Metals and Neurotoxicology[1,2]

Robert O. Wright[3]* and Andrea Baccarelli[4,5]

[3]Department of Pediatrics, Children's Hospital, Boston Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115; [4]Department of Occupational and Environmental Health, Maggiore Policlinico Hospital, Mangiagalli and Regina Elena IRCCS Foundation, and University of Milan, Milan 20122, Italy; and [5]Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115

Abstract

Metals are ubiquitous and play a critical role in neurobiology. Transition metals are important because they alter the redox state of the physical environment. Biologically, transition metals catalyze redox reactions that are critical to cellular respiration, chemical detoxification, metabolism, and even neurotransmitter synthesis. Many metals are both nutrients and neurotoxicants, such as iron, zinc, copper, and manganese. Other metals, such as lead and cadmium, are metabolized similarly to these metals, particularly iron. Iron metabolism and genes that regulate iron metabolism may be the key to understanding metal toxicity. Finally, recent evidence demonstrates that early life exposures may program later life and adult disease phenotypes via processes of epigenetics. Parallel work in metals demonstrates that epigenetics may be a critical pathway by which metals produce health effects.   J. Nutr. 137: 2809–2813, 2007.

Introduction

The biological effects of metals are linked to their chemical properties. Transition metals (such as Cu, Fe, and Mn) are particularly adept at catalyzing redox reactions within biological systems. Zn is a nutrient metal that in high dosage can paradoxically promote oxidative toxicity. Heavy metals (Pb, Cd) and metalloids (As) can also induce oxidative toxicity but more likely work by binding to proteins and interfering with metal transport and protein function. Although Pb and methylmercury neurotoxicity is well established, the effects of other metals on brain development have only recently drawn attention. Unfortunately, it appears that excess metal exposure may be a common source of neurotoxicity in multiple populations around the world.

Although metals have multiple effects on biological systems, an understudied effect is their role in programming gene expression. A growing body of evidence suggests that metals may influence epigenetic phenomena which regulate the expression of genes and ultimately their protein products. In this article, we focus on the neurotoxic properties of metals and their ability to mimic the pathways of Fe metabolism. In addition, we review the data on the effects of metals on DNA methylation and discuss how these properties might explain fetal origins of adult disease.

Neurotoxicity of Fe

Research on Fe and neurodevelopment has focused primarily on the effects of Fe deficiency anemia. Nevertheless, there is evidence that excess Fe stores in pregnancy and newborns may be toxic.

Several studies have noted a "U"-shaped association between maternal hemoglobin or serum ferritin (SF) and low birth weight. This association has been attributed by some investigators to a failure of normal plasma volume expansion in pregnancy (1) or to inflammation from undiagnosed perinatal infection, as serum ferritin is a well-known acute-phase reactant. Goldenberg et al. (2) showed that high trimester-2 maternal Fe stores not only predicted low birth weight and prematurity but also predicted decreases in IQ in children who were followed until age 5 y. More recently, Tamura et al. (3) demonstrated a U-shaped association between infant umbilical cord SF and lower IQ scores at age 5 y in an Alabama birth cohort. Subjects in the highest quartile of SF at birth were 3.3-fold more likely than the middle 2 quartiles (95% confidence interval: 1.2–9.1) to score below the 15th percentile in full-scale IQ. Similar findings were reported for subjects in the lowest quartile for SF, suggesting that both high and low Fe stores are associated with poor developmental outcomes. Animal studies also support these findings. Fe supplementation of rats produced a decrease in motor activity and exploratory and stereotyped behaviors similar to that of late iron deficiency (ID) anemia (4). Another report by Fredriksson et al. showed that mice administered large oral doses of Fe at postnatal d 10–12 had long-term effects on spontaneous motor behavior, with the animals showing a lack of habituation of spontaneous activity and poorer performance in the radial maze test at 3 mo of age (5).

Such findings, if validated, will undoubtedly complicate public health efforts at eradicating Fe deficiency but should not be dismissed as confounding from the effects of ferritin as an acute-phase reactant. Excess Fe is known to be neurotoxic in adults, and the possibility that it may also produce health effects in pregnant women and newborns must also be investigated.

Neurotoxicity of Mn

Unlike As and Pb, Mn is not only a toxic metal but also an essential nutrient and is required for many essential enzymatic

reactions (6,7). Although Mn deficiencies are possible, they rarely occur in humans. The primary mechanisms of Mn neurotoxicity are not well understood but appear to involve increased oxidative damage to neuronal cells (8,9). With respect to potential developmental neurotoxicity, Tran et al. demonstrated that increased dietary Mn supplements fed to lactating dams were associated with decreased striatal dopamine levels as well as significant increases in passive avoidance errors (6,10). Excessive Mn intake from environmental and occupational settings is associated with several negative health outcomes including lethargy, tremor, and psychological and neurological disorders resembling both schizophrenia and Parkinson's disease (11). Numerous occupational studies document memory loss, anxiety, nervousness, impulsive-compulsive behaviors, psychotic experiences, fatigue, and sleep disturbances (12–14).

The main sources of environmental exposure to Mn are through the diet, inhalation, and drinking contaminated water. At least 4 studies have reported toxicity from excess Mn in children. In Chinese children, exposure to elevated Mn concentrations in drinking water were associated with lower scores on tests of short-term memory, manual dexterity, and visual-perceptual speed (15). A pilot study of children with attention deficit hyperactivity disorder demonstrated an association with this condition and higher hair levels of Mn (16). Wright et al. demonstrated an inverse association between hair Mn and IQ in grade-6 children in Oklahoma (17). Finally, Wasserman et al. (18) found associations between high water Mn levels and full-scale, performance, and verbal IQ among 142 children 10 y of age in Bangladesh.

Neurotoxicity of As
Arsenic has traditionally been classified as a peripheral neurotoxin with a clinical manifestation of polyneuropathy, but recent evidence from animal studies suggests that As also affects the central nervous system and that prenatal exposures influence neurological phenotypes in offspring. Arsenic has been shown to pass readily through the placenta, and mice born to As-exposed dams had elevated As concentrations in their brain tissue (19). Others have shown that rats born to dams dosed orally with As had learning and behavioral deficits (20). Compared with unexposed controls, rats exposed to As prenatally had increased spontaneous locomotor activity and increased errors on memory test (20). Mechanistically, these deficits may be caused by increased oxidative toxicity. Arsenic in drinking water will produce a dose-dependent decrease in glutathione, superoxide dismutase, and catalase in the brain, indicating neurotoxic oxidative stress (21,22) Among rats exposed to As during pregnancy, fetal brain neurons underwent apoptotic changes and neuronal necrosis (23).

Historical case studies report As neurotoxicity in Japanese infants who survived an outbreak of As poisoning from contaminated milk powder in 1955 (24). Recent epidemiological studies include a cross-sectional study in Mexico that found that higher levels of urinary As were significantly related to poorer performance on verbal memory, verbal comprehension, and long-term memory (25). These findings are consistent with results from studies in Taiwan, the United States, and Bangladesh (26,18).

Neurotoxicity of Cd
The neurotoxicity of Cd in children was investigated in several studies in the 1970s and 1980s but has received little attention since. In most of these studies, the biomarker of exposure was the concentration of Cd in hair. In case-control studies in which the hair concentration of Cd of a clinically defined group was compared with that of a reference group, higher concentrations were reported in children with mental retardation (27–29), and learning difficulties or dyslexia (30,31). In cohort studies, Thatcher et al. (32,33) reported that the concentration of Cd in hair was inversely related to adjusted IQ. Other investigators (34) have reported associations between hair Cd and children's performance on visual-motor tasks. However, No population-based studies of the neurotoxicity of Cd have been conducted in children to date.

Neurotoxicity of Cu and Zn
Like Fe, most of the literature on the neurotoxicity of Cu and Zn centers around nutritional deficiency and its effect on brain (35). Also, as for Fe, there is evidence of neurotoxicity when these metals are found in excess in the brain. Cu is a transition metal, and consequently, its metabolism and toxicity are similar to those of Fe and Mn. As for Fe, genetic diseases of excess Cu retention are well described and have significant neurologic sequelae. Wilson's disease is the most common of these diseases, and the presenting complaint for this genetic disorder frequently includes neurobehavioral changes resembling schizophrenia (36). These neurologic findings may even precede other findings such as liver disease. Descriptions of environmental or excess dietary Cu producing subclinical neurobehavioral effects are very rare, but these have not been systematically studied. Excess brain Cu is a common finding in neurodegenerative diseases such as Alzheimer's disease.

Zn deficiency has long been known to impact neurodevelopment adversely, but the effects of excess Zn on neurodevelopment are essentially unknown. Excess Zn, like excess Fe and Cu, is a common finding in neurodegenerative disease (37). Zn finger proteins are key transcriptional elements that regulate the cellular response to metal toxicity among other processes. Excess Zn is involved in the neuronal injury observed in cerebral ischemia, epilepsy, and brain trauma. Toxic Zn accumulation may result from either transsynaptic Zn movement or mobilization from intracellular sites, such as Zn flux through receptor-associated calcium channels, voltage-sensitive calcium channels, or Zn-sensitive membrane transporters (38). The mechanisms by which Zn exerts its neurotoxicity include mitochondrial production of reactive oxygen species and the disruption of metabolic enzymes, ultimately leading to activation of apoptotic processes. As with Cu, Fe, and Mn, an exciting new area of research is the role of Zn metabolism in Alzheimer's disease as a trigger for amyloid-β aggregation and neuronal plaque formation.

As we previously noted, excess Fe, particularly during pregnancy, has been associated with neurodevelopmental outcomes later in life, and similar studies of Cu and Zn in pregnancy are sorely needed.

Neurotoxicity of Pb and methylmercury
The literature supporting the neurodevelopmental toxicity of both Pb and methylmercury is extensive, and a comprehensive summary of either metal is beyond the scope of this article. Although controversy still exists regarding the levels at which Pb toxicity manifests itself clinically, there is widespread acceptance that Pb is neurotoxic. With respect to methylmercury, for which the primary exposure source is fish consumption, the most pressing research question at present is how to balance the beneficial effects of fish consumption vs. the toxic effects of methylmercury.

Metal mixtures and neurodevelopment
There are few studies that have reported on the effect of chemical mixtures in humans despite the fact that many metals are

commonly encountered as mixtures in the environment. Initial studies merely reported correlations among markers of internal metal dose, including a positive correlation between blood Pb and Mn (39,40), and a positive correlation between urine As and blood Pb among children (25).

With respect to neurologic outcomes, we are unaware of any clinical data investigating joint exposures to Mn and Pb. However, animal studies provide compelling evidence that exposure to both Mn and Pb lead to synergistic neurological effects. Among rats orally exposed to both Mn and Pb, motor activity and neurotransmitter levels were significantly increased, compared with rats exposed to only 1 metal (41). Exposure to Pb and Mn decreased learning of conditioned avoidance responses more than either Pb or Mn alone, and gestational exposure to both Pb and Mn reduced brain weight to a greater extent than either metal alone (42). Each of these studies also showed that coexposure to Mn and Pb led to increased brain Pb levels, perhaps because of changes in affinity of Pb-binding proteins in the brain (43). In addition, multiplicatively greater changes in monoaminergic neurotransmitter levels occur in the brains of rats exposed to Pb and As jointly, compared either metal alone (44) or to combinations of Pb, Mn, and As. Rodriguez et al. observed that Mn and As had greater accumulation in rat brains relative to controls with single metal exposures (45). The 3 metal concentrations when combined were associated decreases in dopaminergic metabolites and increases in serotinergic metabolites. Overall, these findings are complex, but the data support the concept that coexposure to multiple metals may cause neurotoxic effects not seen with exposure to a single metal at the same dose.

Fetal programming

There is growing evidence that exposure to toxicants in early life may cause later life health effects. The observed phenomenon of "fetal origins of disease" suggests that early environmental exposures, such as metals, program later life gene expression. There is an increasing search for the biological process by which programming occurs. Because DNA sequence is static, genetic susceptibility from DNA sequence variation cannot explain the mechanisms by which prenatal or early childhood metal exposures impact cognition and behavior later in life. One possible mechanistic pathway for this phenomenon, which has yet to be fully explored in humans, is epigenetics. Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. Such changes can have influences as profound as those exerted by mutations but, unlike mutations, are reversible and responsive to environmental influences. DNA methylation is the best studied of the epigenetic processes that regulate gene silencing. In general, increased methylation is inversely associated with gene expression. DNA methylation has been associated with chromosome packaging and heterochromatin formation and determines the 3-dimensional space through which transcription factors can or cannot attach to the DNA sequence. Specifically, the methylated cytosine in DNA promoter regions serves as a "mutation" of a promoter region recognition element, functionally reducing the binding affinity of the response element for its transcription factor. Although in the strictest sense epigenetics refers to changes in germ cell DNA methylation, the process of DNA methylation is critical more globally to cell differentiation and overall child development. Failure of DNA methylation systems in the brain leads to clinical syndromes such as mental retardation and autistic-like behaviors (46). Animal studies increasingly demonstrate that environmental factors can alter DNA methylation patterns and that these changes correlate with animal behavior (47).

The growing interest in epigenetic markers is a result of their potential to explain fetal origins of disease or even simply explain the latency between exposure to toxic substances and subsequent disease phenotypes. Although DNA methylation patterns in different tissues are largely constitutive, DNA methylation patterns are still subject to active regulation in the nervous system in response to environmental stimuli. Endres et al. (48) recently demonstrated that levels of DNA methylation activity in the brain are increased with ischemic injury. Others have shown that diet can impact methylation and behavior. For example, L-methionine treatment can exacerbate psychosis, whereas valproate, a drug producing hypomethylated DNA, reduces such symptoms (49,50). Epigenetic modifications of regulatory DNA sequences in response to subtle variations in environmental conditions might be a critical source of variation in gene expression and function. If so, DNA methylation changes may serve as a process mediating the relationship between genome and environment throughout neurodevelopment. The effects of prenatal/early metal exposure on DNA methylation may program environmental exposures on the fixed genome, resulting in subtle but stable alterations in later life neurophenotypes.

Metals and DNA methylation

Several studies have established an association between DNA methylation and environmental metals, including Ni, Cd, Pb, and particularly As (51–53). Oxidative stress may be a unifying process to explain these findings across different metals. Metals are known to increase reactive oxygen species production in a catalytic fashion via redox cycling (54,55). Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA (56), thus resulting in a generalized hypomethylation of cytosine residues at CpG sites (57). In addition, Takiguchi et al. (58) showed that Cd inhibited DNA methyltransferases in a manner that was noncompetitive with respect to the DNA substrate. This finding is suggestive of interference in enzyme-DNA interaction, possibly through an interaction of Cd with the methyltransferase DNA binding domain (58).

As is the best-studied metal with effects on DNA methylation. Several in vitro studies have shown that As is associated with global DNA hypomethylation (59–61) as well as gene-specific DNA hypermethylation (62,63). This effect might be explained by the overlap between As metabolism and DNA methylation processes. Both consume S-adenosylmethionine, the universal methyl donor, which is a critical cofactor for both DNA methylation and the methylation of xenobiotics. In animal models, global DNA hypomethylation induced during gestation has been shown to perturb the function and survival of central nervous system neurons (64). Metal-induced alterations in methylation metabolism could initiate a cascade of events including gene-specific DNA hypo- or hypermethylation, resulting in aberrant gene expression and also in diminished glutathione activity leaving cells more vulnerable to oxidative stress. Although the results of these epigenetic changes on neurodevelopment have remained unexplored, given the clear importance of DNA methylation to processes of neurodevelopment, the metal-induced disruption of DNA methylation clearly deserves further study.

Neurotoxicity is a common health endpoint for excess metal exposure. Even nutritional metals, such as Fe and Mn, are neurotoxic in excess. Because real-life scenarios include exposures to multiple metals simultaneously, there is a growing need for research on mixtures of metals and their health impact. Finally, there is intriguing evidence that epigenetic phenomena may underlie observed effects of fetal or early life exposure and late onset of disease. Metals appear to alter DNA methylation, an epigenetic

process by which gene expression is regulated. Further research in metals should include the role of epigenetics in determining long-term and late-onset health effects from metal exposure.

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