Temporal Changes in the T1 and T2 Relaxation Rates (ΔR1 and ΔR2) in the Rat Brain are Consistent with the Tissue-Clearance Rates of Elemental Manganese

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Introduction

Manganese (Mn) is an essential biometal that plays an important role as an enzymatic cofactor in the brain. However, excess Mn can damage the CNS by a number of mechanisms, including inhibiting mitochondrial oxidative phosphorylation (1). Manganese can be sequestered by mitochondria via the Ca^{2+} uniporter; however, Mn^{2+} efflux from brain mitochondria is extremely slow (2). For example, the half-life of Mn in the rat brain has been estimated to range from 52-74 days; significantly longer than many other rodent tissues (3). By contrast, the apparent brain Mn bioelimination rate – based on the temporal reduction of Manganese-Enhanced MRI (MEMRI) signal intensity – may be faster, with little residual evidence of T_1 signal enhancement 2-3 weeks after systemic or intrathecal injection of Mn^{2+} (4,5). This possible discrepancy suggests that the temporal reduction of MEMRI signal in the brain may result from more than bioelimination alone. This study tests the hypothesis that over time, a significant fraction of brain Mn is sequestered in a macromolecular environment that is inaccessible to water. In this case, the bound Mn fraction would not contribute to T_1 relaxation; resulting in an apparent reduction in MEMRI signal enhancement that would be indistinguishable from the normal Mn bioelimination process. In this case, Mn that is tightly sequestered in subcellular pools may be partially responsible for the lengthy cerebral retention of Mn observed in earlier studies (2,3).

Methods

All animal work followed the guidelines of the institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (weighing from 140-190 g) were anesthetized with isoflurane and infused with 176 mg/kg of $MnCl_2$ via the tail vein at a rate of 2.25 ml/h. Following the infusion, rats were returned to their cage and allowed normal access to food and water. No adverse reactions to the infusion were observed in any of the rats. T_1 and T_2 relaxation times were measured before, and then at 1, 7, 28, and 35 days after $MnCl_2$ infusion. Images were acquired on an 11.7 T/31 cm horizontal magnet (Magnex Scientific Ltd., Abingdon, UK) interfaced to a Bruker Avance console (Bruker BioSpin, Billerica, MA). During MRI, animals were anesthetized with 2% isoflurane and the body temperature was maintained by a temperature-controlled water bath. A homemade 9-cm birdcage coil was used for RF transmission and a 2.5-cm surface coil was used for signal reception. Twenty-one, 1-mm-thick, MRI slices were acquired across the brain with an in-plane resolution of 0.2 mm X 0.2 mm. T_1 was measured using a Look-Locker sequence (TR=12s, TE=2.5ms, flip angle=25°, interval=400ms, n=25) and the values calculated by a nonlinear 3-parameter fitting algorithm and subsequent post-processing (6). T_2 was measured using a CPMG spin-echo sequence (TR=10s; TE=6.5; and 20 echoes) and the values calculated by a nonlinear 3-parameter fitting algorithm. Following the imaging session for a given time point, the rat was euthanized and the brain removed. The olfactory bulb (OB) was excised as well as a small sample in the cortical region above the striatum. Brain-tissue samples were analyzed for Mn using inductively coupled plasma-mass spectrometry (ICP-MS) (West Coast Analytical Service, Santa Fe Springs, CA). The weight of manganese per gram-wet-weight of brain tissue was converted to molar concentration by assuming 80% water content. For a given animal, anatomical regions of interest in the calculated T_1 and T_2 maps were selected that best corresponded to the size and spatial location of the excised tissue samples. Changes in the R_1 ($1/T_1$) and R_2 ($1/T_2$) relaxation rates relative to the pre-infusion controls (i.e., ΔR_1 and ΔR_2) were compared to the corresponding Mn concentrations in each tissue sample. Statistical analysis was performed using a one-tailed, Student's t-test with unequal variance; $p \leq 0.05$ was considered significant. The data at each time point were compared to the same pre-infusion control group.

Results

For both OB and cortex, Mn concentration, ΔR_1, and ΔR_2 peaked at Day 1 after $MnCl_2$ infusion and gradually returned to near baseline following similar trends (Fig. 1). Mn concentration in the OB was significantly higher at all time points, but only at Day 1 and Day 7 in the cortex. For both OB and cortex, ΔR_1 and ΔR_2 was significantly higher at all time points except at Day 35 for ΔR_2 in the OB. The r_1 and r_2 tissue relaxivities for the OB were calculated from the respective slopes of plots (not shown) of R_1 and R_2 versus Mn concentration and estimated to be 6.5 s^{-1}mM and 65.5 s^{-1}mM, respectively. For the cortex, the analogous plots were not sufficiently well correlated to estimate the r_1 and r_2 tissue relaxivities.

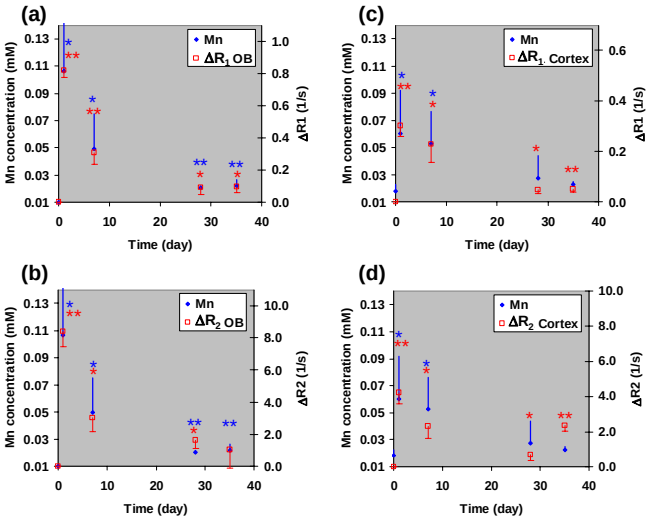

Fig. 1. Temporal changes in ΔR_1, ΔR_2 and Mn concentration. **(a, b)** olfactory bulb (OB); **(c, d)** cortex. *p < 0.05; **p < 0.01; error bar: 1SD.

Discussion

Consistent with earlier studies (4,5), the T_1 and T_2 relaxation times of cerebral tissue water are maximal during the first few days following $MnCl_2$ infusion and then decline to near control levels in the ensuing weeks. The temporal decline in Mn concentration follows a similar trend. In the case of the OB, the Mn concentration at 28 and 35 days post-infusion is approximately twice that – and statistically different from – the controls. However, cortical Mn concentrations were not statistically different from control at the same time points; consistent with a return to pre-infusion Mn levels. By contrast, ΔR_1 and ΔR_2 for both OB and cortex did not return to baseline at the 28- and 35-day time points, except for ΔR_2 at Day 35 in the OB. R_1 and R_2 versus Mn concentration were well correlated for the OB (data not shown) and the r_1 and r_2 relaxivities are in good agreement with those for $MnCl_2$ in water at the same field (6.9 s^{-1}mM). In addition to mitochondria, Mn^{2+} binds tightly to phosphate, nitrate, and carboxylate groups which are abundant in the cellular milieu (1). Mn^{2+} can also bind strongly to ferritin (7), which would reduce water accessibility and thus T_1 contrast. However, the low Mn levels relative to control at 28 and 35 days (even though significant for OB) indicate that only a small fraction of the total Mn dose remains bound in the cell. Consequently, the temporal changes in T_1 and T_2 are mainly due to Mn transport in and out of brain and thus the MEMRI signal intensity largely reflects the underlying tissue Mn concentration. The results of this MEMRI study suggest that efflux of cerebral Mn is significantly faster than that reported in earlier studies using other methods (2,3).

References

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