Environmental Health Perspectives
Vol. 22, pp. 37–41, 1978

General Subcellular Effects of Lead, Mercury, Cadmium, and Arsenic

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This working paper summarizes the known ultrastructural and biochemical effects of lead, mercury, cadmium, and arsenic on subcellular organelle systems following *in vivo* administration. Documented metal-induced alterations in nuclear, mitochondrial, microsomal, and lysosomal functions are discussed in relation to their potential impact on cellular responses to other environmental agents. Each of the above elements has been found to interfere with normal cellular replication and genetic processes. Mitochondrial swelling and depression of respiratory function are discussed in relation to known metal-specific perturbations of mitochondrial heme biosynthetic pathway enzymes. Inhibition of microsomal enzyme activities and protein synthesis by lead and mercury is compared to the apparent absence of such effects following arsenic or cadmium exposure. Lysosomal uptake of all the metals is documented, but biochemical alterations in these structures have been reported for only mercury and cadmium. It is concluded that these toxic metals are capable of interacting with, and biochemically altering major cellular systems at dose levels below those required to produce signs of overt metal toxicity. The impact of these effects on cellular responses to other metals and xenobiotics in complex exposure situations is presently unknown, and further research is urgently needed in this area.

Introduction

Toxic trace metals like lead, mercury, cadmium, and arsenic are present in some fossil fuels and may be emitted in substantial quantities during the course of energy production. Release of these elements into the environment from power plants may lead to their accumulation in soil, water and edible biota at appreciable distances from the original source. Ingestion of pica, water, or food organisms such as fish contaminated with these elements represents a potential human health hazard of unknown magnitude. The problem of assessing biological effects in humans after prolonged low-level exposure to these elements is complicated by a basic lack of understanding concerning the *in vivo* mechanisms of metal toxicity and the impact of trace element exposure on cellular responses to other environmental toxicants such as carcinogens. It is presently clear, however, that many cellular systems affected by trace elements are also sensitive to other classes of toxicants.

The following discussion will attempt to summarize current knowledge of how important subcellular organelle systems respond to prolonged lead, mercury, cadmium and arsenic exposure. It is hoped that this information will provide a basis for discussing possible interactive effects between metals and other environmental agents released during energy production.

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Lead

The effects of lead on subcellular systems have been extensively reviewed by Goyer and Rhyne (*1, 2*). It is clear from these reviews that lead is a broad-spectrum agent which may exert pronounced effects on a number of cellular systems. The following discussion will focus on some of the more salient general effects of lead utilizing an organelle system basis.

Nuclei (Genetic and Chromosomal Effects)

The most prominent early cellular manifestation of prolonged lead exposure is the formation of intranuclear inclusion bodies which are most prominent in the kidney (*3*). These structures which are formed early in the course of lead exposure (*1, 3*) are thought to represent a major intracellular

storage site for lead and in this manner act to reduce exposure of other subcellular systems to this element (*1*). It is worth noting that nuclear exposure to lead may also lead to karyomegaly (*1*), polyploidy, and abnormal mitoses (*4*). Muro and Goyer (*5*) have reported a 13-fold greater incidence of chromosomal gaps in cultured bone marrow cells from mice fed a diet containing 1% lead acetate for 2 weeks in comparison with controls. These authors also noted a 5.4% greater incidence of chromosomal breaks and a 12% greater incidence of chromosome fragments in these cells.

A single low injected dose of lead (5 μg/g) has also been found (*6*) temporarily to stimulate synthesis of new DNA in mouse kidney by a factor of 45 times. This phenomenon was preceded by a general increase in synthesis of new RNA and protein (*7*). It is clear from these studies that lead may interact with cell nuclei *in vivo* and is capable of producing both morphological and functional alteration of nuclear processes in target organ systems.

Mitochondria

The effects of lead on these organelles which are essential to cellular energy production, carbohydrate metabolism, and heme biosynthesis have been extensively studied. Mitochondrial accumulation of lead in the kidney, and the resultant swelling, are early signs of nephrotoxicity from this element (*1*). Biochemical studies of lead-poisoned mitochondria (*2*) have shown NAD-linked substrate mediated respiration in animals fed a 1% lead diet for 10 weeks to be more strongly inhibited than that supported by succinate. Decreases of approximately 23% for mitochondrial control ratios and 23% for P/O ratios were observed. The substrate specificity of this phenomenon is thought to occur via lead inhibition of the lipoic acid dehydrogenase complex. Lead has also been found to inhibit the mitochondrial heme biosynthetic enzymes δ-aminolevulinic acid (ALA) synthetase and ferrochelatase (*1*) which may in part account for depression of mitochondrial cytochrome aa_3 content in lead-poisoned mitochondria (*8*). The important effects of lead on this organelle system are that it may cause cell death by impairment of energy production and compromise cellular detoxification capabilities via inhibition of intracellular hemoprotein function with resultant excretion of coproporphyrin.

Endoplasmic Reticulum (Microsomal Enzyme Activities and Protein Synthesis)

Administration of lead at an injected dose of 5 mg/kg has been found to inhibit the activities of rat hepatic microsomal drug metabolizing enzymes by about 50% and to decrease cytochrome P-450 content by about 40% (*9*). The exact biochemical mechanisms for this effect are unknown, but lead inhibition of cellular heme biosynthesis (*1*) and disaggregation of polyribosomes (*10*) are both potential explanations.

Lysosomes

Studies by Baltrop et al. (*11*) have indicated that some lead is concentrated in lysosomes following prolonged exposure. It is unclear whether this effect occurs as a primary mechanism for metal sequestration or whether it is secondary to autophagy of lead-poisoned organelles such as mitochondria.

Mercury

Mercury is a potent cellular poison whose mode of action and target organ systems are somewhat dependent upon the chemical form of mercury involved. Methylmercury is the mercurial of greatest general environmental concern and hence the following discussion will focus on available data concerning the subcellular effects of this agent.

Nuclei (Genetic and Chromosomal)

Brubaker et al. (*12*) have studied the effects of methylmercury on DNA, RNA, and protein synthesis in several organ systems following subacute exposure to injected doses of 10 mg/kg. These authors observed generally greater synthesis of DNA, RNA, and protein in the livers of treated animals. DNA synthesis increased about 45% at low tissue levels of mercury; however, at higher levels of tissue mercury, DNA synthesis returned to control levels while both protein and RNA synthesis were increased about 77 and 83%, respectively. Similar increases were noted in the brains of these animals. In contrast, renal DNA and protein synthesis were decreased by 36 and 28%, respectively, while RNA synthesis was elevated by about 20%. The authors concluded that, in general, *in vivo* methylmercury treatment did not appear to impair normal nuclear DNA or RNA synthetic processes markedly.

Mitochondria

Prolonged exposure to methylmercury has been found to produce *in situ* mitochondrial damage in both liver and kidneys (*13–17*). These morphological changes are associated with moderate changes in mitochondrial respiratory function and marker enzyme activities (*17, 18*). Hepatic mitochondria from

fetal rats whose mothers were exposed to methylmercury at daily dose levels of 0.42, 0.7, and 1.4 mg/kg for 6 weeks were found to show decreases of 21, 45 and 61%, respectively, in protein synthesis. This effect was associated with decreases of approximately 20–30% in the specific activities of the mitochondrial marker enzymes monoamine oxidase, cytochrome oxidase, and ALA synthetase. Adult renal mitochondria from animals exposed to the same regimen showed a different response pattern (*18*). The most pronounced effect of methylmercury on renal mitochondria appears to entail the selective involvement of mitochondrial heme biosynthesis, leading to a 2.5-fold increase in ALA synthetase and decrease of ferrochelatase to 70% of control values. These enzymatic perturbations result in a porphyrin excretion pattern characterized by an approximately 21-fold increase in urinary coproporphyrin which occurs prior to the onset of overt toxicity (*18*). In summary, the effects of methylmercury on this organelle system may cause cell death and a decrease in intracellular hemoprotein synthesis.

Lysosomes

Mercury derived from methylmercury exposure has been found to accumulate in lysosomes of both liver (*19*) and kidney (*14*), where at dose levels of 0.42, 0.7, or 1.4 mg/kg/day it decreased the activities of β-glucuronidase by approximately 55% and increased the specific activity of acid phosphatase by 80–100% (*15*). The potential biological significance of this effect rests with the fact that lysosomes are also known to accumulate and sequester other foreign compounds such as drugs and dyes (*20*). Perturbation of this system by mercury could presumably alter this cellular defense system in ways which have not been investigated.

Endoplasmic Reticulum (Microsomal Enzyme Activities and Protein Synthesis)

Administration of methylmercury to experimental animals has been found to reduce the activities of hepatic microsomal enzyme systems and cytochrome P-450 content by approximately 50% following injected doses of 5 or 10 mg/kg (*21–23*). Activity of hepatic glucuronyl transferase has been found to be unaffected in these studies (*23*). Methylmercury effects on microsomal drug-metabolizing capability may be attributable to an approximately 2-fold enhancement of CO-binding component degradation (*22*) and/or decrease in protein synthesis at the 10 mg/kg dose level (*12*).

Cadmium

Cadmium is another important toxic metal whose *in vivo* metabolism and cellular mechanisms of toxicity appear to be highly complex.

Nuclei (Genetic and Chromosomal)

The known genetic effects of cadmium have been recently well summarized (*24*). Studies on humans have shown a higher incidence of chromosome abnormalities (about 50–60%) in human populations with known exposure to this element. Data from animal studies have also shown that cadmium can cause malignant tumors at the site of injection. The exact biochemical mechanisms behind these effects and factors which influence the toxic processes are unknown and await further study.

Mitochondria

In vitro studies with Cd^{+2} have shown that this ion becomes strongly bound to mitochondria (*25*) and is capable of inhibiting respiration (by 75%) and oxidative phosphorylation (by 100%) at concentrations as low as $5 \times 10^{-6}M$ (*25, 26*). Complete inhibition of 1-hydroxylation of vitamin D by renal mitochondria at a concentration of $0.025mM$ has also been observed (*27*). Whether these mitochondrial effects of cadmium occur *in vivo* is probably dependent upon the synthesis, availability and degradation of cadmium metallothionein (*27*). The effects of cadmium on other mitochondrial functions have not been studied.

Endoplasmic Reticulum (Microsomal Enzyme Activities and Protein Synthesis)

Cadmium administration is known to result in the synthesis of metallothionein (*28*). This protein appears to play an important role in mediating the *in vivo* metabolism and toxicity of cadmium. The exact mechanisms by which cadmium induces this protein are presently unknown and the effects of cadmium metallothionein synthesis on normal cellular protein synthesis and drug metabolizing function are also unclear.

Lysosomes

Cadmium ions have been found *in vivo* to inhibit normal heterolysosome proteolysis in mice by about 40 percent after a dose of 4.3 mg/kg (*29*). Recent *in vivo* studies (*30, 31*) have shown that the lysosome system may also play an important role in

the metabolism and toxicity of circulating cadmium metallothionein. The impact of cadmium metallothionein on renal lysosome function under chronic exposure conditions remains unknown.

Arsenic

In discussing the subcellular effects of arsenic, it is important to distinguish both chemical form and oxidation state. In general, especially for inorganic arsenic, the trivalent (arsenite) form is considered to be of greatest concern, although the pentavalent (arsenate) is the more common environmental form.

Nuclei (Genetic and Chromosomal)

Arsenicals have been reported *in vitro* to interfere with normal DNA repair (*32–34*). Epidemiological studies have also shown an approximate 5-fold higher incidence of lymphocyte chromosome anomalies (*35*) and 3-fold greater lung cancer incidence (*36*) among smelter workers in high arsenical exposure situations. Animal studies dealing with the carcinogenic effects of arsenic have proven inconclusive to date.

Mitochondria

Arsenicals are known selectively to inhibit NAD-linked mitochondrial respiration and to uncouple oxidative phosphorylation. Konings (*37*) found an approximately 80% decrease in NAD-linked respiration of liver mitochondria incubated with arsenite at a concentration of 0.1*mM*. Similar *in vivo* findings have been recently reported (*38*) in rodents following oral exposure to arsenate at doses of 1.2, 2.2, and 3.5 mg/kg for 6 weeks. Mitochondria from these animals also showed 50–70% increases in the specific activities of the mitochondrial marker enzymes monoamine oxidase, cytochrome oxidase and Mg^{+2}ATPase. Another effect involved perturbation of mitochondrial heme biosynthesis with a resulting porphyrinuria (*39*) which is distinct from that observed with methylmercury or lead. A maximal decrease of heme synthetase activity to 63% of control levels was observed at the 3.5 mg/kg dose level with a resultant increase in urinary uroporphyrin and a lesser increase in coproporphyrin.

Endoplasmic Reticulum (Microsomal Enzyme Activities and Protein Synthesis)

The effects of arsenic microsomal enzyme activities have received relatively little attention but recent studies from our laboratories (*39*) seem to indicate little change in microsomal enzyme activities or cytochrome P-450 content following prolonged oral exposure. A general increase in liver microsomal protein synthesis was also observed in these animals as an apparent sequelae to cell death and replacement.

Lysosomes

Increased numbers of electron dense autophagic lysosomes have been noted (*40*) in renal proximal tubule cells of arsenate-treated rats. Replicate studies (*41*) have failed to demonstrate any significant changes in the activities of lysosomal enzymes.

Summary

It should be clear from the above discussion that toxic metals are capable of interacting with, and biologically altering, major cellular systems at dose levels below those required to produce signs of overt metal toxicity. The impact of these effects on cellular responses to other metals and xenobiotics in complex exposure situations is presently unclear and further research is urgently needed in this area.

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