Visualization of Cerebral Lesions in A Mouse Model of Toxoplasmosis by USPIO-Enhanced Magnetic Resonance Imaging

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Introduction: Toxoplasma gondii is a protozoan parasite found in both animals and humans. Infection is mainly acquired by ingestion of oocysts-contaminated food or water or by eating undercooked/raw meat containing tissue cysts. Infection acquired during pregnancy may cause severe damage to the fetus[1]. In immuno-compromised patients, such as AIDS patients and patients of bone marrow transplantation, reactivation of latent disease can cause life-threatening encephalitis[1-3]. Toxoplasma gondii infection can be diagnosed directly by detection of the parasite and/or indirectly with serological methods[4]. Magnetic resonance imaging (MRI) has also been used for clinical diagnose of toxoplasma-induced encephalitis[5]. Toxoplasmic lesions are often seen as multiple areas with high signal intensities on T_2-weighted images, representing the necrotic component of the abscess and perifocal edema[5]. In this study, a mouse model of cerebral toxoplasmosis was developed, dynamic T_2-weigthed and ultrasmall particles of iron oxide (USPIO)-enhanced MRI was used to monitor the changes in brain induced by toxoplasmosis.

Materials and Methods: Cerebral toxoplasmosis was induced by intracerebral injection of 10 μl tachyzoite containing 8-10 parasites in 24 Kunming mice (20±2 g). The mice were then divided into different groups, and underwent MRI examination at various times (i.e., from 1d to 6d) after tachyzoite injection. All MR experiments were carried out on a 4.7 T/30 cm Bruker Biospec scanner with volume coil excitation and surface coil reception. Multi-slice T_2-weighted (TR/TE 2500/75ms) and T_2^*-weighted (TR/TE 500/25ms) images were acquired with FOV 1.7×1.7 cm^2, matrix size 128×128 and slice thickness 0.8 mm. Poly-ethylene glycol (PEG)-coated USPIO with an average diameter of 9.8±1.7 nm were synthesized by a newly-developed one-pot reaction method and dissolved in saline at a concentration of 0.012 mmol Fe/ml[6]. For USPIO enhanced imaging, T_2^*-weighted imaging was performed before, immediately after and 24 hrs after intravenous (i.e., tail vein) injection of USPIO at a dose of 0.175 mmol Fe/kg body weight.

Results: Figure 1 shows T_2-weighted images acquired from representative mice at various times after induction of cerebral toxoplasmosis. No observable changes were found on the images obtained from 1d to 5d. However, evident periventricular/white matter edema was frequently observed in the infected mice at 6d. Most of the infected mice died at 7d. Figure 2 shows the results of USPIO enhanced imaging obtained from two coronal slices in an infected mouse. USPIO were injected at 4d after infection, and T_2^*-weighted images were acquired before (Fig. 2a and b), immediately after (Fig. 2c and d) and 24 hrs after (Fig. 2e and f) injection. Like the T_2-weighted images (Fig. 1), the pre-contrast T_2^* images (Fig. 2a and b) failed to show any definite toxoplasmic lesions. However, multiple brain lesions, shown as dots with low signal intensities, were evident on the post-contrast images (Fig. 2c and d). T_2^*-weighted images acquired 24 hrs after USPIO injection (Fig. 2e and f) appeared to be similar to those acquired immediately after injection (Fig. 2c and d).

Discussion and Conclusion: In view that toxoplasmosis might play an important role in the pathophysiology of diseases such as AIDS[2] and schizophrenia[8], research interests on this disease are growing rapidly. In this study, a mouse model of cerebral toxoplasmosis was developed, and in vivo MRI was used to monitor the changes occurring in the toxoplasmic mouse brain and to follow the progress of the disease with time. With conventional T_2-weighted imaging, no noticeable abnormalities were found in the infected mouse brain up to 5 days after induction of toxoplasmosis, suggesting that, in this model, the disease is somewhat latent during this period of time. However, after its latency the disease seemed to develop rapidly, as periventricular/white matter edema, sign of encephalitis, became prominent at 6d (Fig. 1). Given that conventional T_2-weighted imaging has little value in detecting latent toxoplasma infection in this model, USPIO-enhanced MRI was therefore examined for its capacity in early diagnosis of the disease. The results show that multiple brain lesions can be clearly identified by USPIO-enhanced imaging as early as 4 days after infection. The lesions appeared as low signal intensity foci on the T_2* weighted images, indicating accumulation of USPIO inside/around the lesions. Previous studies have shown that USPIO can enter into ischemic brain lesions through interrupted blood brain barrier (BBB) and/or by internalized into the infiltrating phagocytic cells in inflammatory responses[7]. In the case of cerebral toxoplasmosis, it has been suggested that, after infection, the parasites first invade vascular endothelium of the capillaries, leading to vasculitis and microcirculatory dysfunction, which in turn cause perivascular edema, perfusion impairment, and tissue anoxia, and eventually result in brain lesions[9]. It is therefore reasonable to hypothesize that accumulation of USPIO inside/around the toxoplasmic lesions is the results of BBB broken down and/or brain tissue inflammation. However, accumulation of USPIO induced by BBB broken-down is more likely given that facts that the appearance of toxoplasmic lesions remained more or less the same immediately after UPPSIO injection and 24hrs later and the PEG coating of USPIO used in this study is resistant to protein adsorption and has low uptake rate by macrophage cells[10].

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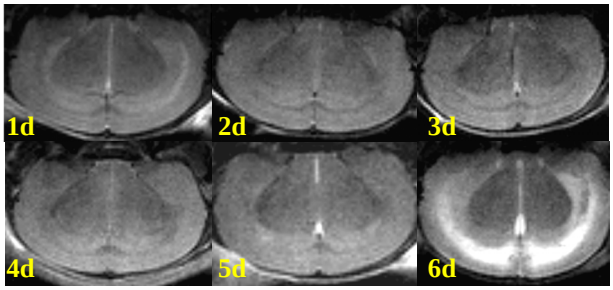

Figure 1. T_2-weighted images of coronal brain slices in infected mice at various times after induction of cerebral toxoplasmosis.

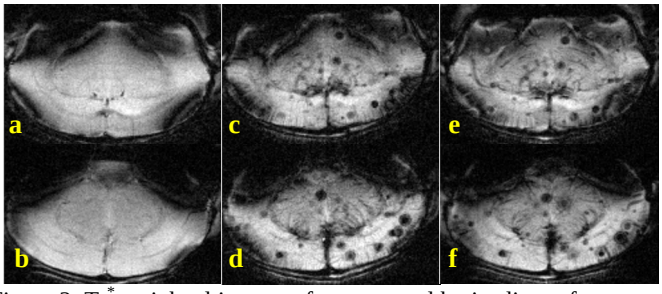

Figure 2. T_2^*-weighted images of two coronal brain slices of a mouse 4 days (a-d) and 5 days (e-f) after toxoplasma infection. The images were acquired before (a-b), immediately after (c-d) and 24 hrs (e-f) after intravenous injection of USPIO.