MRI detection of immune cell infiltration in focal cortical stroke in rats using MPIOs

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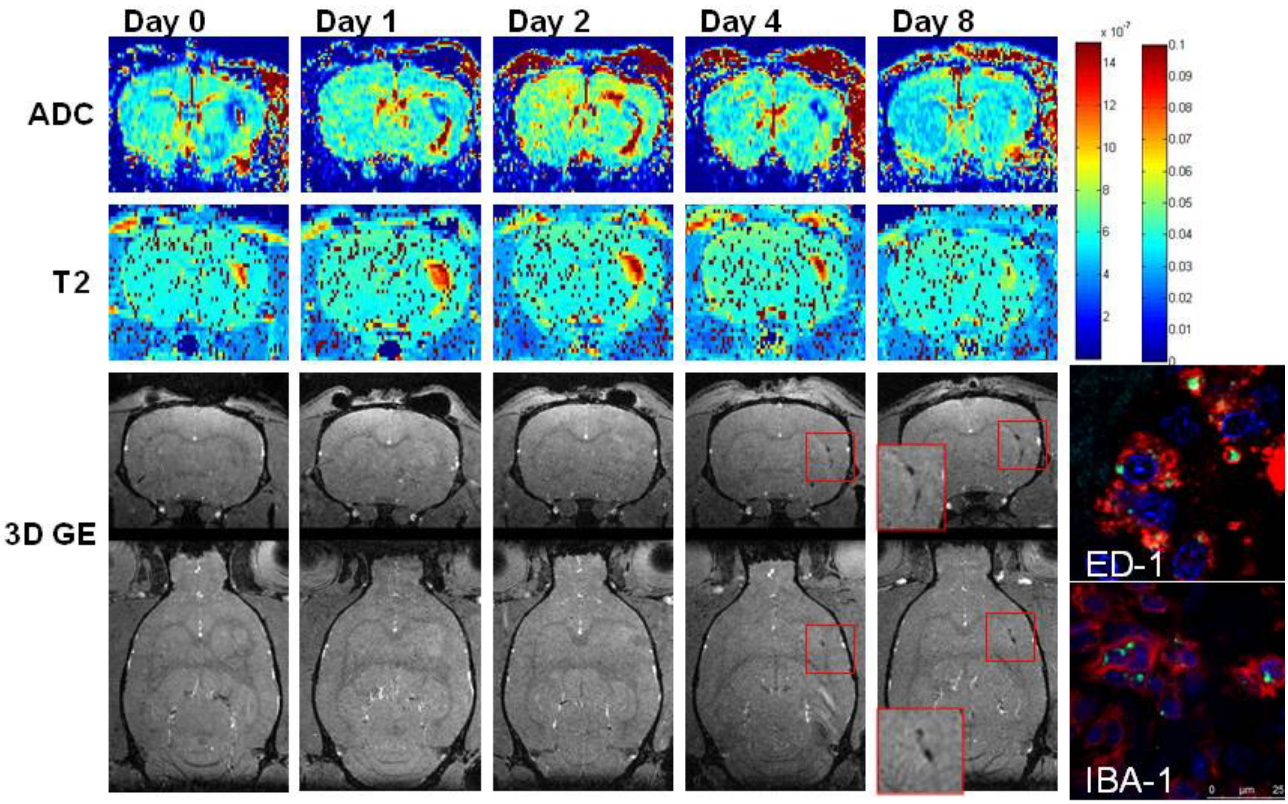
INTRODUCTION: In stroke, significant brain damage is caused by immune cell infiltration. Anti-inflammatory drugs have been largely ineffective against stroke in clinical trials despite some promising results in preclinical rodent studies. Thus, noninvasive tracking of immune cells in stroke may facilitate a better understanding of the infiltration time line and optimal time window for effective treatment. MRI with iron oxide nanoparticles has been shown to be a promising imaging modality for this purpose. Intravenous injection of small iron oxide nanoparticles (SPIOs) after experimental stroke produces focal signal intensity changes on the ischemic boundaries in MRI images. It has been hypothesized that these changes are caused by an influx of blood borne macrophages labeled with iron oxide nanoparticles. Micron sized iron oxide particles, MPIOs, are advantageous over (U)SPIOs for MRI tracking of immune cells because they contain sufficient iron to allow for single particle detection. Indeed, MRI-based cell tracking of infiltrating immune cells using MPIOs has been well documented in organ rejection in rodents (1). The purpose of this study was to investigate the feasibility of using MPIOs to monitor immune cell infiltration into focal cortical stroke.

MATERIALS AND METHODS: Male SD rats (n=3) were given a 400 pmol intracortical infusion of ET-1, a vasoconstrictor, over an hour to produce a focal stroke (2). Laser Doppler flow was used to analyze cerebral blood flow reduction. Animals were then immediately scanned on an 11.7 T Varian system using a stroke MRI protocol consisting of ADC mapping, T_2 mapping, and T_2* weighted 3D gradient echo. Two hours after the end of ET-1 infusion, animals were given 300 µl (1 mg iron) intravenous injection of 1.63 µm green fluorescent, polystyrene/divinyl benzene coated MPIOs (Bangs Laboratories) and scanned again at 1 hr, 24 hr, 48 hr, and 4 and 8 days later. Animals were sacrificed on day 8 and brains were perfused with 10% formalin and extracted for 50 µm isotropic resolution 3D gradient echo imaging at 4.0 T. Brains were then frozen, sectioned, and stained for microglia/macrophage markers IBA-1 and ED-1. Confocal microscopy was used to visualize labeled cells.

RESULTS: ET-1 infusion resulted in a decrease in blood flow of ~ 75% within minutes of infusion, recovering to ~ 40% of baseline at 30 minutes. Ischemic tissue exhibited large increase in T_2 values (from 30 – 40 ms to 70 – 90 ms) and decrease in apparent diffusion coefficient values (from $6e^{-7}$ – $8e^{-7}$ mm^2/s to $3e^{-7}$ - $4e^{-7}$ mm^2/s). These are consistent with previous reports of ET-1 induced cortical stroke (2). Despite this evidence of stroke, T_2* weighted 3D gradient echo images showed no signal voids in the stroke region at days 1 and 2. At day 4, dark contrast was present in the ischemic region, the volume of which increased at day 8. This accompanied reduction in both ADC and T_2 insults. High resolution 3D gradient echo image of the excised brain also displayed the signal intensity decrease in the ischemic region. Histological analysis showed MPIO labeled cells positive for IBA-1 and ED-1, both microglia and macrophage markers, at day 8.

DISCUSSION: It is generally accepted that intense leukocyte infiltration of the brain parenchyma occurs 48-72 hours after stroke with peak monocyte and macrophage accumulation occurring 3 to 7 days after stroke (3). The primary finding in the present study was that MPIO induced signal voids appeared in stroke regions of the brain starting at 4 days post-stroke, consistent with the peak accumulation of macrophages in the ischemic region. Whether these MPIOs are carried in by circulating monocytes or arrive in the stroke region by themselves and are phagocytosed by resident microglia is difficult to answer. While IBA-1 is specific for microglia, it is unclear if our ED-1 staining is truly specific for either microglia or monocytes. Further, a recent report using USPIOs determined that particles were present in a stroke region by themselves (4). Lastly, work has demonstrated that following MPIO iv injection, bone marrow resident monocytes were not labeled with MPIOs even at one week post-injection, despite large accumulation of MPIOs in the marrow (5). Regardless, the presence of MPIO induced signal voids consistent with macrophage infiltration is promising for using MRI-based cell tracking to specifically monitor immune cell infiltration in stroke at low cell numbers.



Top row: ADC maps of a representative slice at Day 0, 1, 2, 4, and 8 following stroke (Left scale bar). **Second row:** T_2 map from same slice (right scale bar). **Third and fourth rows:** axial and coronal slices from 3D gradient echo MRI at the same slice. Red boxes illustrate MPIO induced dark signal voids, with expansion insets at Day 8. **IHC:** Top, ED-1 (red) and DAPI nuclear (blue) staining. MPIOs are green. Bottom, IBA-1 (red) and DAPI nuclear (blue) staining. MPIOs are green.

References: 1) Wu, et al *PNAS* 2006 2) Windle, et al *Exp Neurol* 2006 3) Brown (Ed.) *Focus on Stroke Research* 2005 4) Farr et al *NMR Biomed* 2010 5) Tang et al *Mol Im Bio* 2010.
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