Brain redox imaging using nitroxide contrast agents in pentylenetetrazol-kindled mice with EPR imaging

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Target audience: Neuroscientists and biochemists interested in epilepsy and oxidative stress

INTRODUCTION

Epilepsy is a central nervous system disorder in which nerve cell activity in the brain is disturbed, causing seizures. The precise molecular mechanisms of seizure-induced neuronal death remain unknown; however, several studies have reported experimental evidence of involvement of oxidative stress by reactive oxygen species (ROS) in the pathogenesis of epileptic seizures. Many studies have described the role of antioxidants and the production of ROS in various experimental seizure models, but most were *in vitro* studies and little *in vivo* experimental evidence exists. In the present study, the change in the redox status was visualized in a pentylenetetrazol (PTZ)-induced seizure mouse model using an improved three-dimensional (3D) EPR imaging system [1] in order to attempt to locate the precise site that is affected by the repeated seizures. EPR images of the PTZ-treated mouse brain clearly showed severe changes in redox status in the hippocampus after repeated seizures, which was also confirmed by *in vitro* biochemical assays.

METHODS

***Redox-sensitive EPR imaging probe*:** 3-Methoxycarbonyl-2,2,5,5-tetramethyl-piperidine-1-oxyl (MCP) was obtained from Radical Research Inc. (Tokyo, Japan).
Animal study: Male c57BL/6 mice (aged 6 to 7 weeks, 20 mice) were used. In the kindling experiments, the sub-convulsive doses (40 mg/kg) of PTZ (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneal injections were repeated once a day to produce kindled mice. Reduction rates of MCP were evaluated in kindled mice after three convulsive seizures.
Biochemical assay: Levels of ascorbic acid, cysteine, and glutathione (GSH) were analyzed by HPLC with electrochemical detection.
Imaging experiments. *EPR*: Images were acquired using an in-house built 750-MHz CW-EPR imager [1]. ***MRI*:** Images were acquired using an MRmini scanner (MRTechnology, Tsukuba, Japan) with a 0.5 T permanent magnet.

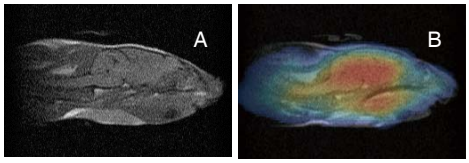

Fig. 1 Co-registered image (B) of MCP distribution in mouse head obtained by EPR and pre-injection MRI (A)

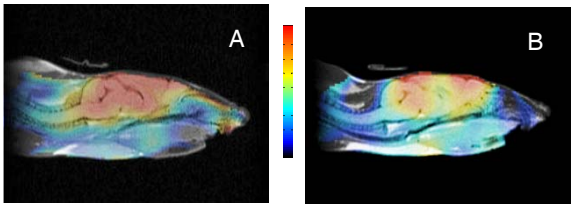

Fig. 2 Co-registered images of redox map obtained by EPR imaging and anatomical image by MRI for control (A) and kindled (B) mouse heads.

RESULTS AND DISCUSSION

The distribution of a redox-sensitive nitroxide probe, MCP, in the kindled mouse head was visualized with EPR images, which were co-registered to pre-injection MRI of the same mouse (Fig. 1). The co-registered images indicate that MCP is successfully localized within the brain and can report the redox status. No appreciable differences in MCP distribution were found in control and kindled mice. The rate constant of the reduction reaction of MCP in mice was measured as an index of redox status *in vivo*. The pixel-based reduction rates of MCP in control and kindled mouse heads were calculated from a series of temporal 3D EPR images of mice. The obtained reduction rates at each pixel in the EPR images were displayed as a "redox map". The co-registered image of the redox map and MRI for both control and kindled mice are shown in Fig. 2. The co-registered images clearly show that the severe difference in the reduction rates were found in and around the hippocampus. Figure 3A shows that the reduction rates of MCP in the hippocampus of kindled mice, 0.281±0.080 min-1 (n=4), were significantly lower than the value in control mice, 0.428±0.033 min-1 (n=4) (**: $P << 0.01$), and there were no differences in reduction rates in the cerebellum of both mice ($P>0.5$). The levels of several antioxidants (ascorbic acid, cysteine, GSH) were measured in the hippocampus of control and kindled mouse brains, and in Fig. 3B the level of GSH is shown. The concentration of GSH in the hippocampus of the kindled mouse was significantly lower than in control mice, strongly indicating that the redox status is apparently changed in the hippocampus after repeated seizures. No difference in the levels of three antioxidants was found in the cerebellum of both mouse brains. A decrease was found in the level of GSH reduction rates of MCP in the hippocampus of kindled mice. These results provide strong *in vivo* evidence of increased oxidative stress in kindled mouse brains during seizure activity.

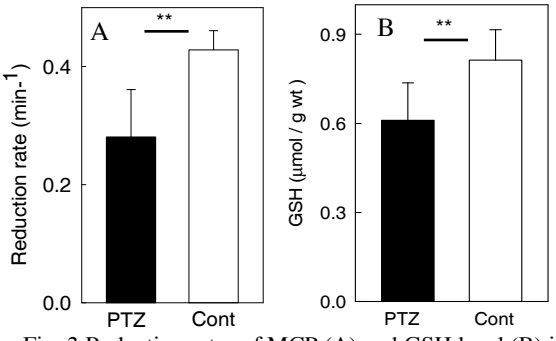

Fig. 3 Reduction rates of MCP (A) and GSH level (B) in hippocampus of control (Cont) and kindled (PTZ) mice

CONCLUSIONS

The change in redox status in the PTZ-treated kindled mouse brain was visualized with EPR imaging. In particular, a remarkable change in redox status in hippocampus of kindled mice was detected by EPR. An *in vitro* assay showed decreased concentrations of GSH in the hippocampus after repeated seizures. Both results supported the involvement of ROS generation in epileptic-seizure mouse brain.

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References: [1] Emoto MC et al., Free Rad Biol Med 2014; 74: 222-228.