Disparate Neuronal and Glial Signal Enhancement in MEMRI

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Introduction. The use of manganese (Mn) enhanced MRI (MEMRI) to facilitate disease diagnosis and elucidate mechanisms of neuroinflammatory and neurodegenerative disorders is an area of active investigation. Based on prior MEMRI rat ischemia studies[1-3], signal enhancement in and around ischemic regions occurred as a result of activated glia (astrocytes and microglia). However, concordant pathobiologic events are operative including neuronal excitation during lesion development and calling into question its mechanisms of action. Thus, we investigated Mn glial uptake *in vitro* and *in vivo* rodent models of human diseases to assess the roles of glia and neurons in the signal enhancement in MEMRI.

Materials and Methods. PC 12 cells differentiated to mimic neurons[4] were co-cultured with astrocytes and microglia. The glial cells were treated with interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) at the following concentrations: 25 and 2.5, 50 and 5 and 100 and 10 ng/ml IFN-γ and TNF-α (labeled low, medium and high) to induce activation. After 9 hours, $MnCl_2$ solution administered at 80, 160 and 320 μM was added to the media. A control cell group was used for baseline measurements. Cells were recovered at 2, 6, and 15 hours after $MnCl_2$ was applied. Inductively coupled plasma mass spectrometry (ICP/MS) was used to measure the Mn sample concentration. Experiments were performed with triplicate samples with each containing 10^5 PC 12 and astrocytes or PC 12 and microglia (both at 1:1 cell ratios).

For the animal studies, 3 eight-week-old male C.B.-17 SCID mice were injected with lipopolysaccharide (LPS, serotype: *e. coli* 055:B5). The LPS was injected intraperitoneally (i.p.) at a 2 mg/kg dose. $MnCl_2$ was administered at 120 mg/kg at a rate of 250 μl/hour after LPS. The control mouse group (n = 3) was injected only with $MnCl_2$. Mice were scanned after 24 hours using T1 mapping (fast spin echo with variable TR from 0.5 s to 10 s, 20 slices, slice thickness = 0.5 mm, in-plane resolution = 0.1x0.1 mm^2) and T1-wt MRI (gradient recalled echo, TR = 15 ms, flip angle = 20°, 3D isotropic resolution = 0.1 mm). Mice were euthanized after scanning for histological analyses.

Results and Discussion. Mn uptake by co-cultured PC 12 and astrocytes are shown in Fig.1A and B, respectively. Uptake with 80, 160 and 320 μM of Mn was concentration dependent at 2 and 6 hours, ($p < 0.01$). Mn uptake by PC 12 cells was significantly increased from 2 hours to 6 hours at all concentrations. From 6 hours to 15 hours, Mn uptake was not changed at 80 and 160 mM and decreased at 320 μM. The correlation of the Mn uptake and cytokine (IFN-γ and TNF-α) concentration was significant only at 2 and 15 hours. At 2 hours, the correlation ratio (R^2) was 0.99 and 0.98 at 160 and 320 μM, respectively. At 15 hours, R^2 was 0.87 and 0.78 at 160 and 320 μM, respectively. At 15 hours, the Mn uptake was inversely proportional to the cytokine concentration. Fig. 1B shows the measured Mn concentrations in astrocytes. The Mn concentrations were 100 times higher than in the PC 12 samples. However, no difference was found between the baseline and the measurements at other Mn concentrations. The results of the co-cultured PC 12 and microglia were similar to that of co-cultured PC 12 and astrocytes.

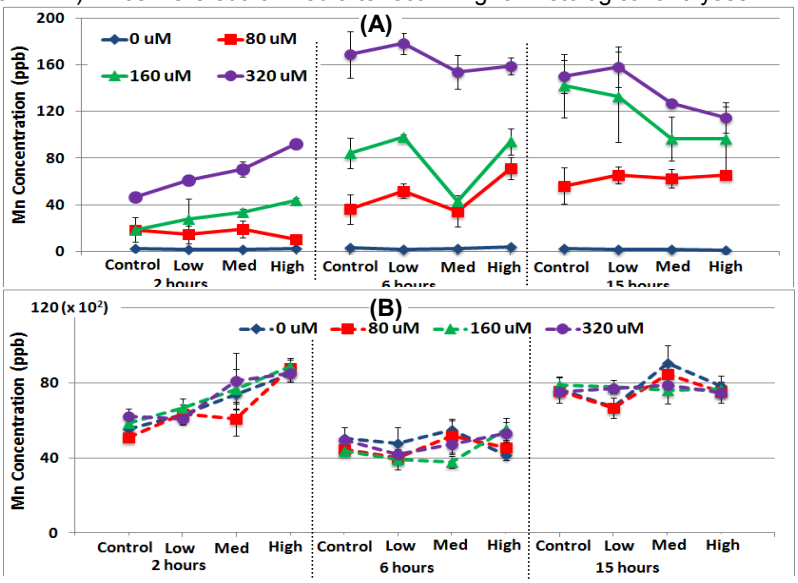

Fig. 1. (A) Mn concentrations measured in PC 12 samples. (B) in astrocytes

The T1 values on the neurostructures in the control and LPS treated mice are shown in the table (mean±SE, ms). No significant difference was found between the two mouse groups. The T1-wt images were shown in Fig. 2. The image on left is from a control mouse, and the right one is from a LPS treated mouse. Mn enhancement can be clearly seen on the layers of olfactory bulbs and cerebellar cortex, and on sub-hippocampal structures in the control mouse. The LPS mouse showed similar signal enhancements.

	CTX	CP	TH	HY	MB
Control	1357±18	1280±37	1275±7	1146±36	1127±33
LPS	1391±58	1345±41	1225±93	1100±81	1258±78

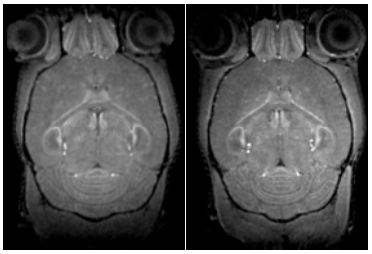

Fig. 2. T1-wt MRI

Our *in vitro* results showed that the Mn uptake by PC 12 cells was dependent on a number of factors including the concentrations of Mn and cytokine, and the time of cytokine treatment and Mn incubation. The similar quantity of Mn in astrocyte and microglia in the baseline samples and the samples mixed with $MnCl_2$ indicated that the measured Mn was from endogenous sources, and the uptake by astrocytes and microglia was not affected by activation. The MRI results supported our *in vitro* findings demonstrating no significant signal enhancement contributed by glial activation.

Refs: 1. M. Wideroe, et al., Neonatology, 2011; 2. M. Wideroe, et al, Neuroimage, 2009; 3. Y. Kawai, Neuroimage, 2010; 4. S. Reaney, Chem. Res. Toxicol., 2002;