Brain Redox Mapping in Methamphetamine-treated Mice using Three-Dimensional EPR Imaging

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INTRODUCTION

The psychostimulant methamphetamine (METH) is a lipophilic molecule that has potent action on the central and sympathetic nervous systems. METH is known to exert its toxic effects in part by causing oxidative stress, which is related to METH-induced dopaminergic neurotoxicity [1]. Once nerve activation takes place, the mitochondrial respiratory chain is activated, and the enhanced activity of the respiratory chain may induce oxidative stress by production of reactive oxygen species (ROS). Several biochemical studies of METH-treated animals and cells have reported involvement of ROS in METH-induced damage. However, little *in vivo* experimental evidence exists concerning redox reactions and redox status under the enhanced mitochondria respiratory chain reaction under METH treatment. In order to examine redox status in experimental animals under oxidative stress, nitroxide compounds are widely used as redox-sensitive contrast probes in magnetic resonance imaging (MRI) and electron paramagnetic resonance (EPR) imaging. A rapid three-dimensional (3D) EPR imaging system has been recently developed that allows acquisition of anatomical and functional 3D EPR images of mouse heads in less than 15 s. In this study, we noninvasively evaluated and visualized redox status of METH-treated mouse heads using a redox-sensitive blood-brain-barrier (BBB) permeable nitroxide probe and an improved 3D EPR imaging system.

METHODS

Paramagnetic nitroxide compounds*:** 3-Methoxycarbonyl-2,2,5,5-tetramethyl-piperidine-1-oxyl (MCP) was obtained from Radical Research Inc. (Tokyo, Japan). The MCP solution was prepared in phosphate-buffered saline (PBS). ***Animal study: Male c57BL/6 and BALB/c mice (aged 6 to 7 weeks, body-weight 20 ~ 25 g, 20 mice) were used. Methamphetamine (Dainippon Sumitomo Pharma Co., Ltd., Osaka Japan) was injected intraperitoneally (1 - 5 mg/kg) once a day for one week. ***EPR imaging measurements*:** All EPR images were acquired using an in-house built 750-MHz CW-EPR imager [2]. The fastest data acquisition time for 3D-EPR imaging was ~ 15 s with 126 projections and 0.1 s field scanning (6 mT field scan). ***MRI measurements*:** MRI was acquired using an MRmini scanner (MRTechnology, Tsukuba, Japan) with a 0.5 T permanent magnet and an operating frequency of 21.8 MHz.

RESULTS AND DISCUSSION

To examine redox status in METH-induced mice, BBB-permeable redox-sensitive nitroxide (MCP) was injected. Figure 1A shows the percent signal intensity increase after injection of MCP relative to that of pre-injection MRI of a mouse head. In Fig. 1B, a 2-dimensional (2D) slice-selected EPR image of a mouse head after injection of MCP is shown. Both images clearly indicate that MCP is localized mainly within the brain and tongue. The half-life of the reduction reaction of MCP was used as an index of redox status *in vivo*.

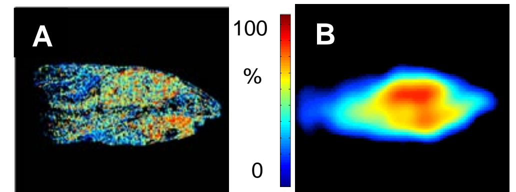

Figure 1: Percent increase in T1-weighted MRI (**1A**). 2D EPR image of mouse head (**1B**).

To localize the half-life of MCP in control and METH-treated mouse heads, a series of temporal 3D EPR images of MCP were taken at an interval of 20 s, and from these data half-life maps were calculated. The obtained half-life maps in Fig. 2 clearly show that the half-life of MCP in the METH-treated mouse brain is shorter than in controls. The averaged half-life of MCP in the ROI selected in controls and METH-treated mouse brains were calculated (Fig. 3). Reduction of MCP was much greater in the brain after METH treatment compared to controls. Pretreatment of mice with a dopamine synthesis inhibitor, alpha-methyl-p-tyrosine (AMPT), suppressed the accelerated reduction reaction of MCP in the METH-treated mouse brain. Together, these *in vivo* results clearly indicate that administration of METH to mice resulted in induction of dopaminergic nerve activation and oxidative stress.

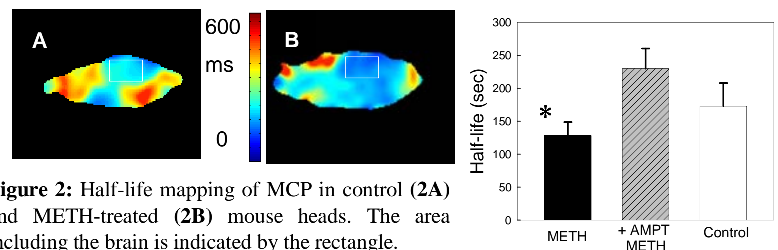

Figure 2: Half-life mapping of MCP in control (**2A**) and METH-treated (**2B**) mouse heads. The area including the brain is indicated by the rectangle.

Figure 3: Effects of METH and AMPT on the half-life of MCP in the mouse brain. Four mice were used for each experiment. Each value represents the mean ± SD. * : $P < 0.05$ compared with control and +AMPT mice.

CONCLUSIONS

The half-life of BBB-permeable MCP in the METH-treated mouse brain was measured noninvasively with a 3D EPR imaging system as an index of redox status. Half-life mapping of MCP clearly visualized dopaminergic nerve activation by METH.

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References : [1] Yamato M. et al., NeuroImage 2011; 57: 866-872. [2] Fujii H. et al., Mag Reson Med 2011; 65: 295-303.