Role of Neuroinflammation in MEMRI signal enhancement

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Introduction: Mn^{2+} enhanced MRI (MEMRI) can be used to detect a number of neuropathological events that include glial cell activation and neuronal excitation and death. However, the mechanisms for Mn^{2+} signal enhancement reflects such pathobiological states remains controversial[1-2]. We posit that signal enhancements are directed by activated glial cells which serve to stimulate neurons leading to elevated neuronal Mn^{2+} uptake[3]. MEMRI signal intensities were investigated during lipopolysaccharide (LPS)-induced neuroinflammation. The overarching idea was to assess the role of glial cell activation in MEMRI signal enhancement.

Materials and Methods: Eight-week-old C57BL/6 mice were injected intracranially with 10 µg LPS in 2µl PBS volume. A control group was injected with 2 µl PBS. Both groups were injected with $MnCl_2$ at 60 mg/kg i.p. for 4 days before the MRI scan. MRI scans were conducted at 7 day after LPS injection 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^o, 3D isotropic resolution = 0.1 mm). Animals were euthanized and brains recovered to immunohistological validation following paraffin embedding. Five µm thick sections were labeled with mouse monoclonal antibodies for glial fibillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1). Images were captured with a 10× objective by using Nuance EX multispectral imaging system (Cambridge Research Instruments), and reflective image analysis software (Media Cybernetics). Stained section were quantitated within regions of interest (in and around the injection line) as reflected by intensity/μm^2. MRI Data Analysis: Field inhomogeneity was first corrected using the N3 algorithm. Enhanced values were measured on a slice-by-slice basis in the axial direction. On each slice, the injection site was first identified. The mean value and standard deviation of the signal intensity about the corresponding location of the needle hole on the contra-lateral hemisphere was measured (in a large ROI), then the intensity threshold was defined as the mean value plus 2 standard deviations (SD). This threshold was applied on the ipsi-lateral hemisphere as the lower boundary to identify enhanced area about the needle hole. The enhanced volume was the summation of the enhanced areas multiplying by the slice thickness. The enhanced volume was then normalized by the needle depth. The enhancement was defined as the ratio of the mean signal intensity on the enhanced volume and mean intensity on the contra-lateral side.

Results and Discussion:

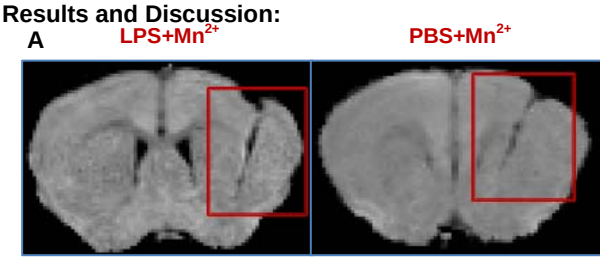

Fig.A. T1-wt MRI images. Signal enhancement around the injection line (encased by red box).

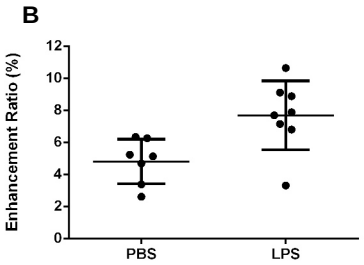

Fig.B. Enhancement Ratio. LPS group showed significantly higher signal enhancement than PBS group (p<0.01).

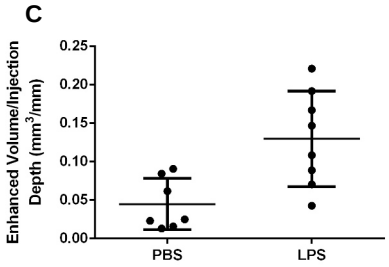

Fig.C. Enhanced Volume. Enhanced volume measurement normalized by dividing it by respective Injection depth. LPS group showed significantly higher enhanced volume (p<0.01).

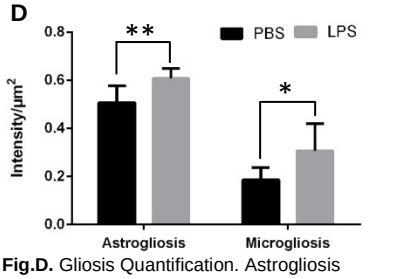

Fig.D. Gliosis Quantification. Astrogliosis (p<0.01) and microgliosis (p<0.05) count (Intensity/μm^2) significantly higher in LPS group.

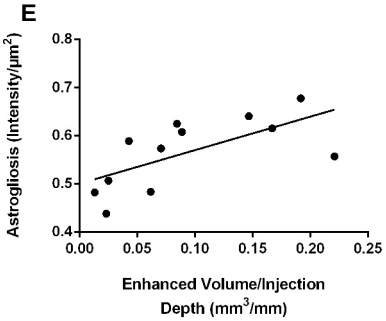

Fig.E. Correlation plot in between Enhanced volume and Astrogliosis (Pearson's correlation,r=0.66, p<0.05).

At day 7, after LPS/PBS and Mn^{2+} injections, T1- wt images showed signal enhancement around the injection line. (Fig. A.) The signal was enhanced in several regions at the injection line compared to the surrounding tissue and the regions in the contralateral hemisphere. Enhancement ratio and enhanced volume were significantly higher in LPS group than in PBS group, where p-value is p<0.01 for both variables (Fig.B. and C.). In Immunohistological analysis, astrocyte (p<0.01) and microglial (p<0.05) activation was significantly higher in the LPS group than in the PBS group (Fig.D.). Correlation comparisons showed correlation between astrogliosis and enhanced volume (Pearson's correlation coefficient = 0.66, p<0.05) (Fig.E.), and between astrogliosis and microgliosis (Pearson's correlation coefficient = 0.62, p<0.05) are significant (figure not shown). Results from our in-vitro comparative study of Mn^{2+} uptake by glia and neurons showed increase in neuronal Mn^{2+} uptake when co-cultured with activated astrocytes but minimal uptake by activated astrocytes or microglia alone[3]. The current in-vivo LPS study combined with our previous study suggests that activated glia do not directly induce signal enhancement in MEMRI, but signal enhancement in MEMRI results from the increased neuronal activity as a result of gliosis, mainly astrogliosis, stimulating neuronal Mn2+ uptake.

References: 1. M. Wideroe, et al., Neonatology, 2011; 2. M. Wideroe, et al., Neuroimage, 2009; 3. Y. Liu, et.al., ISMRM, 2012(Australia). 4. Y. Liu, et.al., ISMRM, 2012(Australia).