Short duration of AcPAS treatment accelerates MEMRI signal decline but not manganese washout

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Introduction: Quantitative manganese uptake provides measures of neuronal and glial activities making MEMRI a valuable tool for assessment of neurodegenerative processes. However, the prolonged half-life ($t_{1/2}$) of manganese (Mn^{2+}) in brain (51–74 days)[1] limits serial quantitative MR assessments. Previous studies[2,3] have suggested that N-acetyl-para-aminosalicylic acid (AcPAS), a chelater of manganese may provide an answer. Thus, we determined whether AcPAS could affect Mn^{2+} enhancement decline and permit its frequent administration for longitudinal studies.

Methods and Materials: Mn^{2+} brain concentration following AcPAS treatment was determined by inductively coupled plasma mass spectrometry (ICP-MS). AcPAS was synthesized from PAS (para-aminosalicylic acid). C57BL/6 mice (n = 9) were administrated $MnCl_2$ (50 mM, 60 mg/kg) i.p. for four days. MRI was performed 24 hrs after the last $MnCl_2$ injection (Day 0). This was followed by the administration of PBS (n = 3) or AcPAS at 100 (n=3) or 200 mg/kg (n=3) for 14 days. PBS/AcPAS was injected i.p. three times/day every 8 hrs. Mice were scanned 7 and 14 days after the first PBS/AcPAS injection on a Bruker 7 T/21 cm MRI system using T_1 mapping and 3D T_1-wt MRI. T_1 values calculated from the T_1 mapping calibrated the T_1-wt MRI to limit variations from system settings. Brain volumes were extracted from T_1-wt MRI[4]. Day 7 and 14 T_1-wt images were registered to start (Day 0) images. Mn^{2+} enhancement change with time was calculated using Eq.1: (Day 7 or 14 – start)/ start. Mice were immediately euthanized after MRI tests. Brain tissue was collected to measure manganese concentration. Another group of mice (n = 3) was injected with AcPAS (200 mg/kg) for HPLC analysis.

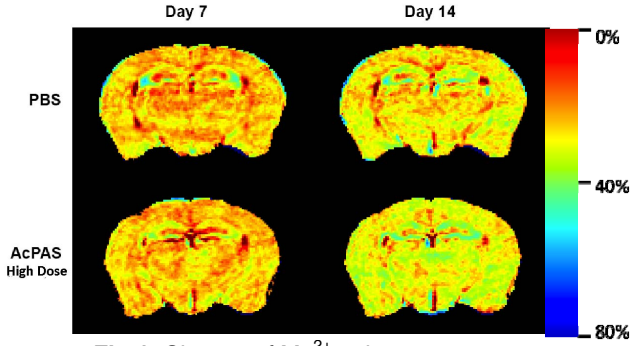

Fig.1. Change of Mn^{2+} enhancement

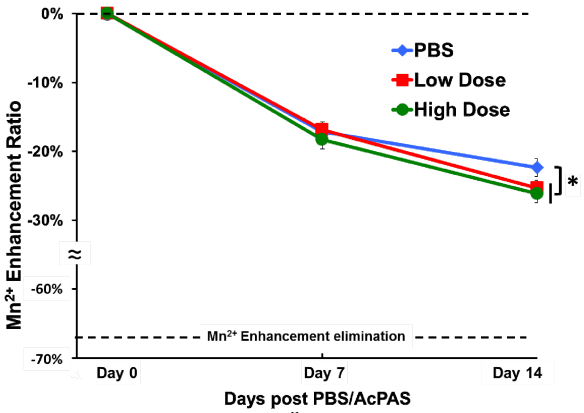

Fig.2. Decline of Mn^{2+} Enhancement

Results: Mn^{2+} enhancement declined after PBS/AcPAS injections (Figure 1). It can be seen that signal enhancement declined in the PBS injected mouse from Day 7 to 14 due to native Mn^{2+} washout. In the AcPAS injected mouse, MRI signal was lower compared to the PBS mouse. Statistical analysis showed that signal enhancement ratio was decreased at day 14 at all AcPAS doses compared to PBS mice ($p<0.05$), whereas at Day 7, no difference was found (Figure 2). From Eq.1, and T_1 values measured on pre-Mn^{2+} administrated mice, a line indicating complete Mn^{2+} enhancement elimination is included in Figure 2. The HPLC analysis showed that AcPAS was initially high in plasma and eliminated at later times, whereas AcPAS steadily increased in hippocampus. ICP-MS analysis showed at Day 14 no difference in Mn^{2+} concentration between PBS and AcPAS mice.

Discussion: HPLC results demonstrated that AcPAS enters freely and has long $t_{1/2}$ in the brain, in which, Mn^{2+} ions are chelated by AcPAS. ICP/MS results indicated that AcPAS chelation did not accelerate Mn^{2+} washout in short period of time probably due to high tissue affinity of AcPAS. However, the chelation by AcPAS does interfere with the interaction of water molecules and Mn^{2+} ions, and therefore suppresses Mn^{2+} T_1 shortening effect as demonstrated in Fig. 1 and 2. Nevertheless, the study suggests that AcPAS may be useful for serial MEMRI studies, as AcPAS chelation may be able to reduce Mn^{2+} toxicity from multiple studies over time.

References: 1. Takeda, et. al., Brain Res, 1995; 2. Zheng, *et. al.*, Neurotoxicology, 2009; 3. Hong, *et. al.*, DMD, 2011; 4. Uberti et al, 2007