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Quantitative neuropathology associated with chronic manganese exposure in South African mine workers

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ABSTRACT

Manganese (Mn) is a common neurotoxicant associated with a clinical syndrome that includes signs and symptoms referable to the basal ganglia. Despite many advances in understanding the pathophysiology of Mn neurotoxicity in humans, with molecular and structural imaging techniques, only a few case reports describe the associated pathological findings, and all are in symptomatic subjects exposed to relatively high-level Mn. We performed an exploratory, neurohistopathological study to investigate the changes in the corpus striatum (caudate nucleus, putamen, and globus pallidus) associated with chronic low-level Mn exposure in South African Mn mine workers. Immunohistochemical techniques were used to quantify cell density of neuronal and glial components of the corpus striatum in eight South African Mn mine workers without clinical evidence of a movement disorder and eight age–race–gender matched, non-Mn mine workers. There was higher mean microglia density in Mn mine workers than non-Mn mine workers in the globus pallidus external and internal segments [GPe: 1.33 and 0.87 cells per HPF, respectively ($p = 0.064$); GPi: 1.37 and 0.99 cells per HPF, respectively ($p = 0.250$)]. The number of years worked in the Mn mines was significantly correlated with microglial density in the GPi (Spearman's rho 0.886; $p = 0.019$). The ratio of astrocytes to microglia in each brain region was lower in the Mn mine workers than the non-Mn mine workers in the caudate (7.80 and 14.68; $p = 0.025$), putamen (7.35 and 11.11; $p = 0.117$), GPe (10.60 and 16.10; $p = 0.091$) and GPi (9.56 and 12.42; $p = 0.376$). Future studies incorporating more detailed occupational exposures in a larger sample of Mn mine workers will be needed to demonstrate an etiologic relationship between Mn exposure and these pathological findings.

1. Introduction

Manganese (Mn), an essential trace element, physiologically acts as a cofactor for multiple enzymes, including pyruvate carboxylase and Mn superoxide dismutase (Bowman et al., 2011; Milatovic et al., 2009; Sidoryk-Wegrzynowicz et al., 2009). When ingested, Mn is tightly regulated and excreted in the bile; however, when absorbed through the respiratory tract, this homeostatic regulation is bypassed (Teeguarden et al., 2007a, 2007b). The original reports of workers exposed to very high levels of Mn described workers with an atypical parkinsonian phenotype, including dystonia, early gait impairment, behavioral dysfunction, and cognitive impairment (Couper, 1837; Rodier, 1955; Wang et al., 1989). However, modern occupational exposures are an order of magnitude lower than these historical exposures, and the resulting clinical phenotype appears to be substantially different

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(Rodier, 1955; Racette et al., 2012, 2005; Flynn and Susi, 2010; Cooper, 1984; Liu et al., 2011; Hobson et al., 2011). Clinical–pathological studies provide the most definitive pathophysiological data to explain the observed phenotypic differences between past and current exposures to Mn. However, these studies are very difficult to conduct due to limited access to neuropathology expertise, long periods between working in an occupation where Mn exposure occurs and time of death, and reluctance of families to pursue autopsies.

To the best of our knowledge, since the first clinical description of manganism in 1837, only eight gross neuropathological examinations have been reported in subjects with high occupational Mn exposures (Couper, 1837; Rodier, 1955; Ashizawa, 1927; Canavan et al., 1934; Casamajor, 1913; Stadler, 1936; Trendtel, 1936; Yamada et al., 1986). Seven of these were qualitative histopathological examinations without immunohistochemical preparations. A 1913 report described a symptomatic Mn separating-mill worker with normal gross neuropathology, but no histopathological examination was performed (Casamajor, 1913). From 1927 to 1954, four case studies were reported, having undergone qualitative histopathological examination: a symptomatic brownstone miller with neuronal loss in the globus pallidus and an intact substantia nigra; a symptomatic dock worker with neuronal loss and concomitant gliosis of the corpus striatum; a symptomatic brownstone miller with putaminal and pallidal neuronal loss and gliosis with preservation of the substantia nigra; and a symptomatic brownstone miller with significant neuronal death in the globus pallidus with a grossly well-pigmented substantia nigra (Ashizawa, 1927; Stadler, 1936; Parnitzke, 1954; Trendtel, 1936). In the only Mn neuropathology study that included a reference subject for comparison, a 55-year-old male mill worker with manganism demonstrated gross atrophy of the basal ganglia and compensatory ventriculomegaly compared to a 55-year-old woman who died of tuberculosis (Canavan et al., 1934). Histopathological comparison revealed greater density of glia and neurons in the caudate and lenticular nuclei in the mill worker. However, this study preceded the current availability of more precise immunohistochemical methods. Subsequently, post mortem gross examination of a 67-year-old woman exposed to manganese dioxide as a battery factory worker, who developed manganism, revealed atrophy of the pallidum as well as microscopic cortical, rubral, and striatal astrogliosis with spotty degeneration of the substantia nigra pars compacta and occasional nigral Lewy bodies (Bernheimer et al., 1973). The most recent histopathological report described a symptomatic Mn ore-crushing factory worker with gross atrophy and discoloration of the globus pallidus as well as histopathological evidence of neuronal loss, a moderate increase in astrocytes in the corpus striatum, and normal substantia nigra (Yamada et al., 1986).

Despite recognition of an Mn neurotoxicity syndrome for nearly 200 years, very few post mortem neuropathological studies in humans with documented Mn exposures have been reported in the literature. These reports comprise qualitative post-mortem histopathological evaluations which lack quantitative data, with the exception of one case report, or immunohistochemical/immunofluorescent evaluations. While these reports provide some insight into the neuropathological effects of Mn exposure, they ultimately describe the end-stage changes of the Mn neurotoxicity syndrome that is rarely, if ever, seen in modern times. In order to address a critical need in the Mn neurotoxicity literature, we designed and implemented a cross-sectional exploratory neuropathological study of the corpus striatum in Mn and non-Mn mine workers in The Republic of South Africa, which contains over 80% of the world's Mn reserves.

This study is the largest neuropathology series of Mn exposed individuals, the first study to evaluate the neuropathology of chronic low-level exposure, and the first quantitative histopathological/immunohistochemical study using a carefully matched reference group. Pathological characterization of the pre-clinical stage of chronic low-level exposure in human tissues is important for further disease characterization, biomarker discovery, and therapy development.

2. Methods

2.1. Sample acquisition

This study was approved by the Washington University Human Research Protection Organization (Saint Louis, MO, USA) and the University of the Witwatersrand Human Research Ethics Committee (Republic of South Africa; RSA). Under the Occupational Diseases in Mines and Works Act of the RSA, deceased mine workers have the right to a cardio-pulmonary autopsy, regardless of the cause of death, on condition that consent is provided by the next of kin (Myers et al., 1987). The families of deceased mine workers are eligible for financial compensation for specific mining-related pulmonary diseases. We built on this existing program by using a regionally based occupational health nurse to recruit potential brain donors from Mn and other mines. The Mn mines are located in a remote region of South Africa in the Northern Cape Province close to the Botswana border. There are several Mn mines located in close proximity to each other as the economically viable Mn field covers an area of approximately 35 km $\times$ 15 km (Gutzmer, 1996). The subjects in this study were employed by various mines in this region.

Upon notification of the death of a mine worker, the occupational health nurse consented and interviewed 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 (Seattle, WA, USA) for pathological analysis. Investigators performing the cell density examinations, immunohistochemical stains, and cell density quantifications were blinded to exposure status.

2.2. Brain specimen processing and examination

At the University of Washington, a certified neuropathologist performed an external gross examination, including assessment of cerebral cortical atrophy. The cerebrum and posterior fossa contents were embedded in a 3% agar solution and sliced coronally and axially, at 4 mm intervals. A standard gross examination was undertaken, including an assessment of atrophy or discoloration of the corpus striatum and degree of pigmentation of the substantia nigra and locus coeruleus. Tissue sampling of several brain regions was performed, including but not limited to, bilateral cortices, hippocampi, basal ganglia, midbrain, pons, and cerebellum. These samples were processed for 24 h in an automated tissue processor and were subsequently embedded in paraffin wax to produce formalin-fixed paraffin embedded (FFPE) tissue blocks.

2.3. Immunohistochemistry

FFPE tissue blocks were sectioned with a microtome producing 4 μm thick tissue sections which were placed on positively-charged glass slides. Deparaffinized rehydrated slides were stained with hematoxylin and eosin (H&E) to examine morphology and to note any neurohistopathological changes. Utilizing previously optimized conditions, automated immunohistochemistry was performed on tissue sections from the basal ganglia, using a Leica Bond III Fully Automated IHC and ISH Staining System (Leica Biosystems, USA). Mouse monoclonal antibodies for glial fibrillary

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