Imaging the Cellular Inflammatory Response in Experimental Allergic Encephalomyelitis Using 3DFIESTA at 1.5T

A. Oweida[1], E. Dunn[1], P. J. Foster[1]

[1]Imaging Research Labs, Robarts Research Institute, London, Ontario, Canada

Introduction

Cellular imaging is a newly emerging field with the potential to allow visualization of early events in inflammation, at the cellular level, before the consequences of disease are apparent by conventional MRI. The use of iron oxide based contrast agents for cell-specific imaging by MRI has now been demonstrated in a number of different disease applications. Dousset et al. (1999) were the first to show that intravenously (i.v.) administered iron oxides, taken up by macrophages in the brain of EAE animals, could be visualized with MRI. This approach has been termed "active labeling" of cells. The presence of iron oxide is indicated by hyperintense regions in T1W images or abnormal signal hypointensities in T2 or T2* weighted images, depending on the magnetic field strength and the concentration of iron. The large magnetic susceptibility of these particles can affect an area much larger than the actual size of the particles; this effect is known as a 'blooming artifact' and leads to an exaggeration of the region occupied by iron oxides. The purpose of this paper was to determine if the macrophage labeling strategies could be used together with new tools and concepts for cellular and microimaging at low field, developed in our lab, to enable the visualization of perivascular cuffing in EAE.

Our results show that small, discrete regions of signal void corresponding to iron accumulation in EAE brain can be detected with 3DFIESTA at 1.5T. Our histological data shows that the numbers of iron labeled cells associated with perivascular cuffs in these EAE brains are small; tens of cells. These small areas of inflammation may go undetected with conventional T2wSE and T2*w GE methods. What was originally considered a negative aspect of the SSFP imaging sequences, the high sensitivity to off-resonance effects, has made it very effective for cellular imaging with iron oxide nanoparticles. At 1.5T the FIESTA sequence is uniquely suited to studies of iron-oxide labeled cells in tissue since the signal response exhibits many of the blooming artifact suppression traits intrinsic to spin echo sequences while maintaining the sensitivity to iron-oxide labeled cells intrinsic to gradient echo sequences. This work provides early evidence that cellular abnormalities that are the basis of disease can be probed and supports our earlier work which indicates that single-cell tracking, will be possible using clinical MRI scanners.

Methods

EAE was induced in Lewis rats. Feridex was administered iv diluted in saline on day 10, in animals with hind limb paralysis. Animals were sacrificed 24 hours later and the brains removed and prepared for ex vivo imaging. Imaging was performed at 1.5T using a customized microimaging protocol, which consisted of a high-powered gradient coil insert (inner diameter 11cm, peak slew rate 2000 T/m/s and maximum gradient strength 1200mT), custom built solenoidal RF coils, and a prototype 3D SSFP sequence (FIESTA) previously optimized in our lab for single cell imaging. FIESTA parameters were TR/TE 10/5ms, 30 flip angle, 21 kHz bandwidth. H&E and Perl's Prussian Blue (PPB) staining was used to visualize morphology and iron labeling of cells.

Results and Discussion

The 3DFIESTA imaging sequence has proven to be highly sensitive to the superparamagnetic effects of iron oxide. Small, localized areas of signal loss were visualized in the brains of all EAE rats that were injected with SPIO. Multiple brain regions were affected (Figure 1). In Figure 2, 3DFIESTA images are compared to the more commonly used T2w FSE. For FIESTA spatial resolution is 200 (B) or 100 (C) µm isotropic. T2w FSE images were acquired with SNR and voxel size matched to the 200µm FIESTA. The area of signal loss is exaggerated in the FSE image as compared to FIESTA and most distinct in the higher resolution FIESTA image (C). Histological analysis showed that patterns of signal loss in the FIESTA images of EAE rat brain matched patterns of perivascular cuffing that were identified in corresponding H&E stained sections (Figure 3). PPB staining showed that regions of signal void represent the presence of SPIO particles in cells (Figure 4).

Figure 5 illustrates some of the challenges associated with using PPB staining to locate small numbers of iron labeled cells which correspond to the regions of signal void in the FIESTA images. First, multiple perivascular cuffs are often represented by a single region of signal void in the image. A single discrete area of signal void identified in a 100µm FIESTA image (labeled 1 in 6A), corresponds to three perivascular cuffs in the brain (3 asterisks in 6D). Second, it is clear that not all of the infiltrated cells present in EAE brain are labeled with iron after i.v. injections, which results in a percentage of the cuffs going undetected with any MR imaging pulse sequence. In 6C arrows indicate cuffs that were not found to contain iron labeled cells. Third, multiple contiguous PPB stained slices may need to be analyzed to account for the iron labeled cells which contribute to a region of signal void which represents a single perivascular cuff. A careful analysis of PPB stained sections reveals that for one cuff (cross in 6D) individual iron labeled cells located in different parts of the single cuff were found in 3 contiguous 8 µm histological images (6E-G). Since it is practically impossible to obtain evenly sliced contiguous slices through an entire brain specimen new methods for correlative microscopy are necessary.

Our studies have shown that not all of the cells in an individual cuff need to be iron labeled to allow the visualization of that cuff in high-resolution 3DFIESTA images. The concept of identifying only a portion of the infiltrated cells in active inflammation may at first seem futile. However, clinically, there is tremendous value in noninvasive markers that demonstrate changes in the degree of inflammation, during disease progression, or in response to anti-inflammatory therapies or treatments. In addition, the possibility for imaging leukocyte activity raises the possibility of understanding the natural history of numerous diseases.

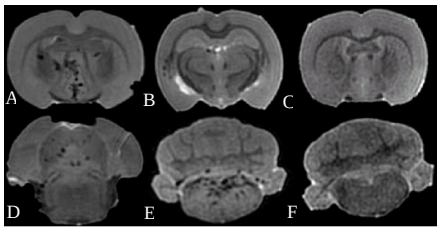

Figure 1. (a,b,d,e) show images from 4 different EAE animals with areas of signal loss observed throughout the brain; (c) normal rat injected with Feridex; (f) EAE rat with no Feridex.

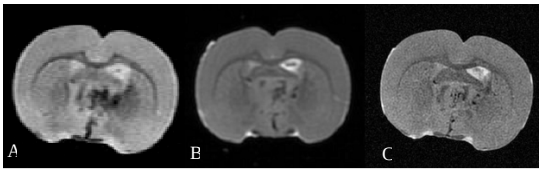

Figure 2. Comparing 2D T2-weighted FSE (a) and 3DFIESTA with 200 µm (b) and100 µm (c) isotropic spatial resolution

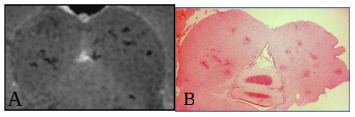

Figure 3. Correspondence between perivascular cuffing in EAE brain identified with (a) 3DFIESTA and (b) H&E stained tissue.

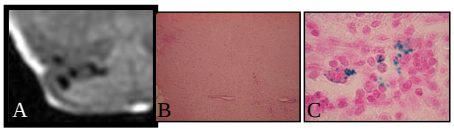

Figure 4. Regions of signal void correspond to iron accumulation in perivascular cuffs. (a) 3DFIESTA image, (b) PPB 4x, (c) PPB 40x.

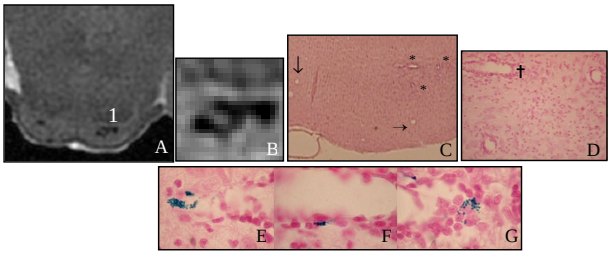

Figure 5. (A) 100 µm FIESTA image shows regions of signal void in the brain tissue, (B) magnified view of void#1, (C) 4x and (D) 10x PPB stained histological images show that void #1 corresponds to three perivascular cuffs (asterisks), (D) only a small number of the infiltrated cells associated with one of the cuffs (†) contain iron, and three contiguous PPB stained sections (E-G) are required to see all of the iron associated with this single perivascular cuff (†).