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Role of cellular antioxidants in metal-induced damage

M. Sugiyama
Department of Medical Biochemistry, Kurume University School of Medicine, Kurume 830, Japan

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Abbreviations: 8-OHG, 8-hydroxy-2′-deoxyguanosine; CAT, catalase; DEM, dimethyl maleate; DMSO, dimethyl sulfoxide; G6PD, glucose-6-phosphate dehydrogenase; GlyGlyHis, glycylglycyl-L-histidine; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, GSH *S*-transferase; MT, metallothionein; SOD, superoxide dismutase; V79, Chinese hamster V79 cells

Introduction

Certain metals such as chromium, nickel, cadmium and mercury and their compounds have been reported to be potent carcinogenic and/or toxic agents in humans and animals. Although these metals produce DNA damage and lipid peroxide *in vitro* and/or *in vivo*, the molecular mechanisms of their toxicity have not been adequately studied. Recent studies have increasingly implied that the generation of free radicals such as active oxygen species, which are well known to produce a number of toxic effects, may, at least in part, be involved in the process of metal-induced carcinogenicity and/or toxicity. Thus, the cellular antioxidant defense system, which removes free radicals, may play an important role in the genotoxicity and toxic effects of metal compounds.

This article reviews the recent studies of the role of antioxidants in the biological effects caused by chromium, nickel, cadmium and mercury based upon *in vitro* studies, mammalian cell studies, or studies in experimental animals. The results will emphasize the importance of the antioxidant system for the induction of metal-induced toxicity in living cells and tissue.

Antioxidants

Prior to discussing the effect of antioxidants on metal toxicity, active oxygen species and antioxidant defense systems are briefly summarized in this section. It is well established that active oxygen species such as superoxide anion (O_2^-), hydroxyl radical (·OH), singlet oxygen ($^1O_2$) and H_2O_2 have a wide potential for causing cell injury (Meneghini, 1988). These species have been shown to cleave DNA, modify DNA bases such as 8-hydroxy-2′-deoxyguanosine (8-OHG) (Floyd, 1990), peroxidize lipid and alter enzyme activity. Among the active oxygen species, ·OH is particularly reactive, and is formed via a metal (M) catalyzed reaction such as the Fenton and/or Haber–Weiss system as follows:

$$M^n + H_2O_2 \rightarrow M^{n+1} + OH^- + \cdot OH \text{ (Fenton reaction)}$$

$$M^{n+1} + O_2^- \rightarrow M^n + O_2$$

$$\text{Net: } O_2^- + H_2O_2 \xrightarrow{\text{Metal}} O_2 + OH^- + \cdot OH \text{ (Haber–Weiss reaction)}$$

Several carcinogenic metals have been reported to react with H_2O_2 and/or O_2^-, in Fenton/

Haber–Weiss type manner to produce ·OH (see below).

The protection of cells from oxidative damage can be accomplished through enzymatic and nonenzymatic systems. Superoxide dismutase (SOD) and catalase (CAT) or glutathione peroxidase (GPx), which eliminate O_2^- and H_2O_2 respectively, are considered the primary antioxidant enzymes. GPx catalyzes the oxidation of reduced glutathione (GSH) to an oxidized form (GSSG) at the expense of H_2O_2 and/or lipid peroxide (LOOH) and this enzyme can be divided into selenium (Se)-dependent GPx and Se-independent GPx. The Se-independent form is GSH S-transferase (GST), which has relatively low activity towards lipid hydroperoxides and none at all towards H_2O_2. GSSG can be changed back to its reduced form by the enzyme glutathione reductase (GR). This enzyme needs NADPH as a coenzyme, and one major source for NADPH is the enzyme glucose-6-phosphate dehydrogenase (G6PD) of the pentose phosphate pathway. Therefore, GST, GR and G6PD are secondary antioxidant enzymes that help in the detoxification of active oxygen species by decreasing lipid peroxide or by maintaining the activity of the primary antioxidant enzyme. These primary and secondary antioxidant enzymes can be summarized as shown in Figure 1 (Sun, 1989).

The nonenzymatic small molecular antioxidants include nonprotein sulfhydryls (NPSH) such as GSH, *N*-acetylcysteine and thiols, vitamins C, A and E, metallothionein (MT) (see later), and so on (Hochstein and Atallah, 1988). These low-molecular-weight compounds have been shown to scavenge free radical species directly. However, it should be noted that some of these compounds are also able to directly react and/or complex with these metals; thus, not all antioxidants may influence the metal-induced toxicity in the same way.

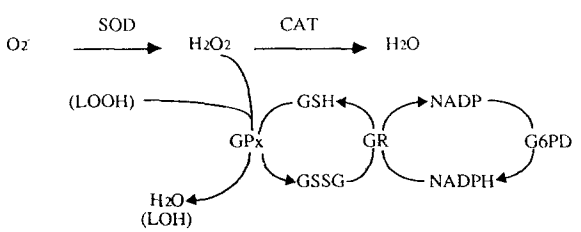

Figure 1.

Chromium

Chromium(VI) compounds have been shown to exert serious toxic and carcinogenic effects in humans and are potent inducers of tumors in experimental animals (De Flora et al., 1990). Chromium(VI) compounds have been reported to be more toxic and carcinogenic than chromium(III) compounds since chromium(VI), in contrast to chromium(III), readily enters cells by the sulfate transport system because at physiological pH it exists as an oxy anion (De Flora and Wetterhahn, 1989). However, once inside, chromium(VI) is reduced through reactive intermediates such as chromium(V) and (IV) to the trivalent form by cellular reductants including NPSH, H_2O_2 vitamins C and B_2 and flavoenzymes, as reviewed previously (De Flora and Wetterhahn, 1989; De Flora et al., 1990). Thus, the formation of trivalent chromium and other intermediate oxidation states may play an important role in inducing adverse biological effects of chromium(VI).

The role of antioxidants such as the active oxygen scavengers, GSH, vitamin B_2, E and C in chromium(VI)-induced cellular injury, such as DNA lesions, chromosomal damage, lipid peroxidation, enzyme inhibition, cytotoxicity and so on, has recently been reviewed by the present author (Sugiyama, 1992). In addition, Standeven and Wetterhahn (1991) also reviewed the role of reactive oxygen species in the mechanism of chromium(VI) carcinogenesis. Therefore, the present report will focus on studies not discussed previously.

Earlier *in vitro* studies have shown that chromium(VI) reacts with H_2O_2 to form

chromium(V), leading to the generation of ·OH, which causes DNA breaks and 8-OHG in DNA (Kawanishi et al., 1986; Aiyar et al., 1990). In addition, biologically generated chromium(V) complex has been reported to react with H_2O_2 in a Fenton-type manner, to produce more ·OH than a similar reaction with chromium(VI), resulting in the induction of DNA damage *in vitro* (Aiyar et al., 1990; Shi an Dalal, 1990). A recent study reported that O_2^- (produced using xanthine/xanthine oxidase) can reduce chromium(VI) to generate chromium(V), which can react with H_2O_2 to produce ·OH and regenerate chromium(VI), indicating that chromium(VI) can catalyze the Haber–Weiss cycle to form ·OH *in vitro* (Shi and Dalal, 1992). Furthermore, metal-chelating agents such as deferoxamine and *o*-phenanthroline were found to inhibit the chromium(V) complex that formed during reduction of chromium(VI) *in vitro*, resulting in a decrease of chromium(V)-mediated ·OH formation and less 8-OHG (Shi et al., 1992; Sugiyama et al., 1993b). Although there are no reports examining the formation of 8-OHG in cultured cells or in experimental animals treated with chromium(VI), chromium(VI) and chromium(V) compounds have been reported to increase the concentration of 8-OHG in isolated calf thymus DNA and this increase of 8-OHG was inhibited by CAT, but not by GSH (Faux et al., 1992). In cultured Chinese hamster V79 (V79) cells, treatment with *o*-phenanthroline decreased the cellular level of chromium(V), DNA strand breaks and/or alkali-labile sites as well as GR inhibition caused by the metal (Sugiyama et al., 1993b). Recently, H_2O_2-resistant Chinese hamster ovary (CHO) cells have been shown to exhibit an increase of CAT activity and GSH, and to have less total chromium(V) and simultaneously fewer DNA breaks, as compared with wild-type cells; however, the cytotoxicity caused by chromium(VI) as well as cellular uptake of the metal did not differ in the parent and resistant cells (Sugiyama et al., 1993a). Collectively, these studies suggest that chromium(V) must be the critical form that is responsible for the induction of DNA breaks and/or alkali-labile sites, and indicate that active oxygen species, in particular H_2O_2 are closely related with the DNA breakage as well as the 8-OHG caused by this metal.

Previous studies demonstrated that chromium(III) is the critical form that is responsible for the DNA–protein crosslinks induced by chromium(VI) (as reviewed by Sugiyama, 1992). Salnikow et al. (1992) suggested that trivalent chromium creates DNA–protein crosslinks by binding with reactive amino acids such as cysteine or histidine and then linking these to the phosphate backbone of DNA. Recent *in vitro* studies have suggested that chromium(III) may also be involved in the generation of active oxygen. First of all, Ozawa and Hanaki (1990) showed that, at pH 2, chromium(III) was reduced to chromium(II) by biological reductants such as L-cysteine and NADH, and then chromium(II) reacted with H_2O_2 in a Fenton type manner to generate ·OH *in vitro*. Sugden et al. (1992) demonstrated that, in contrast to chromium(III) salts, the mutagenic chromium(III) complexes such as bipyridyl and phenanthroline complexes display characteristics of reversibility and positive shifts of the chromium(III)/(II) redox couple consistent with the ability of these chromium(III) complexes to serve as cyclical electron donors in a Fenton-like reaction, and these complexes caused the relaxation of supercoiled DNA in the presence of ascorbate and H_2O_2 *in vitro*. However, nonmutagenic chromium(III) complexes have no such activity. Thus, mutagenesis of chromium(III) complexes is associated with the generation of active oxygen species, and the activity of chromium(III) complexes is dependent on the associated ligands. Shi et al. (1993) reported that incubation of chromium(III) with H_2O_2 and lipid peroxide (LOOH) at pH 7.2 generated ·OH and lipid peroxide-derived free radicals, such as alkyl radical (L·) and alkoxyl radical (LO·), respectively, and that the generation of ·OH is

enhanced at higher pH and inhibited by the metal-chelator diethylenetriamine pentaacetic acid while deferoxamine has only a small effect, and cysteine, GSH and NADH do not have any significant effect. As was the case with chromium(VI) and (V), these results indicate that chromium(III) and/or (II) may generate free radical species *in vitro*; however, it is not clear what role they play *in vivo*.

Although previous studies reported that chromium(VI) inhibited the activity of GR in human erythrocytes without affecting 16 other enzymes such as glycolytic and pentose-phosphate pathway enzymes (Koutras et al., 1964), the *in vivo* modification of antioxidant enzyme activity by chromium(VI) compounds has been reported by Sengupta et al. (1990), who showed that acute oral administration of chromate to rats led to an increase of lipid peroxide, and to a decrease in GSH and activity of G6PD, GPx, GR, GST, SOD and CAT of intestinal epithelial cells, while chronic oral administration of the metal led to an increase in lipid peroxide, SOD and GPx activity and to a decrease in GST activity, without affecting G6PD, GR and CAT activity. The mechanism of this modification by chromium(VI) is obscure. The authors indicate that under acute treatment the antioxidant system is more susceptible to chromium-induced oxidative damage as compared to chronic treatment.

We previously reported that pretreatment of V79 cells with vitamin C resulted in the decrease of alkali-labile sites caused by Na_2CrO_4 (Sugiyama et al., 1991). Wise et al. (1993) also recently showed that in CHO cells chromosomal aberrations induced by lead chromate, a highly insoluble metal salt, were inhibited by simultaneous treatment with vitamin C. Although chromium(III) complex was formed immediately following the incubation of V79 cell homogenates with chromium(VI) and ascorbate at a 1:10 mole ratio (Sugiyama et al., 1989), Lefebvre and Pezerat (1992) showed that the reaction of chromate and ascorbate in a 1:1 molar ratio in the absence of H_2O_2 produces free chromium(V), but not ·OH, with concomitant formation of ascorbate radical, and that the addition of formate to this reaction mixture resulted in the formation of carboxylate radicals ($COO^{\cdot-}$). The formation of $COO^{\cdot-}$ was decreased either in the absence of O_2 or in the presence of 2-ethyl-2-hydroxybutyric acid (EHBA), which produces chromium(V)–EHBA complex, leading to an inhibition of the chromium(V) redox activity. Furthermore, the addition of SOD produced an increase of $COO^{\cdot-}$ formation while CAT caused an inverse effect, indicating the generation of both $O_2^{\cdot-}$ and H_2O_2. It was suggested that the formation of $COO^{\cdot-}$ during the reaction of chromium(VI) and ascorbate is probably due to the reaction of chromium(V) and oxygen to form a chromium(V) superoxo complex *in vitro*.

The important role of vitamin C in the extracellular and/or intracellular reduction of chromium(V) in rat lung and in cultured cells has been reported previously (reviewed in Sugiyama, 1992). Recently, Standeven and Wetterhahn (1992) examined chromium(VI) reduction in rat lung ultrafiltrates and cytosols, and chromium–DNA binding in nuclei suspended in lung cytosols, in the presence and absence of L-ascorbate oxidase and *N*-ethylmaleimide (NEM) to selectively deplete ascorbate and thiols. They found that depletion of ascorbate caused an inhibition of about 95% of chromium(VI) reduction while depletion of thiols blocked less than 15% of the reduction in lung ultrafiltrates and cytosols. Chromium–DNA binding following the incubation of chromium(VI) and nuclei in rat lung cytosols was completely inhibited by the depletion of ascorbic acid and 60% of this binding was inhibited by the thiol depletion. The authors suggested that ascorbate and/or ascorbate-dependent factors in the lung are the principal intracellular reductants of chromium(VI) and play an important role in the induction of genotoxicity by chromium(VI). We also showed that the elevation of vitamin C level in V79 cells accelerated chromium(VI) reduction as evaluated

by the decrease of chromium(V) and the increase of chromium(III) (Sugiyama et al., 1991). In contrast, Capellmann and Bolt (1992) reported that, in human plasma incubated with chromium(VI), no changes in the instrinsic contents of ascorbic acid were observed, and that the reduction capacities for chromium(VI) in different plasma samples did not correlate to their ascorbic levels, indicating that the reduction of chromium(VI) by native human plasma is a complex feature that is not determined by the plasma ascorbic acid levels. Since the concentration of ascorbic acid in lung is much higher than that in blood, the contribution of vitamin C to chromium(VI) reduction appears to be dependent on the tissue or organs used.

Nickel

Nickel compounds have been clearly established as human carcinogens and potently induce tumors in experimental animals (Sunderman, 1984; Kasprzak, 1991; Klein et al., 1991). They induced DNA damage, lipid peroxidation, chromosomal aberrations, sister chromatid exchanges as well as mutation and transformation in cultured mammalian cells (see reviews in Coogan et al., 1989; Christie and Katsifis, 1990). The water-insoluble nickel compounds, such as Ni_3S_2 and NiS, were more potent transforming agents than the soluble compounds, such as $NiSO_4$ and $NiCl_2$, and this differential activity was ascribed to the mode of delivery of nickel ion into the nucleus (Costa, 1991). NiS particles, which were phagocytized by cells, aggregated around the nucleus where they were dissolved by lysosome-facilitated acidification. Therefore, the observed phagocytosis of NiS particles was thought to represent an efficient means of attaining high intracellular concentrations of nickel ions, leading to the formation of DNA damage.

The generation of active oxygen species by nickel complexes *in vitro* was reported by Inoue and Kawanishi (1989), who showed that Ni(II) complexes of oligopeptides such as glycyl-glycyl-L-histidine (GlyGlyHis) reacts with H_2O_2 to produce $O_2^{\cdot-}$, ·OH and $^1O_2$ *in vitro*. The addition of SOD to the reaction mixture of Ni(II) GlyGlyHis with H_2O_2 resulted in an inhibition of $^1O_2$ formation, indicating the involvement of $O_2^{\cdot-}$ in $^1O_2$ generation. The production of ·OH may simply be explained by a Fenton-type reaction of Ni(II) complex with H_2O_2. However, ·OH scavengers, such as ethanol and formate, had inhibitory effects in this system without the sufficient formation of α-hydroxyethyl radical and $COO^{\cdot-}$, respectively. Since free ·OH reacts with ethanol and formate to form α-hydroxyethyl and $COO^{\cdot-}$, they suggested that other active species such as nickel–oxygen complexes including an oxo-Ni(IV) complex or Ni(III)-peroxide complex, which may be capable of releasing ·OH, are involved in the reaction of Ni(II) oligopeptide complexes with H_2O_2. Other studies also suggested that Ni(II)–oligopeptide complexes react with H_2O_2 to form a reactive Ni-oxene radical (NiO^{2+}) (Nieboer et al., 1989; Cotelle et al., 1992). Similarly, Ni(II) oligopeptide complexes have been shown to react with lipid peroxide (LOOH) *in vitro*, resulting in the generation of peroxyl radical (LOO·), alkyl radical (L·) and/or alkoxyl radicals (LO·). Interestingly, antioxidant oligopeptides such as GSH generated more L· and LO· formation than other oligopeptides tested in the reaction of Ni(II) with LOOH (Shi et al., 1992). In these studies, neither Ni(II) alone nor the oligopeptides alone exhibited any reactivity toward H_2O_2 and LOOH. Other studies also demonstrated that nickel can undergo redox cycles between Ni(III)/Ni(II) valance states when Ni(II) is complexed to protein (Nieboer et al., 1984; Sunderman et al., 1987), and that the complex of Ni(II) with DNA is necessary for reacting with H_2O_2 *in vitro* (see below). Thus, the complexation of Ni(II) with biological ligands such as DNA, protein and oligopeptides including antioxidants such as GSH may be the critical factor for the production of free-radical

species inside cells.

With respect to DNA damage, Kawanishi et al. (1989) reported that $NiCl_2$ in the presence of H_2O_2 caused DNA cleavage *in vitro*. The addition of diethylenetriamine-*N*,*N*,*N'*,*N''*,*N''*-pentaacetic acid or Mg(II) ion to the reaction mixture of H_2O_2 and $NiCl_2$ inhibited the DNA damage, indicating that Ni(II) binds to DNA and the complex formed reacts with H_2O_2. $^1O_2$ scavengers such as sodium azide and dGMP inhibited the DNA damage, but 1,4-diazabicyclo[2,2,2]octane and dimethylfuran did not. Among ·OH scavengers, dimethyl sulfoxide (DMSO), methionine, methional and sodium formate inhibited DNA cleavage, whereas ethanol and mannitol did not. Additionally, SOD did not inhibit the DNA damage. Thus, the formation of $^1O_2$ and ·OH, but not that of O_2^-, may be involved in the induction of DNA cleavage by Ni(II) plus H_2O_2. However, it is speculated that active species other than $^1O_2$ and free ·OH, such as nickel–oxygen complexes, are involved, because $^1O_2$ and ·OH scavengers did not inhibit DNA damage. The effects of nickel compounds on DNA base modification, especially 8-OHG, was also reported *in vitro* and *in vivo*, as summarized recently by Kasprzak (1991). It should be noted that Ni_3S_2 and $NiCl_2$ markedly enhanced 8-OHG production by either H_2O_2 or H_2O_2 plus vitamin C *in vitro* (Kasprzak and Hernandez, 1989). The ability of Ni(II) in the presence of H_2O_2 to cause the formation of typical ·OH-induced modification of DNA bases was also shown in chromatin extracted from cultured cells of human origin, because this ability was enhanced by SOD and decreased by ·OH scavenger DMSO, but GSH had little effect on the DNA base modification (Nackerdien et al., 1991). Most recently, Change et al. (1993) examined whether $NiCl_2$ is able to induce bulky DNA adduct formation *in vitro* and *in vivo* as assayed by ^{32}P-postlabeling method. The results showed that $NiCl_2$ in the presence of H_2O_2 caused two major DNA-adducts *in vitro* that were inhibited by ·OH scavengers such as sodium formate and *p*-nitrosodimethylamine, and by the $^1O_2$ scavenger sodium azide. In contrast, these DNA-adducts induced by $NiCl_2$ plus H_2O_2 were enhanced by vitamin C and by replacement of H_2O_2 with D_2O. The lifetime of $^1O_2$ is found to be longer in D_2O than in H_2O_2. Thus, the DNA-adducts may be formed via ·OH and $^1O_2$. Furthermore, these adducts were similar to those induced by $FeSO_4$ and H_2O_2, indicating the involvement of Fenton-type reaction in the induction of DNA-adducts by $NiCl_2$ and H_2O_2. In addition, the increase of two major DNA-adducts was similarly observed in the kidney after the administration of Ni(II) acetate to mice. Although the effects of antioxidants on DNA damage, such as breakage, base modification and adducts, have not been evaluated *in vivo*, these results implicate the involvement of active oxygen in the DNA damage caused by Ni(II) compounds.

The generation of H_2O_2 by insoluble nickel compounds has been reported to occur in human polymorphonuclear leukocytes (PMNs) in a manner similar to that caused by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Zhong et al., 1990). Insoluble sulfides such as Ni_3S_2, NiS_2, and NiS stimulated PMNs to form H_2O_2, while the soluble salts such as $NiSO_4$ did not. The level of H_2O_2 production was dependent on the type of sulfide used. The addition of CAT inhibited H_2O_2 production by PMN treated with NiS and Ni_3S_2, and the addition of SOD also suppressed the level of H_2O_2 produced by NiS, but not by Ni_3S_2, indicating that the insoluble NiS induces formation of both O_2^- and H_2O_2, whereas Ni_3S_2 produces only H_2O_2. In our experiments with CHO cells, pretreatment with vitamin E succinate was found to inhibit some but not all of the chromosomal aberrations induced by insoluble NiS, but not those induced by the soluble $NiCl_2$ (Lin et al., 1991). Similarly, pretreatment with vitamin E succinate protected against the cytotoxicity of NiS while there was only a small reduction in cytotoxicity of CHO cells treated with $NiCl_2$. In general, $NiCl_2$ has

been shown to exert a weak mutagenic effect at the hypoxanthine–guanine phosphoribosyl transferase locus in V79 cells (Miyaki et al., 1979) and the insoluble Ni_3S_2 is not very mutagenic in V79 cells (Arrouijal et al., 1990). However, recent studies have shown that NiS and Ni_3S_2 are quite mutagenic at the bacterial guanine phosphoribosyl transferase locus in transgenic V79-derived G12 cell lines (Christie et al., 1992; Kargacin et al., 1993), which have been shown to be hypersensitive to mutagenesis by oxidative agents such as X-rays and bleomycin. Although $NiCl_2$ induced a slight mutagenic response in G12 cells, pretreatment of the cells with vitamin E succinate was also found to have a protective effect on the cytotoxicity and mutation caused by several insoluble nickel compounds such as NiS, Ni_3S_2 and NiO, while no such effect was observed for $NiCl_2$ (Kargacin et al., 1993). These results suggest that the phagocytosis of insoluble nickel compounds, which allows for high concentrations of the potentially reactive Ni(II) in cells, produces more active oxygen species that may be associated with the induction of chromosomal aberrations and mutation than the soluble nickel compounds. The results also demonstrated the ability of vitamin E to suppress some of the cytotoxic, clastogenic and mutagenic activity of the nickel compounds and supports the important role of vitamin E in the induction of nickel-induced toxicity and genotoxicity.

Recent studies have shown that nickel compounds modify the antioxidant system. Iscan et al. (1992) studied the effect of nickel on hepatic GSH level and GST activity in mice, rats and guinea-pigs after the administration of $NiCl_2$. The hepatic GSH level was significantly reduced in mice whereas an increase and no alteration were observed in rats and guinea-pigs, respectively. Furthermore, the hepatic GST in these animals is differentially regulated by nickel. In another study, the administration of Ni(II) to rats caused a decrease of GSH level, followed by a rebound in GSH levels in liver, but not in kidney, and a decrease of hepatic G6PD and GR activity was also observed (Cartana et al., 1992). Apparently, the effect of nickel on GSH level varies depending on the animal species and the organ. In cultured mouse 3T3 cells, treatment with Ni(II) was reported to cause a dose-dependent decrease in cellular GSH content (Li et al., 1993). In the case of guinea-pig alveolar macrophages, nickel hydroxycarbonate (NiHC) decreased the cellular levels of GSH at low doses of the metal, while a decrease of SOD and CAT activity was observed for high doses (Arsalane et al., 1992). Only at the low dose of nickel did the chemiluminescence increase, indicating the generation of free radicals at low concentrations of NiHC. Novelli et al. (1990, 1992) showed an increase of SOD activity in lungs, pancreas and erythrocytes of rats after intratracheal administration of $NiCl_2$. Rodriguez et al. (1990) showed that Ni(II) inhibited the CAT activity in a concentration-dependent manner *in vitro*, possibly through its direct interaction with the CAT-protein moiety, and that administration of Ni(II) to rats subsequently decreased the activity of CAT in liver and kidney. However, there was no direct relationship between the enzyme inhibition and Ni(II) concentration in these tissues, suggesting that the enzyme inhibition by Ni(II) *in vivo* is more complex than that *in vitro*.

The role of antioxidants in lipid peroxidation caused by nickel compounds has been discussed. Athar et al. (1987) showed that the administration of $NiCl_2$ to rats resulted in enhanced levels of lipid peroxide and GSH with a concomitant decrease in GPx activity in liver, and this enhanced lipid peroxidation was inhibited by the exogenous addition of ·OH scavengers such as benzonate and ethanol, but not by SOD and CAT. The increase of hepatic lipid peroxide and GST activity as well as a decrease of free radical reductase, as shown by membrane-bound heat-labile protein, were also reported in mice following the administration of $NiCl_2$ (Srivastava et al., 1990). In another study, $NiCl_2$ administration was shown to deplete hepatic GSH in 8–12-week-old mice but not in

the younger age groups, and mortality and hepatic lipid peroxidation caused by $NiCl_2$ were correlated with the decreased level of GSH (Andersen and Andersen, 1989). Misra et al. (1990) also showed that the administration of Ni(II) acetate to rats increased lipid peroxide and decreased GSH levels as well as antioxidant enzyme activity such as CAT, GPx and GR, but did not affect SOD activity in liver and kidney, although a decrease and increase of GST activity were observed in liver and kidney, respectively. They also reported, using four mouse strains, that the magnitude of nickel-induced lipid peroxide in kidney was greatest in the strain that is lowest in GSH and GPx, but which is not low in SOD and CAT (Misra et al., 1991). In addition, Ni(II) acetate increased hepatic lipid peroxide, which showed a reverse correlation with the extent and direction of nickel's effect on GSH, GPx and GR, but no correlation with CAT, SOD or GST (Rodriguez et al., 1991). The antioxidant system, in particular GSH and GPx, appears to play an important role in the prevention of nickel-induced lipid peroxidation.

Cadmium

Cadmium is a highly toxic metal, and human exposure to cadmium is generally increasing. In Japan, Itai-itai disease, which is characterized by osteomalacia and multiple renal tubular dysfunction, occurred when cadmium was discharged from a mine into a river (Hagino and Yoshioka, 1961; Friberg et al., 1974). Cadmium compounds have also been shown to be human carcinogens for the lung and possibly the prostate (IARC, 1987), and to induce a variety of tumors in organs including the lung (Takenaka et al., 1983), testes (Gun et al., 1963; Waalkes et al., 1989) and prostate (Waalkes et al., 1989) in experimental animals (see review in Waalkes and Oberdörster, 1990).

The generation of H_2O_2 by cadmium compounds has been shown directly in human PMNs (Zhong et al., 1990). Insoluble cadmium such as CdS stimulated PMNs to form H_2O_2 while the soluble $CdSO_4$ did not. Another study (Amoruso et al., 1982) also showed that $CdCl_2$ enhanced the production of O_2^- in digitonin-stimulated phagocytes, human granulocytes or rat alveolar macrophages. Additionally, based upon the effect of antioxidants, the involvement of active oxygen species in the induction of DNA single-strand breaks, chromosomal aberrations, and the inhibition of cell growth by $CdCl_2$ was also reported in cultured V79 cells by Ochi et al. (1983, 1985, 1987). They showed a complete inhibition of DNA breaks in cells treated with $CdCl_2$ in the anaerobic culture condition, and found that the presence of SOD suppressed DNA breaks and inhibition of cell growth by the metal. Furthermore, the metal-induced chromosomal aberrations in V79 cells were found to be inhibited by the presence of CAT or the diffusible radical scavenger butylated hydroxytoluene (BHT), but not by SOD and $^1O_2$ scavengers such as dimethylfuran. The enhancement of chromosomal damage was also detected in cells treated with a CAT inhibitor, aminotriazole. They further showed using V79 cells that the metal inhibition of cell growth was suppressed by treatment with BHT and ·OH scavenger mannitol, but CAT treatments had a marginally suppressive effect on this inhibition. The inhibition of DNA synthesis by $CdCl_2$, as estimated by [^{3}H]thymidine incorporation, was also suppressed by BHT, but not by SOD or CAT treatment. In another study (Snyder, 1988), DNA breakage induced by $CdCl_2$ was suppressed by the addition of CAT and ·OH scavengers such as KI and mannitol, but not by SOD, in cultured human cells. Similarly, we showed that H_2O_2-resistant CHO cells were cross-resistant to $CdCl_2$ with respect to DNA breaks and cytotoxicity (Sugiyama et al., 1993). Martins et al. (1991) reported that cadmium-resistant V79 were cross-resistant to the induction of DNA breaks and the mortality induced by H_2O_2. Although cadmium was

shown to neither induce DNA breaks nor enhance H_2O_2-induced DNA breaks in testicular Leydig cells (Koizumi et al., 1992), these results suggest that active oxygen may, in part, be involved in the induction of genotoxic, clastogenic and cytotoxic actions of cadmium compounds.

The protective role of intracellular GSH on cadmium toxicity has been extensively investigated. It has been shown that the addition of GSH ameliorates cadmium toxicity in isolated rat hepatocytes (Stacey, 1986). Recent studies (Kang and Enger, 1987, 1990) using cultured human lung carcinoma cells showed that the depletion of most cellular GSH by buthionine sulfoximine (BSO) or diethyl maleate (DEM) prior to cadmium exposure caused an enhancement of cytotoxicity by this metal, and found a high correlation between cadmium cytotoxicity and GSH content. Similarly, enhanced cadmium toxicity was reported in mice pretreated with BSO (Singhal et al., 1987). Protective elevation of GSH was also reported in cultured rat mesangial cells treated with low levels of $CdCl_2$ (Chin and Templeton, 1993). In addition, the increase of GSH content by cadmium was detected in mouse peritoneal macrophages, and this increase was attributed to the induction of cysteine transport activity (Bannai et al., 1991). Cadmium-induced acute testicular toxicity and testicular interstitial cell tumors in rats can be prevented by low-dose cadmium pretreatment *in vivo*, which caused an increase of GSH without altering levels of low-molecular-weight testicular cadmium-binding protein (Wahba et al., 1990). Since the testes in rat and mouse have been shown to be deficient in MT (Waalkes and Perantoni, 1986; Waalkes et al., 1988), GSH in the testes may play a key role in protecting them from cadmium toxicity. In addition, the cross-resistance to oxidative stress exhibited by cadmium-resistant cells was reported to be responsible, in part, for increased cellular GSH content (Chubatsu et al., 1992). The mechanism of action of GSH appears to be related to its ability to form a complex with GSH (Sheabar and Yannai, 1989; Almar and Dierickx, 1990; Kadima and Rabenstein, 1990a,b) and to scavenge radical species.

The role of MT, a cytosolic, low-molecular-weight, cysteine-rich, metal binding protein, in the development of resistance to cadmium has also been well established in cultured systems (Rugstad and Norseth, 1975, 1978; Hildebrand et al., 1979; Beach and Palmiter, 1981; Gick et al., 1981). These studies demonstrated that selection for cadmium resistance is associated with a cadmium-mediated increase in the synthesis of MT. The induction of MT synthesis by exposure to metals such as zinc has also been shown to confer resistance in animals against subsequent lethal doses of cadmium (Webb, 1972; Probst et al., 1977), and to protect rat hepatocyte cells from toxicity caused by various metals including cadmium, mercury and nickel (Liu et al., 1991). In addition, the development of resistance is associated with an amplification of the MT gene as well as with an increase in the rate of transcription of this gene in cultured cells (Beach et al., 1981; Walters et al., 1981). The protective mechanism of MT against cadmium toxicity was based upon the chelation of the metal ion by MT, because of its high content of cysteine, which has a great affinity for certain toxic metals: $Hg^{2+} < Cu^{2+} < Cd^{2+} < Zn^{2+}$ (Holt et al., 1980). However, MT has been shown to have an extremely high ·OH scavenger capacity (Thornalley and Vasak, 1985; Abel and Ruiter, 1989), thus protecting against various forms of oxidative stress. Müller et al. (1991) demonstrated the ability of cadmium, when bound to MT, to induce DNA strand breaks in isolated supercoiled plasmid DNA *in vitro*. Coogan et al. (1992) showed that the increase of MT levels by the pretreatment of zinc in cultured rat hepatocytes resulted in a marked decrease of DNA breaks as well as of cytotoxicity caused by $CdCl_2$, without decreasing cadmium accumulation. In another study (Mello-Filho et al., 1988) cadmium-resistant V79 cells, which contained an

enrichment in MT content, were significantly more resistant than parental cells to oxidative stress caused by either H_2O_2 or a mixture of H_2O_2 and O_2^- generated by xanthine oxidase plus acetaldehyde, while there were no differences between the two cells in their total H_2O_2-decomposing or O_2^- dismutating activity. In addition, V79 cells exposed to zinc were reported to exhibit an increase in MT, but not in GSH, and these cells become more resistant to the DNA damaging effect of H_2O_2 (Martins et al., 1991). Preexposure of rats to cadmium aerosols has been found to produce a marked degree of tolerance to hyperoxia and to induce 400-fold higher levels of MT in the lung while GPx, CAT, GR, and SOD activity were also augmented in the lung by this pretreatment with cadmium (Hart et al., 1990). The administration to mice of DEM alone, which caused depletion in GSH levels, has been also shown to be quite effective at increasing hepatic concentrations of MT, and this pretreatment with DEM protected against cadmium-induced hepatotoxicity (Bauman et al., 1992). In another study (Hirano et al., 1990), the intratracheal instillation of cadmium oxide caused the induction of MT in lung, and a slight decrease of SOD, without affecting GPx and GR activity, although a slight increase of GSH and an increase of G6PD were observed. This suggested that MT played a key role in the detoxification of instilled cadmium, but that the antioxidant enzymes had a minimal role. As was the case with GSH, these results suggest that intracellular MT could possibly protect against toxicity, possibly due to its ability to chelate metal and to scavenge active oxygen species.

Recent studies have compared the role of GSH and MT in the tolerance to cadmium. Kang et al. (1989) showed that the depletion of GSH in human lung carcinoma cells caused an increase of MT synthesis, while cadmium cytotoxicity was enhanced. The administration of $CdCl_2$ to fasted rats was reported to decrease total hepatic GSH concentration while hepatic MT concentration was markedly higher in fasted rats than in fed rats, resulting in an enhancement of cadmium toxicity in fasted rats, as shown by a markedly decreased LD_{50} (Shimizu and Morita, 1990). These results suggest that GSH plays an important role in protection against cadmium toxicity before the onset of MT synthesis. In contrast, Chan and Cherian (1992), using an *in vitro* system, showed that both GSH and MT play a similar and important role in the protection against cadmium toxicity in rat liver slices, but that MT serves as a specific and effective defense against cadmium toxicity even when the intracellular GSH level is significantly low, because the enhanced cadmium toxicity in liver slices resulting from the depletion of GSH by BSO can be totally overcome by the induction of MT by zinc pretreatment. Kang et al. (1990) showed that the difference in cadmium toxicity response between two cloned human lung carcinoma cells could not be explained either by the alteration of MT synthesis or by the difference in initial GSH content. Ochi (1991) also showed that the resistance to cadmium in V79 cells was not attributable to either increased inducibility of MT or increase in intracellular levels of GSH.

The role of antioxidant enzymes in cadmium-induced lipid peroxidation has been discussed. Shukla et al. (1987) reported that cadmium acetate administration to rats increased lipid peroxide in brain with a concomitant decrease in SOD activity. Similarly, cadmium caused strong inhibitory effects on brain and purified bovine blood SOD, and increased lipid peroxide in fresh brain homogenate. A similar decrease in SOD activity has been reported in erythrocytes and lung lavage taken from rats exposed to cadmium fumes (Minami et al., 1982). This suggests that cadmium-stimulated lipid peroxidation may be the consequence of either high levels of O_2^- resulting from increased production and inhibited SOD activity, or a direct action of the metal ion on the peroxidative reaction. An *in vitro* study showed that cadmium inhibition of SOD activity appears to be the replacement by cadmium of

zinc in Cu–Zn SOD (Bjerrum et al., 1991; Kofod et al., 1991). A previous study (Omaye and Tappel, 1975) also reported that $CdCl_2$ administration to rats resulted in an increase of testicular lipid peroxide, with a concomitant decrease of GPx, and a similar inhibition of testicular GPx activity by $CdCl_2$ was observed *in vitro*. In addition, another study (Koizumi and Li, 1992) showed that treatment of rats with $CdCl_2$ caused the elevation of lipid peroxide, H_2O_2 production, and xanthine oxidase activity in testicular Leydig cells, while the activity of GPx, GR, and CAT as well as the levels of GSH were decreased, but SOD activity was unchanged. The intragastric administration of $CdCl_2$ and ethanol to rats has been shown to increase lipid peroxide and cadmium accumulation in liver, which was associated with a greater inhibition of GPx, GR, and SOD than cadmium treatment alone (Sharma et al., 1991). The levels of GSH and total thiols in liver also decreased under the same conditions. The administration of $CdCl_2$ to rats was also shown to stimulate lipid peroxidation in liver, kidney, brains, lungs, heart and testes, and the most sensitive organs were brains and lungs. The investigation of antioxidant enzymes (GPx, GR, G6PD, SOD) in liver, lungs, and heart revealed that GPx appears to play important role in the cadmium-induced lipid peroxidation in lungs and heart tissue (Manca et al., 1991). Cadmium has been shown to interfere with the absorption of selenium (Flagel et al., 1980), and selenium given to rats treated with cadmium overcomes the inhibition of GPx (Omaye and Tappel, 1975; Jamall and Smith, 1985) (see later), indicating that this inhibition by cadmium *in vivo* may be due to selenium deficiency caused by the metal. In contrast, $CdCl_2$ administration to rats was also reported to elevate the levels of lipid peroxide in the testes, liver, and kidney, with a particularly remarkable increase in the testes, while SOD and GPx activities in these organs were not changed and CAT activity in the testes was increased, suggesting a relatively small contribution of SOD, GPx, and CAT in cadmium-induced lipid peroxidation in the testes (Kojima et al., 1990). Thus, the role of antioxidant enzymes in lipid peroxidation caused by cadmium remains controversial.

Previous studies have shown that selenium protected against cadmium-induced toxicity such as testicular lesions (Mason et al., 1964; Gunn et al., 1968), pancreatic changes, and alterations in gluconeogenic enzymes (Merali and Singhal, 1975), and a decrease in the plasma and testicular GPx activity in rats (Omaye and Tappel, 1975; Prohaska et al., 1977). A protective effect of selenium on cadmium-induced chromosomal aberrations in bone marrow cells of mice was also reported by Mukherjee et al. (1988). The mechanism of detoxification of cadmium by selenium is thought to be related to either the formation of cadmium–selenium complexes in association with MT or the alteration of the tissue distribution of cadmium by selenium (Margos and Webb, 1976; Gasiewicz and Smith, 1978; Early and Schnell, 1981; Jamall and Sprowls, 1987). The feeding of cadmium to rats caused significant reductions in ocular selenium levels and GPx activity (Jamall and Roque, 1989), and there was a negative correlation in cadmium-treated rats between ocular selenium concentration and lipid peroxidation (Shinno, 1989). In rat hepatocytes, the decrease in GSH levels induced by cadmium was found to be lowered when selenium was concurrently added to the incubation medium (Bell et al., 1991). Sugawara et al. (1989) showed that cadmium injections in mice resulted in an increase of testicular lipid peroxidation and a decrease of GSH, and that the selenium injection restored their normal levels. In this case, no changes of testicular concentration of metals such as Zn, Mg, Ca, and Fe induced by cadmium were observed with cadmium plus selenium treatment, although selenium stimulated the cadmium uptake into the testes. Thus, selenium appeared to act as an indirect antioxidant for preventing lipid peroxidation.

The role of antioxidant vitamins in cadmium toxicity has also been demonstrated. Shukla and Chandra (1989) showed that the vitamin E levels in the brain, liver, kidney, testes, red blood cells, and plasma were decreased in rats treated with cadmium acetate for 60 days while exposure to this metal for 15 days decreased the levels of vitamin E in the liver and testes only, and 30 days treatment caused a decrease of the vitamin in brain and plasma. They also showed a duration-dependent decrease of vitamin C levels in liver. Another study (Pharikal et al., 1988) reported that the concentration of vitamin C decreased in plasma, liver, kidney, spleen, adrenal, pituitary, and brain, but increased in testes and thyroid, though no significant alteration was observed in the pancreas after cadmium treatment to rats in their dietary regimen. These results suggest that alteration of vitamins C and E in the different tissues and organs has some correlation with the extent of cadmium toxicity. The enhancement of lipid peroxide in the kidney, liver, and serum of $CdCl_2$-treated guinea pigs was also reported with a low intake of vitamin C, whereas a high intake of the vitamin decreased the levels of lipid peroxide (Hudecová and Ginter, 1992). The preventive effect of high doses of vitamin C against cadmium deposition in the testes, heart, brain, and kidney, but not in the liver, after the administration of cadmium to guinea pigs, was also reported (Kadrabova et al., 1992). Fariss (1991) reported that the exogenous administrations of vitamin E succinate protected rat hepatocytes from cadmium-induced injury and lipid peroxidation. Simultaneous administration of vitamin E to rats has also been shown to reduce cadmium-induced biochemical alterations such as a decrease of the activity of hepatic and renal glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase, and to decrease the accumulation of cadmium in blood, liver, and kidney (Tandon, 1992). In a previous study, complete protection against muscle lesions produced by cadmium in ducklings was provided by vitamin E and selenium (Vleet et al., 1981). Similarly, lipoic acid and lipoate have been shown to protect rat hepatocytes from intracellular toxic responses to cadmium, such as a decrease in thiols including GSH or an increase of lipid peroxide (Müller, 1989; Müller and Menzel, 1990). Concerning vitamin D, cadmium is an osteotoxin and the mechanism of toxicity is suspected to be associated with dietary deficiencies in calcium or vitamin D and phosphaturia induced by the renal tubular toxicity of cadmium (Nomiyama et al., 1979); a protective effect of 25-dihydroxyvitamin D_3 on cadmium toxicity was reported in rat osteosarcoma cells (Angle et al., 1990). Collectively, these vitamins appear to have a protective role in cadmium-induced cellular injury.

Mercury

Organic and inorganic mercury compounds have polluted our environment and have proven to be extremely toxic. Organic mercury compounds, with a higher lipophilicity, can pass the placenta and the blood–brain barrier through an amino acid carrier (Kerper et al., 1992), and most likely target the nervous system. In Minamata, Japan, people were poisoned as the result of consuming fish that had acquired high concentrations of organic mercury due to contamination of local waters with industrial waste (Study Group of Minamata Disease, 1968; Kojima and Fujita, 1973; Kudo and Miyahara, 1988). A more recent concern has been the level of mercury exposure as a result of dental amalgam, a mixture of mercury and other metals (Vimy and Lorscheider, 1985; Molin et al., 1990). Organic and inorganic mercury compounds have been shown to cause genotoxicity such as DNA single-strand breaks (Costa et al., 1991) and chromosomal aberrations in mammalian cells (Betti et al., 1992; Watanabe et al., 1982).

Early studies reviewed the protective effect of vitamin E and selenium on methylmercury

toxicity in animal and nervous tissue culture (Ganther, 1978, 1980), and suggested the involvement of radical species in the toxicity of organic mercury, since the biotransformation of organic mercury by a homolytic process may produce an alkyl radical and a metal radical. Recent studies reported that ·OH and $^1O_2$ appeared to be the active oxygen species mainly responsible for the biotransformation of organic mercury compounds (Suda et al., 1991; Suda and Takahashi, 1992). In the case of inorganic mercury, Woods et al. (1990a,b) showed that the mercuric ion interacts with GSH in the presence of either H_2O_2 alone or H_2O_2 produced by mitochondria to promote formation of multiple free-radical species, including ·OH, glutathionyl radical, and carbon-centered radicals, which oxidize reduced porphyrins *in vitro*. Cantoni et al. (1982, 1984) showed that the DNA strand breaks and GSH depletion caused by this metal were similar to those caused by X-rays in CHO cells, and that an increase of extracellular formation of O_2^-, as measured indirectly by the reduction of cytochrome *c*, was detected in $HgCl_2$-treated CHO cells. Furthermore, they examined the effects of extracellular radical scavengers such as SOD, CAT, mannitol, and vitamins C and E on DNA breaks caused by $HgCl_2$. However, the effects of these extracellular scavengers were difficult to estimate, since similar suppressive effects were obtained using the autoclaved enzymes and since other scavengers caused an inhibition of $HgCl_2$ uptake that was proportional to alterations in DNA breaks. In contrast, pretreatment of cells with SOD inhibitor diethyl dithiocarbamate or GSH depleter DEM resulted in an increase of DNA breakage caused by $HgCl_2$. Similarly, we recently showed that H_2O_2-resistant CHO cells, which have high levels of GSH and CAT activity, were also cross-resistant to the DNA breaks and cytotoxicity caused by $HgCl_2$ (Sugiyama et al., 1993). In human oral fibroblasts, depletion of cellular GSH by BSO was found to enhance the toxicity and to potentiate the depletion of protein thiols caused by Hg^{2+} (Liu et al., 1992). Thus, intracellular SOD, CAT, and GSH may have a protective effect on DNA breaks and cytotoxicity caused by $HgCl_2$.

A protective role of antioxidants in lipid peroxidation induced by mercury compounds has been reported. Lund et al. (1991) showed that $HgCl_2$ increased H_2O_2 formation and depleted GSH in rat kidney mitochondria *in vitro*, both of which are associated with the increase of lipid peroxide, suggesting that mercury-induced oxidative stress in the kidney may be partially due to impairment of the structure and function of the mitochondrial inner membrane. Benov et al. (1990) found that mercury poisoning in rats was associated with increased lipid peroxidation in the liver and in the kidneys and with inactivation of SOD and CAT, and that thiol antidotes such as 2,3-dimercapto-1-propanesulfonic acid and D-penicillamine, which act as O_2^- scavengers and lipid peroxidation inhibitors *in vitro*, suppressed mercury-induced lipid peroxidation in the liver and/or kidneys of rats. Methylmercury was also reported to induce a concentration-dependent increase in lipid peroxidation and a rapid decline in GSH in cerebellar granule cells prepared from rats, and methylmercury-induced lipid peroxidation was not associated with the cell death because this peroxidation could be blocked by vitamin E without appreciably protecting against cell mortality, although significant protection from methylmercury-induced cell death was observed with EGTA, deferoxamine and KCN (Sarafian and Verity, 1991).

The effects of NPSH including GSH in mercury accumulation in the kidney have been extensively studied *in vivo*. It is clear that accumulation of mercury in the kidney is dependent upon cellular NPSH, primarily GSH (Richardson and Murphy, 1975; Johnson, 1982). A recent study has shown that renal accumulation of methylmercury and inorganic mercury in mice is correlated with the hepatic and plasma GSH level, because pretreatment of

the mice with 1,2-dichloro-4-nitrobenzene (DCNB), to deplete hepatic GSH without affecting renal nonprotein thiol level, led to a decrease of hepatic and plasma GSH levels that correlated with decreased mercury levels in the kidney and consequently reduced the renal damage caused by this metal (Tanaka et al., 1991). Furthermore, coadministration of GSH with $HgCl_2$ resulted in an increase in the renal mercury content in mice, suggesting that mercury is transported to the kidney as a mercury–GSH complex (Tanaka et al., 1990). Accumulated methylmercury and inorganic mercury bound to ligands other than MT in renal cytosol appear to be secreted into the lumen of proximal tubules with intracellular GSH (Yasutake et al., 1989; Tanaka-Kagawa et al., 1993). The importance of the role of renal γ-glutamyl-transpeptidase (GGP) in mercury accumulation was recently reported in mice treated with methylmercury or inorganic mercury (Yasutake et al., 1989; Di Simplicio et al., 1990; Tanaka et al., 1990, 1991). Intra- and extracellular GSH levels not only in the kidney but also in other tissues such as liver appear to play important roles in the accumulation of mercury in kidney.

The protective effect of NPSH on the cellular toxicity induced by mercury compounds has been demonstrated. In the kidney, Naganuma et al. (1990) showed that depletion of GSH by treatment of mice with BSO markedly enhanced the lethality and renal toxicity of $HgCl_2$, and that the administration of GSH monoester, which is transported to cells and split intracellularly to GSH, prevented the metal lethality in mice. Another study showed that pretreatment of rats with GSH monoester, which produces high intrarenal levels of GSH, resulted in the moderation of $HgCl_2$-induced acute renal failure, possibly by the chelation of this metal with the excess intracellular GSH, resulting in a reduction of the mercury reactivity with endogenous cellular proteins and enzymes (Houser et al., 1992). Furthermore, depletion of renal NPSH with DEM and BSO, which decreased mercury content in the kidney, was found to cause a greater mortality rate in rats injected with $HgCl_2$, indicating the mortality was not related to the renal damage caused by this metal (Baggett and Berndt, 1986). They suggested that a reduced renal level of mercury may make more mercury available at other target organs, leading to a higher mortality produced by mercury. The depletion of NPSH in the kidney of rats by DEM was reported to cause the enhancement of $HgCl_2$-induced nephrotoxicity while *N*-acetylcysteine pretreatment, which increased cellular NPSH pools, had a protective effect on the nephrotoxicity caused by the metal (Girardi and Elias, 1991). As an initial adaptive response to mercury-induced oxidative stress, GSH synthesis at the genetic level was also shown to be increased in the kidney of rats during prolonged treatment with mercury as methylmercury hydroxide (Woods et al., 1992). In the case of rat lung exposed to mercury vapors, treatment with *N*-acetylcysteine resulted in an increase of survival time and in the percentage of surviving animals, and in a decrease of mercury levels in blood and lung (Livardjani et al., 1991).

NPSH such as cysteine and GSH are well known to be endogenous biliary constituents that form mercury complexes in bile. It has been demonstrated that mercuric and methylmercuric ion may be mainly excreted into bile as complexes with GSH (Gyurasics et al., 1991). After secretion, these complexes are further metabolized by GGP, and then the complexes of methylmercury, but not of inorganic mercury, appear to be extensively reabsorbed from the gallbladder, providing evidence for the biliary–hepatic recycling of methylmercury (Dutczak et al., 1991; Dutczak and Ballatori, 1992). Thus, NPSH in bile plays an important role in the elimination of mercury compounds.

Conclusion

The results presented in this review suggest that the carcinogenic and/or toxic metals induce oxidative damage to DNA and lipids, and that the extent of this damage is influenced by antioxidants *in vitro* and *in vivo*. The decrease of any one of these antioxidants, including enzymes, may lead to the induction of oxidative damage caused by the metal compounds. Below, evidence for the role of active oxygens is summarized under each metal.

Chromium

In vitro studies indicated that not only chromium (VI)/(V) but also the chromium (III)/(II) redox couple serve as cyclical electron donors in a Fenton-like reaction to produce active oxygen species, and that chromium(VI) can catalyze the Haber–Weiss type reaction. In addition to DNA breakage, the *in vitro* formation of 8-OHG in DNA by chromium(VI) and (V) is also involved in the generation of active oxygen species. In mammalian cell studies, the induction of DNA stand breaks by chromium(VI) appears to be associated with active oxygen species and/or chromium(V) formation. However, the relationship between active oxygen and chromium(V) formation in intact cells is obscure. *In vivo* study also showed that the antioxidant systems are modified by treatment with chromium(VI). Among cellular antioxidants, vitamin C plays a particularly important role in the reduction of chromium(VI) in tissues such as lung that contain high levels of vitamin C. However, as discussed previously (Sugiyama, 1992), it is still difficult to reach a final conclusion regarding the mechanism of chromate-induced cellular damage and the mechanism of protection, because of the varied effects of antioxidants on the chromium(VI)-induced damage (see Sugiyama, 1992). It is possible that the differences in experimental conditions, such as in the concentration of chromate and/or antioxidant in cells and tissues used, may be critical factors affecting the reduction of chromium(VI). It is not fully understood what kind of antioxidants, including antioxidant enzymes, play roles in the formation of active oxygen and/or chromium(V) and (III) in a variety of animals and cultured cells.

Nickel

In vitro studies indicated that the complexation of nickel(II) with biological ligands such as protein and oligopeptides such as GSH may undergo redox cycles between the Ni(III)/Ni(II) valance states, leading to generation of active oxygen species and/or nickel–oxygen complexes that may induce oxidative DNA damage such as DNA breakage and/or 8-OHG. However, the formation of nickel(III) has not been identified in these systems. Oxidative DNA adducts were also increased by nickel(II) *in vivo*. Insoluble nickel compounds, but not soluble nickel compounds, appear to induce the production of active oxygen species directly or indirectly through stimulation of phagocytes in PMN, and vitamin E had a protective effect on the cytotoxic, clastogenic and mutagenic actions of the insoluble nickel compounds in cultured mammalian cells. In cultured cells and animal studies, nickel compounds modified the cellular antioxidant system differentially, depending upon the animal species and the organ as well as on the concentration of nickel administration. *In vivo* studies showed that the induction of lipid peroxidation by nickel compounds is associated with cellular levels of antioxidants, in particular GSH and GPx activity. In spite of this evidence, there have been only a few studies that examined the effect of antioxidants such as GSH and vitamins on nickel-induced lethality and genotoxicity in mammalian cells and animal studies.

Cadmium

In cultured cell studies, cadmium-induced lethality and genotoxicity such as DNA breaks and chromosomal aberrations are inhibited by antioxidants. However, the mechanism of the generation of active oxygen species by cadmium compounds is not clear. In addition, the formation of oxidative DNA base modification has not been examined. Many studies suggest that intracellular antioxidants, in particular GSH and MT, play an important role in the prevention of cadmium toxicity, presumably due to their ability to chelate metals and scavenge radicals, and the reduction of cellular antioxidant enzymes by cadmium appears to be related to the induction of lipid peroxidation. Selenium, vitamin D and antioxidants such as vitamins E and C, lipoic acid, and lipoate have been shown to protect against cadmium-induced injury, but the mechanism of their protection remains obscure.

Mercury

Inorganic mercury compounds induced DNA strand breaks that are inhibited by intracellular SOD, CAT, and GSH in cultured mammalian cells. *In vitro* studies indicated that mercuric ion can interact with GSH in the presence of H_2O_2, leading to the generation of active oxygen species. However, the mechanism of the generation of radical species including the redox cycle of the metal and the formation of oxidative DNA modification by mercury compounds has not been studied. The induction of lipid peroxidation by inorganic and organic mercury compounds was blocked by vitamin E and thiol. This lipid peroxidation is associated with the decrease of cellular antioxidants such as GSH, SOD, and CAT. *In vivo* studies show that cellular levels of antioxidants, in particular NPSH including GSH, play important roles in the accumulation and/or elimination of mercury compounds, and in the prevention of mercury-induced cellular injury. Although vitamin E and selenium have been shown to have a protective effect on mercury-induced cellular damage *in vivo*, the mechanism of the protective effects of these antioxidants is not clear.

The effects of antioxidants clearly demonstrate the generation of active oxygen species by these metals. It is likely that the effects of antioxidants on the metal-induced damage are dependent upon many factors such as the animal species, tissue, and cell types, as well as the molar ratio of antioxidants and metals inside cells. More work is needed to elucidate the precise role of physiological antioxidants in the toxic and/or carcinogenic processes of these metal compounds, in order to attain a better understanding of the mechanisms of metal toxicity as well as the mechanisms behind the protective effects of antioxidants.

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Address for correspondence: Masayasu Sugiyama, Department of Medical Biochemistry, Kurume University School of Medicine, Kurume 830, Japan