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Dose and scanning delay using USPIO for central nervous system macrophage imaging

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Abstract

Rationale and objectives: In experimental allergic encephalomyelitis (EAE), central nervous system (CNS) macrophage imaging is achievable by MRI using AMI-227 an ultra-small particle iron oxide contrast agent at a dose of 300 μmol/kg Fe. The objective was to test the feasibility at the human recommended dose of 45 μmol/kg Fe. *Methods*: Two groups of EAE rats were tested with AMI-227 using 45 and 300 μmol/kg Fe respectively. Following i.v. injection of AMI-227, they were scanned after a delay of 4–6 and 20–24 h. *Results*: With a high dose of AMI-227, all animals showed low signal intensity related to iron-loaded macrophages in the CNS. At low dose no abnormalities were found in the CNS. Furthermore, a delay of 4–6 h failed to demonstrate abnormalities even at high dose. *Conclusions*: Dose, scanning delay after administration and blood half-life are major parameters for T2* CNS macrophage imaging.

Keywords: MRI; Allergic encephalomyelitis; Macrophages; Contrast agents

1. Introduction

Ultra-small particle iron oxide (USPIO) accumulates within the cytoplasm of mononuclear phagocytic cells [1,2]. In a magnetic field, these particles are responsible for a **T2**/T2* effect which allows to detect the presence of macrophages in tissue. Several hours after intravenous administration they can be found in the macrophages of normal lymph nodes [3]. 24 h after administration of AMI-227, a USPIO contrast agent [4], in experimental allergic encephalomyelitis, a model of central nervous system inflammation, spots of decrease signal intensity were found in the CNS on T2-weighted images at 4.7 and 1.5 T. Histologically, cells with iron burden were detected nearby vessel walls among other infiltrating inflammatory cells. Those cells had the morphological appearance of macrophages. The work demonstrated for the first time the ability to perform in vivo CNS macrophage imaging by MRI. For this experiment, a dose of 300 μmol/kg Fe was used.

AMI-227 is currently in a phase III trial for human MR lymphography, administrated at a dose of 45 μmol/kg Fe which is the dose recommended for human use.

CNS macrophages are involved in numerous human CNS diseases including trauma, inflammation, demyelinating diseases such as multiple sclerosis, infectious diseases, and tumor-associated inflammation [5–9]. Thus, MRI macrophage activity imaging in humans could be of interest.

To test the ability of performing CNS macrophage imaging in human, we designed the present work to compare the imaging findings at doses of 45 and 300 μmol/kg Fe in rat EAE. We performed the imaging studies at several times from 4 to 24 h after AMI-227 administration in order to determine whether the variation of the dose of AMI-227 would influence the delay of enhancement. All imaging studies were done at 4.7 Tesla.

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2. Materials and methods

2.1. EAE model

An EAE model was induced in 16 female Lewis rats (EAE rats) using guinea pig spinal cord [10]. Immunization was performed with 0.1 g of CNS guinea pig together with 0.1 ml complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, MI, USA) and 2.5 mg H37RA mycobacterium tuberculosis (Difco Laboratories, Detroit, MI, USA). Under general anesthesia using halothane, inoculation of 0.1 ml was performed intradermically in both hind feet, one cm above the ankle at day 0. A control group was composed of four normal rats. Clinical examination was performed every day after day 7, with grading according to the following clinical scale: 0, normal healthy animal; 1, loss of tail tonus; 2, hind limb weakness; 3, complete hind limb paralysis; 4, complete paralysis, incontinence and moribund state.

2.2. Contrast agent administration

The USPIO contrast agent, AMI-227, was provided by Guerbet laboratory (Laboratoire Guerbet, Aulnay, France). It is a dextran-coated iron particle of 20–40 nm. Its blood half-life is about 2 h at a dose of 45 μmol/kg Fe and 5–6 h at 300 μmol/kg Fe in Lewis rats.

AMI-227 was given i.v. in the tail vein at a dose of 300 μmol/kg Fe, 1 day after the clinical onset of the disease in 10 EAE rats, and the same day in two normal rats. Injection of 45 μmol/kg Fe was given in six EAE rats, and the same day in two normal rats. At a given dose, half of the EAE and normal rats were imaged between 4 and 6 h after AMI-227 injection, and half were imaged between 20 and 24 h.

2.3. MRI protocol

Imaging studies were performed under general anesthesia (intraperitoneal injection of 60 mg/kg of pentobarbital) on a Bruker Biospec 4.7-T/50-cm system (Bruker, Rheinstetten, Germany), equipped with a 120 mm shielded gradient insert (maximum strength 193 mT/m, rise time 250 μs). The animal head was positioned prone in a custom-made bird-cage type head coil of 5 cm inner diameter. The localizer was a sagittally oriented spin-echo T1-weighted sequence with TR = 500 ms and TE = 20 ms. A RARE T2-weighted sequence was used with the following parameters: TR, 4300 ms; TE, 68 ms; matrix size, 256 × 160; FOV, 5 cm; slice thickness, 3 mm; number of slices: 9, 4 averages; RARE factor, 40; resulting in a total imaging time of 69 s. Slices were coronally oriented with the second slice being tangential to the occipital bone in order to ensure anatomical reproducibility of imaging between the rats.

2.4. Analysis

Images were read by three observers which determined the presence or absence of areas of low signal intensity related to a magnetic susceptibility effect in the rats brain.

3. Results

3.1. Clinical findings

All EAE rats showed clinical abnormalities starting between days 10 and 12 with maximum scores between 1 and 4. None of the rats from the control group had clinical abnormalities.

3.2. MRI findings

MRI studies performed on five EAE rats 20–24 h after i.v. injection of AMI-227 at a dose of 300 μmol/kg Fe showed multiple focal abnormal low signal intensities in the brain (Fig. 1). These abnormalities were mainly located in the brainstem and the cerebellum, but were also present to a lesser extent in the forebrain.

On five EAE rats which received a dose of 300 μmol/kg Fe, MRI studies performed 4–6 h after i.v. injection of AMI-227 were negative.

On six EAE rats which received a dose of 45 μmol/kg Fe, images performed 4–6 and 20–24 h after i.v. injection of AMI-227 failed to demonstrate any abnormalities in the brain Fig. 2.

The animals of the control group which received AMI-227 at doses of 45 and 300 μmol/kg Fe did not display abnormalities on MRI studies.

MRI results are summarized in Table 1.

4. Discussion

In a recent study, we have investigated the feasibility of MR imaging of the CNS using USPIO [4]. USPIO are contrast agents that accumulate in the macrophagic cells of the mononuclear phagocyte system [1,2]. Macrophages are cells involved in the response to damage to the CNS like on EAE in the rat, a model of multiple sclerosis (MS), characterized by inflammation and demyelination mediated by T-cells and macrophages [11]. Using a high dose of USPIO and a delay of 24 h after i.v. injection, all EAE animals demonstrated areas of low signal intensity related to magnetic susceptibility induced by iron-loaded macrophagic cells infiltrated in the CNS. The present study confirms the high sensitivity of this technique using a dose of 300 μmol/kg Fe and a scanning delay of 20–24 h.

Macrophages are involved in the physiopathological stages of numerous human CNS diseases including trauma, inflammation, demyelinating diseases such as multiple sclerosis, infectious diseases including HIV brain infection, tumor-associated inflammation and Wallerian degeneration [5–9,12]. Macrophages play a central role in the CNS. Thus, macrophage activity imaging raises the possibility of understanding in vivo the natural history of numerous diseases, and of testing drug efficacy with regard to macrophage response.

The goal of the work was to test the feasibility of CNS macrophage imaging at a dose of 45 μmol/kg Fe appropriate for human use. We failed to demonstrate any abnormalities at this dose after a scanning delay of 20–24 h. USPIO blood half-life is lower at lower doses. At a dose of 45 μmol/kg Fe, the blood half-life is about 2 h, whereas at a dose of 300 μmol/kg Fe the blood half-life is about 5 h. Given this difference, we tested a reduced scanning delay of 4–6 h after injection of 45 μmol/kg Fe. The results were negative. Furthermore,

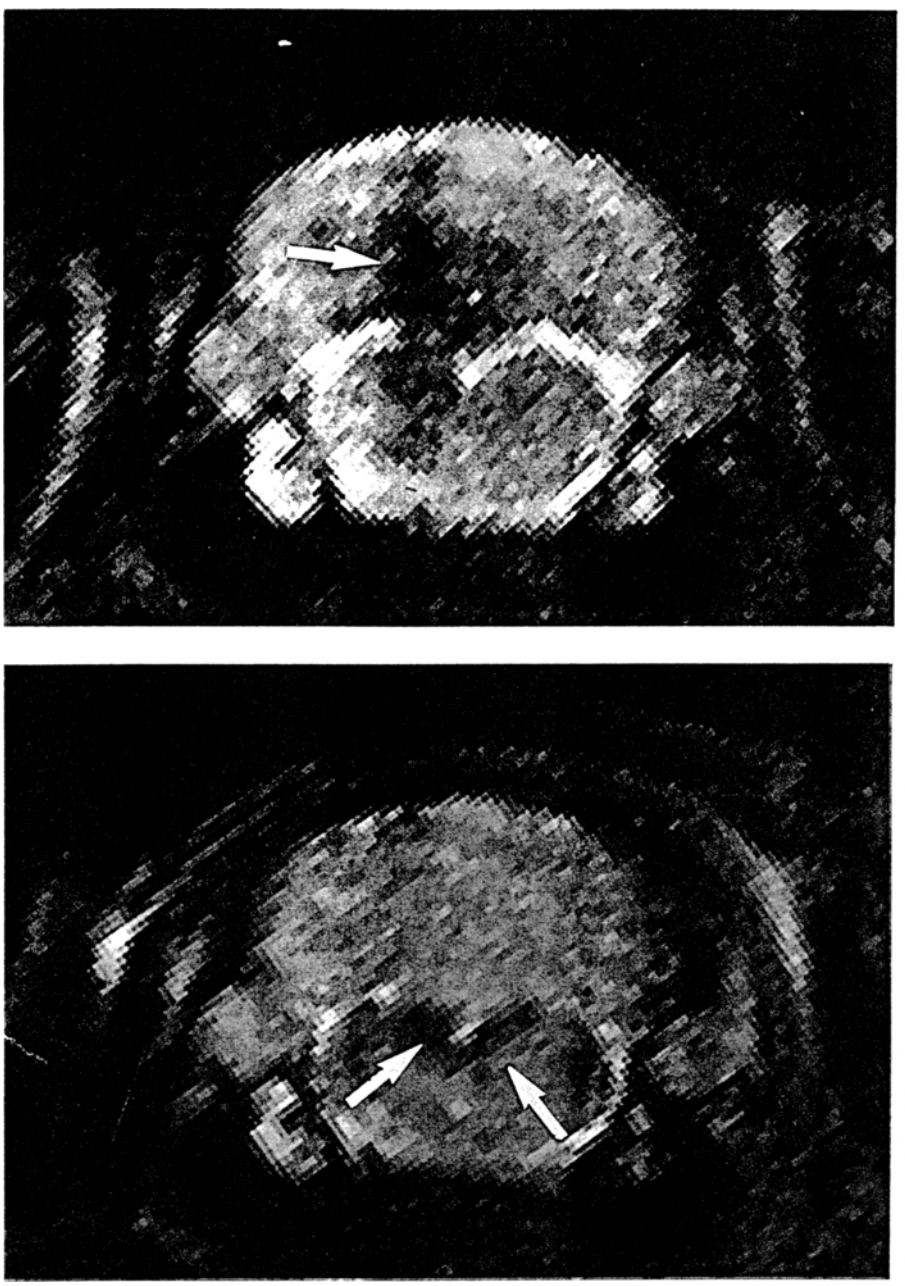

Fig. 1. Coronal RARE T2-weighted images of two rat brains with clinical EAE (A, B) 24 h after i.v. administration of AMI-227 at a dose of 300 μmol/kg Fe. Numerous sites with low signal intensities related to magnetic susceptibility effects due to iron-loaded macrophages are seen on the EAE rat brain parenchyma (arrows).

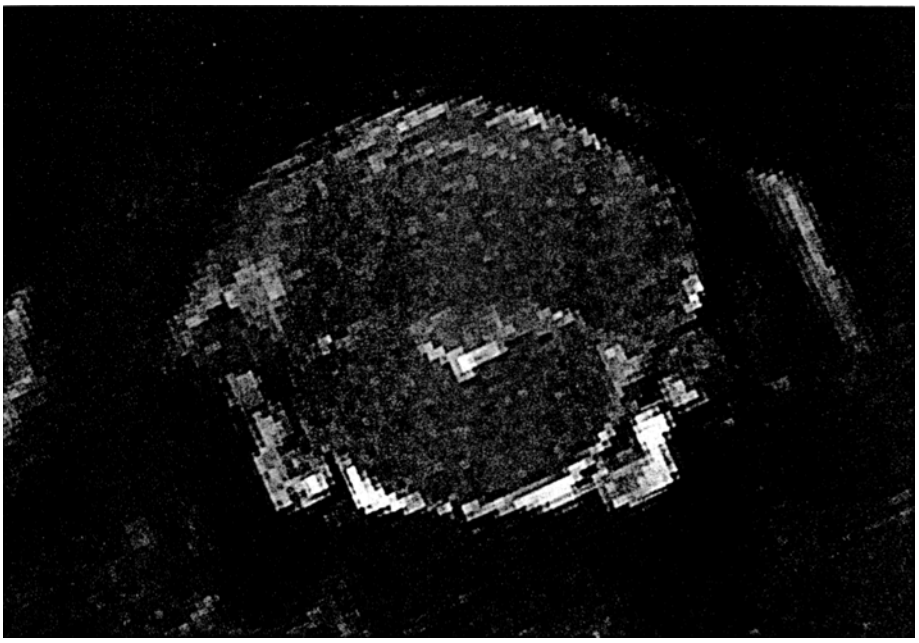

Fig. 2. Coronal RARE T2-weighted images of one rat brain with clinical EAE 24 h after i.v. administration of AMI-227 at a dose of 45 μmol/kg Fe. No abnormalities are seen.

with a dose of 300 μmol/kg Fe, imaging studies 4–6 h were also negative.

Thus, dose and scanning delay after administration are major parameters for the CNS macrophage imaging method.

4.1. Role of the dose

It is likely that for a low dose, the uptake of all USPIO nanoparticles occurs in the tissular cells of the MPS, like in macrophages from the spleen, the liver, the bone marrow [13]. At high dose, a saturation in the capability of phagocytosis may occur. Thus either circulating macrophagic cells like monocytes can probably take up and transport USPIO in the CNS or CNS macrophagic cells can increase the uptake of USPIO directly from the blood. The failure to demonstrate abnormalities at a dose of 45 μmol/kg Fe in EAE rats does not predict a failure in human because the blood half-life of AMI-227 is longer than in rats. It is about 24 h at a dose of 45 μmol/kg Fe (data provided by Guerbet Laboratory). Thus, a longer half-life increases the probability of USPIO uptake by circulating macrophagic cells or from CNS macrophagic cells. We are currently investigating patients with multiple sclerosis using USPIO at a dose of 45 μmol/kg Fe. USPIO at lower dose may produce a T1 enhancement effect that is currently tested at our institution.

4.2. Role of scanning delay

The time between i.v. injection and scanning is an important parameter that must be established in order to allow sufficient time for the iron particles to accumulate in the macrophage phagolysosomes and for circulating macrophagic cells to reach the CNS. In their study, Weissleder et al. demonstrated that 24 h after i.v. injection, all particles had accumulated in the macrophages [2]. In human MRI lymphography, a delay of 24 h is also used to obtain a sufficient filling of the normal lymph nodes by iron-loaded macrophages. Our study shows that a delay of 20–24 h gives a high positive rate for CNS macrophage imaging. It is likely that at intermediate times such as 4–6 h after injection, both for low and for high dose application at AMI-227, there are not enough iron-loaded macrophages in the CNS to be detectable by MRI.

Another important issue for human MRI of CNS macrophages is the magnetic field. The present study was performed at 4.7 Tesla because it allowed increased spatial resolution for the small rat brain due to a higher signal-to-noise ratio compared to lower fields. Nevertheless, macrophage activity imaging is achievable at lower fields as demonstrated by studies on experimental lymphography at magnetic field strengths ranging from 0.5 to 9.4 T [14]. Furthermore, in a previous study we have obtained similar results to those at 4.7 T using an imaging field of 1.5 T on rat EAE [4].

In conclusion, in vivo CNS macrophage imaging performed on experimental EAE in rats requires a dose of AMI-227 which exceeds the human recommended dose of 45 μmol/kg Fe. A dose of 300 μmol/kg Fe and a scanning delay of 20–24 h produce a high rate of

Table 1
Number of rats with MRI low signal intensity related to magnetic susceptibility in the brain, at different time and dose after AMI-227 i.v. administration

Delay (h)	Dose			
	45 μmol/kg Fe		300 μmol/kg Fe	
	EAE rats ($n = 6$)	Control rats ($n = 2$)	EAE rats ($n = 10$)	Control rats ($n = 2$)
4–6	0/3	0/1	0/5	0/1
20–24	0/3	0/1	5/5	0/1

positivity. A shorter delay of 4–6 h is insufficient to show iron-loaded macrophages in the CNS.

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