

## Redox-mapping MRI in rodent brain with nitroxide contrast agents

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### INTRODUCTION

Overproduction of reactive oxygen species (ROS) and decreased antioxidant defense system may contribute to numerous brain disorders such as stroke, epilepsy, Parkinson's disease, and Alzheimer's disease. Non-invasive evaluation and visualization of oxidative stress is important to elucidate the role of ROS in brain diseases. In order to visualize the effect of ROS in vivo, nitroxide compounds have been used as redox-sensitive contrast agents in EPR imaging, and recently these compounds have also been started to be used as a redox-sensitive T1 contrast agents in MRI [1,2]. Paramagnetic nitroxide works as an MRI contrast agent, but not when reduced to its hydroxylamine derivatives. Therefore the ratio of paramagnetic nitroxide / diamagnetic hydroxylamine has been shown to be dependent on the redox state of the tissues. With this, MRI can show the mapping image of the redox status of the animal disease models being examined. The purpose of this study was to examine the possibility and ability of nitroxide compounds with different lipophilicities as a redox-mapping tool in MRI of brain disease animal models such as epilepsy and transient cerebral ischemia. The results were discussed with reference to the data obtained by EPR spectrometry.

### METHOD

**Paramagnetic nitroxide compounds:** 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3CA-P), 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3CO-P), 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3HM-P), methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MC-P), and 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl (Cat1) were used in this study. All nitroxide solutions were prepared in phosphate-buffered saline (PBS), and were injected by tail vein cannulation in examined animals under anesthesia. **Brain disease model:** Two rodent brain disease models, epileptic-seizure model and ischemia-reperfusion model, were employed in this study. Epileptic seizure was induced by the injection of pentylenetetrazole (PTZ) into rodents (50 mg/kg) under anesthesia using isoflurane or nembutal (50 mg/kg, Dainippon Pharmaceutical). For ischemia-reperfusion animal model, transient middle cerebral artery occlusion (MCAO) was employed. **MRI measurements:** MRI data were acquired using MRmini (MRTechnology, Tsukuba, Japan), consisting of a 0.5-Tesla permanent magnet made of Nd-Fe-B [2]. After appropriate positioning was confirmed on localizer images, axial, horizontal, and sagittal MR images were obtained using a T1-weighted multi-slice sequence. Typical imaging parameters were TR = 450 ms; TE = 14ms; matrix = 256 × 128, NEX = 2-4; FOV: 30 mm; slice thickness: 1-2 mm.

### RESULTS AND DISCUSSION

Figure 1 shows T1-weighted MR images of mice injected with nitroxides with different lipophilicities. Figure 1 (top) shows MR images of mouse head before and after injection of water-soluble nitroxide, 3CO-P. These images indicate that the image intensity outside the brain increased with injection of 3CO-P, but not within the brain. Similar images were obtained with Cat1, highly water-soluble nitroxide. On the other hand, when a relatively hydrophobic nitroxide (3HM-P, MC-P, and 3CA-P) was injected into mice, the MRI signal intensity in the cortex increased significantly, and 3HM-P penetrated blood brain barrier (BBB) of brain tissue, as shown in Figure 1 (bottom). In the cerebral cortex, the MRI signal intensity increased for all the BBB-permeable nitroxides, and the maximum concentration in the cortex was about 4.5 mmol/L when 3HM-P was used. The results in Figure 1 clearly indicate that nitroxides with different lipophilicities work as site-specific paramagnetic contrast agents. To show the possibility of these nitroxides working as redox-sensitive contrast agent in neurologic disease model, epileptic-seizure model mice were examined by MRI. The reduction rates of 3HM-P and MC-P were remarkably large after the convulsive seizure was induced by PTZ, suggesting a change in redox balance due to the oxidative stress. These results were discussed with reference to the results of EPR.

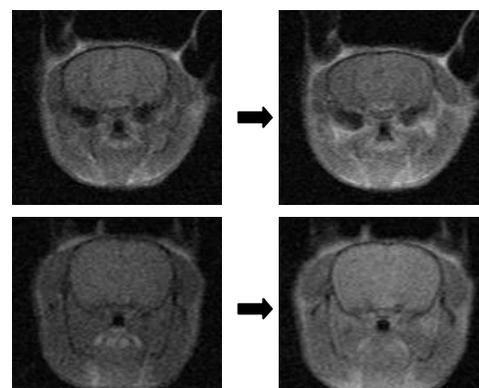


Figure 1 T1-weighted MR images of mouse head after injection of 3CO-P (top) and 3HM-P (bottom)

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### REFERENCES

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