Detection of toxoplasmic lesions in mouse brain by USPIO-enhanced magnetic resonance imaging

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Abstract

The objective of this study was to examine the feasibility of detecting toxoplasmic brain lesions in a mouse model of cerebral toxoplasmosis by ultrasmall superparamagnetic particles of iron oxide (USPIO)-enhanced magnetic resonance imaging (MRI). Toxoplasmosis encephalitis was induced in Kunming mice by intracerebral injection of Toxoplasma gondii tachyzoites. T2- and T2*-weighted MRI was performed 1, 3, 4, 5 and 6 days after infection before USPIO injection; immediately after USPIO injection; and 24 h later. A comparison of USPIO enhancement and Gd-DTPA enhancement was made in three toxoplasmic mice 4 days after infection. Hematoxylin and eosin staining and Prussian blue staining were performed to detect inflammatory reactions and presence of iron in and around the toxoplasmic brain lesions. Nonenhanced T2-/T2*-weighted imaging detected few abnormalities in the brain up to 5 days. Most mice developed prominent hydrocephalus at 6 days. Gd-DTPA-enhanced imaging showed prominent enhancement of the cerebral ventricles but revealed only few space-occupying lesions in the parenchyma. USPIO-enhanced T2*-weighted imaging showed improved detection of toxoplasmic brain lesions that were invisible to nonenhanced T2-/T2*-weighted imaging and gadolinium-enhanced imaging. Most of the enhancing lesions showed nodular enhancement immediately after USPIO injection, some of which changed appearance 24 h later, having a ring enhancement at the outer rim. It can be concluded that USPIO enhancement of the toxoplasmic lesions may reflect blood–brain barrier impairment and/or inflammatory reactions associated with these lesions. USPIO-enhanced imaging may be used in combination with gadolinium-enhanced imaging to provide better characterization of toxoplasmic brain lesions and, potentially, improve the differential diagnosis of toxoplasmosis encephalitis.

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Keywords: Magnetic resonance imaging; Toxoplasmosis encephalitis; USPIO; Mouse; Brain

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite primarily infecting immunocompromised subjects such as AIDS patients and patients who have undergone bone marrow transplantation (BMT) [1–4]. Clinical diagnosis of toxoplasmosis can be established by serologic tests, detection of parasites and/or relevant DNA in body fluids (i.e., cerebrospinal and bronchoalveolar fluids, blood and urine, etc.) and histological/immunohistological demonstration of the presence of the parasite and/or its antigens in tissue sections [5]. The central nervous system (CNS) is the site most typically affected by T. gondii infection [6]. Toxoplasmosis encephalitis is a rare but fatal complication in BMT patients [7] and is a frequent cause of space-occupying brain lesions in AIDS patients [4]. Magnetic resonance imaging (MRI) has been used in the clinical diagnosis of toxoplasmosis encephalitis. Toxoplasmic brain lesions often present as multiple foci with hyperintensity on T2-weighted images and with isointensity/hypointensity on T1-weighted images [1,2]. Enhancement of these lesions by contrast agent Gd-DTPA is useful for diagnosis but can be variable among different patient populations [1,8,9].

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Table 1
Summary of the MRI results in the first study

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Precontrast ( T_2 )-weighted images</th>
<th>Postcontrast ( T_2^* )-weighted images</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass lesion</td>
<td>Hydrocephalus</td>
</tr>
<tr>
<td>1</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
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<tr>
<td>4</td>
<td>1/4</td>
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<td>5</td>
<td>0/7</td>
<td>6/7</td>
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<tr>
<td>6</td>
<td>0/7</td>
<td>6/7</td>
</tr>
</tbody>
</table>

USPIO-enhanced \( T_2 \)-weighted imaging has a much higher sensitivity in detecting toxoplasmic brain lesions than conventional nonenhanced \( T_2 \)-weighted imaging. Cells that have no entry indicate that precontrast \( T_2 \)-weighted images were not taken.

\(^a\) The data in this column show the number of mice that showed enhancing lesions immediately after USPIO injection.

\(^b\) The data in this column show the number of mice that showed enhancing lesions either immediately or 24 h after USPIO injection.

\(^c\) 0/5 means that a total of five mice were imaged at this time point, and none of these five mice showed abnormalities.

Ultrasmall superparamagnetic particle of iron oxide (USPIO) is a novel MRI contrast agent that does not penetrate intact blood–brain barrier (BBB) but can be internalized by macrophages and other activated mononuclear cells through phagocytosis/endocytosis [10,11]. Taking advantages of these properties, USPIO-enhanced MRI has been applied to monitor the inflammatory responses in various CNS diseases including cerebral ischemia, multiple sclerosis and acute disseminated encephalomyelitis [12–15]. Few studies, however, have evaluated the usefulness of this technique in detecting toxoplasmic brain lesions. A mouse model of toxoplasmosis encephalitis was developed and monitored by nonenhanced and USPIO-enhanced \( T_2 \) and \( T_2^* \)-weighted MRI to examine the feasibility and effectiveness of detecting toxoplasmic brain lesions by USPIO-enhanced MRI. Furthermore, we compared the enhancement of the toxoplasmic lesions following USPIO and Gd-DTPA administration.

2. Materials and methods

All animal protocols were approved by the institutional animal care committee. \( T. gondii \) infection was induced in 33 male Kunming mice (20±2 g) by injecting 10 µl of physiologic saline containing 8–10 tachyzoites intracerebrally at a location about 3 mm below the bregma. After injection, the mice were housed in a temperature- and humidity-controlled environment, having free access to standard mouse chow and tap water. Two MRI studies were performed: one is to examine the feasibility and effectiveness of detecting toxoplasmic brain lesions by USPIO-enhanced MRI at different times after infection and the other is to compare the enhancement of toxoplasmic lesions with USPIO and that with Gd-DTPA. The first study was performed on 30 mice 1, 3, 4, 5 and 6 days (\( n=5, 3, 9, 6 \) and 7, respectively) after infection. The second study was performed on three toxoplasmic mice 4 days after infection. For comparison, Gd-DTPA-enhanced imaging was also performed in three control mice.

All MRI experiments were carried out on a 4.7 T/30 cm Bruker Biospec scanner equipped with actively shielded gradients. A 12-cm-diameter Helmholtz volume coil was used for excitation, and a 2.5-cm-diameter single-loop surface coil was used for reception. Anesthesia was maintained with 1.5% isoflurane in a 2:1 N\(_2\)O/O\(_2\) gas mixture administered via a nose cone. Multislice \( T_2 \)-weighted (TR/TE=2500/75 ms), \( T_2^* \)-weighted (TR/TE=500/25 ms) and \( T_1 \)-weighted (TR/TE=300/19 ms) images were acquired with the following parameters: field of view=1.7×1.7 cm, matrix size=128×128, slice thickness=0.8 mm. The flip angle of the excitation pulse was 90° in \( T_2 \)- and \( T_1 \)-weighted imaging and 45° in \( T_2^* \)-weighted imaging.

For the mice utilized in the first study, a saline solution of custom-made polyethylene-glycol-coated USPIO (0.012 mmol Fe/ml, 0.175 mmol Fe/kg body weight, average diameter of the USPIO particles=9.8±1.7 nm) was injected via the tail vein after acquiring the precontrast \( T_2 \)- and \( T_2^* \)-weighted images [16]. Postcontrast \( T_2 \)- and \( T_2^* \)-weighted images were acquired immediately after USPIO injection and 24 h later. The control mice and the infected mice utilized in the second study received an intravenous injection of 0.015 mmol/ml Gd-DTPA solution at a dose of 0.1 mmol/kg after acquiring the precontrast \( T_1 \)- and \( T_2^* \)-weighted images. Postcontrast \( T_1 \)-weighted images were acquired immediately after Gd-DTPA injection. Thirty minutes later, a dose of USPIO (i.e., the same as that used in the first study) was injected intravenously into the toxoplasmic mice, followed by immediate acquisition of postcontrast \( T_2^* \)-weighted images.

After the MR experiments, the mice were sacrificed by either decapitation or trancardial perfusion (i.e., for Prussian blue staining) with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline. The brains were quickly removed, fixed, embedded in paraffin wax and serially cut into 5-µm-thick sections, which were then mounted and stained with either hematoxylin and eosin (H&E) only or Prussian blue plus H&E. For H&E and Prussian blue staining, the brain sections were first deparaffinized, hydrated with distilled water and stained for 20 min in a mixture solution composed of equal parts of 20% hydrochloric acid and 10% potassium ferrocyanide prepared immediately before use. After washing thoroughly in distilled water, the brain sections were counterstained with hematoxylin for 10 min and eosin for 30 s. Between the two staining steps, the sections were destained with 1% acid ethanol for 10 s and rinsed with distilled water for 20 min.

3. Results

The MRI results for the first study are summarized in Table 1. Few abnormalities were observed on the precontrast...
$T_2$-weighted images obtained up to 5 days after infection (Fig. 1A–D). There were only two exceptions: one mouse showed mass lesions with hyperintensity at 4 days and the other developed hydrocephalus at 5 days. Prominent hydrocephalus was observed in almost all the mice imaged at 6 days (Table 1, Fig. 1E). Similarly, few obvious abnormalities were found on the precontrast $T_2^*$-weighted images, except in four mice (i.e., two in the 4-day group and two in the 6-day group) that had hypointensive lesions near the lateral ventricles (black arrow, Fig. 2B). H&E staining showed that such lesions were associated with para-ventricular hemorrhage and inflammation (black arrow, Fig. 2C). All three toxoplasmic mice (i.e., at 4 days after infection) examined in the second study showed significant Gd-DTPA enhancement in and around ventricles (white arrows, Fig. 2E), demonstrating that the brain–cerebrospinal fluid (CSF) barrier of these animals was impaired and permeable to Gd-DTPA. The control mice did not show Gd-DTPA enhancement in the CSF space.

Fig. 3 shows $T_2$-weighted (A) and $T_2^*$-weighted images (B and C) of a brain slice in an infected mouse at 4 days acquired before (A and B) and immediately after (C) USPIO injection. No evident abnormalities were found on the precontrast images. Immediately after USPIO injection, multiple lesions shown as solid hypointensive foci were observed on the $T_2^*$-weighted images (Fig. 3C), some of which had unclear rims with higher signal intensities than those at the centers. Some of the enhanced lesions had the same appearance on the $T_2$-weighted images 24 h later (Fig. 3E). However, there were also lesions (indicated by arrows, Fig. 2E), demonstrating that the brain–cerebrospinal fluid (CSF) barrier of these animals was impaired and permeable to Gd-DTPA. The control mice did not show Gd-DTPA enhancement in the CSF space.

Fig. 2. $T_2$-weighted (A) and $T_2^*$-weighted (B) images of a coronal brain slice from a mouse 6 days after *T. gondii* infection. Prominent hydrocephalus is seen on the $T_2$-weighted image (TR/TE=2500/75 ms), while the $T_2^*$-weighted image (TR/TE=500/25 ms) shows lesions around the ventricles with hypointensity (black arrow). H&E stain (C, ×100) of a brain section from the same mouse demonstrates that hydrocephalus and the lesions observed on the MR images are associated with paraventricular hemorrhage (black arrow), accumulation of inflammatory cells and parasites and neuronal death in the region. Such pathological changes may also lead to impairment of brain–CSF barrier. Pre-Gd-DTPA (D) and post-Gd-DTPA (E) contrast-enhanced $T_1$-weighted images (TR/TE=300/19 ms) were obtained from a mouse 4 days after infection. The postcontrast image shows significant enhancement of the CSF space after Gd-DTPA administration (E, white arrows).
arrows) that evolved with time, resulting to a torus-like appearance 24 h after injection of USPIO, comprising of a hypointense ring at the outer rim, an annular region with more or less the same signal intensity as that of the normal tissue and a dark core that has the lowest signal intensity. In this particular mouse, USPIO-enhanced lesions were also clearly visible on the postcontrast $T_2$-weighted images (Fig. 3D), although they appeared to have much smaller sizes than those observed with $T_2$-weighted imaging (Fig. 3E).

With the postcontrast $T_2^*$-weighted images acquired either immediately or 24 h after USPIO injection, enhanced toxoplasmic lesions were observed, by visual inspection, in 22 out of the 30 mice examined in the first study. The number, location and size of the enhancing lesions varied from mouse to mouse. The brain regions where enhancing lesions could be found included the cerebellum, middle brain, cerebral cortex, thalamus and paraventricular areas.

The enhancing lesions shown on the $T_2^*$-weighted images were not observable on the $T_2$-weighted images in most cases, except in three mice (i.e., two at 4 days and one at 5 days) including the one shown in Fig. 3D.

Fig. 4 compares the enhancement of toxoplasmic lesions following Gd-DTPA and USPIO administration in the same mice after 4 days of $T. gondii$ infection. Gd-DTPA was injected first, followed by USPIO 30 min later. The most prominent feature of Gd-DTPA enhancement was the strong and homogeneous enhancement of the enlarged CSF space indicating hydrocephalus (black arrows, Fig. 4B). However, only few, if any, space-occupying brain lesions could be detected in this animal with Gd-DTPA enhancement. In comparison, USPIO-enhanced $T_2^*$-weighted imaging showed patterns of inhomogeneous negative enhancement in the paraventricular regions (white arrows, Fig. 4D), reflecting extravasation of the contrast agent from impaired BBB. No
negative enhancement was detected in the CSF space after USPIO administration, possibly indicating that the particles did not traverse the impaired brain–CSF barrier in large amount. A number of toxoplasmic lesions in the cortex and thalamic regions were found to enhance following USPIO (white arrows, Fig. 4 F and H) but not Gd-DTPA administration.

The results of H&E staining demonstrated that toxoplasmic mouse brain was characterized by massive inflammatory reactions occurring inside the blood vessels and in the parenchyma. Inflammatory cells were often found activated and aggregated around the inner wall of blood vessels (Fig. 5A). In the parenchyma, localized lesions were frequently observed, as evidenced by the signs of accumulation of inflammatory cells, glial proliferation and neuronal degeneration (Fig. 5B). In and around toxoplasmic brain lesions, there were mainly two different patterns of iron labeling: (a) intravascular iron deposition (white arrow, Fig. 5C) and (b) intracellular accumulation of iron in the cells surrounding areas showing microhemorrhage, inflammation and/or tissue degeneration (black arrows, Fig. 5C and D).

4. Discussion

In human, toxoplasmic brain lesions are often observed in the basal ganglia and at the corticomedullary junction of the cerebral and cerebellar hemispheres. These lesions appear as hypointense/isointense foci on $T_1$-weighted images and isointense/hyperintense foci on $T_2$-weighted images, representing the necrotic component of the abscess and perifocal edema and the possible presence of hemorrhagic transformation [1–3,9]. Typically, the lesions show ring or nodular enhancement after Gd-DTPA injection [17]. Detection of toxoplasmic lesions by nonenhanced $T_1$- and $T_2$-weighted imaging and Gd-DTPA-enhanced imaging might be negative during the early or dormant stage of infection, especially in BMT patients [9], perhaps due to absent or delayed cellular-mediated immunity [3].

With nonenhanced $T_2$- and $T_2^*$-weighted imaging, only few noticeable abnormalities were found in the infected brain up to 5 days after infection, suggesting that, in this model, the infection is somewhat dormant during this early period of time. Almost all the mice examined developed prominent hydrocephalus at 6 days (Fig. 1E), suggesting that there was a fulminant progress of the infection in the late period, agreeing with what has been observed in BMT patients [1,9]. Hydrocephalus is a major pathological alteration in cerebral toxoplasmosis [18–20], which can result from the blockage of the subarachnoid space by leptomeningeal inflammation, blockage of ventricular foramina or stenosis of the aqueduct of Sylvius [20–23]. In this study, we found that the development of hydrocephalus at 6 days was histologically often associated with aggregation of inflammatory cells and parasites, hemorrhage and neuronal death occurring around the ventricles (Fig. 2C). Such pathological changes may lead
to impairment of the brain–CSF barrier, changes in the permeability of which would have also played a role in causing hydrocephalus.

USPIO is an MRI contrast agent that does not penetrate intact BBB but can be taken up by blood-borne monocytes and traffic with these cells [11]. It is generally accepted that USPIO-enhanced imaging can be used to not only monitor changes in BBB permeability but also characterize the distinct cellular and inflammatory events involved in different types of brain lesions. This technique has been applied to visualize and study brain lesions associated with many inflammatory and vascular neurological diseases including multiple sclerosis, vasculitis, stroke, acute disseminated encephalomyelitis and primary central nervous lymphoma [15].

Toxoplasmic brain lesions are known to be associated with both inflammation and BBB impairment. It has been shown that T. gondii can actively cross extracellular matrix and BBB in brain tissue [24], infect and migrate with blood-borne leukocytes via a Trojan-horse-type mechanism. Upon arriving at immunologically privileged sites, the parasites are capable of triggering nonspecific activation of microglia [25–27], vascular-cell-adhesion-molecule-1-mediated recruitment, adhesion and transendothelial migration of leukocytes [28,29] and consequent BBB impairment [8,30]. It was found in this study that the toxoplasmic brain lesions were negatively enhanced on the T2*-weighted images immediately after USPIO injection, likely reflecting contrast agent extravasation through a compromised BBB.

Interestingly, some of the enhancing lesions changed appearances to have a well-defined hypointensive ring at the outer rim when imaged 24 h later. Similar ring enhancement has also been observed in subacute ischemic lesions that resulted from experimental stroke [11,12]. Given that the blood half-life of the UPSIO used in this study is more than 10 h [16], the distinct late enhancement may be attributed to the phagocytic activities of activated microglia around the lesions with BBB impairment and/or infiltrated iron-load inflammatory cells from the blood. The association of USPIO enhancement with BBB impairment and tissue inflammation was further substantiated in this study by histological assessment. H&E and Prussian blue costaining showed that iron deposition in and around enhancing toxoplasmic brain lesions was related to either vascular abnormality or internalization of USPIO particles into cells via pathocytosis and/or endocytosis (Fig. 5).

It has been shown previously that, in a variety of neurological diseases, the number and enhancement pattern of brain lesions observable to USPIO-enhanced imaging do not necessarily coincide with those found by Gd-DTPA-enhanced imaging [15,31]. It has been suggested that it may be useful to combine the two enhanced imaging techniques for differential diagnosis of different types of brain lesions. It was found in this study that the two contrast agents enhance toxoplasmic brain differentially. Compared to USPIO-enhanced T2*-weighted MRI, gadolinium-enhanced MRI appeared to be less sensitive for detecting the lesions in the parenchyma (Figs. 2 and 4). For instance, some of the toxoplasmic lesions showed enhancement with USPIO but not with Gd-DTPA (Fig. 4). There are a number of reasons that might have contributed to the observed differences between the two contrast agents. First, in the CNS, Gd-DTPA enhancement only reflects permeability changes in the BBB and other barriers, while USPIO enhancement can result not only from changes in the permeability of the barriers but also from inflammation reactions involving phagocytic activity. Secondly, Gd-DTPA is a small molecule chelate that has a much smaller size than USPIO particles so that it may be able to cross the impaired brain–CSF barrier and BBB more effectively. This might explain the enhancement of the CSF space with Gd-DTPA in the toxoplasmic mice (Fig. 2). Finally, it is well-known that T2*-weighted imaging has higher sensitivity than T1- and T2*-weighted imaging in detecting the presence of paramagnetic contrast agents, and in part, this is due to the fact that the lesions containing contrast agents were artificially enlarged. Thus, USPIO-enhanced T2*-weighted imaging could potentially be more sensitive for detecting toxoplasmic lesions as compared to Gd-DTPA-enhanced T1-weighted imaging. Nevertheless, our results suggest that USPIO-enhanced imaging might be used in combination with gadolinium-enhanced imaging to provide better characterization of toxoplasmic brain lesions and, potentially, improve the differential diagnosis of toxoplasmosis encephalitis.

In summary, the results obtained in this study demonstrate that, in the mouse model of toxoplasmosis encephalitis, toxoplasmic brain lesions invisible to nonenhanced T2*/T2*-weighted imaging can be detected by USPIO-enhanced T2*-weighted MRI. The enhancement patterns observed immediately after USPIO injection and 24 h later might reflect, differentially, BBB impairment and inflammatory responses associated with these lesions. It is also shown that USPIO-enhanced MRI can provide additional and complementary information on toxoplasmic lesions to gadolinium-enhanced MRI, and the two techniques may be used in combination to improve the differential diagnosis of toxoplasmosis encephalitis.

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