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Non-invasive imaging of the levels and effects of glutathione on the redox status of mouse brain using electron paramagnetic resonance imaging

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ABSTRACT

Glutathione (GSH) is the most abundant non-protein thiol that buffers reactive oxygen species in the brain. GSH does not reduce nitroxides directly, but in the presence of ascorbates, addition of GSH increases ascorbate-induced reduction of nitroxides. In this study, we used electron paramagnetic resonance (EPR) imaging and the nitroxide imaging probe, 3-methoxycarbonyl-2,2,5,5-tetramethylpiperidine-1-oxyl (MCP), to non-invasively obtain spatially resolved redox data from mouse brains depleted of GSH with diethyl maleate compared to control. Based on the pharmacokinetics of the reduction reaction of MCP in the mouse heads, the pixel-based rate constant of its reduction reaction was calculated as an index of the redox status *in vivo* and mapped as a "redox map". The obtained redox maps from control and GSH-depleted mouse brains showed a clear change in the brain redox status, which was due to the decreased levels of GSH in brains as measured by a biochemical assay. We observed a linear relationship between the reduction rate constant of MCP and the level of GSH for both control and GSH-depleted mouse brains. Using this relationship, the GSH level in the brain can be estimated from the redox map obtained with EPR imaging.

1. Introduction

Under normal physiological conditions, oxidative damage is prevented due to regulation of reactive oxygen species (ROS) by the antioxidant defense system, endogenous antioxidant compounds, and enzymatic systems. The major endogenous antioxidant materials in the brain are ascorbic acid (AsA) and glutathione (GSH), and the levels of these antioxidants change depending on the disease state and type of disease, including cancer and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease [1–3].

Nitroxides are nontoxic imaging probes with a single unpaired electron. Once infused into the body, nitroxides are readily reduced depending on the redox status of the body during oxidative stress, and their reduction rate can be used as an index of the *in vivo* redox status. Therefore, electron paramagnetic resonance (EPR) imaging with nitroxide imaging probes has been used to visualize changes in the redox status of animal disease models during oxidative stress by mapping the reduction rates of injected nitroxide probes [4–7].

Low levels of AsA and GSH have been implicated in neurologic damage. Therefore, measuring the reduction rates of nitroxides catalyzed by antioxidants gives useful information for assessment of the disease state during oxidative stress. Because AsA but not GSH can directly reduce nitroxide probes *in vitro*, the role of AsA in the reduction reaction of nitroxides *in vivo* seems clear. However, the contribution of GSH to *in vivo* redox reactions of nitroxides has not been clearly elucidated, even though the importance of GSH in regulation of the intracellular redox status is clear.

In the present study, using the mouse model of GSH depletion with diethyl maleate (DEM), the brain redox status was examined

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non-invasively with EPR imaging using the nitroxide imaging probe, MCP. We demonstrated that EPR images were capable of visualizing changes in the brain redox status depending on the level of brain GSH. From the quantitative analysis of the reduction rate constant of MCP in mouse brains, we found a linear correlation between the reduction rate constant of MCP and the GSH level in mouse brain. Using this relationship, estimation of brain GSH levels in living mouse brain is possible with EPR imaging.

2. Materials and methods

2.1. Chemicals

The paramagnetic nitroxide imaging probe 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MCP, Fig. 1A) was obtained from NARD Chemicals, Ltd. (Osaka, Japan). DEM was obtained from Wako Pure Chemicals (Osaka, Japan).

2.2. Animals

The protocols for all animal experiments were approved by the Sapporo Medical University Animal Care Committee and were carried out in accordance with the National Institutes of Health Animal Care and Use Protocol (NIH, Bethesda, MD, USA). Male c57BL/6 mice aged 5–8 weeks with body weights of 20–25 g were purchased from Japan SLC Inc. (Shizuoka, Japan) and used in this study. Mice were housed three per cage in climate-controlled, circadian rhythm-adjusted rooms, and were allowed access to food and water *ad libitum*.

2.3. DEM-treated mice

Mice were given a single intraperitoneal injection of DEM in corn oil (4 mmol/kg, 100 μL). Magnetic resonance imaging (MRI) of mouse heads was performed before EPR measurements with or without DEM administration, and EPR images of the same mice were carried out 2 h after DEM treatment.

2.4. In vivo EPR imaging of mouse brains

All EPR images were acquired using an in house-built 750-MHz CW-EPR imager [8]. Mice were anesthetized with 1.5% isoflurane in air at 200 mL/min. MCP in saline (1.0 μmol/g body weight) was injected via the tail vein. Using a rapid magnetic field scan system, the data acquisition time for 3D-EPR images used in this study is about 9 s for 50-ms field scanning (6-mT field scan) and 181 projections. EPR images were obtained by the filtered back-projection algorithm, and two-dimensional (2D) images were generated from the reconstructed 3D images [9]. The matrix for EPR images was 128 × 128 × 128, and the field of view was 50 × 50 × 50 mm.

***MRI measurements*:** MRI of mouse heads was performed using an MR mini scanner (MR Technology, Tsukuba, Japan) with a 0.5-T permanent magnet. MRI was performed using a spin-echo multi-slice T1-weighted sequence. Imaging parameters used in this study were: repetition time, 450 ms; echo time, 12 ms; field of view, 60 × 30 mm; matrix, 256 × 128; number of excitations, 10; slice thickness, 1.5 mm. MRI data were calculated using ImageJ software (http://rsb.info.nih.gov/ij/) and a custom-written program running in MATLAB (MathWorks Inc., Natick, MA, USA) [10].

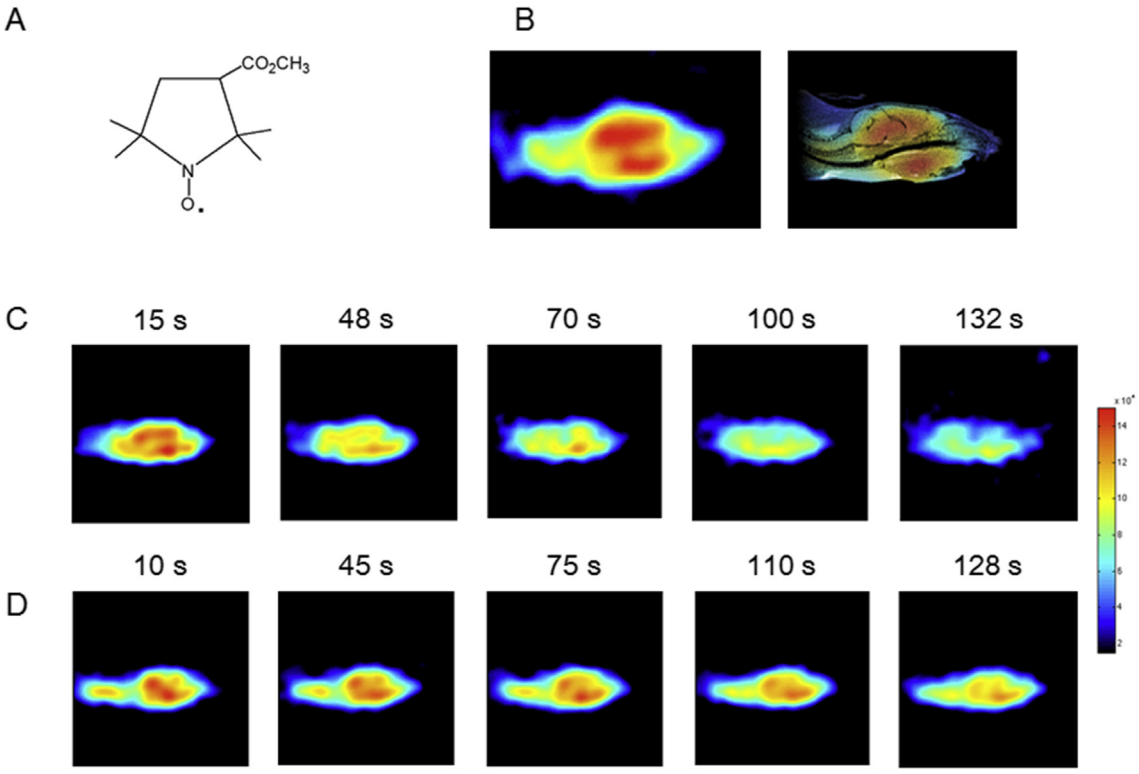

Fig. 1. Distribution of MCP in mouse heads and temporal changes in 2D EPR images of mouse heads with or without GSH depletion. (A) Chemical structure of the nitroxide imaging probe, MCP. (B) Co-registration of 2D EPR images of MCP-injected mouse heads and the anatomical images of the same mouse heads obtained with MRI before EPR measurements. Left; 2D EPR image, right; co-registered image. (C and D) Temporal changes in 2D EPR images of mouse heads in the absence (C) or presence (D) of DEM treatment. Five selected EPR images of control (C) and DEM-treated (D) mouse heads are shown with the corresponding approximate time after MCP injection.