A Novel Method for Dynamic Manganese-Enhanced MRI

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Introduction Manganese-enhanced magnetic resonance imaging (ME-MRI) has been successfully applied to map neuronal response to a variety of stimuli (1-6). However, manganese (Mn^{2+}) has very low permeability through the blood-brain barrier (BBB). For studies focusing on structures that have no or low BBB, such as the olfactory tubercle, superior colliculus nucleus, hypothalamus (7), ME-MRI can be performed by systemic administration of Mn^{2+}. However, for studies employing pharmacological or other manipulations, where multiple cortical and subcortical structures are expected to be activated, temporal disruption of BBB appears to be necessary for whole brain imaging (5). The BBB disruption method of Lin et al. (8) requires catheterization of the carotid artery and bolus injection of hyperosmolar mannitol via the internal carotid artery. Since the circle of Willis in the rat provides incomplete vascular collateralization, it appears technically and anatomically difficult for mannitol to be distributed homogenously within both hemispheres with a single bolus injection. Additionally, such factors as the amount of mannitol, the speed of injection, and the temperature of the drug solution can influence BBB disruption (9). Furthermore, carotid artery catheterization practically limits this technique to non-survival experiments. Thus, finding new methods that overcome these technical difficulties is of great interest for functional ME-MRI experiments.

The endothelial barrier antigen (EBA) is a membrane protein expressed by the endothelial cells of rat BBB. Ghabriel et al. (10) suggested that immunological targeting of EBA by I.V. administration of a monoclonal antibody (anti-EBA) could acutely open the BBB to exogenous tracers. This BBB opening method avoids traumatic surgical preparation and provides a potentially novel Mn^{2+} delivery method for whole brain functional imaging using ME-MRI. In the present study, we evaluated the feasibility of using an anti-EBA agent to facilitate ME-MRI experiments.

Methods Twenty-two S.D. rats weighing 339±24 g were used in this study. Animals were anesthetized with urethane (1.2g/kg), intubated and artificially ventilated. Femoral artery and veins were catheterized for pressure monitoring and drug delivery. Scan procedures generally followed a previous report (5). Briefly, five baseline T1-weighted images were acquired followed by continuous I.V. infusion of 1% $MnCl_2$ solution. Twenty min. later, I.V. infusion of anti-EBA agent SMI-71 (30 µl in 0.4ml saline, 0.08ml/min) was initiated. Since we observed dramatic blood pressure increase in the first group of animals (n=10) immediately following SMI-71 infusion, a second group of animals (n=6) received isoflurane (1.5%) to mitigate the blood pressure effect, which was started 5 min before the initiation of SMI-71 infusion, lasting for about 20min. A third group of animals (n=6) received vehicle (no SMI-71) as a control. MRI scans were performed at a Bruker 9.4T scanner. Scan parameters were: traditional spin echo sequence (MSME), TR=450 ms, TE=8ms, matrix size=128×128, 13 slices with a slice thickness of 1mm. The effect of BBB disruption was evaluated in a real-time fashion using an in-house developed program. Images were registered to a common space and detrended as needed. Results were quantified by calculating fractional MRI signal enhancement at the end of the scans relative to baseline scans, each averaged with 3 data points. Pre- and post-SMI fractional signal changes were compared using one-tailed paired t-test on a voxel-wise basis.

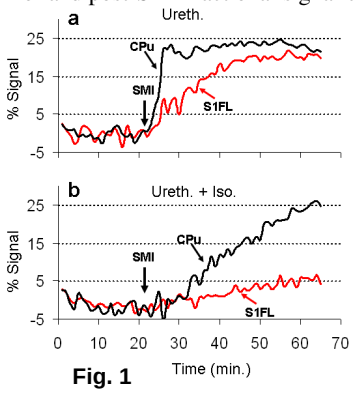

Fig. 1

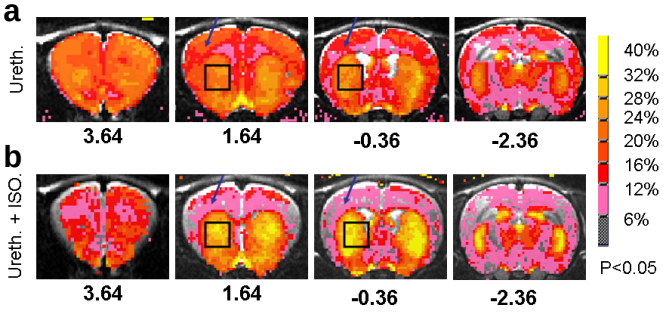

Fig. 2

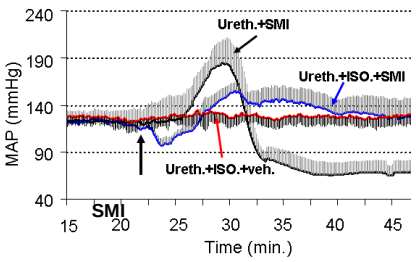

Fig. 3

Fig. 1. Representative time courses from CPu and S1FL regions under urethane (**a**) and urethane + isoflurane anesthesia (**b**). Fig.2. ME-MRI signal enhancement as a result of BBB disruption by I.V. infusion of SMI. Notice the different outcomes under the two different anesthesia protocols, as indicated by arrows and squares (n=10 for Ureth. Group, n=6 for Ureth.+ISO group). Numbers below images are coordinates relative to bregma. Fig. 3. The effects of SMI injection on mean arterial blood (MAP). MAP profile during vehicle injection (without SMI) is also shown.

Results Following SMI infusion, all animals in the urethane anesthesia group had BBB disruption. Fig. 1a shows representative time courses in the caudate putamen (CPu) and the primary somatosensory cortex of the forepaw (S1FL) region. Group analysis results are shown in Fig. 2. Whole brain BBB disruption was achieved, with CPu and hippocampus demonstrating the highest signal enhancement. However, most animals in this group had abrupt increases in mean arterial blood pressure (MAP) with a peak value of ~190 mmHg, followed by irreversible blood pressure drop to ~60mmHg (Fig. 3), significantly compromising animal physiological conditions. The second group of animals receiving SMI under isoflurane demonstrated a smaller peak but longer duration of MAP effect (Fig 3), presumably due to the vasodilatory effect of isoflurane. In general, there was a smaller signal enhancement in the cortex but similar magnitude in the subcortical structures in this group (Fig. 1b and 2b). Animals receiving vehicle injections did not show enhancement in ME-MRI signal or increase in MAP.

Discussion We present a novel BBB opening method that is potentially useful for dynamic ME-MRI experiments. It appears that BBB remains open for at least 2 hrs once it is disrupted (data not shown). Robust BBB disruption, especially in subcortical structures, suggests this technique may be particularly promising for imaging the pharmacological effects of drugs of abuse. The mechanism of BBB disruption by SMI remains unknown, but it appears to be related to MAP increase.

References 1. Duong TQ et al. MRM 2000;43:383–392. 2. Pautler RG et al. Neuroimage 2002;16:441–448. 3. Aoki I et al. MRM 2002;48:927-33. 4. Yu X et al. Nat Neurosci. 2005;8:961–968. 5. Lu H et al. PNAS 2007;104:2489–2494. 6. Kuo YT et al. NMR Biomed. 2006;19:1028-34. 7. Kolb B and Whishaw IQ. Fundamental of human neuropschology 2003, p.119-20. 8. Lin YJ et al. MRM 1997;38:378-88. 9. Aoki I et al. NMR Biomed 2004;17:569–80. 10. Ghabriel MN et al. Brain Res. 2000;878:127-35.