

Detection of Blood-brain barrier disruption in a mouse model of transient cerebral ischemia by EPR imaging

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INTRODUCTION

EPR imaging using nitroxides is a powerful non-invasive method for visualizing the redox status modulated by oxidative stress in vivo. Recently we successfully reduced the scan time of three-dimensional (3D) EPR imaging of nitroxides in rodents. This system enables us to obtain 3D images of nitroxide probes with a half-life of a few minutes in living mice [1]. Another advantage of using the rapid field-scanning system is that, compared with other EPR imaging systems, we can collect a greater number of projections, before the signal becomes undetectable. The benefit of which is to obtain a better quality of reproduction for the resulting EPR images. In this study, using the improved EPR imaging system we assessed the redox status of an ischemia-reperfusion (IR) model mouse brain, as well as the effect of oxidative stress on its infarcted hemisphere. The results obtained in this study clearly show that through the use of blood-brain barrier (BBB)-permeable and BBB-impermeable nitroxides, rapid field scanning EPR imaging can be used to assess BBB permeability and time course of changes in the BBB-permeability of the wounded hemisphere of IR model mice. The infarcted hemisphere was visualized by three-dimensional surface-rendered EPR images.

METHOD

Paramagnetic nitroxide compounds: The BBB-impermeable nitroxide, 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (COP) was obtained from Sigma, and the BBB-permeable nitroxide, 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3HM-P) was obtained from Toronto Research Chemicals, Inc. All nitroxide solutions were prepared in phosphate-buffered saline (PBS), and were injected by tail vein cannulation in examined mice under anesthesia. **Brain disease model:** For the ischemia-reperfusion model, transient middle cerebral artery occlusion (MCAO) model mice were prepared (Male ICR mice aged 6 to 7 weeks, body-weight ~30g). **EPR measurements:** All EPR images were acquired using an in-house built 750-MHz CW-EPR imager [1]. Using our rapid field scan system, the fastest data acquisition time is ~28 sec in cases of 0.3 sec field scanning (6mT field scan) and 81 projections. EPR images were reconstructed using a filtered back-projection method. **MRI measurements:** MRI mice data was acquired using a MRmini scanner (MRTechnology, Tsukuba, Japan) with a 0.5T permanent magnet and 21.8 MHz operating frequency.

RESULTS AND DISCUSSION

3D EPR images of mice were visualized every 28 sec using 81 projections with a 0.3 sec scan time. Figure 1 shows 3D EPR images of mouse head after injection of BBB-permeable nitroxide, HMP (B), and BBB-impermeable nitroxide, COP (C). The surface-rendered images clearly show the location of brain tissues in the mouse head.

Twenty-four hours after reperfusion following 2 h of MCAO, BBB-impermeable COP was injected into MCAO mouse. Figure 2 shows a T2-weighted MR image of MCAO mouse head (A) and 2D slice-selected EPR images of MCAO (B, C) and control mice

(D). The MR image of each mouse brain was used to locate the corresponding region in EPR image, shown by the dotted line. In the control mouse (D), COP does not enter the brain tissues, due to the impermeability of BBB to this nitroxide. However, in the case of MCAO mouse brain with a right hemisphere infarction, the BBB became permeable to COP, and the image intensity in right hemisphere (Fig. 2 B and C) increased gradually, compared to the control image (D). The infarcted right hemisphere where COP entered could be visualized by 3D surface-rendered images (not shown). This technique provides a useful method

for locating the disrupted region of BBB, which takes place in the infarcted hemisphere of MCAO mouse head. And through repeated EPR experiments, it is possible to follow the time course of BBB permeability changes shown in Fig. 2 B and C, where the time dependence of COP penetration into the right hemisphere of MCAO mouse brain is observed. We believe this technique may be particularly relevant for the study of pharmacokinetics of COP in mouse head, whereby half-life mapping of COP may allow visualization of the redox status of MCAO mouse under oxidative stress.

Acknowledgments : This work was supported in part by grants from the Japan Society for the promotion of Science (19390322) and SENTAN, JST. **References :** 1 Fujii H., Akaba H., Kawanishi K., Hirata H. Mapping of Redox Status in a Brain-Disease Mouse Model by Three-Dimensional EPR Imaging. *Mag Reson Med.* (2010) in press.

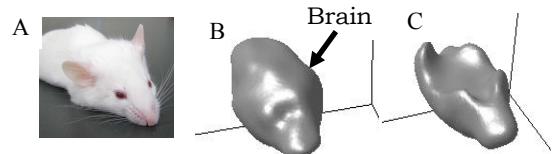


Figure 1 3D Surface-rendered EPR image of mouse head. A: mouse photo, B: HMP-injected image C: COP-injected image.

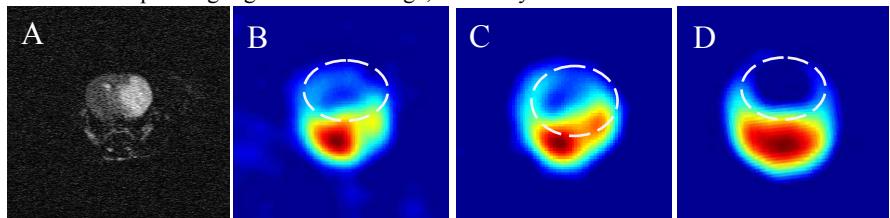


Figure 2 T2-weighted MR image of MCAO mouse (A), and EPR images of MCAO (B, C) and control (D) mouse heads. Images B and C were obtained 3 and 7.5 min after COP, respectively. The dotted line in B and C showed the mouse brain region.