Use of short chain oligo-glycerolipids to improve BBB permeability: application to Managanese-Enhanced MRI (MEMRI)

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Introduction

Manganese ions (Mn2+) can be used as an indicator of calcium (Ca2+) influx, owing to their ability to enter cells through voltage-gated Ca2+ channels [1]. As Mn^{2+} possesses paramagnetic properties, its accumulation in cells affects MR contrast, and manganese-enhanced MRI (MEMRI) methods have been applied to elucidate anatomical information and to identify regions of increased neuronal activity in the brain [2,3]. Paramount to the successful application of MEMRI is the efficient delivery of Mn^{2+} to the tissue of interest at an appropriate concentration. Systemically administered Mn^{2+} does not efficiently cross the blood brain barrier (BBB), but can readily enter the cerebrospinal fluid (CSF) through the choroid plexus[2], producing a slow and spatially heterogeneous distribution of the ions in the brain, with high concentrations in periventricular structures, and low or undetectable uptake in parenchymatic areas. To overcome this problem, previous MEMRI studies have been performed using invasive approaches such as osmotic disruption of the BBB [4]. However this technique is highly invasive and induces mono-hemispheric, inhomogeneous increase of BBB permeability [5,6]. Here we examined the effect of pre- or co-administration of short chain oligoglycerolipids, which are thought to modulate tight junctions and facilitate translocation of exogenous substances through the BBB [7], as a means to enhance the BBB permeability to Mn^{2+} in a MEMRI protocol.

Methods

Experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985). **Animal preparation**: Male Sprague-Dawley Rats (347±8g) were anaesthetised with isoflurane and tracheotomised. Left femoral vein and arteries, and common carotid artery (CCA) were cannulated to allow continuous blood pressure monitoring, measurement of arterial blood gases and administration of compounds. The right external carotid artery was closed to avoid the perfusion of extra-brain organs. Image acquisition was performed under 1.2% isoflurane anaesthesia, neuromuscular blockade and artificial ventilation. **Experimental groups**: Two short chain oligoglycerolipidic isoforms were tested: 2-Lipobridge (2-LB) and 1-Lipobridge (1-LB; Genzyme, UK). **(1)** 2-LB is thought to induce a transient (≈20 min) opening of BBB tight junctions [7]. 2-LB (85 mM, n=8) or saline (n=6) were injected through the CCA (6 ml/min; 1 ml) followed by a 35 min infusion of 12.5 mg/ml $MnCl_2$ (6 ml/hr) through the femoral vein. **(2)** 1-LB is thought to form microparticles that can enclose and translocate compounds across the BBB. 1-LB (75 mM n=4) was co-administered with 10 mg/Kg of $MnCl_2$ through left CCA (6 ml/hr, 1 min). Control (baseline) rats (n=4) were administered $MnCl_2$ without LB. **MEMRI acquisition protocol:** MEMRI time series data were acquired on a Bruker Biospec 4.7T system using a T1-weighted FLASH sequence [8] (matrix 256x256; FOV 40mm; slice thickness 1mm; 24 contiguous coronal slices; α=90, TE=4.9; TR=400ms; δt=120s). **Data analysis:** time series data were spatially normalised to a reference study template [9]. Signal changes were normalised to pre-injection baseline. Fractional signal enhancement maps were obtained by plotting the relative signal increase (average of 8 post-injection and 3 pre-injection time points). The map was thresholded at $p<0.01$ (paired *t* test). Data are presented as mean ± SEM.

Results

(1) Effect of 2-LB. $MnCl_2$ infusion produced robust (+80%) signal increases in periventricular areas in both vehicle and 2-LB treated animals consistent with ability of Mn to enter the CSF. Brain parenchymal regions in control animals showed weak but significant signal increases (+8-10%), indicative of a poor but non-negligible BBB permeability to this ion. 2-LB induced weak signal enhancements only in the ipsilateral somatosensory cortex, and a trend for a slight enhancement in the striatum. The overall effect was very small, spatially heterogeneous, and rather variable across animals. No signal enhancement was observed in the contralateral hemisphere. **(2) Effect of 1-LB.** Acute $MnCl_2$ challenge *per se* produced robust (+60%) signal increases in periventricular areas, but negligible signal increases in brain parenchyma (Fig. 1b). Co-administration of 1-LB+$MnCl_2$ induced robust and reproducible signal enhancements in brain parenchyma regions across both the hemispheres (+45-75% in the Cpu, +40-70% in the SS Ctx). The intra-hemispheric distribution of the effect was highly homogeneous. The magnitude of the MEMRI signal was slightly more pronounced in the ipsilateral hemisphere.

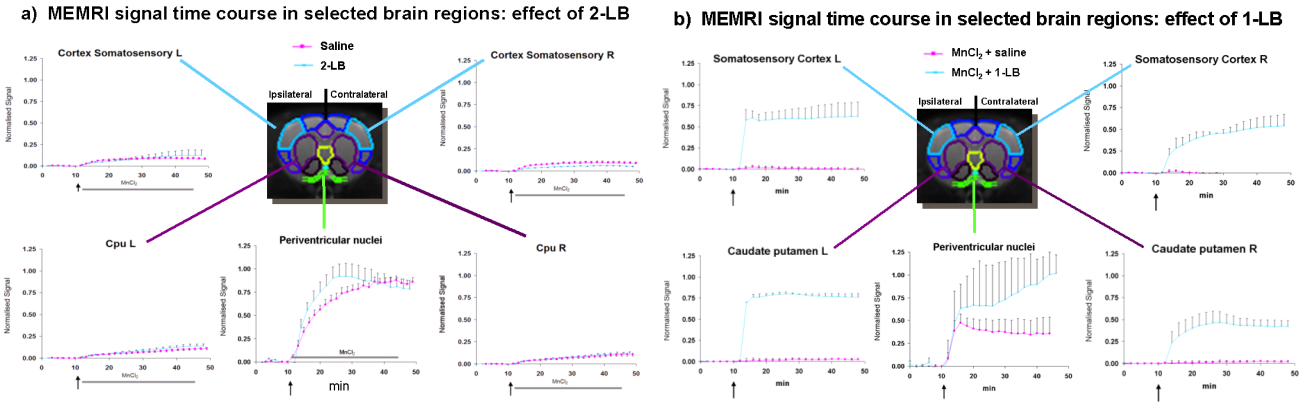

Figure 1 a) *Time course of fractional MEMRI signal in representative brain regions. Arrows indicate time of injection of 2-LB or saline through the CCA. Grey line illustrates period over which $MnCl_2$ was infused i.v.* **b)** *Time course of fractional MEMRI signal in representative brain regionsArrows indicate time of injection of $MnCl_2$ or $MnCl_2$ + 1-LB through the CCA.*

Conclusions

2-LB did not produce significant signal enhancement in any of the parenchymatic regions analysed. By contrast, 1-LB gave rise to spatially homogeneous signal increases in both brain hemispheres. These findings make 1-LB an attractive alternative to hyperosmolar disruption of the BBB. Indeed, previous studies have shown that the effect of osmotic agents is strictly mono-hemispheric, spatially heterogeneous and often scarcely reproducible [6]. Moreover, these agents produce a prolonged opening of the tight junctions that is of limited use for pharmacological studies, as this can alter the pharmacokinetics and brain penetration of the drug of interest. The use of 1-LB can overcome these problems by inducing a rapid and transient translocation of manganese across the BBB, giving rise to reproducible and homogeneous bi-hemispheric MEMRI signal increases.

References: [1] K. Narita *Brain Res* 510, 289-295 (1990) **[2]** AC Silva, *NMR Biomed.* 17, 532-543 (2004) **[3]** RG Pautler *Methods Mol.Med.* 124, 365-386 (2006) **[4]**H Lu *PNAS* 104, 2489-2494 (2007) **[5]** C Zimmer *Radiology* 196, 521-527 (1995) **[6]** GL Remsen et al., *Anesth Analg* 88, 559 (1999) **[7]** P Hoffmann, *Proc. Of 34th Annual Meeting of Controlled Release Society (Long Beach)* 19, 19 (2007) **[8]** J Haase, *JMRI* 67, 258--266 (1986) **[9]** AJ Schwarz et al., *NeuroImage* 32, 538-550 (2006)