Co-registered EPR and MRI of the mouse head indicates detailed distributions of piperidine nitroxides

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INTRODUCTION

Electron paramagnetic resonance (EPR) imaging is a noninvasive imaging method for visualizing redox status using nitroxide as an imaging probe.[1] Nitroxides are known to have unique antioxidant properties, since they are able to mimic the catalytic activities of important antioxidant enzymes such as superoxide dismutase and catalase. These useful nitroxides, such as TEMPOL and TEMPONE, could be used as imaging probes in EPR imaging studies, but a few of them have been imaged in live mice because of the low temporal resolution of EPR images.[2] To overcome these problems, an improved three-dimensional (3D) EPR imaging system was developed that employs rapid field scanning. This system enables faster data acquisition with larger projection data before disappearance of the nitroxide. By using the improved EPR imaging system, it is possible to use a variety of nitroxide compounds for EPR imaging probes, regardless of their short *in vivo* lifetimes. In the present study, EPR images of TEMPONE and TEMPOL in mice were acquired in about 15 s, and 3D EPR images of mouse heads were reconstructed from 181 projections. 2D EPR images of the mouse head were overlaid on MR images, which clearly visualized the localization of piperidine nitroxide in the mouse brain.

MATERIALS AND METHODS

Control sequence for field scanning and field gradient: To reduce the total acquisition time, analog signals were used to drive the Helmholtz coil pair for field scanning, and field gradient coils were used. These signals were generated simultaneously with four 12-bit digital analog converters (MCP4822) controlled by a Field Programmable Gate Array (FPGA) developing board (DE0-nano) in the sequence controller. Each converted analog signal (see Fig. 1) was filtered and amplified up to ±10 V, which was fed to the bipolar power supplies for field scanning and field gradients. For 3D imaging, the control sequences for four different projections (81, 126, 181, 246) and six different durations of field scanning (54~132 ms) were installed in the FPGA board. ***Animals*:** Male c57BL/6 mice aged 5 to 7 weeks with body weights of 20–25 g were used. ***Paramagnetic nitroxides*:** TEMPONE and TEMPOL were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitroxide solutions were injected by tail vein cannulation under isoflurane anesthesia. ***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 aquired using an in-house built 750-MHz continuous wave EPR imaging system. Using a rapid field scanning system, the shortest 3D data acquisition time for 181 projections was about 15 s at the field scan width of 6 mT. EPR images were reconstructed using a filtered back-projection method.

RESULTS AND DISCUSSION

For 3D EPR images of the living mouse head, the total imaging time for a field scan (T) of 54 ms with 181 projections was 12.9 s, which included the stabilizing time for the field scan and the field gradient when the direction of the projection changed. Immediately after injection of piperidine nitroxide, TEMPOL and TEMPONE, via the tail vein, a 3D EPR data set was obtained every 15 s. A surface-rendered 3D EPR image of TEMPONE in the mouse head (image matrix, 128×128×128) is shown in Fig. 2A. To show the detailed distribution of TEMPONE in mouse heads, 2D EPR images (Fig. 2B) were obtained from 3D EPR imaging data. MR images of mouse heads were taken in advance for co-registration, on which the EPR images (Fig. 2B) was overlaid. Fig. 2D shows the co-registered image of the EPR images and MR images, which clearly indicates that TEMPONE can enter the brain through the blood–brain barrier (BBB) and spread through the brain, spinal cord, and tongue. This is the first co-registered image of EPR and MR images confirming the detailed distribution of piperidine nitroxides in the live mouse brain. Fig. 2D also shows that TEMPONE seems to be more distributed in the posterior area (cerebellum) than frontal area (olfactory bulb). Less of the nitroxide was localized in neck muscle and nose tissues. With the present improved EPR imaging system, it is likely that a wide variety of nitroxides can be used to estimate the redox status *in vivo* under oxidative stress.

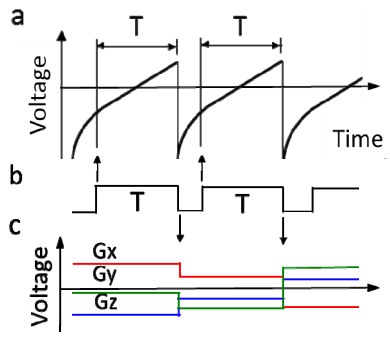

Fig. 1. Timing chart and control signals for the (a) field scan, (b) trigger pulse used for the data acquisition window with a period of T, and (c) 3D field gradients: Gx, Gy and Gz.

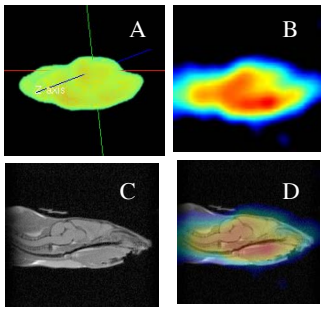

Fig.2. Coregistration of EPR of TEMPONE and MRI. (A) 3D EPR image (B). Slice-selected 2D EPR image in the sagittal plane (C). MRI in the sagittal plane of mouse head. (D) is coregistration of (B) and (C).

CONCLUSION

An improved EPR imaging system using rapid field scanning allows aquisition of 3D EPR images of a piperidine nitroxide, TEMPONE, in mouse heads in 15 s with 181 projections. The co-registered MR images and 2D EPR images reveal in detail the distribution of TEMPONE in mouse heads, especially within the brain.

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References: 1 Fujii GH. et al., Mag.Res.Imag 31, 2013; 130-138

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