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Full Length Article

Longitudinal T1 relaxation rate (R1) captures changes in short-term Mn exposure in welders

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ABSTRACT

Objectives: We demonstrated recently that the T1 relaxation rate (R1) captured short-term Mn exposure in welders with chronic, relatively low exposure levels in a cross-sectional study. In the current study, we used a longitudinal design to examine whether R1 values reflect the short-term dynamics of Mn exposure.

Methods: Twenty-nine welders were evaluated at baseline and 12 months. Occupational questionnaires estimated short-term welding exposure using welding hours in the 90 days prior to each study visit ($HrsW_{90}$). In addition, blood Mn levels, the pallidal index (PI; globus pallidus T1-weighted intensity (T1WI)/frontal white matter T1WI), and R1 values in brain regions of interest (ROIs) were determined as Mn biomarkers at each visit. Associations between changes in estimated welding exposure and changes in purported Mn biomarkers were assessed by Spearman's correlations with adjustment for age and baseline R1, $HrsW_{90}$, and blood Mn values.

Results: Changes in welding hours ($HrsW_{90}$: the short-term welding exposure estimate), was associated significantly with changes in R1 values in the putamen ($r = 0.541$, $p = 0.005$), caudate ($R = 0.453$, $p = 0.023$), globus pallidus ($R = 0.430$, $p = 0.032$), amygdala ($R = 0.461$, $p = 0.020$), and hippocampus ($R = 0.447$, $p = 0.025$), but not with changes in blood Mn levels or the PI.

Discussion: Changes in R1 values correlated with changes in the short-term welding exposure estimate, but not with more traditional measures of Mn exposure (blood Mn levels or PI). These results suggest that R1 may serve as a useful marker to capture the short-term dynamics in Mn brain accumulation related to welding exposure.

1. Introduction

Manganese (Mn) is an essential nutrient that can be toxic at high doses, causing neurological effects such as parkinsonism and dystonia (Racette et al., 2005; Cersosimo and Koller, 2006), as well as cognitive and behavioral deficits (Dobson et al., 2004; Bowler et al., 2006; Flynn and Susi, 2009). There is uncertainty, however, regarding the occupational and public health consequences of Mn exposure, especially at low exposure levels. This is due partly to the lack of an objective and sensitive *in vivo* marker of Mn concentration in human brain, and partly because of insufficient data about the dynamic relationships between proposed

Abbreviations: GP, globus pallidus; HrsW, hours spent welding, brazing, or soldering in the 90 day period preceding MRI; MRI, magnetic resonance imaging; R1, T1 relaxation rate; ROIs, regions-of-interest; T1, MRI longitudinal relaxation time; T1WI, T1-weighted intensity; TE, echo time; TR, repetition time; UPDRS, Unified Parkinson's Disease Rating Scale; YrsW, cumulative lifetime years welding.

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biomarkers and exposure. In addition, the toxicokinetics of Mn accumulation in brain is complex and not well understood.

Mn blood levels are increased in welders (Lu et al., 2005) and have been suggested as markers for exposure. The relationship between Mn blood levels and exposure, however, is weak and often non-significant (Lu et al., 2005; Baker et al., 2014). Mn has paramagnetic properties and can shorten the MRI longitudinal relaxation time (T1) and increase T1-weighted intensity (T1WI). Thus, T1WI imaging has been used to determine the pallidal index [PI (T1WI in globus pallidus/T1WI in orbitofrontal white matter)] as an estimate of Mn accumulation in the globus pallidus (GP) (Pal et al., 1999; Sen et al., 2011; Baker et al., 2015a). Although useful, it has been hypothesized that the PI may not capture Mn brain accumulation sensitively when the exposure level is low because Mn deposition in frontal white matter regions (Dorman et al., 2006; Lee et al., 2015) will affect the "calibration" critical for the PI.

T1 relaxation rate (R1), a quantitative estimate of Mn, may overcome the need to use such a "calibration." We recently studied the relationship between R1 (1/T1) values and welding, using the welding hours in the 90 days prior to imaging ($HrsW_{90}$) as an estimate of short-term welding exposure. We found an association between R1 and $HrsW_{90}$ in all basal ganglia areas, as well as in regions outside the basal ganglia (amygdala, hippocampus, and frontal cortex). In contrast, the correlations between R1 values and long-term exposure measures were limited to the caudate and putamen regions of the basal ganglia (Lee et al., 2015). The current study is a follow-up of this cohort assessing blood metal levels, exposure estimates, and imaging longitudinally. We tested the hypothesis that changes in R1 values, but not blood metal levels or the PI, would be associated with changes in short-term welding exposure (estimated by $HrsW_{90}$), thus providing R1 as a marker of short-term dynamics of brain Mn exposure.

2. Methods

2.1. Subjects

Originally (Lee et al., 2015), 35 welders were recruited from regional unions in Philadelphia and Harrisburg, PA, USA, and from the community around the Penn State Milton S. Hershey Medical Center. Twenty-nine welders returned for this longitudinal cohort study and had full blood, exposure, and imaging data both at the baseline and follow-up (12 months later) visits. A detailed questionnaire at each visit confirmed that welders were welding actively and that controls had no history of welding. All subjects were male and denied past diagnosis of Parkinson's disease or related disorders. Welders represented several different trades and industry groups (*e.g.*, boilermakers, pipefitters, pile drivers, railroad welders, and a variety of different manufacturing jobs).

Detailed demographic information was collected at baseline and updated at the follow-up visit, including age, education, history of smoking, and history of current and/or past major medical/neurological disorders. All subjects were examined and ascertained to be free of any obvious neurological and movement deficits using the Unified Parkinson's Disease Rating Scale-motor scores (UPDRS-III) with a threshold score of <15 (Racette et al., 2012). Blood samples were collected from all subjects on the day of the study visit (thus, not directly after welding exposure occurred). All subjects had normal liver function, blood calcium and magnesium levels, and no Fe deficiency. All welders underwent an orbital radiograph to rule out any metal fragments around the orbit. Written informed consent was obtained in accordance with guidelines approved by the Internal Review Board/Human Subjects Protection Office of the Penn State Milton S. Hershey Medical Center.

The mean time between study visits was 12.9 ± 0.9 months for welders. The subjects included in the current study (*i.e.*, with both baseline and follow-up visits) did not differ significantly from those excluded (due to loss at follow-up) in terms of age, education, welding exposure, blood metal levels, or imaging measures at baseline (p's > 0.06).

2.2. Exposure assessment

A supplementary exposure questionnaire SEQ (Lee et al., 2015) focused on the 90-day period prior to the MRI and determined the time spent welding, type of metal welded, and various types of welding performed. The exposure metrics derived from the SEQ were: hours spent welding, brazing, or soldering in the 90-day period preceding the MRI. That is, $HrsW_{90}$ = (weeks worked) * (h/week) * (fraction of time worked related directly to welding) (Lee et al., 2015). The E90 also was used to provide an estimate of the 90-day time-weighted cumulative exposure for each subject in the 90 days prior to their study visit (baseline or follow-up). This estimate is designed to account for both work exposure (based on the Mn exposure data for welders from OHSA) and ambient (non-work) exposure (Lee et al., 2015). A detailed description and the calculations used have been published (Lee et al., 2015) and are included in the Supplementary data.

2.3. Blood analysis

Whole blood was analyzed for metal levels by Inductively Coupled Plasma Mass Spectrometry [ICP-MS; (Lee et al., 2015)]. Digestion was performed by microwave methods using the Discovery SPD digestion unit (CEM, Matthews, NC). After digestion, the samples were analyzed for trace minerals using the Thermo (Bremen, Germany) Element 2 SF-ICP-MS equipped with a concentric glass nebulizer and Peltier-cooled glass cyclonic spray chamber. Bulk mineral concentrations were determined by ICP-OES (Optical Emission Spectrometry) analysis on the Thermo iCAP equipped with a polypropylene cyclonic spray chamber.

2.4. MRI image acquisition and analysis

Images were acquired using a Siemens 3 T scanner (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany) with an 8-channel head coil using the same parameters at both the baseline and 12-month follow-up visits. Namely, high-resolution T1-weighted and T2-weighted images were acquired for anatomical segmentation. T1W images were collected using an MPRAGE sequence with Repetition Time (TR) = 1540 ms, Echo Time (TE) = 2.3 ms, FoV = 256 × 256 mm, matrix = 256 × 256 mm, slice thickness = 1 mm, slice number = 176 (with no gap), and voxel spacing 1 × 1 x 1 mm. T2-weighted images were collected using a fast-spin-echo sequence with TR/TE = 2500/316, and the same spatial resolution as the T1W images.

For whole brain fast T1 mapping, images were acquired using a spoiled gradient recalled echo (SPGR) with two flip angles and transmit field (B1) correction. Image acquisition parameters for T1 mapping were as follows: TR = 15 ms, TE = 1.45 ms, flip angles = 4/25, FoV = 250 × 250 mm, matrix = 160 × 160, slice thickness = 1 mm, slice number = 192 50% overlap, and voxel spacing = 1.56 × 1.56 × 1 mm; and for the B1 field mapping: TR = 1000 ms, TE = 14 ms, flip angles = 45/60/90/120/135, FoV = 250 × 250 mm, matrix = 32 × 32, slice thickness = 5 mm, and slice number = 22.

2.5. Defining brain regions of interest

Bilateral basal ganglia structures [GP, putamen, caudate nucleus], amygdala, and hippocampus were selected as ROIs