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Neurochemical changes in welders revealed by proton magnetic resonance spectroscopy

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ABSTRACT

Background: Occupational and environmental exposure to manganese (Mn) is associated with various neurobehavioral and movement dysfunctions. However, few studies have systemically examined the neurochemical effects of Mn exposure.
Objectives: We examined typical changes in cerebral metabolite ratios in welders chronically exposed to Mn, compared with control individuals, using proton magnetic resonance spectroscopy (MRS), investigated whether an abnormality in brain metabolism is associated with neurobehavioral changes, and assessed possible implications of chronic Mn exposure.
Methods: Thirty-five welders chronically exposed to Mn and 20 age-matched healthy subjects underwent single-voxel MRS at short echo time to assess the *N*-acetylaspartate (NAA), myoinositol (mI), total choline (tCho), and glutamine plus glutamate (Glx) levels, each of which was expressed as a ratio to total creatine (tCr). Neurobehavioral tests were also performed to define cognitive status.
Results: NAA/tCr, Glx/tCr, and tCho/tCr ratios in the frontal gray matter (anterior cingulate cortex; ACC) and parietal white matter did not differ significantly between welders and control subjects. These metabolite ratios did not correlate significantly with blood Mn concentration or neurobehavioral parameters. However, mI levels in the ACC, but not in the parietal white matter, were significantly reduced in welders compared with control individuals ($P < 0.01$). Furthermore, in the frontal lobe of the brain, the mI/tCr ratio was significantly correlated with verbal memory scores as well as blood Mn concentration ($P < 0.05$).
Conclusions: The cognitive decline observed in welders exposed to Mn was associated with a decreased mI/tCr ratio in the ACC. The depletion of mI in welders may reflect possible glial cell swelling and/or detoxification processes associated with long-term exposure to Mn.

1. Introduction

Chronic exposure to an excess of manganese (Mn) in mining, milling, and other manufacturing settings has been known to be associated with an increased risk of manganism since the first reported case of manganism in 1837 (Couper, 1837). However, the first studies in welders were not performed until after 1980, and only about 15 case reports of clinical neurological impairment in welders have been reported over the past 100 years (Santamaria et al., 2007). There have been even fewer cross-sectional studies on the subclinical neurobehavioral effects of Mn exposure in welders (Bowler et al., 2003, 2006, 2007; Chandra et al., 1981; Ellingsen et al., 2008; Sinczuk-Walczak et al., 2001; Sjogren et al., 1990, 1996).

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In the central nervous system (CNS), the increase in Mn concentration in the brain is a critical step in the development of Mn-induced neurotoxicity. Mn ions are known to enter the brain via the cerebral capillaries in the blood–brain barrier or the cerebrospinal fluid in the choroid plexus (Crossgrove et al., 2003), and once within the CNS, Mn ions can be transported along nerve fibers. Excessive accumulation of Mn in the brain is known to cause variable combinations of behavioral and cognitive dysfunction, and movement disorders (Iregren, 1999). Severe Mn intoxication, known as manganism, can phenotypically resemble Parkinson's disease, but is distinct in pathology, neuroimaging, and disease progression (Sadek et al., 2003; Kim, 2006; Feldman, 1999).

In vivo proton magnetic resonance spectroscopy (^{1}H MRS) is an image-guided, noninvasive method to monitor neurochemical metabolites in the brain (Rosen and Lenkinski, 2007). Currently, ^{1}H MRS is the most commonly used biomedical technique for obtaining metabolic information as diagnostic indicator in many neurological diseases, and also follow the progression of disease and evaluate the response to treatment (Ross et al., 2006). Although MRS allows noninvasive, *in vivo* measurement of brain metabolites, to date only a few MRS investigations have been performed to assess the neurological effects of heavy metals in the environmental or occupational health. Recently, a few reports have assessed the impact of lead exposure on brain metabolism *in vivo*, including children (Meng et al., 2005; Trope et al., 2001; Weisskopf, 2007; Weisskopf et al., 2004, 2007). However, little is known about the effects of chronic Mn exposure on brain metabolites *in vivo*. To date, only two reports have used MRS to investigate the potential neurotoxic effects of chronic Mn exposure in the brain (Guilarte et al., 2006; Kim et al., 2007). Guilarte et al. (2006) assessed the toxic effects of chronic Mn exposure on levels of brain metabolites in non-human primates. This ^{1}H MRS study found a decrease in the *N*-acetylaspartate/creatine (NAA/Cr) ratio in the parietal cortex and frontal white matter at the end of the Mn-exposure period relative to baseline, indicating ongoing neuronal degeneration or dysfunction. Kim et al. (2007) investigated the potential neurotoxic effects of chronic Mn exposure in welders. Using point-resolved spectroscopy (PRESS) at 1.5 T, they measured the NAA/Cr, choline/creatine (Cho/Cr), and NAA/Cho ratios in the basal ganglia and found no significant differences between welders and control subjects.

In this study, we sought to determine whether there were metabolic differences between welders chronically exposed to Mn and healthy age-matched control individuals by measuring the brain metabolites using ^{1}H MRS. Five brain metabolites — NAA; Glx complex including both glutamine (Gln) and glutamate (Glu); total creatine (tCr); total choline (tCho); and myoinositol (mI) — were measured in the anterior cingulate cortex (ACC) and parietal white matter. Furthermore, we investigated correlations between neurochemical changes in the ACC of the brain with neurobehavioral changes to assess possible associations between chronic Mn exposure and cognitive deficits.

2. Subjects and methods

2.1. Subjects

Males aged more than 40 years, who were fulltime current welders with more than 5 years welding experience in factories making mild steel blocks for shipbuilding in the southeast of Korea were recruited to the present study. The welders were contract workers rather than permanent employees of a single factory. The main type of welding performed by the workers over the past 10 years was gas metal arc welding (GMAW) using CO_2 as a shielding gas. Exposure in the workplace was assessed by qualified industrial hygienists and welding was shown to be the only source of Mn in the workplace. Age- and gender-matched, non-welding production workers from the same workplaces, who were not exposed to other hazardous material such as paint, were recruited as a control group in the present study. We selected these control workers rather than office workers because, although office workers were not exposed to Mn, the socioeconomic status and behavioral patterns of office workers differed to those of the welders. Workers with a university education (more than 12 years of education) were excluded from the present study to avoid differences in neurobehavioral performance among study participants. Workers with a recent history of head injury, hand injury or other physical impairment such as history of chronic liver disease, iron deficiency anemia, carbon dioxide poisoning, stroke, depression or seizure disorders were also excluded. Of the subjects recruited, three welders and five control individuals failed to complete all the examinations, so a total of 55 workers (35 welders and 20 control individuals) were included in the final analyses. Workers visited a university hospital during the weekend. The time between the previous work shift and examination was at least 12 h. Each participant completed a questionnaire, provided blood samples, and underwent neurobehavioral tests and a ^{1}H MRS examination on the day of the visit. Written informed consent was obtained from all subjects before the examination, and the study protocol was approved by the Institutional Review Board of Ulsan University Hospital.

2.2. Exposure

We analyzed airborne Mn levels that had been measured twice-yearly at each workplace since 2006. Airborne Mn released by the welding process was collected on mixed cellulose ester membrane filters (0.8-μm pore size, 37-mm diameter; SKC Corp., USA) in personal air samplers (AirLite, SKC, USA). All pumps were calibrated before and after use. Sampling was performed for at least 6 h, excluding workers' breaks, with flow rates of 1–2.5 L/min. Samples were measured in the laboratory involved in quality control programs organized by the Korea Occupational Safety and Health Agency (KOSHA). Analyses were performed using inductively coupled plasma atomic emission spectroscopy (ICP-AES; ULTIMA2, Horiba Jobin Yvon, France) according to NIOSH analytical methods 7300 (NIOSH, 1994).

2.3. Questionnaires and blood measurements

Each individual completed a questionnaire and underwent blood sample collection during a single day. The questionnaire assessed basic demographic information (such as age and education level), information about smoking, alcohol consumption, medications, medical history, subjective symptoms, and job type including type and duration of welding. Each participant was asked detailed questions about his work history. Blood samples were obtained by venipuncture of the antecubital vein. Special care was taken to avoid contamination of skin and equipment with workplace dust. Mn levels were measured using a graphite furnace atomic absorption spectrophotometer (Varian AA240Z, Varian Techtron Pty, Victoria, Australia). All analyses were performed in the laboratory involved in KOSHA quality control programs. Each blood sample was also analyzed for complete blood counts, hemoglobin and hematocrit levels, as well as liver function (aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase levels).

2.4. Neurological examinations

Each subject underwent neurological examinations to detect clinical signs of manganism. Manganism was defined as a gross neurological symptom complex including symptoms such as