Brain redox imaging using nitroxide contrast agents and blood-brain barrier function in methamphetamine-treated mice

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INTRODUCTION

Methamphetamine (METH) is a potent stimulant with strong euphoric properties and has long-lasting neurotoxic effects. The toxic effects induced by METH are known in part to be caused by oxidative stress, which is related to METH-induced dopaminergic neurotoxicity. Once nerve activation takes place, the enhanced activity of the respiratory chain may induce oxidative stress, followed by production of excess reactive oxygen species (ROS). Excessive generation of ROS and inadequate antioxidant defenses result in mitochondrial oxidative impairment and also alter the function of the blood–brain barrier (BBB). Although many studies have described the involvement of oxidative stress in METH-mediated neurotoxicity, little in vivo experimental evidence exists concerning the change in cellular redox balance and BBB dysfunction in METH-treated animals. In this study, visualized redox status of the METH-treated mouse brain was noninvasively evaluated using a redox-sensitive nitroxide probe, 3-Methoxycarbonyl-PROXYL (MCP) and a three-dimensional (3D) EPR imaging system. EPR images of the METH-treat mouse brain clearly detected acceleration of the reduction reaction of MCP, indicating a shift in redox balance by the effects of METH. Impairment of the BBB in the METH-treated mouse brain was also detected by MRI.

METHODS

**Paramagnetic nitroxide compounds:** 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. METH (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) was injected intraperitoneally (5 mg/kg) once a day for 1 week. **EPR imaging measurements:** All EPR images were acquired using an in-house built 750-MHz CW-EPR imager \([1]\). MRI measurements: MRI was acquired using an MRmini scanner (MRTechnology, Tsukuba, Japan) with a 0.5 T permanent magnet. Gadovist (Bayer Inc., Berlin, Germany) was used as a Gadolinium-based contrast agent.

RESULTS AND DISCUSSION

The distribution of a redox-sensitive nitroxide probe, MCP, in the METH-treated mouse head was visualized with EPR images, which were co-registered to pre-injection MRI of the same mouse (Fig. 1). These 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 mice. The rate constant of the reduction reaction of MCP in mice was measured as an index of redox status in vivo. The rate constants of MCP in control and METH-treated mouse heads were calculated from a series of temporal 3D EPR images of MCP. The obtained rate constants at each pixel in the EPR images were used to reconstruct a 2D map of rate constants, called a “redox map”. Figure 2 shows the redox map of MCP in a METH-tread mouse and a control mouse head. The averaged MCP reduction rate (0.33±0.05 min\(^{-1}\), n=4) in the ROI selected in the METH-treated mouse brain (Fig. 2A) was faster than in controls (0.25±0.05 min\(^{-1}\), n=4, Fig. 2B).

 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 (0.20±0.08 min\(^{-1}\), n=4). The effect of oxidative stress induced by METH on BBB permeability was examined by MRI. Difference MR images of METH-treated mice (Fig. 3) obtained by subtracting the MR image without the gadolinium contrast agent (Gd) from that with Gd shows that the signal intensity in cerebral parenchyma was enhanced by BBB-impermeable Gd, indicating BBB disruption in the METH-treated mouse brain. Dysfunction of the BBB in METH-treated mice gradually recovered after a 1-week METH withdrawal period. Together, these in vivo results clearly indicate that administration of METH to mice resulted in induction of oxidative stress in the whole brain and dysfunction of the BBB.

CONCLUSIONS

The change in redox status in mouse brain induced by METH was visualized with EPR imaging. Under oxidative stress induced by METH, dysfunction of the BBB in live mouse brain was detected by MRI.

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**Fig. 1.** MCP distribution in mouse head taken by EPR (B) was coregistered to pre-injection MRI (A).

**Fig. 2** Redox map of MCP in METH-treated (A) and control (B) mouse heads. The ROI is shown by the rectangle.

**Fig. 3.** Difference T1-weighted MR image of METH-treated mouse after Gd injection (0.4 μmol/g. IV). Dotted line shows increased signal intensity in brain parenchyma.