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Clinical Studies

Variance components of short-term biomarkers of manganese exposure in an inception cohort of welding trainees

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ABSTRACT

Various biomarkers of exposure have been explored as a way to quantitatively estimate an internal dose of manganese (Mn) exposure, but given the tight regulation of Mn in the body, inter-individual variability in baseline Mn levels, and variability in timing between exposure and uptake into various biological tissues, identification of a valuable and useful biomarker for Mn exposure has been elusive. Thus, a mixed model estimating variance components using restricted maximum likelihood was used to assess the within- and between-subject variance components in whole blood, plasma, and urine (MnB, MnP, and MnU, respectively) in a group of nine newly-exposed apprentice welders, on whom baseline and subsequent longitudinal samples were taken over a three month period. In MnB, the majority of variance was found to be between subjects (94%), while in MnP and MnU the majority of variance was found to be within subjects (79% and 99%, respectively), even when controlling for timing of sample. While blood seemed to exhibit a homeostatic control of Mn, plasma and urine, with the majority of the variance within subjects, did not. Results presented here demonstrate the importance of repeat measure or longitudinal study designs when assessing biomarkers of Mn, and the spurious associations that could result from cross-sectional analyses.

Introduction

Elevated Mn exposures have long been an occupational health concern, as chronic elevated exposures have been implicated in the development of "manganism", a Parkinson's-like syndrome [1]. However, even workers with exposures considerably lower than those historically associated with manganism have demonstrated subtle neurological effects, making Mn a relevant occupational and environmental health concern [2–5].

Various biomarkers of exposure have been explored as a way to quantitatively estimate an internal dose of Mn. In a literature-based analysis of the relationship between manganese in whole blood (MnB) and manganese in air (MnA) we hypothesized that there may be a point above which blood begins to act as a biomarker of inhalation exposure to Mn, but this still requires additional time-specific analyses, and knowledge of non-occupational exposure to Mn and its physiological variability in absorption or excretion [6]. For some biological media, notably Mn in plasma (MnP) and Mn in urine (MnU), limits of detection could be problematic. Hoet et al. [7] found 80% of control and 65% of welder urine samples to be below their limit of quantification (0.20 ng/mL) for MnU, making further analysis impossible. The majority of biomarker studies related to occupational inhalation of Mn are cross-sectional, typically only analysing a single biosample. Only a few studies have looked at biomarkers of Mn longitudinally or with repeat measures [7–11], but none have thoroughly assessed the within-individual or between-individual variability components. Järvisalo et al. [11] briefly discussed the variability in six MnU measurements taken over a 24 h period in five shielded metal arc welding (SMAW) welders, noting that Mn showed diurnal variation throughout the day, with lower MnU values tending toward the late afternoon or evening, even with increased exposure to Mn over the workday. However, no formal assessment of variability components was carried out in this manuscript.

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In proposing a framework for developing and applying biological guidance values, Bevan et al. [12] recommended that inter- and intra-variability in biomarker measurements be considered when developing guidelines for a specific chemical. However, often these data are not available. Similarly, Albertini et al. [13] in a mini-monograph called for research to advance the understanding of biomonitoring to assess human exposure and health risks and specifically study designs to better assess variance components in biomonitoring measurements.

The assessment of variance components and exploration of which external factors may affect them can help to better characterize relationships between exposure, dose, and effects. Thus, the purpose of this manuscript is to investigate the factors associated with variability in MnB, MnP, and MnU in a newly-exposed group of apprentice welders.

Material and methods

Occupational setting and study population

The focus of this manuscript is nine welder trainees enrolled in a technical college in Washington state, who are a subset of a larger inception cohort study (that is, all subjects joined our cohort prior to any reported occupational exposure to Mn) which we have described elsewhere [6]. All study protocols were reviewed and approved by the University of Washington Institutional Review Board and subjects provided written informed consent. Data collection for the inception cohort study began in April 2011, but this manuscript is restricted to nine welder trainees in the first academic quarter (September 2012–December 2012) of their traineeship, who entered the welding training program with no prior welding experience or reported occupational exposure to Mn.

The welding training program consists of five academic quarters, where students progress through a schedule of different welding processes, though time on each process may vary between subjects. While the nine trainees described here were followed for all five academic quarters, this analysis is restricted to their first academic quarter period of study. During this first quarter of the program, all nine trainees started with oxyacetylene welding (very low Mn exposure) before progressing to SMAW (moderate Mn exposure) after about a months' time. Trainees were in class Monday through Friday, with time split between welding practice and classroom lectures. Each welding booth in the facility has a dedicated adjustable exhaust ventilation hood to control fume, and trainees were given the choice whether or not to wear respiratory protection. No subjects wore respiratory protection while doing oxyacetylene welding, but six of the nine subjects (66.7%) consistently wore respiratory protection during SMAW welding.

Trainees were asked to provide a baseline blood and urine sample on the morning of their first day enrolled in the traineeship (a Tuesday), and at seven additional times over the course of their first quarter: the afternoon of their first day, the morning and afternoon of their first Friday, the morning and afternoon of their last Monday of the quarter, and the morning and afternoon of their last Friday of the quarter. On each biosampling day, subjects were also fitted with a personal air pump for the entire school day to measure airborne exposure to Mn. At the end of each biosampling day, trainees completed a questionnaire to assess their welding activity, use of respiratory protection, and any confounding sources of Mn exposure.

Demographic and work characteristics for these nine trainees are summarized in Table 1. Measured total Mn concentration in the air (total suspended particles) were normalized to eight-hour time weighted averages (TWA) and Mn exposure from SMAW welding was on average about 10 times higher than from oxyacetylene welding. No oxyacetylene or SMAW exposure measurements exceeded the National Institute for Occupational Safety and Health (NIOSH) eight hour TWA recommended exposure limit (REL) of 1 mg/m^3. However, based on self-report, subjects welded on average less than 4 h each work day (221.2 ± 81.8 min).

Table 1
Subject demographic and exposure characteristics.

n	9
Age (years)	22.1 ± 2.9
Male, *n* (%)	9 (100)
Current smoker, *n* (%)	1 (11.1)
Respirator user, *n* (%)	6 (66.7)
Time welding/day (mins)	221 ± 82
Oxy welding 8 h TWA Mn	3.6 ± 3.5 μg/m^3
SMAW welding 8 h TWA Mn	39 ± 46 μg/m^3
n blood samples/subject	7.1 ± 1.1
n plasma samples/subject	6.9 ± 1.4
n urine samples/subject	7.0 ± 1.2

Blood Mn analysis

At the beginning and end of sampling days, 6 mL of whole blood were collected in plastic Vacutainer® evacuated tubes containing 10.8 mg K_2EDTA anticoagulant (BD, Franklin Lakes, NJ) and transported on ice to University of Washington Environmental Health Laboratory (UW EHL). One mL of whole blood was transferred to a 15 mL Corning CentriStar polypropylene centrifuge tube (Corning, NY) and stored at 4 °C until analysis for trace metals. Microwave assisted acid digestion prepared samples for multi-element analysis of whole blood by ICP-MS (Agilent 7500 CE) [14]. A control material (ClinChek Level 1 Whole Blood Control, lyophilized for trace elements (Lot 038), Recipe Chemicals, Munich, Germany) was analyzed periodically. The results obtained for Mn in ClinChek materials were within the manufacturer's acceptable reference range of 5.6–8.4 ng/mL (found mean test value 7.3 ng/mL, SD: 0.1 ng/mL, *n* = 6). The limit of detection for Mn was 0.5 μg/L, and is based on three times the standard deviation of the analysis-batch-specific field blanks. Limit of quantification for Mn in blood (10 times the standard deviation of the blanks) was 1.7 μg/L. No blood samples fell below the limit of detection or limit of quantification for Mn.

Plasma Mn analysis

The remaining whole blood was centrifuged for 20 min at 600 CFM to allow for the extraction of 1 mL plasma, which was pipetted into 2.0 mL Eppendorf tubes (Hauppauge, New York). Aliquoted plasma samples were sent on dry ice for analysis at the UK Health and Safety Laboratory (HSL, Harpur Hill, Buxton, UK) where instrumentation allowed for an improved limit of detection compared to the UW EHL. Samples were still frozen upon arriving at HSL, and were stored at 4 °C until analysis for trace metals. The samples were diluted twenty-fold and analyzed using ICP-MS (Thermo X Series 2) in collision cell gas mode. The instrument was calibrated 0.05–20 ng/mL for Mn. Certified reference materials (Seronorm serum CRM Levels 1 (Lot 0903106) and 2 (Lot 0903107), Sero, Billingstad, Norway) and a check standard were analyzed periodically throughout the analysis. The results obtained for Mn in Seronorm serum were on average within the manufacturer's acceptable reference range of 14.1–15.9 ng/mL (found mean test value 15.17, SD: 0.52, *n* = 6) for Level 1, and on average within the manufacturer's acceptable reference range of 18.8–21.0 ng/mL (found mean test value 20.4, SD: 0.66, *n* = 6) for Level 2. Given there are no plasma certified reference materials for trace elements, a blank and a spiked plasma sample were analyzed as well as a spiked