Neurochemistry International 97 (2016) 109–116

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Brain effects of manganese exposure in mice pups during prenatal and breastfeeding periods

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ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form
2 February 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Manganese
Mouse
Brain effects
Pups
Parental exposure

ABSTRACT

Manganese (Mn) is a trace element essential for brain development and functioning of the central nervous system. However, there is a lack of information concerning the neurotoxicity of Mn under realistic doses in early stages of development, though excess of Mn results in a progressive disorder of the nervous system called manganism. In the current study, adult mice were exposed to three doses of Mn for 60 days through daily gavages, while mice pups were exposed to the same Mn doses during developmental period (gestational and breast-feeding). From the latter group of mice, a group was exposed for more 60 days to the same Mn doses. Chemical analysis revealed a dose-dependent bio-accumulation of Mn in mice's brain. Biochemical parameters revealed that (1) Mn affects non-protein thiol levels, glutathione *S*-tranferase and acetylcholinesterase activities, as well as the levels of oxidized lipids and proteins in mice brain, though lipids and proteins alterations were found only after exposure to high and unrealistic doses; (2) Realistic doses of Mn affects the activity of brain AChE and finally; (3) Pups' brain were affected by Mn even whether only the parental females had been previously exposed. The current study shows evidences of chemical stress in mice exposed to Mn during the early period of development and an efficient mechanism of Mn elimination under higher doses. These findings open new lines of investigation regarding manganese toxicity in vertebrates mainly in the early stages of development.

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1. Introduction

Manganese (Mn) is an abundant transition metal essential to mammalian physiological processes (Santos et al., 2014). Eleven chemical oxidation states are found, where di, tetra and heptavalent states are the most important for cell physiology. Particularly, Mn plays vital roles on the mitochondrial enzymes manganese-superoxide dismutase (Mn-SOD), glutamine synthetase, alkaline phosphatase and arginase (Takeda and Avila, 1986).

Mn uptake is more efficient by inhalation, but ingestion and skin surface are also potential routes. In general, a human (~70 kg) is exposed to about 2–9 mg Mn.day^{-1} mainly through food (Aschner et al., 1999); but according to Cornelis and Nordberg (2007) the level of exposure is higher if water and food contamination, due to human activities, is considered. According to Martinez-Finley et al. (2013), an overexposure or accumulation of Mn causes a pathological state known as manganism. Perl and Olanow (2007) described this disturb as a consequence of increased production of reactive oxygen species and toxic metabolites, or depletion of cellular antioxidant defenses.

The brain is particularly vulnerable to oxidative damage due to its high rate of oxygen consumption, intense production of reactive radicals and high levels of transition metals, such as iron and manganese. According to Ansari et al. (2008), both metals can catalyze the production of the very toxic hydroxyl radical ($OH^{\cdot}$) through Fenton's reaction. Mitochondria, nucleus and synaptosomes of neuron and astrocytes of *globus pallidus* are described as the primary sites of Mn accumulation and toxicity in the brain (Martinez-Finley et al., 2013). Additionally, many studies reported

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http://dx.doi.org/10.1016/j.neuint.2016.03.009
0197-0186/Published by Elsevier Ltd.

that the sub-clinical neurological effects are the decrease of intellectual functions and increased risk of mortality of infants during their first year of life (Hafeman et al., 2007).

The neurotoxicity of Mn is characterized by neuropsychiatric, cognitive, behavioral and motor disorders (Erikson et al., 2007; Perl and Olanow, 2007). According to Mergler et al. (1999), the most important effects in vertebrates are poor hand-eye coordination, motor slowing, increased tremor, reduced response speed, olfactory enhancement and mood changes with possible memory and intellectual deficits. Cholinergic afferents are crucial for locomotion, cognition, emotion and behavioral response, and, therefore, anatomical selectivity of most manganese-induced cholinergic effects is compatible with the clinical symptoms of manganism (Finkelstein et al., 2007). Additionally, manganese influences astrocytic choline transport systems and astrocytic acetylcholine-binding proteins, affecting the reciprocal relationship between astrocytes and cholinergic neurons. A controversial point is whether the effects of manganese are more related to dopaminergic dysfunction in detriment of cholinergic one.

Neonates may be subject to a greater risk for Mn-induced neurotoxicity than adults (Torrente et al., 2002), and Mn crosses the placenta barrier exposing fetal tissues (Domingo, 1994; Fechter, 1999). Thus, in the current study, the neurological risk of exposure to manganese was investigated in mice chronically exposed to low daily doses of manganese as studies utilizing realistic doses in vertebrates are very scarce. Particularly, the effects of Mn at prenatal and breastfeeding periods were investigated through biochemical biomarkers in mice pups in order to elucidate some important aspects chemical stress in the early stages of development of vertebrates.

2. Material and methods

2.1. Animal manipulation

All experiments were conducted in accordance with the Ethics Animal Experiment Committee from Sector of Biological Sciences at Federal University of Paraná (protocol 467/2010) (http://www.bio.ufpr.br/portal/comissao-de-etica-para-o-uso-de-animais/) in accordance with the experimental Animal Brazilian Council (COBIEA) and Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care).

2.2. Experimental design

Experiment I. In order to confirm the effect of manganese chloride in adults and validate the experimental design, twenty-one-day adult male mice (Swiss) were exposed to 0 (control), 7.5, 15 and 30 mg kg^{-1}.day^{-1} of $MnCl_2$ through daily gavages during 60 days (n = 15 mice per group). After exposure, the liver and brain were sampled to chemical and biochemical analyses.

Experiment II. To evaluate the effects of low doses of manganese in adults and to compare the bioavailability of two different chemical forms, a group of twenty-one-day old male mice were exposed to 0 (control), 0.013, 0.13 and 1.3 mg kg^{-1}.day^{-1} of either manganese chloride or manganese acetate through daily gavages during 60 days. After this period, mice (n = 15 per experimental group) were sacrificed by cervical dislocation, and the liver and brain were sampled to biochemical analyses. The intermediate dose was based on a potential human daily exposure to Mn (9 mg Mn/70 kg individual) (Aschner et al., 1999).

Experiment III. Initially, 21-day old male (n = 5 per group) and female (n = 10 per group) mice were exposed to 0 (control), 0.013, 0.13 and 1.3 mg kg^{-1}.day^{-1} of $MnCl_2$ through daily gavages during 60 days. For mating, male and female mice were placed together in a proportion of 1:2. Pregnant female mice continued to be exposed to the nominal $MnCl_2$ doses throughout pregnancy and breastfeeding periods. After weaning, fifteen mice pups per group were sacrificed by cervical dislocation for liver and brain sampling. The remaining pups were utilized in the experiment IV.

Experiment IV. Mice puppies parentally exposed to $MnCl_2$ in *Experiment III* were exposed to 0 (control), 0.013, 0.13 and 1.3 mg kg^{-1}.day^{-1} of $MnCl_2$ through daily gavages during 60 days (n = 15 mice per group). Then, the liver and brain were sampled to biochemical analyses.

2.3. Chemical procedures for manganese analysis

Sample extraction: the samples were lyophilized in a Terroni LD1500A lyophilizer. The extractions were carried out in triplicate in Microcontrolled digestion block Tecnal Model 40/25. Each sample extracted of about 0.3 g was weighed in analytical scales Shimadzu AY 220 in borosilicate with the addition of 10 ml nitric acid and left to rest for 2 h. Then, they were heated up to 120 °C for 4 h. The extract obtained was transferred to 50 ml volumetric flasks through filtration in Whatman number 540 quantitative filter, followed by successive washing and assessment with ultrapure water after Reverse Osmosis procedure. The extracts were then stored under refrigeration at 4 °C up to the reading.

Flame atomic absorption spectroscopy: the readings were carried out in the Flame Atomic Absorption Spectrometer – FAAS Varian, model 240FS, using the SIPS automatic dilution system, equipped with deuterium lamp background correction and Mn multi-element hollow cathode lamp with 5.0 mA of current intensity, 279.5 nm wavelength and 0.2 nm fissure width. The ideal working band used was from 0.02 to 5.00 μg ml^{-1}. An oxidant flame air/acetylene was used, with 13.50 L min^{-1} and 2.00 L min^{-1} flow, respectively. In order to prepare the standard solution, the 1000 mg l^{-1} manganese stock solution Qhemis High Purity (PAMN 1000-0125) tracked by SRM/NIST-USA 928, prepared with ultrapure water. The analytical curve was built from this pattern at the concentration 1.00 mg l^{-1}, obtaining dilution at 0.75, 0.50, 0.25 and 0.00 mg l^{-1}. The correlation coefficient (r) obtained by analytical curve was 0.9987, with a value close to the reference value considered excellent (r = 1.0000). The blank reading was carried out, where we calculated the limit of detection (LOD), the lowest concentration of analyte in a sample that can be detected, of 0.2988 mg l^{-1} (Eq I), and the limit of quantification (LOQ), the lowest concentration of analyte that can be determined with a reasonable degree of precision and accuracy, of 0.9963 mg l^{-1} (Eq II). After the analysis, results were converted into mass and used in each sample.

$$\text{LD}: \ 3 \times \text{Sb}/\text{b} \tag{1}$$

$$\text{LQ}: \ 10 \times \text{Sb}/\text{b} \tag{2}$$

Where

Sb: White analysis triplicate standard deviation
b: Straight linear coefficient

Validation Method: the accuracy of the proposed method was verified from the certified reference material analysis ERM-CE278 (mussel tissue) from the European Measure and Reference Material Institute – IRMM. The method described revealed recovery of 90.2%, and the certified amount of Mn was 7.69 ± 0.23 μg g^{-1} while the amount obtained through the method was 6.94 ± 0.18 μg g^{-1}, showing that the method can be used as the matrix of this study.