

Redox map of mouse brain by three-dimensional EPR imaging with six-membered nitroxyl radicals

Miho C Emoto¹, Hideo Sato-Akaba², Hiroshi Hirata³, and Hirotsada G Fujii¹

¹Center for Medical Education, Sapporo Medical University, Sapporo, Hokkaido, Japan, ²Department of Systems Innovation, Osaka University, Toyonaka, Osaka, Japan, ³Division of Bioengineering and Bioinformatics, Hokkaido University, Sapporo, Hokkaido, Japan

INTRODUCTION

Electron paramagnetic resonance (EPR) imaging is a noninvasive imaging method for visualizing the distribution of nitroxides, which are used as redox sensitive imaging probes. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) has been widely used in *in vivo* EPR; however, due to its short lifetime *in vivo*, examination of the redox status of the heads of small animals based on EPR imaging using six-membered ring nitroxides such as TEMPOL has not been much reported. We recently developed an improved three-dimensional (3D) EPR imaging system that employs rapid field scanning. In the present study, 3D EPR imaging data was sequentially acquired from 126 projections over about 16 s, and the 3D distributions of TEMPOL and 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPONE) in mouse heads were visualized. The distribution and reduction reaction of TEMPOL and TEMPONE in the brain were compared to other previously reported blood-brain barrier (BBB)-permeable nitroxides, which were reported previously [1]. Obtained EPR images clearly reveal that both TEMPOL and TEMPONE were able to enter the brain through BBB, and TEMPONE collected in the brain more than TEMPOL. Calculated half-life maps indicate that, compared to other areas, the reduction reactions of these nitroxides were remarkably short in 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. **Paramagnetic nitroxides:** TEMPOL and TEMPONE were purchased from Sigma-Aldrich (St. Louis, MO, USA). All nitroxide solutions were injected by tail vein cannulation into the mice under isoflurane anesthesia. **Magnetic resonance imaging (MRI) measurements:** MRI of mouse heads was 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 126 projections was about 16 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 1(B) show MRI of the percent signal increase after injection of TEMPOL and TEMPONE, respectively, relative to that of pre-injection

MRI. These images indicate that these compounds can pass through the BBB, and that TEMPONE is more concentrated in the brain than TEMPOL. The surface-rendered 3D EPR images of mouse heads of TEMPOL and TEMPONE are shown in Fig. 2(A) and 2(B), respectively, indicating that both nitroxides pass the BBB and are spread out within the brain. This is supported by the calculated percent MRI signal increase seen in Fig. 1.

To study the distribution of these nitroxides within the brain in more detail, 2D slice-selected EPR images of TEMPOL and TEMPONE in the sagittal plane are

shown in Fig. 3(A) and 3(B). TEMPOL was distributed both inside and outside the mouse brain, whereas TEMPONE was mainly localized within the brain. The results in Fig. 3 are consistent with Fig. 1. Since the half-life of nitroxide can be used as an index of redox status, position-specific half-lives of TEMPOL and TEMPONE were calculated using the temporal 2D slices (Fig. 4). The averaged half-life of TEMPOL in the ROI of examined mouse brain (Fig. 4(A)) was 43.9 ± 13.4 s, similar to the value of TEMPONE (Fig. 4(B)) of 40.3 ± 6.0 s. Compared to other BBB-permeable nitroxides reported previously [1], the present results indicate that TEMPONE, which is localized mainly in the brain, resembles methoxycarbonyl-PROXYL (MCP), whereas TEMPOL, which is distributed in all regions of mouse head, is more similar to hydroxymethyl-PROXYL (HMP). The half-lives of TEMPOL and TEMPONE were much shorter than those of MCP and HMP, suggesting that TEMPOL and TEMPONE have higher sensitivities to reduction reactions in the brain.

CONCLUSION

This improved EPR imaging system using rapid field scanning allows noninvasive acquisition of 3D EPR images of six-membered ring nitroxides in mouse heads. Half-life maps of TEMPOL and TEMPONE in mouse heads were obtained from a series of EPR images at an interval of 16 s, which clearly indicated the redox status within the mouse brain.

Acknowledgments: This work was supported by a grant from the Japanese Society for the Promotion of Science (24791218).

References: 1 Emoto M C, Sato-Akaba H, Hirata H, Fujii H G, Proc. Intl. Soc. Mag. Reson. Med. 20, 2012; 1709

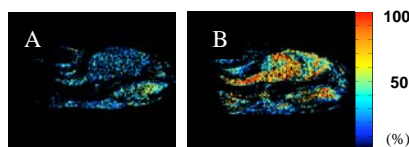


Figure 1: Percent signal increase in T1-weighted MRI of the mouse head, calculated from images obtained before and after injection of (A) TEMPOL, (B) TEMPONE.

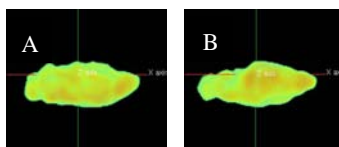


Figure 2: 3D Volume-rendered EPR images of the mouse head from 126 projections. 3D image data consisted of $128 \times 128 \times 128$ voxels. (A) TEMPOL, (B) TEMPONE

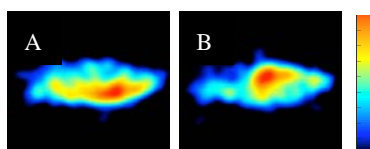


Figure 3: Slice selective EPR images of the mouse head in the sagittal plane. (A) TEMPOL, (B) TEMPONE

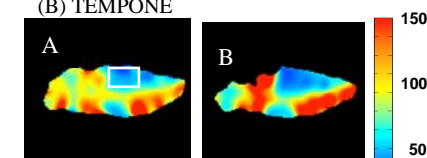


Figure 4: Half-life maps in the mouse head. (A) TEMPOL, (B) TEMPONE

(s)