INTRODUCTION
EPR imaging using nitroxides is a powerful non-invasive method for visualizing the redox status produced by free radicals, which play important roles in many biological processes and various physiological conditions in vivo. We recently developed a new three-dimensional (3D) EPR imaging system that employs rapid field scanning and we successfully applied this system to mice [1]. Compared with other EPR imaging systems, this system enables us to collect more projections, resulting in higher quality reconstructed EPR images. Two blood–brain barrier (BBB)-permeable nitroxides, 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (HMP) and 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MCP), were used for EPR imaging of small rodent brains. However, information about their distribution and in vivo properties has not been compared in detail. In this study, the detailed distributions and time courses of HMP and MCP in mouse heads were examined using our improved EPR imaging system and MRI. EPR images of mouse heads obtained using our system clearly reveal that HMP and MCP have different distributions and time courses for entering the brain.

MATERIALS AND METHODS

**Animals:** Male c57BL/6 mice aged 5 to 7 weeks with body weights of 20–25 g were used in this experiment. Animals were housed in a temperature and circadian rhythm controlled room with unlimited food and water. **Paramagnetic nitroxide compounds:** The BBB-permeable nitroxides HMP and MCP were obtained from Toronto Research Chemicals, Inc. and Radical Research, Inc., respectively. All nitroxide solutions were prepared in phosphate-buffered saline, and were injected by tail vein cannulation into the mice under isoflurane anesthesia. **MRI measurements:** MR images of mouse heads were acquired using an MRmini scanner (MR Technology, Tsukuba, Japan) with a 0.5-T permanent magnet. **EPR imaging measurements:** All EPR images were taken using an in-house built 750-MHz continuous wave EPR imaging system. Using our rapid field scanning system, the shortest 3D data acquisition time for 181 projections was ~30 s in the case of field scanning for 6 mT. EPR images were reconstructed using a filtered back-projection method.

RESULTS AND DISCUSSION

Figures 1(A) and (B) respectively show images of the percentage (%) that the signal intensity increased on the injection of HMP and MCP relative to that of pre-injection MR images of mouse heads. The images indicate that HMP was distributed in all regions of the mouse head including the brain, whereas MCP was preferentially concentrated in the brain and the tongue. The injection of HMP and MCP increases the signal intensity in the brain by about 30% (A) and more than 50% (B), respectively. This indicates that MCP is more concentrated in the brain than HMP.

EPR imaging experiments were performed to investigate the time courses for localization of nitroxides in mouse heads. 3D EPR images of mouse head were obtained every 45 s from 246 projections with a scan time of 0.1 s. Figures 2(A) and (B) show surface-rendered 3D EPR images of mouse heads after intravenous injection of HMP and MCP, respectively. They reveal that MCP was more concentrated in the brain than HMP. To observe the localization of BBB-permeable nitroxide in brain in more detail, EPR imaging was performed using faster data acquisition. EPR images of mouse heads were recorded in less than 30 s. Figures 3(A) and (B) respectively show 2D slice-selected EPR images of HMP and MCP. HMP was localized in the head, whereas it was mainly located outside the brain (Fig. 3(A)) immediately after injection. However, MCP showed remarkable localization within the brain. For the slower data acquisition (i.e., 246 projections with a scan time of 0.1 s), we did not observe any differences in the distributions of HMP and MCP in the brain. These results suggest that MCP can enter the brain through the BBB more rapidly than HMP. The newly developed 3D EPR imaging system allows us to visualize differences in distributions and time courses of BBB-permeable nitroxides in the brain.

**Acknowledgments:** This work was supported in part by a grant from the Japan Society for the Promotion of Science (22390236).