Transition Metal Homeostasis in the Mouse Brain following High-dose Manganese Injections

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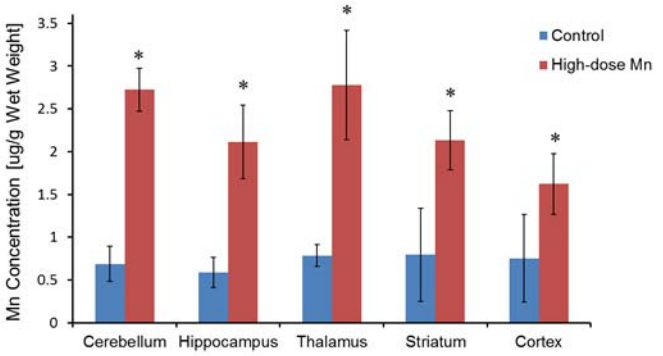
INTRODUCTION: MRI is sensitive to the presence of transition bio-metals in the brain, such as manganese (Mn), iron (Fe), and copper (Cu), if they are in a paramagnetic form. Manganese-enhanced MRI (MEMRI) is widely used in animal brain imaging with divalent manganese (Mn^{2+}) providing T_1 contrast [1, 2]. Given that metal homeostasis in the brain is tightly regulated [3] and Mn shares transporter proteins with Fe and Cu [4], the contrast seen in MEMRI may be a result of disrupted transition metal homeostasis in the brain rather than Mn accumulation alone. Here we investigate the source of MEMRI contrast by measuring the regional Mn, Fe, and Cu metal levels in rodent brains treated with fractionated high-doses of Mn which have been shown to produce good MEMRI contrast [5].

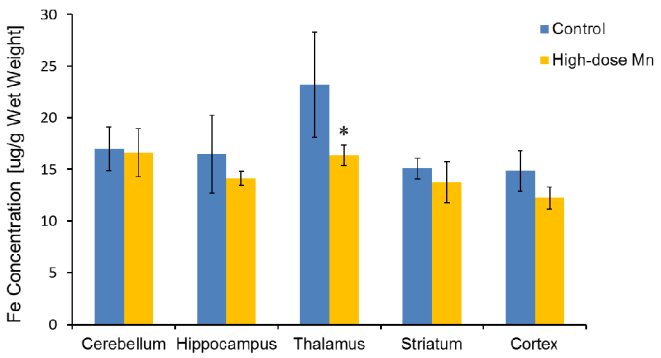
METHODS: **Animals:** All experiments were approved by the Animal Research Ethics Board at McMaster University. Experiments were carried out in ten, 16-week-old adult male C57Bl/6 mice weighing (30±2 g) (Jackson Laboratories, ME). Five mice received intraperitoneal injections of 30 mg $MnCl_2 4H_2O$/kg Mn solution in bicine buffer spaced 24 hours apart for four consecutive days. Five control mice received no injections. Animals were sacrificed 24 hours following the final Mn injections in the treated animals and the brains were removed and dissected into five regions: cerebellum, hippocampus, thalamus, striatum, and cortex. Metal levels in these tissue samples were measured using x-ray fluorescence (XRF) and neutron activation analysis (NAA). **XRF:** Fe and Cu content in the regional brain tissue specimens was measured using XRF. Fresh brain tissue was mounted onto XRF sample holders of 4 mm sample diameter. Measurements were done on an XRF system equipped with an XFlash LE SDD Detector (Bruker AXS GmbH, Karlsruhe, Germany) and a molybdenum target x-ray tube. The K-alpha fluorescence photopeaks of Fe and Cu were analyzed using PeakFit spectrometry analysis software package (PeakFit™ SPSS Inc., AISN Software Inc.). The ratio of fluorescence to scatter peak areas and linear calibration equations were used to quantify the Fe and Cu concentrations in the five brain regions. **NAA:** The concentration of Mn in freeze-dried brain tissue samples was quantified using comparative NAA. Mn standards (J.T. Baker 6458-04 Mn stock solution) and tissue samples were irradiated at the McMaster Nuclear Reactor and counted using an Aptec HPGe spectroscopy system. The area of the 847 keV photopeak corresponding to ^{56}Mn decay was determined using the Aptec software and the ratio of the specimen to standard peak area divided by the wet weight of the sample was used to calculate the Mn concentration.

RESULTS: Figures 1, 2, and 3 show the Mn, Fe, and Cu concentrations (mean ± std. dev., n=5 for each group) respectively in different brain regions of control mice and mice treated with a dose of 30 mg $MnCl_2 4H_2O$/kg. There was significant uptake of manganese into all of the brain regions at the p=0.05 level as expected. The iron levels remained the same in the cerebellum, hippocampus, striatum, and cortex of Mn treated mice, while there was a decrease in iron concentration in the thalamus of treated mice at the p=0.05 level. There was no significant change in the copper levels in the brains of mice treated with a high dose of Mn.

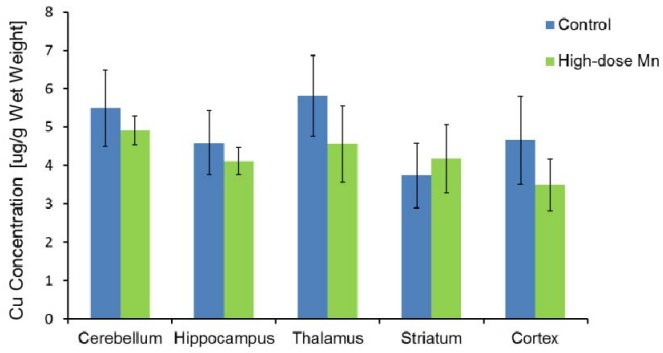
1 **Mn Concentration in Control vs 30mg/kg $MnCl_2$ Mouse Brain Regions**



2 **Fe Concentration in Control vs 30mg/kg $MnCl_2$ Mouse Brain Regions**



3 **Cu Concentration in Control vs 30mg/kg $MnCl_2$ Mouse Brain Regions**



DISCUSSION: Fractionated injections of Mn at the high dose commonly used in MEMRI did not in general disrupt overall metal homeostasis in the rodent brain, except for decreasing Fe levels in the thalamus. In terms of absolute metal amounts, Mn in that region increased by 2.0 µg/g while Fe decreased by 6.8 µg/g. This large absolute decrease in the Fe amount could result in a significant change in MEMRI contrast, depending on the relaxivities (T_1, T_2, and T_2*) of the form of Fe which is present [6]. While more experiments are needed to determine the effect of the change in Fe on MEMRI contrast, this experiment should serve as a caution that signal changes in MEMRI may not always be strictly associated with increases in Mn.

References:

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