lack of protective effects by vitamin E and EDTA raises questions regarding this conclusion. If oxidative processes are involved, oxygen radical production by iron and/or other cations may contribute to the cytotoxicity of methylmercury. The methyl mercury may cause displacement of cations from binding sites, resulting in a more catalytically active form of the iron or other cations.

Suda et al.[91] have examined the degradation of methyl mercury and ethyl mercury into inorganic mercury by oxygen free radical-producing systems in vitro. Copper ascorbate, xanthine oxidase-hypoxanthine, and iron-EDTA plus hydrogen peroxide were used to produce hydroxyl radical. Both methyl mercury and ethyl mercury were readily degraded by these three systems. The degradation appeared to be unrelated to either superoxide anion production or hydrogen peroxide production, indicating that hydroxyl radical might be the oxygen free radical primarily responsible for degradation of the two forms of organic mercury.

Nickel

Nickel is widely used in numerous metal products including jewelry and coins. This cation has long been known to produce nasal and lung cancers in nickel refinery workers. Nickel dermatitis is one of the most common forms of allergic contact dermititis, with 4–9% of persons with contact dermatitis responding positively to nickel.[75]

The administration of nickel to rats results in enhanced lipid peroxidation, decreased glutathione peroxidase activity, and increased tissue iron levels.[92] The carcinogenicity of nickel compounds may be related to enhanced production of reactive oxygen species, presumably through the formation of oxidative tissue damage including damage to DNA. It is possible that the nickel-induced accumulation of iron may be directly responsible for the formation of reactive oxygen species and the subsequent enhancement of lipid peroxidation.

Based on the fact that inorganic nickel chloride forms DNA adducts, induces hepatic DNA strand breaks, produces chromosome aberrations, and induces lipid peroxidation, Stinson et al.[93] have examined the relationship between nickel chloride-induced lipid peroxidation and DNA strand breaks in rat liver. At an SC dose of 0.52 nmol/kg, nickel chloride induced DNA strand breakage at 4 h and lipid peroxidation at 12 h posttreatment in rat liver. DFX (1 g/kg, IP, 15 min prior to nickel chloride injection) completely inhibited DNA strand breakage but had no effect on lipid peroxidation. Thus, the nickel-induced lipid peroxidation is not causally related to genetic damage, while inhibition of DNA damage by DFX suggests a role for iron in this process. Nickel chloride-induced DNA strand breakage may be caused by the induction of the Fenton reaction, generating hydroxyl radicals. The DFX may have inhibited DNA strand breakage by either acting as a chelator or as a peroxyl radical scavenger.[27] However, the rapid excretion of DFX may prevent it from inhibiting the lipid peroxidation that was observed 12 h after treatment with nickel. Furthermore, additional investigations may be required and other explanations may be possible.

Shi et al.[94] have shown that the incubation of nickel with cumene hydroperoxide or *tert*-butyl hydroperoxide in the presence of glutathione, carnosine, homocarnosine, and anserine resulted in the formation of alkyl, alkoxyl, and peroxyl radicals. Glutathione, carnosine, homocarnosine, and anserine are normally considered to be cellular antioxidants. These studies suggest that instead of protecting against oxidative damage, these oligopeptides may facilitate nickel-mediated free radical production, possibly via the "radical sink" effect described by Winterbourn for glutathione.[45] These studies provide another potential mechanism for both the carcinogenicity and toxicity of nickel.

The incubation of Ni(II) with cultured 3T3 cells results in a dose-dependent decrease in cytoskeletal protein sulfhydryls as well as cellular glutathione content.[57] Furthermore, aggregation of microtubules occurs in these cells in the presence of nickel, which is believed to be due to sulfhydryl oxidation with the formation of disulfide bonds between individual microtubular polymers. These studies support the role of oxidative mechanisms in the cytotoxicity of nickel.

Further evidence for the involvement of oxidative mechanisms in the carcinogenesis and toxicity of nickel compounds has been provided by Huang et al.,[95] who exposed cultured Chinese hamster ovary cells to nickel salts. Formation of the fluorescent oxidized compound, dichlorofluorescein (DCF), was used as an index of the formation of oxidants within the intact cells. Both Ni_3S_2 and $NiCl_2$ increased the degree of fluoresence in intact cells. The greatest increase in fluorescence was produced by the relatively water-insoluble Ni_3S_2 as compared to the water soluble $NiCl_2$.

Vanadium

Studies by Younes and Strubelt[96] suggest that a strong correlation exists between vanadate-induced hepatotoxicity and the induction of lipid peroxidation. Both processes are inhibited in parallel by antioxidants, suggesting a role for lipid peroxidation in vanadate-induced hepatotoxicity. Previous studies had shown that vanadate induces lipid peroxidation in isolated hepatocytes,[97] and depletes glutathione.[98] Furthermore, vanadate stimulates the exhalation of ethane in rats.[99] Taken together, these investigations provide evidence that vanadate induces an oxidative stress and lipid peroxidation.

Zaporowska et al.[100] have examined the effect of chronic vanadium administration in drinking water to rats. Ammonium metavanadate at a concentration of 0.2 or 1.0 mM for a period of 4 weeks resulted in a decrease in plasma L-ascorbic acid levels and an increase in blood MDA concentrations. In a similar feeding study by Thompson and McNeill,[101] rats were given vandyl sulfate (1.00 to 1.25 mg/ml) in drinking water for 3 weeks. Lipid peroxidation (MDA/g tissue) was markedly elevated, while significant decreases occurred in total body weight, kidney weight, and heart weight. Oster et al.[102] also treated rats with sodium metavanadate in the drinking water (1.2 mM) and observed that tissue vanadium concentrations were positively correlated with lipid peroxidation (TBARS production). These investigations indicate that vanadium induces an oxidative stress that may participate in the toxic manifestations of this transition metal.

Shi and Dalal[103] have conducted mechanistic studies to investigate the formation of hydroxyl radicals by rat liver microsomes in the presence of vanadate. ESR spin trapping techniques were used. The addition of catalase to the incubation mixture decreased the hydroxyl radical signal while increasing a V(IV) signal. Addition of superoxide dismutase also decreased hydroxyl radical production. The results support the conclusion that a one-electron reduction of V(V) occurred in the presence of microsomes and NADH with the reduction of molecular oxygen to hydrogen peroxide, which subsequently reacted with V(IV) to generate hydroxyl radical. Present evidence indicates that vanadate generates the hydroxyl radical via a Fenton-like reaction rather than a Haber–Weiss reaction.[104] This mechanism is consistent with the evidence which has been presented for other transition metal ions concerning the formation of hydroxyl radical.

$$V(V) + O_2^{\cdot -} \rightarrow V(IV) + O_2$$

$$V(IV) + H_2O_2 \rightarrow V(V) + {}^{\cdot}OH + OH^-$$

Shi and Dalal[105] have reported that a V(IV) species accumulates when a mixture of V(V), NADPH, and a flavoenzyme, such as glutathione reductase, lipoyl dehydrogenase, or ferredoxin-$NADP^+$ oxidoreductase, is incubated in a phosphate buffer. Thus, the above-mentioned flavoenzymes can act as NADPH-dependent vanadate (V[V]) reductases.

Lead

Lead is a major environmental toxin that causes hematological, gastrointestinal, and neurological dysfunction. Prolonged exposure to lead may also cause reproductive impairment, hypertension, and nephropathy. Furthermore, lead slows nerve conduction, alters calcium homeostasis, inhibits enzymes, and stimulates synthesis of binding proteins. Major sources of lead exposure are dust, water, paint, cosmetics, folk remedies, and food supplements.[106]

Although lead is not a transition metal, the catalysis of peroxidative reactions by lead may be a major contributor to the toxic effects of this metal. Donaldson and Knowles[107] have reviewed the role of oxidative tissue damage and altered fatty acid composition in the toxicity of lead, and based on the evidence presented, oxidative mechanisms appear to be involved in some of the toxic effects of lead. Dose- and time-dependent increases in peroxides in hepatic microsomal membranes and arachidonic acid content occur in response to lead.[108] Ramstoeck et al.[109] have shown that the administration of lead to rats results in the production of ethane, an index of lipid peroxidation, while administration of vitamin E to these animals prevents enhanced lipid peroxidation.

The administration of lead compounds to rats also results in an increase in lipid peroxidation in brain.[110,111] Administration of a dose of triethyllead as low as 1.75 mg/kg body weight daily for 5 days resulted in a significant increase in lipid peroxidation in the frontal cortex of rat brain as early as one day after the last injection, with a significant increase still evident 21 days after the last injection. However, hippocampal and cerebral membranes showed no increases in peroxidative capacity at the time points that were examined.[112] As with some transition metals, such as cadmium, lead results in an increase in glutathione levels in tissues including liver, kidney, and erythrocytes. This increase in tissue glutathione appears to be a compensatory response to ameliorate lead toxicity.[90] Results involving the role of ractive oxygen species and oxidative stress in the toxicity of lead are consistent with results for other heavy metals.

Zinc

Various investigators have proposed that a biochemical function of zinc is the maintenance of membrane structure and function.[113] Dietary zinc deficiency has been shown to increase the susceptibility of rat hepatic microsomes to lipid peroxidation both in vivo[113] and in vitro.[114,115] The ability of a dietary zinc deficiency to stimulate the production of carbon-centered free radicals in lung microsomes was reported by Bray et al.[116] Hammermueller et al.[117] have demonstrated that a zinc deficiency causes leakage of hydrogen peroxide from a NADPH-dependent cytochrome P450 enzyme system.

Xu and Bray[118] have examined the effects of increased microsomal oxygen radicals on the function and stability of cytochrome P450 in dietary zinc deficient rats. Based on their results, they concluded that severe dietary zinc deficiency in rats causes a functional and structural impairment of liver microsomal cytochrome P450 function via a free radical-mediated mechanism. The removal of zinc from thymocytes results in DNA fragmentation, which is believed to occur by one of several possible mechanisms. Endonuclease may contain a zinc-binding site, and zinc inactivates the enzyme possibly by competing with calcium. Alternatively, zinc and other metal ions may regulate DNA fragmentation via a direct mechanism that does not involve endonuclease.[119]

The most likely mechanism for the ability of redox inactive zinc to reduce oxidative stress is the displacement of redox active metal ions from site-specific loci where damage occurs. Chevion et al.[120] have shown that the administration of excess zinc inhibits the toxicity of paraquat through a mechanism that may involve the displacement of copper from its binding sites, and thus prevent free radical production at that site. Similarly, zinc has been shown to compete for iron and copper binding sites, providing protection against metal-mediated DNA single and double strand breakage.[121]

Chevion[122] has proposed two models for interventions in transition metal ion mediated oxidative processes, which are referred to as "pull" and "push" mechanisms. The first mechanism involves the use of specific chelators that "pull" redox active and available metals out of binding sites, while the second mechanism involves the use of redox inactive metals such as zinc to "push" redox active metals from binding sites. Evidence in support of both mechanisms and approaches to the production of oxidative tissue damage exists.[123]

In summary, although most cations facilitate the formation of an oxidative stress, zinc appears to act as a membrane stabilizer and prevents the formation of reactive oxygen species through a mechanism that may involve protection of sulhydryl groups against oxidation,[124] and the inhibition of the production of reactive oxygen species by transition metals by the displacement of redox metal ions from site-specific loci. However, other critical antioxidant functions for zinc may still be found.

DISCUSSION

The preceding studies provide insight into the role of reactive oxygen species in the toxicity of transition metals. Similar mechanisms involving the Fenton-like production of superoxide anion and hydroxyl radical appear to be involved for iron, copper, chromium, and vanadium. However, with some metal ions, such as mercury, nickel, lead, and cadmium, depletion of glutathione and protein-bound sulfhydryls may play a primary role in the overall toxic manifestations.

$$M(X) + O_2^{\cdot -} \rightarrow M(X - 1) + O_2$$

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

$$M(X - 1) + H_2O_2 \rightarrow M(X) + {}^{\cdot}OH + OH^-$$

Increasing evidence indicates that multiple mechanisms may be involved in the production of reactive oxygen species. Fenton-like reactions may be commonly associated with most membranous fractions as mitochondria, microsomes, and peroxisomes (Fig. 1). Phagocytic cells may be another important source of reactive oxygen species. Thus, a single transition metal may initiate formation of reactive oxygen species by more than a single mechanism, involving more than one organelle or cell type. The ability of zinc to reduce oxidative stress may be due to its ability to displace redox active metal ions from site-specific loci.

Based on the evidence that has accumulated to date, the involvement of reactive oxygen species and/or free radicals may be common to the toxic mechanisms of most xenobiotics, although differences will exist in whether the formation of reactive oxygen species is an early, intermediate, or late event in the sequence of events leading to irreversible cell damage and death (Fig. 1). It is becoming increasingly clear that reactive oxygen species are formed and play a role in the toxic manifestations of most xenobiotics.

Figure 1 summarizes the interactions that are currently known regarding the involvement of reactive oxygen species and free radicals in the toxicity of various xenobiotics. The figure summarizes the damage to lipids, proteins, and DNA, the role of thiols and antioxidants, including vitamins A, E, and C, as well

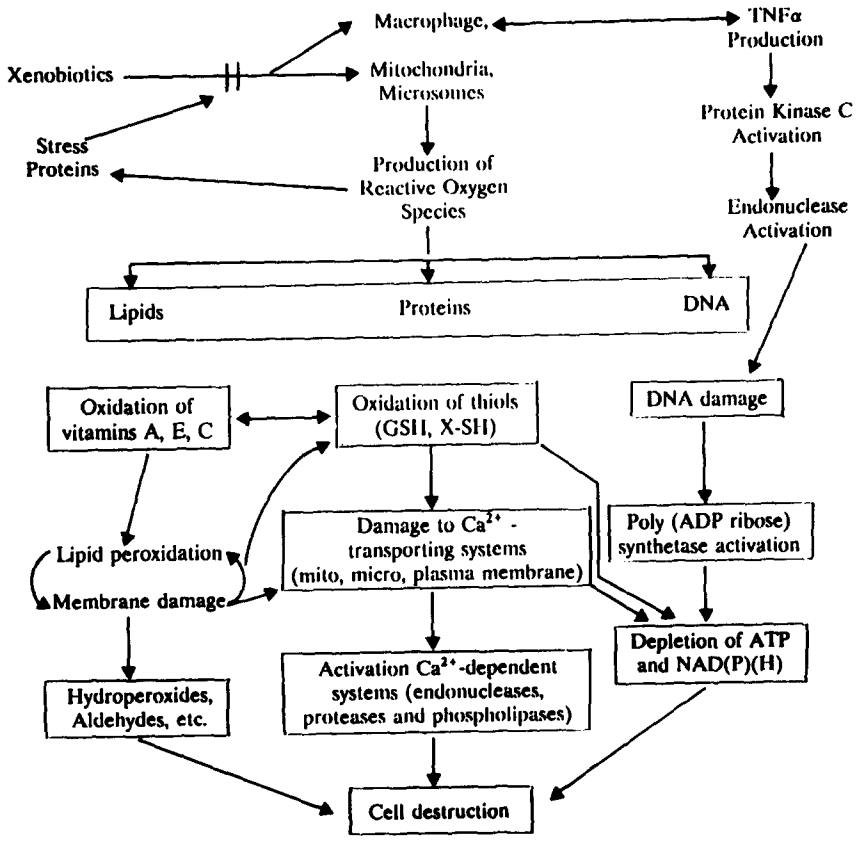

Fig. 1. Summary of the interactions that are known to exist involving reactive oxygen species and tissue damaging effects.

as the altered calcium homeostasis that occurs as a result of membrane damage, leading to activation of various calcium-dependent systems including endonucleases. Furthermore, the amplification loop associated with TNFα and the roles of protein kinase C and stress proteins are depicted. Future studies will provide greater clarification of the complex interrelationships that are involved.

Many of the inconsistencies and contradictions that appear to exist with respect to the role of reactive oxygen species in the toxicities of many xenobiotics, including transition metals, may be explained on the basis of the toxicokinetic properties of each of the xenobiotics in question. The absorption of various xenobiotics, their distribution, compartmentation within cells, site-specific loci, microenvironments, localization of enzyme systems, and the cellular distribution of antioxidant defense mechanisms all contribute to differences that are observed with respect to the production and tissue-damaging effects of reactive oxygen species.

With the advent of new technologies, numerous questions regarding reactive oxygen species, their transport, tissue-damaging effects, and relative roles in the mechanistic sequences associated with toxicity and carcinogenicity will be answered.

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