NeuroToxicology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

NeuroToxicology

Neurofunctional dopaminergic impairment in elderly after lifetime exposure to manganese

Roberto G. Lucchini [a,b,f,*], Stefano Guazzetti [c], Silvia Zoni [a], Chiara Benedetti [a], Chiara Fedrighi [a], Marco Peli [a], Filippo Donna [a], Elza Bontempi [d], Laura Borgese [d], Serena Micheletti [e], Roberta Ferri [a], Serena Marchetti [a], Donald R. Smith [f]

[a] Section of Occupational Health, University of Brescia, Italy
[b] Department of Preventive Medicine, Mount Sinai School of Medicine, NY, USA
[c] Public Health Service, Reggio Emilia, Italy
[d] INSTM and Chemistry for Technologies Laboratory, University of Brescia, Italy
[e] Department of Brain and Behavioral Sciences, University of Pavia, Italy and Child Neurology and Psychiatry Unit, Spedali Civili of Brescia, Italy
[f] Microbiology and Environmental Toxicology, University of California, Santa Cruz, USA

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 20 May 2014
Accepted 21 May 2014
Available online xxx

Keywords:
Ferroalloy emission
Heavy metals
Manganese
Lead
Odor identification
Motor coordination
Cognitive abilities
Prolactin control
Elderly

ABSTRACT

Background: Manganese (Mn) is an essential element that can become neurotoxic through various exposure windows over the lifespan. While there is clear evidence of Mn neurotoxicity in pediatric and adult occupational populations, little is known about effects in the elderly who may exhibit enhanced susceptibilities due to compromised physiology compared to younger adults. In the province of Brescia, Italy, the Valcamonica area has been the site of three ferroalloy plants operating from 1902 to 2001. Metal emissions of Mn and to a lesser extent lead (Pb) have impacted the surrounding environment, where a high prevalence of Parkinsonism was previously observed. This study aimed to assess neurocognitive and motor functions in healthy elderly subjects residing for most of their lifetime in Valcamonica or in a reference area unimpacted by ferroalloy plant activity.

Methods: Subjects were enrolled for extensive neurobehavioral assessment of motor, cognitive and sensory functions. Exposure was assessed with 24 h personal air sampling for PM10 airborne particles, surface soil and tap water measurement at individual households, Mn levels in blood and urine and Pb in blood. Dose–response relationships between exposure indicators and biomarkers and health outcomes were analyzed with generalized (linear and logistic) additive models (GAM).

Results: A total of 255 subjects (55% women) were examined; most (52.9%) were within the 65–70 years age class. Average airborne Mn was 26.41 ng/m^3 (median 18.42) in Valcamonica and 20.96 ng/m^3 (median 17.62) in the reference area. Average Mn in surface soil was 1026 ppm (median 923) in Valcamonica and 421 ppm (median 410) in the reference area. Manganese in drinking water was below the LDL of 1 μg/L. The GAM analysis showed significant association between airborne Mn ($p = 0.0237$) and the motor coordination tests of the Luria Nebraska Neuropsychological Battery. The calculation of the Benchmark Dose using this dose–response relationship yielded a lower level confidence interval of 22.7 ng/m^3 (median 26.4). For the odor identification score of the Sniffin Stick test, an association was observed with soil Mn ($p = 0.0006$) and with a significant interaction with blood Pb ($p = 0.0856$). Significant dose–responses resulted also for the Raven's Colored Progressive Matrices with the distance from exposure point source ($p = 0.0025$) and Mn in soil ($p = 0.09$), and for the Trail Making test, with urinary Mn ($p = 0.0074$). Serum prolactin (PRL) levels were associated with air ($p = 0.061$) and urinary ($p = 0.003$) Mn, and with blood Pb ($p = 0.0303$). In most of these associations age played a significant role as an effect modifier.

Conclusion: Lifelong exposure to Mn was significantly associated with changes in odor discrimination, motor coordination, cognitive abilities and serum PRL levels. These effects are consistent with the hypothesis of a specific mechanism of toxicity of Mn on the dopaminergic system. Lead co-exposure, even at very low levels, can further enhance Mn toxicity.

* Corresponding author at: Division of Occupational and Environmental Medicine, Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1057, New York, NY 10029, USA. Tel.: +1 212 824 7052; fax: +1 212 996 0407.
E-mail addresses: roberto.lucchini@mssm.edu, lucchini@med.unibs.it (R.G. Lucchini).

http://dx.doi.org/10.1016/j.neuro.2014.05.006
0161-813X/

1. Introduction

While it is clear that the aged are at greater risk and more susceptible to the deleterious effects from exposure to environmental agents compared to younger adults (Geller and Zenick, 2005; Risher et al., 2010), few studies have investigated manganese (Mn) pathophysiology in the elderly as a specific sensitive population. A large body of evidence, now further confirmed by meta- (Meyer-Baron et al., 2011) and pooled analyses (Meyer-Baron et al., 2013), shows that prolonged occupational exposure to Mn, even at relatively low levels causes motor neurotoxicity which may persist into retirement (Bouchard et al., 2008). Non-occupational studies with adults have shown both neuromotor and cognitive abnormalities (Kim et al., 2011; Roels et al., 2012), including increased frequency of Parkinsonism associated with Mn in airborne particles (Finkelstein and Jerrett, 2007) and deposited dust (Lucchini et al., 2007). Moreover, a high prevalence of Parkinsonism has also been observed in Mn-exposed welders (Racette et al., 2012), though the symptomatology of Mn-associated Parkinsonism in welders may differ from both idiopathic Parkinson Disease and the Parkinsonism associated with environmental Mn exposure (Racette, 2013). This difference in symptomatology may be due to the particular type of exposure in welding operations, which are mainly characterized by fine and ultra-fine respirable particles.

Over the past decade, there has also emerged evidence of health deficits associated with elevated Mn in newborns (Claus Henn et al., 2012), and older children exposed via drinking water (Bouchard et al., 2011; Wasserman et al., 2011; Khan et al., 2012) and airborne particulates (Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2011; Lucchini et al., 2012; Zoni et al., 2012; Vivas-Carvalho et al., 2013). In particular, a recent study from our group showed an association between environmental Mn exposure and deficits in fine motor and olfactory discrimination in children 11–13 years of age (Lucchini et al., 2012) – both Mn-related health effects similar to those reported in adult studies of Mn-associated Parkinsonism (Zoni et al., 2012). Overall, these studies support the concept that exposure to Mn over different temporal windows throughout the lifespan, even at relatively low levels of exposure, may lead to similar long-lasting neurotoxic endpoints (Lucchini and Zimmerman, 2009).

In light of this new evidence, we investigated the relationship between environmental Mn contamination and neurological health outcomes in aged subjects living in regions impacted by ferromanganese plant emissions in northern Italy. Previously we have reported a higher than expected prevalence of Parkinsonism in this region in relation to Mn emissions from ferroalloy plants (Lucchini et al., 2007), and Mn-related neurological deficits in both adolescents from this region and adult ferromanganese plant workers (Lucchini et al., 1999, 2012).

2. Methods

2.1. Target areas

The industrial sources of Mn in the study area are three former ferromanganese plants located in ValCamonica (VC), a valley of the pre-Alps that runs for about 50 miles in the NE-SW direction with an average width of about 2 miles, and is delimited by mountains of about 10,000 feet elevation. The industries operated from 1902 to 2001 in the municipalities of Darfo (lower Valcamonica, population 13,200), Breno (mid Valcamonica, population 5000), and Sellero (upper Valcamonica, population 1500). The Garda Lake (GL) tourist area of the Province of Brescia, with no history of metal industry was used as a reference group community. More detailed information on the study areas were published previously (Lucchini et al., 2007, 2012). Environmental levels of Mn and other metals have been thoroughly characterized in the study regions, showing that levels of Mn are significantly higher in Valcamonica compared to the Garda Lake reference area for airborne particles (Borgese et al., 2011, 2012), deposited outdoor dust (Zacco et al., 2009), indoor house and attic dust (Pavilonis et al., 2014), soil (Borgese et al., 2013), and locally cultivated leafy vegetables (Ferri et al., 2012).

2.2. Study design

Elderly subjects residing in the historically exposed area of Valcamonica and in the reference area of Garda Lake were enrolled in the study. This research was part of a large project funded by the European Union 6th Frame Program called PHIME (Public Health Impact of Mixed element Exposure in susceptible populations) that targeted various age groups in the community including pregnant women, adolescents, adult workers and elderly. Based on a community approach, the PHIME study was designed with a strong collaborative interaction with various community stakeholders. Subjects were recruited through public social centers, trade unions, and cultural and religious associations, and then invited to attend *ad hoc* meetings where the study aims and methodology were explained in detail. Inclusion criteria included men and women aged 65–75 years and locally residing since at least the 1970s. Eligible participants were interviewed for the assessment of the following exclusion criteria: (i) exposure to neurotoxic agents through occupation or hobbies; (ii) alcohol consumption >80 g/day; (iii) clinical neurologic, hepatic, or psychiatric disease; (iv) medical therapies active on the nervous system; (v) joint diseases of the hand and fingers; (vii) visual deficits not adequately corrected. Once properly informed, participants signed an informed consent that was approved by the Ethical Committee of the Local Public Health Agency of Brescia. The health assessment was conducted on different days over 2 consecutive weeks. Trained medical doctors and neuro-psychologists conducted the testing within facilities made available by the local Public Health Agency. Socio-demographic data, consumption of alcohol and smoking habits, clinical, occupational and residential histories were collected with *ad-hoc* questionnaires specifically designed to assess this cohort. A questionnaire for the screening of Parkinson's disease was also administered (Panisset et al., 1996), which included 10 items that were weighted in order to obtain a final score for the classification of "unlikely", "possible", or "probable" Parkinson's disease. Anthropometric data were measured for the calculation of Body Mass Index (BMI), and a food frequency questionnaire weighted for portion sizes was administered to estimate the daily oral intake of Mn. Each participant filled a personal diary with complete records of their activities and time spent in indoor/outdoor locations during the air-sampling period. Data on atmospheric conditions during the sampling period were obtained by the online meteorological system of the local Environmental Protection Office (ARPA Lombardia).

2.3. Neuropsychological battery

The health assessment test battery aimed to assess cognitive and motor functions and was identified based on a review of specific reported in the literature for Mn neurotoxicity (Zoni et al., 2007). It included the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) based on 30 simple questions and problems in a number of areas: the time and place of the test, repeating lists of words, arithmetic, language use and comprehension, and basic motor skills. The Italian version of the Story Recall Test (Spinnler and Tognoni, 1987) was used to evaluate long-term verbal memory; for this, the examiner read a short story and asked the

Please cite this article in press as: Lucchini RG, et al. Neurofunctional dopaminergic impairment in elderly after lifetime exposure to manganese. Neurotoxicology (2014), http://dx.doi.org/10.1016/j.neuro.2014.05.006