Brain Redox Imaging Using Blood Brain Barrier Permeable Nitroxide MRI Contrast Agent

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Introduction; Reactive oxygen species (ROS) and compromised antioxidant defense may contribute to numerous brain disorders such as stroke, amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease. Nitroxides are nontoxic stable organic free radicals having a single unpaired electron and therefore are capable of providing MRI contrast via shortening the longitudinal relaxation time (T_1). In addition, nitroxides exhibit catalytic antioxidant activity. One of the nitroxides, methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MC-P; Fig. 1A), has been used as a contrast agent for brain imaging in electron paramagnetic resonance (EPR) imaging experiments. MC-P is a low molecular weight (MW; 200) compound with lipophilic nature (Po/w; 14). Therefore, MC-P can be used as a blood brain barrier (BBB) permeable MRI contrast agent that can participate in redox reactions. Furthermore, T_1 mapping has the potential to quantify the tissue concentration of the contrast agent with the knowledge of the relaxivity of the agent used. We reported fast T_1 mapping based on the Look-Locker (LL) sequence that can significantly reduce scanning time without sacrificing accuracy. In this study, the ability of MC-P, as an MRI contrast agent for brain tissue redox imaging was examined. In addition, MC-P relaxation in the rodent brain was quantified by MRI at 4.7 T using a fast LL T_1 mapping.

Methods; Rats were anesthetized with isoflurane and carefully controlled to keep constant respiration rate. The tail vein was catheterized, and body core temperature was maintained at 37^0C with a heated air. MRI measurements were performed at 4.7 T. A series of T_1-weighted spoiled gradient echo (SPGR; TR = 75 ms, TE = 3 ms, flip-angle (FA) = 45°, N_{EX} = 2) was employed to observe T_1 effects. Multi-slice LL data were acquired by segmented gradient-echo echo planar imaging (EPI) with TR = 10 s, TE 6.7 ms, FA = 20°, acquisition interval = 400 ms, and number of LL time points = 20. With a matrix size of 64 × 64 (0.4 mm in-plane resolution), a set of 13, 1.5 mm slices could be obtained in 20 s.

Results and Discussion; T_1 changes were readily detected throughout the brain at a well-tolerated dose of MC-P. MRI signal intensity in the cerebral cortex and thalamus increased up to 50 % after MC-P injection in T_1 weighted MRI (Fig. 1B). Even when blood flow was stopped by KCl injection, MC-P reduced in the brain tissue. On the other hand, there was little enhancement (2.7 %) when a BBB-impermeable nitroxide, 3-carboxy PROXYL (3CxP) was used. T_1 relaxation time in the brain tissue clearly decreased from 1 577±19.9 to 1034±70. 4 ms in cerebral cortex and from 1315±11. 9 to 779 ±70. 2 ms in thalamus after administration of MC-P (Fig. 1C). The maximum concentration of thalamus and cerebral cortex after MC-P injection was calculated to be 1.9 ± 0.35 mM and 3.0 ± 0.50 mM based on the in vitro relaxivity of MC-P. These values were consistent with the ex-vivo data of brain tissue and blood concentration obtained by electron paramagnetic resonance (EPR) spectroscopy. Interestingly, clearance of total MC-P (oxidized + reduced form) was slow and total MC-P level was maintained over 1 mM level during the experiments, although the MR signal enhancement of MC-P in T_1 weighted image disappeared within 7 min suggesting that MRI signal disappearance represents reduction ability (redox-status) of the brain tissue. These results demonstrate the possibility of using BBB permeable nitroxides as MRI contrast agents and antioxidants to evaluate the role of ROS in neurological diseases.

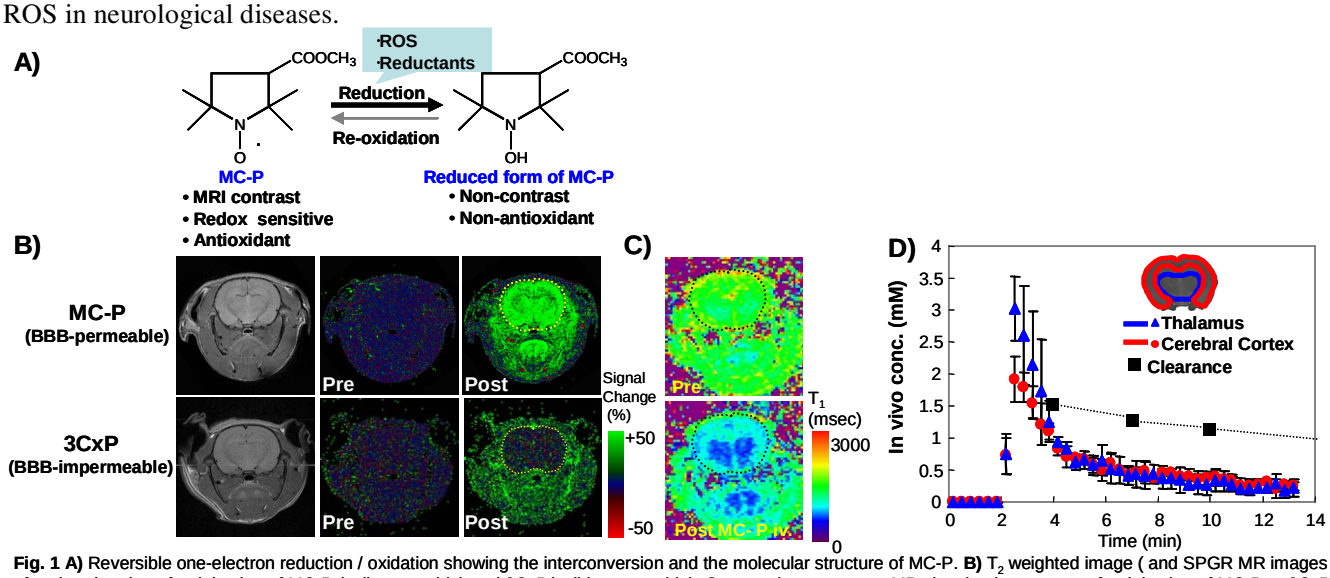

Fig. 1 A) Reversible one-electron reduction / oxidation showing the interconversion and the molecular structure of MC-P. **B)** T_2 weighted image (and SPGR MR images of rat head region after injection of MC-P (cell-permeable) and 3CxP(cell-impermeable). Green color represents MR signal enhancement after injection of MC-P or 3CxP. **C)** T_1 maps of pre and post injection of MC-P by LL sequence. **D)** In vivo MC-P concentration in cerebral cortex (red) and thalamus (blue) region and clearance of MC-P from the brain tissue (black) were shown. The MC-P concentration was calculated from T_1 maps by LL sequence. Error bar is standard deviation (± SD, number of experiment was 3).