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Research Capacity Development in South African Manganese Mines to Bridge Exposure and Neuropathologic Outcomes

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Abstract

Manganese (Mn) is a common occupational exposure worldwide. Recent studies indicate clinical and imaging evidence of neurotoxicity in chronically exposed workers. The pathologic significance of these findings is unclear. South Africa produces over 80% of the world's Mn from mines from a desert region in the Northern Cape Province. An autopsy program at the National Institute for Occupational Health (NIOH) in South Africa has provided compensation to families for mining-related lung diseases for almost 100 years. Building on this, we implemented a brain autopsy program to investigate the feasibility of obtaining brains from South African Mn miners and non-exposed reference miners to investigate neuropathologic consequences of chronic Mn exposure. Employing an experienced occupational health nurse, we identified deceased miners within 100 square km of the Mn mines. The nurse was notified of any Mn (case) or other (reference) miner or ex-miner death by local medical practitioners, occupational health and mine physicians, and community members, and families were approached for consent to remove the brains in addition to the cardio-respiratory organs. Families of deceased miners who had an autopsy at the NIOH in Johannesburg were also approached. To confirm exposure in Mn miners, mean pallidal indices were compared between Mn miners and non-exposed reference miners. Sixty-eight potential brain donors were identified; we obtained consent from the families to remove 51 (75%). The mean autopsy interval was seven days. With optimized fixation methods, the tissue quality of the brains for gross and regular microscopic examination was excellent. Ex-vivo MRI demonstrated increased pallidal index in Mn miners compared to reference miners. We conclude that obtaining brain tissue from deceased miners in South Africa is highly successful

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with only a modest investment in local infrastructure. Tissue quality was excellent and should be ideal to investigate the neuropathologic consequences of chronic occupational Mn exposure.

Keywords

Manganese; parkinsonism; neuropathology; MRI

1. Introduction

Manganese (Mn) is an essential trace metal required for amino acid, lipid, protein, and carbohydrate metabolism and is a co-factor for superoxide dismutase(Hurley and Keen CL, 1987) and enzymes involved in neurotransmitter synthesis(Golub et al., 2005). Sources of Mn are dietary or respiratory. Environmental exposure to Mn occurs primarily through occupational exposure, in Mn miners, smelters, workers in dry-cell battery factories, and welders. Mn was first recognized as a neurotoxin in the 19th century with the report of four Mn ore crushers developing a syndrome of a lower extremity predominant “muscular weakness”, festination, postural instability, facial masking, hypophonia, and sialorrhea (Couper, 1837). The syndrome was more clearly delineated by Rodier in 1955 when he described a group of Moroccan Mn miners with a neurologic illness characterized by parkinsonism, gait disorder, dystonia, psychosis and emotional lability (Rodier J, 1955).

There are very limited human autopsy data on subjects with manganism but pathology affecting primarily the basal ganglia appears to be characteristic. Yamada et al.(2006) reported a 52 year old man with “increased muscle tone”, weakness, hyperreflexia, and a mood disorder attributed to working in a Mn ore crushing plant. Autopsy demonstrated cell loss in the pallidum with astrocytosis but normal substantia nigra. Bernheimer et al. reported a patient with a long history of a parkinsonian illness, associated with markedly elevated blood Mn levels that developed while exposed to Mn dioxide in a battery factory (Bernheimer et al., 1973). Autopsy revealed pallidal atrophy, marked degeneration of the substantia nigra pars compacta, and occasional Lewy bodies in nigral neurons. Older reports commented primarily on cell loss in the putamen and pallidum (Casamajor, 1913; Canavan MM et al., 1934). All of these studies predate the modern immunohistochemical markers for Lewy bodies and none provided quantitative cell counts in the basal ganglia. Clearly, additional pathologic material studied with modern techniques and stains will be necessary to more clearly define the pathologic spectrum of Mn exposure in humans and to understand the relationship between Mn exposure and neurodegenerative diseases such as Parkinson disease (PD).

The objective of this study was to ascertain the feasibility of obtaining brains of Mn exposed miners and reference miners for neuropathologic assessment of the consequences of chronic occupational Mn exposure.

2. Material and Methods

This study was approved by the Human Research Protection Organization at Washington University School of Medicine and the Human Research Ethics Committee at the University of Witwatersrand.

2.1. Autopsies

Using a regionally based occupational health nurse, we identified potential brain donors from Mn and other mines in the Northern Cape Province, as well as gold miners coming to the NIOH in Johannesburg for autopsy. The families provided consent for removal of the

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brain and cardio-respiratory organs. The brains were floated in formalin for a minimum of three weeks and were then sent to the NIOH with the cardio-respiratory organs. The cardio-respiratory organs were examined at the NIOH for occupational disease and the brains were couriered to Washington University.

2.2. Ex-vivo MRI

MRI imaging of the brains was performed on a 3.0T Siemens Trio scanner (Erlangen, Germany) using a standard 12 channel head coil Structural anatomic scans included a T1-weighted sagittal, magnetization-prepared rapid gradient echo (MPRAGE; repetition time (TR) = 1900 ms; inversion time (TI) = 1000 ms, echo time (TE) = 3.52 ms, flip angle = 8°, 0.8 ×0.8 ×0.8 mm voxels). To prevent any bias in analysis, a reviewer blinded to the clinical status of the subject outlined volumes of interest (VOIs) in the globus pallidus and white matter reference regions on individual MR images. The intensity of the pallidal signal in the VOI was compared by calculating a pallidal index for each subject as previously described (Spahr et al., 1996).

2.3. Pathologic Analysis

Brains were examined grossly for evidence of maceration, inadequate fixation, putrefaction or significant mechanical artifacts. If the brains were in satisfactory condition, the integrity of the meninges and the degree of cortical atrophy and atherosclerosis were noted, and the olfactory bulbs were removed and embedded in paraffin. Coronal cuts through the entire cerebrum, and axial and parasagittal cuts through the agar-embedded brainstem and cerebellum were performed at 4mm intervals. Detailed gross examination directed at identification of any sort of pathologic process involving the cortex, white matter, deep gray matter structures, brainstem or cerebellum was conducted.

Representative sections, including the cortices, basal ganglia, thalamus and hippocampi, midbrain, pons, and medulla were embedded in paraffin for histopathologic and immunohistochemical studies. The entire basal ganglia and brainstem were blocked in sequence for future stereological analysis.

The sections were stained with Hematoxylin and Eosin to identify frank necrosis, neuronal loss and gliosis, as well as any other pathologic processes. Immunohistochemical studies with antibodies to GFAP were performed to qualitatively assess for astrogliosis.

2.4. Statistical Analysis

Mean pallidal indices were compared with a 2-tailed t-test using $p<0.05$ as the threshold for significance.

3. Results

3.1. Autopsies

We identified 51 potential brain donors from Mn and non-Mn miners from the Northern Cape Province, and 17 from gold miners coming to the NIOH in Johannesburg for autopsy (68 in total). Fifty-one brains were obtained after the families agreed to autopsy (21 from Mn miners and 30 from non-exposed reference miners), giving a 75% consent rate. Four families refused to consent to any organs being removed; nine consented to the removal of the hearts and lungs only; for one case, there was no-one available to remove the brain at the time; for two cases, the body was too far away to be transported to the place of autopsy; and for one case, no employment history was available.

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Only seven of the Mn miners (33.3%) had been employed exclusively in the Mn mining sector. The others also had asbestos, iron ore, lime, diamond, platinum, copper and/or gold exposure. Sixteen of the reference subjects (53.3%) had most exposure in asbestos mines; the remaining 14 (46.7%) had most exposure in the gold mines. Many of the reference subjects had mixed exposures in that they had also been employed in other mining sectors, e.g. iron ore and platinum. Ages of the Mn miners ranged from 39 to 71 years and reference miners ranged from 44 to 89 years. (Table)

3.2. Ex vivo MRI

To calculate the pallidal index for Mn exposed miners, we matched exposed miners with non-exposed reference miners by race and gender. After excluding the four non-exposed women miners and six brains that were not adequately fixed, we completed ex-vivo MRI scans on 13 workers who had worked in a Mn mine at some time during their career and 10 miners who had never worked in a Mn mine. The mean pallidal index for Mn miners (124.2 ± 11.12) was higher than non-exposed reference miners (114.72 ± 7.22; p=0.03). Within the Mn miners, there was no relationship between the time interval from last exposure and the pallidal index; however, 7/13 Mn miners were working in the mines up to the time of death. Interestingly, three former Mn miners had stopped working in the mines from 15–25 years prior to death but still had elevated pallidal indices (Table). The pallidal index was 120.5 (± 5.7) in those with Mn only exposure (N=3) and 125.3 (± 12.3) in those exposed to Mn and one or more other heavy metals (N=10). These differences were not statistically significant.

3.3. Neuropathology

Gross examination of brain did not reveal apparent alterations in Mn miners. Conventional histology, i.e. H&E, and GPAP immunostaining, of control cases provided similar results to brain autopsies performed in the Seattle area. Qualitatively, there appeared to be a slight increased gliosis in the basal ganglia of miners exposed to Mn.

4. Discussion

This preliminary report demonstrates feasibility of obtaining neuropathologic tissue from chronic Mn exposed workers. Most families agreed to brain autopsy even though the Occupational Diseases in Mines and Works Act provides for compensation for only occupational respiratory disease. Logistical issues of coordinating resources in this remote region accounted for a minority of the “non-removals”. The quality of the tissue was excellent after extending the fixation time and modifying the storage conditions. Most importantly, the ex vivo MRI provides evidence that these Mn miners do have exposures to Mn and that these exposures result in Mn deposition in the brain that persists after death. The high quality of the tissue and documented exposure on MRI demonstrate that this population is ideally suited to address unanswered questions about the pathologic effects of Mn neurotoxicity.

Much of the current knowledge about human Mn neurotoxicity is based upon in-vivo clinical assessments and neuroimaging. Understanding the pathophysiology of the neurotoxic effects of Mn has the potential to inform potential therapeutic studies and preventative measures for workers. Moreover, pathologic tissue provides an opportunity to understand the relationship between chronic Mn exposure and neurodegenerative diseases like PD. The most recent neuropathological examination in a worker with high Mn exposure was in 1986, predating knowledge of the role of α-synuclein in PD. In this cohort of subjects, we did find qualitative differences in gliosis between Mn miners vs controls; but unbiased stereology will be needed to provide accurate neuronal counts and extent of gliosis, astrocytes or microgia throughout the basal ganglia. Furthermore, it will be essential to

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investigate whether there is increased α-synuclein aggregation in the basal ganglia. Regardless of the outcome, the large number of available brains from deceased Mn miners provides an unprecedented opportunity to investigate dose-response relationships between cumulative Mn exposure and neuropathologic endpoints.

There are no prior studies that have investigated ex-vivo imaging in Mn exposed workers. High levels of Mn exposure can result in deposition in the brain with a characteristic increased signal on T1-weighted MR imaging in the globus pallidus (Nelson et al., 1993). The intensity of the pallidal signal may correlate with occupational exposure (Dietz et al., 2001) and Mn blood levels (Kim et al., 1999) but is less clearly linked with clinical symptomatology including parkinsonism. However, the relationship between signal intensity and neuronal pathology is unknown. An interesting finding from this study is that increases in the pallidal index may persist years after cessation of exposure. This contrasts to prior studies in patients with Mn deposition in the pallidum due to end stage liver disease, in which the increased pallidal index returned to normal after 10–20 months (Pujol et al., 1993). However, the pathophysiology of the MRI abnormalities may be different in airborne occupational exposed subjects as compared to end-stage liver subjects with presumably oral sources of Mn. Most importantly, this study provides a unique opportunity to understand the pathologic correlates to this well described MRI abnormality and to investigate extra-pallidal Mn deposition.

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Table

Exposure details of 13 Mn miners on whom MRI scans were completed

Case no.	Age at death	Mn mining history		Other mining history		Time between death and last Mn exposure (years)
		Dates	No. years	Commodity	No. years	
1	60	1987-2006	20.0	Asbestos	10	4
				Lime	1	
2	57	1978-2009	21.5	-	-	1
3	67	1976-1993	16.0	Asbestos	1	17
4	53	2008-2010	1.2	Diamond	29	0
5	39	1996-2010	14	-	-	0
6	54	1984-1989	5	Asbestos	0.5	21
7	65	1979-1980 1982-1988	7	Asbestos Gold Platinum	5 1 7	22
8	50	1995-1999 2003-2005	6	Asbestos	12	5
9	67	1974-1975 1985	2	Asbestos Diamond	2 10	25
10	46	2002-2009	7.5	Asbestos	2	0
11	62	1977-2010	31	Asbestos Copper	2 1	
12	70	1981[a]	unknown	Asbestos	Unknown	0
13	46	1988-2011	22	-	-	0

[a]Only information available indicates working in Mn mine in 1981; start and end dates and duration unknown.