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Considerations on manganese (Mn) treatments for *in vitro* studies

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Abstract

Manganese (Mn) is an environmental risk factor for neuronal dysfunction and neurodegeneration of the basal ganglia and other brain regions. Aberrant brain Mn levels have been linked to Manganism, Parkinson's disease (PD), Huntington's disease (HD) and other neurological disorders. Research on the cellular basis of Mn neurotoxicity has relied upon *in vitro* or non-human model systems. However, an analysis of relevant Mn concentrations for *in vitro* studies is lacking – and few studies have examined intracellular Mn levels. Here we perform calculations to evaluate *in vitro* exposure paradigms in relation to relevant *in vivo* levels of Mn post-exposure.

Introduction

Exposure to high manganese (Mn) levels in occupational or environmental settings or disease conditions is accompanied by Mn accumulation in brain regions highly sensitive to oxidative injury, namely the substantia nigra (SN), globus pallidus (GP) and striatum (Newland, 1989; Cersosimo et al., 2006; Olanow, 2004; Guilarte et al., 2010). Excessive Mn deposition in these regions leads to dopaminergic (DAergic) neuronal loss accompanied by an extrapyramidal syndrome referred to as manganism. Manganism patients exhibit rigidity, tremor, dystonic movements and bradykinesia, all characteristic features of Parkinson's disease (PD) (Cersosimo et al., 2006; Olanow, 2004; Guilarte et al., 2010; Calne et al., 1994). Exposure to Mn also represents a risk factor for PD (Gorell et al., 2004). Indeed, one of the strongest correlations between environmental exposure and PD is noted in Mn-exposed human cohorts (Hudnell, 1999). Parkinsonism in welders (*vs.* non-welders) is clinically distinguishable only by age of onset (46 *vs.* 63 years, respectively) and the prevalence of PD is higher among welders compared with age-standardized individuals in the general population (Criswell et al. 2011; Racette et al., 2005). Alterations in neuronal handling of Mn have also been observed in the context of Huntington's disease (Williams et al., 2010; Madison et al., 2012). Although a myriad of studies examined the cellular effects

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of Mn, surprisingly few have measured intracellular Mn concentrations. To assist the reader in determining relevant Mn concentrations for *in vitro* studies we provide the following calculations.

Results and Discussion

Protein content in cultured astrocytes is 0.006409 mg/million cells (unpublished data). Assuming an average cell radius of 2.25 μm, crystalline protein s=~0.65 (Matthews, 1974), and volume (((4pi)x3) x (radius cubed) x s) of 31.01×10^{-9} μl, we derive a protein content of 0.2067 mg/μl. Normal human brain Mn concentrations are in the range 1.1 – 2.9 ppm (Császma et al., 2003). These estimates may vary by a factor of 2 or more, dependent upon cell size and the established propensity of astrocytes to more readily accumulate Mn (compared to neurons), as well as regional differences in Mn distribution (e.g. basal ganglia are known to contain higher Mn levels *vs.* other brain regions) (Bowman et al., 2011). Nonetheless, if we assume a homogeneous regional and cell distribution, and apply the conversion factor of 206.7 μM/(nmol/mg)) we calculate (Mn brain concentration/conversion factor) normal human brain Mn concentrations at 5.32 – 14.03 ng Mn/mg protein (corresponding to 20.0 – 52.8 μM Mn).

Given that in mammalians, general toxic responses occur when Mn brain concentrations are elevated by ~3 fold (Erikson et al., 2007; Molina et al., 2011), aberrant function would be expected to occur at Mn brain levels of 15.96 – 42.09 ng Mn/mg protein (corresponding to 60.1 – 158.4 μM Mn).

Therefore, if cellular Mn concentrations in your *in vitro* studies capture these ranges of sub-threshold and threshold toxic levels, your studies are within the physiological and pathophysiological levels of Mn in the human brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We perform calculations to evaluate relevant in vitro exposures to Mn.

We establish media levels of Mn in the range of 60.1 – 158.4uM are relevant to testing its toxicity.