Environmental Research 159 (2017) 458–465

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Sex differences in sensitivity to prenatal and early childhood manganese exposure on neuromotor function in adolescents

Yueh-Hsiu Mathilda Chiu[a,b,*], Birgit Claus Henn[c], Hsiao-Hsien Leon Hsu[a], Mathew P. Pendo[d], Brent A. Coull[e,f], Christine Austin[a], Giuseppa Cagna[g], Chiara Fedrighi[g], Donatella Placidi[g], Donald R. Smith[h], Robert O. Wright[a,b], Roberto G. Lucchini[a,g], Manish Arora[a]

[a] Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[b] Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[c] Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA
[d] Center for Medicine, Health and Society, Vanderbilt University College of Arts and Science, Nashville, TN, USA
[e] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[f] Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[g] Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Section of Occupational Medicine, University of Brescia, Brescia, Italy
[h] Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, USA

ARTICLE INFO

Keywords:
Manganese
Tooth biomarker
Motor function
Prenatal and postnatal exposure
Adolescents
Sex difference

ABSTRACT

Introduction: While studies have suggested that exposure to manganese (Mn) may be associated with neurodevelopment in school-age children, there is limited information on prenatal and postnatal Mn exposures and tremor or motor function in children.
Methods: We measured Mn levels in dentine of shed teeth, representing prenatal, early postnatal, and cumulative childhood exposure windows, from 195 children (predominantly right-handed, 92%) in Italy. Pursuit Aiming, Luria Nebraska Motor Battery, as well as Tremor and Sway system from Computerized Adaptive Testing System (CATSYS) were administered at 11–14 years old. We examined the relationships of tooth Mn (ln-transformed) with motor function using multivariable linear regressions and generalized additive models, adjusting for age, sex, and socioeconomic status index. Effect modification by sex was also examined.
Results: We found that higher prenatal Mn was associated with better body stability in boys in a number of sway tests (including mean sway, transversal sway, sagittal sway, sway area, and sway intensity), while Mn was associated with poorer performance in girls on all of these metrics (all p for Mn × sex interaction < 0.05). Higher prenatal Mn was also modestly associated with better hand/finger and eye-hand coordination in boys compared to girls in sex-stratified analyses, although interaction models did not reach statistical significance. For tremor, on the other hand, higher early postnatal Mn was associated with increased right-hand center frequency in girls (p for interaction < 0.01), but increased Mn level at the later postnatal period was associated with increased center frequency in boys (p for interaction $= 0.01$).
Conclusions: This study, which used a direct measure of prenatal and childhood Mn exposure, suggested sex-specific critical windows of early life Mn exposure in relation to neuromotor function in adolescents. The sex-specific associations might be strongest with measures of whole body stability, for which the critical exposure window was during the prenatal period.

1. Introduction

Manganese (Mn) is an essential element necessary for a number of biological processes. It plays crucial roles in healthy body growth, immune system function, and the regulation of metabolism and bone growth (ATSDR, 2012; Yoon et al., 2011). However, Mn has also been

Abbreviations: AUC, area under the curve; BMI, body mass index; BSID, Bayley Scales of Infant Development; CATSYS, Computerized Adaptive Testing System; CHAMACOS, Center for the Health Assessment of Mother and Children of Salinas; GAMs, generalized additive models; LA-ICP-MS, laser ablation inductively coupled plasma mass spectroscopy; Mn, manganese; NL, neonatal line; PDI, psychomotor development index; PHIME study, Public Health Impact of Manganese Exposure study; SES, socioeconomic status

* Correspondence to: Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1057, New York, NY 10029, USA.
E-mail address: mathilda.chiu@mssm.edu (Y.-H.M. Chiu).

http://dx.doi.org/10.1016/j.envres.2017.08.035
Received 14 March 2017; Received in revised form 25 July 2017; Accepted 18 August 2017
Available online 18 September 2017

recognized as a potential neurotoxin when present in excess levels (Kern et al., 2010). The most widely studied neurological health outcome related to Mn exposure is termed manganism, a Parkinson's-like condition observed in adults with high occupational exposure to Mn (Lucchini et al., 2009; O'Neal and Zheng, 2015). The negative neurological health outcomes correlated with Mn overexposure in children include behavioral disinhibition (Ericson et al., 2007), olfactory and motor function (Lucchini et al., 2012), and hyperactivity (Mora et al., 2015). However, investigation of the pediatric health outcomes of Mn effects has also led to inconsistent findings. For example, Ode et al. (2015) found no significant relationship between umbilical cord Mn levels and hyperactivity. Additionally, there is a lack of knowledge specifically on tremor and motor development in children with prenatal and postnatal Mn exposures. Most previous studies on tremor and neuromotor functions focused on elderly or adult populations.

Much of the inconsistency in findings among studies of Mn as a pediatric neurotoxin might be due in part to the lack of an accepted standard biomarker for Mn levels as well as measurements taken at different exposure time points. In recent years, dentine in the shed teeth of children has emerged as a novel validated biomarker that has the ability to accurately measure Mn exposure in more precise periods of development (Arora et al., 2012, 2011; Gunier et al., 2015, 2013). The primary benefit of this novel biomarker is the ability to determine a timeline of exposure that can be differentiated based on developmental periods.

Further, animal studies have shown potential sex differences in the association between Mn and neurodevelopmental outcomes. For example, behavioral damage associated with Mn exposure in rats showed a sex-specific response where male rats were more severely impacted, and female rats did not experience as severe motor damage until the dose was increased (Yamagata et al., 2016). However, to date, limited human research has studied the potential for effect modification by sex on the associations between perinatal Mn exposure and neuromotor functions.

To address these research gaps, the goal of this study was to investigate the relationships of early life exposure to Mn, measured in dentine of shed teeth, with motor function in adolescents. Specifically, we considered tooth Mn levels in different exposure windows, including prenatal, early postnatal, and childhood cumulative periods. The major focus was to examine effect modification by sex, and to disentangle the effects on whole body stability (e.g. sway) from finer/local motor skills.

2. Materials and methods

2.1. Study participants

Participants of the Public Health Impact of Manganese Exposure (PHIME) Study were enrolled from a total of 20 junior high schools through the local public school system in Italy. These schools were located in three communities with different levels of Mn exposure (historical, current and reference areas). Details about the study sites and the recruitment process were described previously (Lucchini et al., 2012). In brief, teachers, parents and children were informed with ad hoc meetings and brochures. Subjects who agreed to participate filled in a screening questionnaire for the evaluation of inclusion and exclusion criteria. The inclusion criteria were: (i) to be born in the study area from resident families living in the study area since the 1970s; (ii) to live in the study area since birth; (iii) to be aged 11–14 years. The exclusion criteria included: (i) pathological conditions potentially affecting neuro-development, including neurological, hepatic, metabolic, endocrine and psychiatric diseases; (ii) consumption of medications with known neuropsychological side-effects; (iii) clinically diagnosed motor deficits of hand and fingers; (iv) clinically diagnosed cognitive impairment and behavioral manifestations; (v) visual deficits not adequately corrected. A total of 720 adolescents were enrolled into the study. In 2013, supplemental funding was obtained to conduct an initiative to assess tooth biomarkers in a subset of these participants. The study design, study aims, and the forms for informed consent had been reviewed and approved by the ethical committees of the local Public Health agencies of the study sites, University of Brescia, and Icahn School of Medicine at Mount Sinai.

2.2. Tooth collection and measurement

For this initiative, we contacted children within tooth shedding age to request that they provide their shed deciduous teeth, and a total of n = 195 participants were able to provide at least one shed tooth that was suitable for analysis at the time of this study. Incisors that were free of obvious defects such as caries and extensive tooth wear were analyzed. Detailed analytical methods have been described elsewhere (Arora et al., 2012, 2011). In brief, we first identified the neonatal line (NL), a histological feature formed in deciduous teeth at birth, using light microscopy. With the NL as a reference point, the concentrations and spatial distribution of Mn in different developmental windows were determined using laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) as detailed previously (Arora et al., 2012, 2011). Prenatally formed primary dentine adjacent to the enamel-dentine junction was sampled to obtain *prenatal Mn exposure* information; postnatally formed primary dentine after the NL was sampled to obtain *early postnatal Mn exposure* information, which reflects the exposure at approximately 0–1 years old. Measurements in secondary dentine, formation of which starts after the completion of the tooth root and proceeds at a slower rate as long as the tooth remains vital, were used to estimate *childhood cumulative Mn exposure* from root completion to the time the tooth was shed. All tooth Mn levels were normalized to measured tooth calcium levels (^{55}Mn:^{43}Ca ratio) to provide a measure independent of variations in mineral density. Multiple measurements were taken in prenatal and postnatal dentin, and thus the area under the curve (AUC) of Mn levels across all sampling points was calculated to estimate cumulative Mn exposure during each developmental period. Final Mn exposure values are (^{55}Mn:^{43}Ca AUC) × 10,000 for prenatal and early childhood exposures, and average ^{55}Mn:^{43}Ca for cumulative childhood levels. Values below the detection limit (n = 2 for early postnatal and childhood cumulative samples) were assigned half of the lowest value among the samples above the detection limit.

2.3. Motor function assessment

The neuromotor test battery was designed based on the review of the tests reported in the Mn neurotoxicity literature (Zoni et al., 2007; Zoni and Lucchini, 2013). We assessed two major domains–whole body related postural measurements and hand related motor measurements. Whole body postural sway/stability was assessed using the force plate test of the CATSYS 2000 system (Danish Product Development, Ltd) (Despres et al., 2000). The participants were asked to stand erect on a force platform with eyes opened and with eyes closed, each for 75 s. The force plate produces signals from three sensors to provide a map of the position of the force center during the test period, and the change in position of the force center can be observed in a X-Y coordinate system. The indices recorded include mean sway (mm), transversal sway (mm), sagittal sway (mm), sway area (mm^2), sway velocity (mm/s), and sway intensity (mm/s^2) (Despres et al., 2000). Hand/finger motor coordination was assessed with the 5 subtasks from the Luria Nebraska Neuropsychological Battery (Golden et al., 1980), which consists of dominant hand clenching, non-dominant hand clenching, alternative hands clenching, thumb-touching of dominant hand, and thumb-touching of non-dominant hand, each lasting 10 s. We then calculated the sum and the mean score for each participant based on the scores of these subtasks. Hand dexterity and eye-hand coordination was assessed using the Pursuit Aiming test (Fleishman, 1954), in which the participants were asked to use a pencil to place one dot inside each circle with 2 mm diameter on a test sheet that consists of a series of circles