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Prenatal manganese levels linked to childhood behavioral disinhibition

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Abstract

Although manganese (Mn) is an essential mineral, high concentrations of the metal can result in a neurotoxic syndrome affecting dopamine balance and behavior control. We report an exploratory study showing an association between Mn deposits in tooth enamel, dating to the 20th and 62–64th gestational weeks, and childhood behavioral outcomes. In a sample of 27 children, 20th week Mn level was significantly and positively correlated with measures of behavioral disinhibition, specifically, play with a forbidden toy (36 months), impulsive errors on a continuous performance and a children's Stroop test (54 months), parents' and teachers' ratings of externalizing and attention problems on the *Child Behavior Checklist* (1st and 3rd grades), and teacher ratings on the *Disruptive Behavior Disorders Scale* (3rd grade). By way of contrast, Mn level in tooth enamel formed at the 62–64th gestational week was correlated only with teachers' reports of externalizing behavior in 1st and 3rd grades. Although the source(s) of Mn exposure in this sample are unknown, one hypothesis, overabsorption of Mn secondary to gestational iron-deficiency anemia, is discussed.

Keywords: Manganese exposure; Tooth biomarker; Developmental neurotoxicology; Dopaminergic systems; Behavioral disinhibition

1. Introduction

Manganese (Mn), an essential nutrient, plays a part in bone mineralization, protein and energy metabolism, metabolic regulation, and cellular protection from damaging free radical species. It is a cofactor for enzymes such as Mn superoxide dismutase, arginase, and pyruvate carboxylase [29]. However, high concentrations of Mn can result in a neurotoxic syndrome affecting monoaminergic systems, in particular dopamine (DA) [15,16]. A number of mechanisms of Mn-induced neurotoxicity have been proposed, including DA receptor destruction mediated by free radicals [26], alteration of membrane processes via Mn-influenced neuronal oxygen reduction [20], increased iNOS mRNA and the release of neurotoxic nitric oxide [46], impaired glutamate uptake due to decreased glutamate/aspartate transporter (GLAST) mRNA [19], decreased metallothionein (MT-I) mRNA impairing the sequestration of oxidants by metallothionein [19], and enhanced expression of the transferrin receptor, inducing iron-induced oxidative stress in sensitive brain regions [65].

Exposure may occur via inhalation of Mn dust, bypassing the hepatic homeostatic process so that Mn remains available for tissue uptake over longer periods of time [4,60]. This exposure route became a matter of particular concern with the introduction of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline additive in Canada, in 1977, and, since 1995, in the US (although its use is prohibited in some states, notably California). It has been shown that increased tissue absorption of Mn, coupled with perturbations of brain chemistry and development, will occur in experimental animals exposed prenatally (via maternal inhalation) and/or neonatally to MnO_2 dust [11,45]. There is also evidence of Mn-induced neurotoxicity from high concentrations of the metal in drinking water [30,61].

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However, food is the major source of absorbed Mn in the general population [54], and certain groups, such as neonates and infants, are more vulnerable than adults to Mn via intestinal absorption [34]. In the young rodent, intestinal absorption of Mn is on the order of 70%, compared to the 1–2% in the adult rat; further, Mn enters the neonatal brain at a much higher rate than in adult animals [17,36]. With respect to prenatal exposure, Mn concentrations in umbilical cord blood have been found to be 33% to 50% higher than in maternal blood, suggesting not only an active transport system, but also a concentrating mechanism [31,35].

Suckling animals exposed to high dietary levels of Mn show not only higher levels of Mn in the brain but also decreased striatal DA and increased behavioral deficits [51–53]. The pups of female rats exposed to high concentrations of Mn in their drinking water during pregnancy absorbed 35% to 150% more Mn into their brains than controls, accompanied by significantly increased locomotor activity [38]. This finding is of interest because perturbations of DAergic networks have been associated with behavioral disinhibition, as found in conditions such as attention deficit-hyperactivity disorder (ADHD), which affects 3% to 5% of all children [48]. Furthermore, higher levels of Mn have been found in the head hair of children with hyperactivity [9,13].

There are several methods for measuring Mn concentrations in humans, each with its unique advantages and drawbacks. Mn levels in whole blood, the most widely accepted measure of Mn status, provide a convenient record of recent Mn exposure (half life ca. 37 days) [34]. A more refined index of Mn exposure in industrial workers can be obtained by neutron activation analysis of liver, indicating recent exposure [5], and bone, showing body burden [6]. Mn exposure over a matter of months can also be determined by analysis of hair, a keratin protein that acts as a reporter for inorganic elements absorbed from the diet [47]. The analysis of tooth enamel provides a reliable measure of the accretion of Mn over a much longer time frame [18,20]. Enamel crystals provide a longitudinal record of absorption, analogous to levels of pollutants recorded in tree rings. Thus, deciduous teeth provide a useful, accessible manner for assessing exposure to Mn at specific epochs in development. The cusp tip of the first molar, formed at 20 weeks gestation and shed at 10–11 years of age, provides a record of Mn exposure at approximately the 20th gestational week; the root tip of the molar provides a record of Mn exposure at approximately the 63rd gestational week (7 months postnatal) [58].

Here, we report relations between prenatal and early postnatal Mn, as reflected in Mn deposits in tooth enamel formed around the 20th and 63rd gestational weeks, and childhood behavioral outcomes. Because both iron (Fe) and lead (Pb) body burdens have been demonstrated to correlate with Mn body burdens [21], and because excess Fe has been shown to be neurotoxic in infants [49], we also assessed the concentrations of these metals. Because Mn neurotoxicity preferentially affects dopaminergic networks, we expected to observe a correlation between Mn and measures of DA-mediated behavior, in particular behavioral disinhibition, which is considered the preeminent symptom of ADHD [7,8].

2. Methods

2.1. Participants

Participants were children from the NICHD Study of Early Child Care and Youth Development (SECCYD), a prospective longitudinal study of development that began in 1991 with 1364 normal newborns [37]. The institutional review boards of the participating university sites approved the original SECCYD study and permitted the use of unlinked archival behavioral data and discarded tissue (i.e., teeth) for the present analysis. From this national sample, shed molars were collected from 400 children who lost a tooth between 11 and 13 years of age. Those providing teeth were not significantly different from the SECCYD sample as a whole in terms of family income, ethnicity or child behavioral outcomes. However, parents in the former group had significantly higher education levels (14.7 years vs. 14.1 years, $p < .05$).

For this exploratory study, 27 teeth were randomly selected, from children at seven of the ten study sites: Little Rock, AR, Lawrence, KS, Boston, MA, Pittsburgh, PA, Philadelphia, PA, Morganton, NC, and Madison, WI. (Sample size was dictated by budgetary limitations; analysis of the full sample will be completed when additional funding becomes available.) The sample contained teeth from 11 boys and 16 girls. Mean maternal education was 14.2 years (S.D. = 2.31), average family income 2.3 times the poverty threshold (S.D. = 1.70), and 89% of the subjects were Caucasian. The representativeness of the selected sample was determined via a quasi Monte Carlo method, in which multiple random samples of $n = 27$ were taken from the population of 400. No demographic characteristic of the study sample exceeded the 90% confidence limits derived from the multiple population samples.

2.2. Measures

Included in the SECCYD database were measures of behavioral inhibition collected at ages ranging from 3 to 9 years (see Table 1 for the age of assessment and statistical properties of each measure). Three measures were based on direct behavioral assessment: The *Forbidden Toy Task* (FTT) was administered at 36 months to assess the child's ability to delay or inhibit play with an attractive toy when asked by the experimenter to do so. The measure of interest was the amount of time spent actively playing with the forbidden toy (intercoder reliability = .98) [42,55]. The *Mirsky Continuous Performance Test* (CPT) was administered at 54 months of age [41]. The CPT is a widely used assessment of sustained attention and impulse control with high construct validity, acceptable test–retest reliability ($rs = .65$ to .74) [27], and established predictive validity for clinical group membership [7,12]. Computer-generated pictures of ten familiar objects (e.g., butterfly, fish, flower) were presented on a 2-inch square screen. The child was asked to press the button "as fast as you can" each time a target stimulus (a chair) appeared on the screen. Errors of commission (impulsivity) were recorded when children pressed the button for a non-target. The *Children's Stroop Test* was administered at