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Ex vivo magnetic resonance imaging in South African manganese mine workers

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ABSTRACT

Background: Manganese (Mn) exposure is associated with increased T1-weighted magnetic resonance imaging (MRI) signal in the basal ganglia. T1 signal intensity has been correlated with occupational Mn exposure but not with clinical symptomatology or neuropathology.
Objectives: This study investigated predictors of ex vivo T1 MRI basal ganglia signal intensity in neuropathologic tissue obtained from deceased South African mine workers.
Methods: A 3.0 T MRI was performed on ex vivo brain tissue obtained from 19 Mn mine workers and 10 race- and sex-matched mine workers of other commodities. Basal ganglia regions of interest were identified for each subject with T1-weighted intensity indices generated for each region. In a pathology subset, regional T1 indices were compared to neuronal and glial cell density and tissue metal concentrations.
Results: Intensity indices were higher in Mn mine workers than non-Mn mine workers for the globus pallidus, caudate, anterior putamen, and posterior putamen with the highest values in subjects with the longest cumulative Mn exposure. Intensity indices were inversely correlated with the neuronal cell density in the caudate ($p = 0.040$) and putamen ($p = 0.050$). Tissue Mn concentrations were similar in Mn and non-Mn mine workers. Tissue iron (Fe) concentration trended lower across all regions in Mn mine workers.
Conclusions: Mn mine workers demonstrated elevated basal ganglia T1 indices when compared to non-Mn mine workers. Predictors of ex vivo T1 MRI signal intensity in Mn mine workers include duration of Mn exposure and neuronal density.

1. Introduction

Occupational Mn exposure is associated with parkinsonism (Racette et al., 2012), cognitive dysfunction (Bowler et al., 2007; Park et al., 2009; Rodier, 1955), and increased signal on T1-weighted magnetic resonance imaging (MRI) in the basal ganglia (Criswell et al., 2012; Huang, 2007; Kim et al., 1999; Nelson et al., 1993). The signal changes are presumed to be caused by Mn deposition and its secondary effects on the magnetic resonance properties of surrounding brain tissue (Newland, 1999; Kang et al., 1984). The intensity of in vivo pallidal signal correlates with Mn exposure (Dietz et al., 2001) and Mn blood levels (Chang et al., 2010) but the relationship with clinical symptomatology has not been clearly established. Moreover, the relationship between Mn tissue concentration and neuropathology has been inconsistent. Previous studies with subcutaneous manganese oxide exposure in monkeys resulted

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in severe loss of pallidal nerve cells and astrogliosis with increased regional Mn deposition (Eriksson et al., 1987). However, rhesus monkeys injected with manganese chloride developed similar findings of pallidal and nigral gliosis with astrocytosis, but no change in tissue Mn (Olanow et al., 1996). More recently, elevated Mn tissue concentrations have been reported throughout the basal ganglia of Mn-injected macaques, but post-mortem immunohistochemistry results were normal (Guilarte et al., 2006, 2008).

Pathologic studies in occupationally-exposed humans are very limited but similarly inconsistent. Autopsy data from a 52-year-old man exposed to Mn in an ore plant describe pallidal cell loss but normal tissue Mn concentration (Yamada et al., 1986). Older reports document cell loss in the putamen and pallidum but did not examine Mn tissue concentrations (Canavan et al., 1934; Casamajor, 1913). All of these human studies in occupationally-exposed subjects predate modern immunohistochemical studies, and none have corresponding imaging. Further evaluation of the relationship between imaging characteristics and neuropathology could provide valuable information on the mechanism of Mn neurotoxicity and a better understanding of the clinical significance associated with the increased T1 signal characteristic of this exposure.

Ex vivo MRI imaging permits correlation of histopathology with MRI signal intensity (Augustinack et al., 2013; Baltes et al., 2011; Garbelli et al., 2011; Riddle et al., 2011). Ex vivo imaging also provides a cost effective method of establishing imaging parameters for future in vivo sequences as these scans can be completed at a fraction of the cost and with no risk to human subjects. However, formalin fixed ex vivo tissue has different physical properties than in vivo tissue and subsequently different T1 imaging properties (Baba et al., 1994; Henriksen et al., 1993; Tovi and Ericsson, 1992). Therefore, the primary objective of this study was to validate the use of ex vivo imaging in Mn neurotoxicity by confirming T1 signal differences persist post-mortem in Mn-exposed subjects. Our secondary objective was to explore the relationships between ex vivo T1 imaging, neuropathology, and tissue metal concentration using post-mortem brain tissue from South African Mn mine workers and matched non-Mn mine workers.

2. Methods

2.1. Brain acquisition

This study was approved by the Washington University Human Research Protection Organization and the University of the Witwatersrand Human Research Ethics Committee. Race- and sex-matched Mn mine workers and non-Mn mine workers were selected from our previously established autopsy program (Nelson et al., 2012) for imaging and analysis. Upon notification of the death of a mine worker, consent was obtained from the next-of-kin. The brain specimens were suspended in 10% neutral buffered formalin for a minimum of three weeks, after which they were shipped to Washington University for ex vivo MRI imaging, and then to the University of Washington for pathology and tissue metal analysis.

2.2. Exposure assessment

After obtaining consent, an occupational health nurse interviewed the family to obtain occupational and medical histories. Permission to access the deceased subject's medical and employment records was also requested to verify accuracy of the historical information. Workers whose medical histories indicated any neurologic diagnosis were excluded. An industrial hygienist reviewed the occupational history and employment records and assigned duration of Mn exposure to each subject.

2.3. MRI studies

Fixed brains were placed in the scanner within MRI compatible, plastic containers filled with 10% formaldehyde solution with plastic supports to prevent movement. Imaging was performed on a 3.0 T Siemens Trio scanner (Erlangen, Germany). A Siemens adapted neuromelanin imaging technique (Astafiev et al., 2010) (repetition time = 600 ms, echo time = 14 ms, flip angle = 120°, voxel dimensions 0.7 mm × 0.43 mm × 2.5 mm, matrix dimensions 314 × 550, 11 axial slices) was used to obtain high-resolution T1-weighted images through the basal ganglia and midbrain. A transverse slice through a Mn miner brain demonstrates the differences in T1 weighted signal from fixed tissue including the including the gray matter to white matter signal reversal that occurs at 11 weeks post fixation (Tovi and Ericsson, 1992) (Fig. 1). A single reviewer, blinded to the clinical status of the subject, outlined volumes of interest (VOIs) including the caudate, globus pallidus, anterior and posterior putamen, and standardized white matter regions on individual images. The intensity of the signal on the T1-weighted image in the VOI was compared by calculating an intensity index for each region in each subject. The regional intensity indices were defined as the ratio of T1 signal in the VOI to a standard frontal white matter reference region following previously used methods for the pallidal index (Spahr et al., 1996).

$$\text{Intensity index} = \frac{\text{VOI}}{\text{Left white matter control region} + \text{Right white matter control region}} \times 100$$

Intensity indices were created for the globus pallidus, caudate, anterior putamen, and posterior putamen using the same reference control regions. A combined basal ganglia intensity index was created by averaging the intensity indices of all four VOIs.

All brains were scanned on the same scanner in the same configuration and with the same pulse sequence. The intensity indices as ratios should thus be largely independent of any underlying signal inhomogeneity. To confirm this, we compared white matter signal intensity in the control region (the denominator of the intensity indices) across all subjects (mean = 926.26, standard deviation 80.04) which corresponded to a very low coefficient of variation of 8.64% with <2% difference between Mn miner workers and non-Mn mine workers.

2.4. Neuropathology

A board certified neuropathologist performed a gross examination. The cerebrum and posterior fossa contents were embedded in a 3% agar solution and sliced coronally and axially, at 4 mm intervals respectively. Formalin fixed paraffin embedded (FFPE) tissue blocks were sectioned and deparaffinized rehydrated slides were stained with hematoxylin and eosin. Automated immunohistochemistry was performed on tissue sections from the basal ganglia using mouse monoclonal antibodies for glial fibrillary acidic protein (GFAP) (Dako, USA) to label astrocytes, microtubule associated protein-2 (MAP-2) (Millipore, USA) to label neurons, and CD68 (Dako, USA) to label macrophages and microglia using a Leica Bond III fully automated immunohistochemistry and in situ hybridization staining system (Leica Bio-Systems, USA). FFPE tissue blocks corresponding to a unilateral coronal section of the