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Effect of environmental manganese exposure on verbal learning and memory in Mexican children

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ABSTRACT

Manganese (Mn) is an essential metal, but in excess it becomes neurotoxic. Children's developing nervous system may be especially vulnerable to the neurotoxic effects of overexposure to this metal. The aim of this study was to assess the effect of Mn exposure on verbal memory and learning in 7- to 11-year-old children. We tested 79 children living in the Molango Mn-mining district and 95 children from a non-exposed community in the same State of Mexico. The Children's Auditory Verbal Learning Test (CAVLT) was administered. Blood and hair samples were obtained to determine Mn concentrations using atomic absorption spectrophotometry. CAVLT performance was compared between the two groups and multilevel regression models were constructed to estimate the association between biomarkers of Mn exposure and the CAVLT scores. The exposed group presented higher hair and blood Mn ($p<0.001$) than the non-exposed group (median 12.6 vs. 0.6 μg/g, 9.5 vs. 8.0 μg/L respectively), as well as lower scores ($p<0.001$) for all the CAVLT subscales. Hair Mn was inversely associated with most CAVLT subscales, mainly those evaluating long-term memory and learning ($\beta=-0.47$, 95% CI -0.84, -0.09). Blood Mn levels showed a negative but non-significant association with the CAVLT scores. These results suggest that Mn exposure has a negative effect on children's memory and learning abilities.

1. Introduction

Manganese (Mn) is an essential metal (Aschner et al., 2007), but under certain conditions, exposure to high amounts can be neurotoxic (Keen et al., 2005). Mn overexposure in adults has been associated with cognitive and psychiatric impairment (Guilarte and Chen, 2007). In highly exposed workers, it can cause a neurological disabling disorder similar to Parkinson's disease, called "manganism" (Calne, 1997). The most important neurotoxic effect caused by Mn exposure is damage to the basal ganglia after chronic inhalation of dust containing Mn in both animals and humans (Aschner et al., 2007; Calne, 1997). Other studies with non-human primates have reported that Mn also affects the prefrontal cortex (Dorman et al., 2006), resulting in motor and executive function impairment (Guilarte et al., 2006; Schneider et al., 2009, 2006).

Results reported for adult populations show motor and cognitive impairment due to Mn exposure. To date, most of the research concerning Mn neurotoxicity has been carried out primarily with occupationally exposed populations (Bowler et al., 2006a; Bowler et al., 2006b, 1999; Mergler et al., 1994; Zoni et al., 2007; Lucchini et al., 1995); few studies of environmental exposure have been performed (Mergler, 1998; Mergler et al., 1994; Rodríguez-Agudelo et al., 2006; Solís-Vivanco et al., 2009). In the workplace, there are high peak-exposure periods, while environmental exposure is lower but continuous (Hudnell, 1999). The neuropsychological effects of

Abbreviations: As, Arsenic; ATSDR, Agency for Toxic Substances and Disease Registry; CAVLT, Children's Auditory Verbal Learning Test; Cd, Cadmium; CI, Confidence Interval; CONAPO, Consejo Nacional de Población (National Population Council); EDTA, ethylene diamine tetraacetic acid; ICC, Intraclass Correlation; INEGI, Instituto Nacional de Estadística, Geografía e Informática (National Institute of Statistics, Geography and Informatics); INNNMVS, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez" (National Institute of Neurology and Neurosurgery); INSP, Instituto Nacional de Salud Pública (National Institute of Public Health); IQ, Intelligence Quotient; g/dL, Grams per deciliter; MeV, Mega Electron Volt; μg/dL, Micrograms per deciliter; μg/L, Micrograms per liter; μg/g, Micrograms per gram; μg/m^3, Micrograms per cubic meter; Mn, Manganese; NMDA, N-methyl-D-aspartate; Pb, Lead; $PM_{2.5}$, Particles less than 2.5 μm in diameter; ppb, Parts per billion; UNAM, Universidad Nacional Autónoma de México (National Autonomous University of Mexico); USEPA, United States Environmental Protection Agency

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chronic environmental exposure to Mn (Mergler et al., 1999; Rodríguez-Agudelo et al., 2006) may be on a "continuum of dysfunction" (Mergler et al., 1999).

Children are considered especially vulnerable to neurotoxic agents (Grandjean and Landrigan, 2006; Weiss, 2000), because damage to specific brain structures in early development can result in impaired cognitive functions (Weiss, 2000). Lead, for example, is a confirmed neurotoxicant, and its detrimental effect on IQ has been the outcome most often studied (Needleman, 2006; Weiss, 2000). To date, there are few reports assessing Mn neurotoxic effects in children, and most of these studies have analyzed intellectual functions (Bouchard et al., 2011; Menezes-Filho et al., 2011; Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2009; Wasserman et al., 2006a, 2011; Wright et al., 2006b).

Some studies have reported impairment of other cognitive functions, such as memory and learning, in children due to Mn exposure. A study carried out in the United States (Wright et al., 2006) with 11- to 13-year-old children living close to waste deposits with high arsenic (As), Mn, and cadmium (Cd) content reported a negative association between As and Mn levels and the Children's Auditory Verbal Learning Test (CAVLT) scores. He et al. (1994) found low scores in short-term memory tasks, hand skills, and perceptual speed in children exposed to Mn-containing drinking water. These studies suggest a probable effect of Mn exposure on children's memory and learning, though the reported routes of exposure to the metal have been different, and this could represent variable effects in terms of cognitive damage. To our knowledge, there are no reports assessing memory and learning in children exposed to Mn by inhalation.

Mexico has one of the main Mn mining deposits in the world, the Molango basin located in the North of Hidalgo State. Inhalation is the main route of exposure in this geographic area, due to high concentrations of Mn-laden particles suspended in the air (Rodríguez-Agudelo et al., 2006). In previous studies of adults from communities close to the Mn deposits and nearby processing facilities, motor and cognitive impairment has been reported (Rodríguez-Agudelo et al., 2006; Solís-Vivanco et al., 2009).

The aim of this study was to estimate the association between verbal memory and learning and biomarkers of Mn exposure, measured as Mn levels of the metal in blood and hair samples, from children whose age ranged from 7 to 11 years living in a mining district of Hidalgo, Mexico, compared to a non-exposed group of children.

2. Methods

2.1. Study design and population

This study was carried out in two communities (Chiconcoac and Tolago) of the Molango mining district in the northern area of Hidalgo State. These communities are close (less than 1 km away) to a Mn-processing facility named Otongo. A cross-sectional study was designed to sample 100 resident boys and girls from 7 to 11 years old, who attended the same elementary school and had lived in the community for a minimum of 5 years. Ninety-five children, also attending elementary school in four communities (Chichicaxtle, El Palizar, Los Cubes, and Plan Grande) within the municipality of Agua Blanca, located 80 km away from the mining district but in the same State, were tested as a comparison group. These communities had similar socio-economic to the exposed group, based on the National Population Council marginalization index (CONAPO, 2005). Children with a diagnosis of neurological or psychiatric illness and/or physical problems that would prevent them from responding to the test were excluded from the study.

The project was approved by the Bioethics Committee from the National Institute of Neurology and Neurosurgery "Manuel Velasco Suárez" (INNNMVS) and the National Institute of Public Health (INSP), both in Mexico. Resident children and parents of the communities were invited to participate voluntarily during school meetings where the study was described. Mothers, as well as participating children, signed an informed-consent letter in which the objective and procedure were explained. Children fulfilling the required criteria and whose parent had signed the informed consent were given an appointment in the community health center where hair and blood samples were obtained and the neuropsychological test battery was administered.

2.2. Cognitive Assessment

The Children's Auditory Verbal Learning Test (CAVLT) was used (Talley, 1997). The Semantic nature of the stimuli makes CAVLT a reliable test, and insensitive to cultural bias (Talley, 1997). The test was translated from English into Spanish and then back translated for this study, and applied in a pilot study to Mexican children to confirm comprehension of the instructions. CAVLT administration and scoring was carried out by a trained psychologist. CAVLT consists of three word lists: two of free recall, which the child must evoke as accurate as possible after the test administrator reads them, and one of recognition, in which the child must tell whether or not the word listened was in the first list. Its administration takes approximately 25–30 min.

CAVLT is composed of the following subscales:

a) *Learning curve*: progression of learning, measured as the number of words, from the original 16-word list, the subject has repeats over the course of five trials. Normally, the curve shows a consistent increase from one trial to the next.
b) *Immediate recall*: susceptibility of new information to be disrupted. Low scores suggest new learning is vulnerable to interference from exposure to other material (an interference word list).
c) *Delayed recall*: long-term memory functioning and retrieval ability. Low scores suggest retrieval deficits.
d) *Recognition accuracy*: reflects recognition memory ability. Low scores suggest difficulty in the initial coding of information into long-term memory.
e) *Immediate memory span*: reflects memory function when the information exceeds short-term storage capacity. Poor performance suggests a deficit in short-term memory function.
f) *Level of learning*: long-term memory coding abilities. Low scores suggest a reduced rate of learning.

2.3. Socio-demographic questionnaire

Mothers of participating children were asked to complete two questionnaires, the first to collect socio-demographic data and the second to inquire about the child's development including information about pregnancy and birth.

2.4. Blood metal measurements

Venous Blood (5 ml) was obtained from the cubital vein in metal-free Vacutainer tubes with EDTA as anticoagulant. Samples were refrigerated during transport and until analysis. Mn and Pb Analysis were performed using an atomic absorption spectrophotometer (Perkin Elmer 3110) equipped with a graphite furnace (HGA-600). Results for Mn are shown in micrograms per liter (μg/L) and for Pb in micrograms per deciliter (μg/dL; Montes et al., 2008). Quality control of the analytical procedure was assured by using the external standard Bovine liver from NIST (1577b); this biological-matrix based, reference material was digested and analyzed in the same session as samples, valid analytical sessions were considered only if metal measurements were 95–100% from those reported from the provider in the analysis certificate. Samples were analyzed in duplicates with less than 10% standard deviation. Quantification limits were 0.5 μg/L for Mn and 1 μg/dL for lead.

2.5. Hair metal measurements

Hair was obtained along with blood samples. Samples of approximately 1 g of hair were taken from the occipital region as close as possible to the scalp and stored in plastic bags for transportation. Then, they were cut into small pieces, washed thrice with non-ionic detergent (2% Triton X-100), rinsed with deionized water, and finally dried at 60 °C. Hair samples of 0.3 g were placed in metal-free polyethylene tubes with 250 μl of nitric acid (Suprapur, Merck, Mexico) and kept at 60 °C with continuous shaking. Results for Mn are given in micrograms per gram of hair (μg/g; Mortada et al., 2002). For hair Mn analysis quality control, we also used biological matrix NIST 1577 material, a 95% confidence interval for mean Mn in the reference material was considered along with the Mn hair determination. Limit of quantification of Mn in hair was 0.01 μg/g.

2.6. Air manganese sampling and measurement

Indoor and outdoor $PM_{2.5}$ air sampling was performed over 24-hour periods using MiniVol samplers (version 4.2; Airmetrics, Eugene, Oregon, USA). Samples