EPR imaging of short-lifetime nitroxyl radicals in mouse head

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Introduction

In vivo electron paramagnetic resonance (EPR) imaging of unstable spin probes has been required to investigate the pharmacokinetics or dynamics of these probes in living organisms (1). However, it is challenging to detect free radicals that have a short lifetime in living organisms. For example, nitroxyl spin probes such as 4-Hydroxy-2,2,6,6-tetramethyl-piperidinooxy (TEMPOL) have a half-life of a few minutes in live animals. This requires an acquisition time of less than a minute. EPR imaging of nitroxyl spin probes in the brains of mice and rats has been reported (2). However, only five-membered nitroxyl spin probes have been used, since their lifetimes in living organisms are relatively longer than those of six-membered nitroxyl spin probes such as TEMPOL. The goal of the present work was to demonstrate *in vivo* 3D CW-EPR imaging on nitroxyl spin probes with a half-life of a few minutes in animals. An acquisition time of 30 s for *in vivo* EPR imaging was achieved by reducing the scanning time for a single spectrum to 0.5 s and using a uniform distribution for 46 projections.

Methods and Materials

A home-built 650-MHz EPR spectrometer was used with three sets of gradient coils that generated magnetic field gradients along the X-, Y-, and Z-directions. A multi-coil parallel-gap resonator with an inner diameter of 24 mm and a length of 34 mm was used to measure phantoms and the head of a mouse. The unloaded quality factor of the resonator was 490, and this decreased to 170 when the head of the mouse was inserted. The efficiency of the resonator for generating an RF magnetic field was 44 $\mu T/W^{1/2}$ at the center of the resonator. For 3D imaging, a uniform distribution of projections, in which angles were defined by the vertices of a geodesic sphere generated from an icosahedron, was used. One-step filtered back projection (FBP) was carried out to reconstruct the 3D images. The current computation time is 5 s for 128x128x128 voxels and 81 projections. From a phantom study, we found that the quality of images generated from 46 projections by the uniform distribution was better than that from 81 projections obtained by a conventional non-uniform distribution for two-step FBP. To decrease the effect of signal decay on a reconstructed image, signal averaging was not performed in the process of data acquisition for 3D EPR imaging. The acquisition time was 30 s. Data acquisition was continued to measure the next set of projections for 3D imaging until the EPR signal from the spin probes in the subject became weak. Anesthetized male ICR mice (6 weeks of age, weight 30 g) were used for the 3D EPR imaging, and they received 0.2 - 0.25 ml of 200 mM nitoroxyl spin probes via the intraperitoneal route.

Results and Discussion

To prove that our data acquisition was faster than the decay of nitroxyl spin probes, we have shown the temporal change in EPR signal intensities in Fig. 1a. Data acquisition of 30 s is reasonably fast for the decay of TEMPOL-d_{17}, -^{15}N in an examined animal. Figures 1b and 1c show the surface-rendered images of TEMPOL-d_{17}, -^{15}N and hydroxymethyl-PROXYL in mouse heads. The axis of the body of the subject mouse was in the Y-direction. The dorsal side of the subject mouse corresponds to the upper side of the surface-rendered images. The left side of the images corresponds to the nose of the subject mouse. Distinct differences between the distributions for two kinds of spin probes were observed in the heads of mice. The images were reconstructed from a data set that was obtained by averaging two successive data sets (46 projections). Thus, the total acquisition time of a 3D image was 60 s.

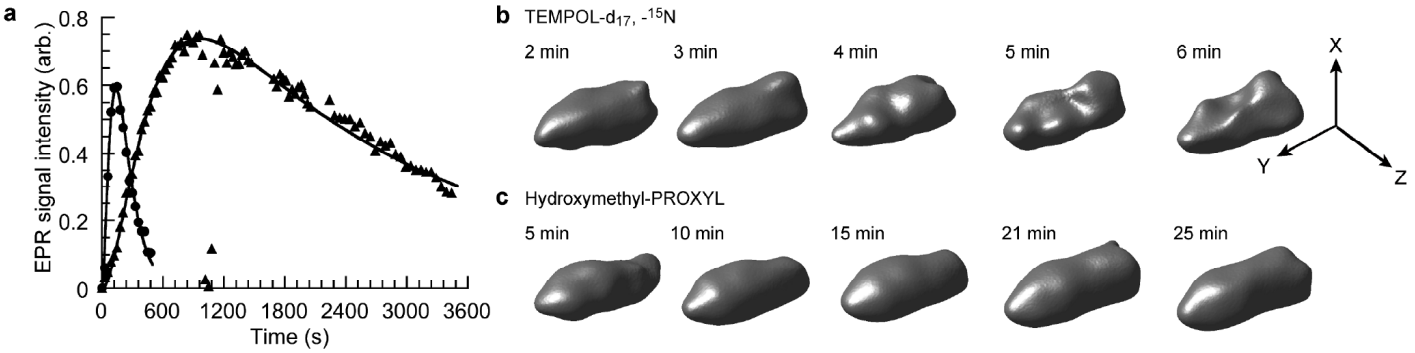

Fig.1. *In vivo* EPR imaging of mouse head. **a:** Decay of EPR intensities for (●) TEMPOL-d_{17}, -^{15}N and (▲) hydroxymethyl-PROXYL in head . 3D surface-rendered images of mouse head with **b:** TEMPOL-d_{17}, -^{15}N and **c:** hydroxymethyl-PROXYL probes. Times beside images mean start times of data acquisition for images. Isosurface level that was used for surface rendering of 3D images was set at half of maximum intensity in each 3D image. Field-of-view (FOV) of both images was 50 mm for 128 pixels.

Conclusion

Temporal change in infused TEMPOL-d_{17}, -^{15}N in mouse heads was successfully visualized with 3D CW-EPR imaging that was previously not possible. Differences between the distributions of two spin probes could be recognized in surface-rendered 3D images; these would be due to the differences in permeability for the blood-brain barrier and the reduction/oxidation reaction. This finding suggests that 3D CW-EPR imaging can be applied to nitroxyl spin probes with a half-life of a few minutes in live animals. This would be useful for studies of pharmacokinetics, drug delivery, and *in vivo* free-radical-related molecular imaging.

References

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