Surprising results in the use of MPIOs to label bone-marrow resident monocytes for immune cell tracking by MRI

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INTRODUCTION: The use of iron oxide nanoparticles to label bone-marrow resident monocytes for MRI-based cell tracking of monocyte infiltration into disease or injury has a long, successful history, including human trials (1). Micron sized iron-oxide particles (MPIOs) for MRI-based cell tracking have become increasingly popular due to the increased iron content per particles (2), and their use has recently been demonstrated for monitoring immune cell infiltration in organ rejection (3) and brain injury (4). Our goal was to systemically deliver MPIOs to mice to label bone marrow-resident monocytes to enable immune cell tracking in vivo. The accumulation and presence of MPIOs in bone marrow was studied over seven days. High resolution, serial in vivo MRI was performed on mice injected with various quantities of MPIOs. MRI signal changes were monitored in bone marrow and muscle to study MPIO trafficking. I*n vivo* labeling efficiency of bone marrow-resident monocytes was then quantified using flow cytometry. Unexpected results were obtained.

MATERIALS AND METHODS: Four week old CD-1 mice were injected intra-ocular with various quantities of different green fluorescent MPIO preparations (1.63 µm COOH, 1.63 µm NH_2, 0.86 µm COOH). Injection volumes ranged from 30 - 150 µl, delivering 10-100 µg iron in a 10 g mouse. Animals then underwent in vivo MRI immediately after injection, at 6 hours post injection, then again at 1, 2, 4 and 7 days post injection. MRI consisted of 3D gradient echo (TR = 100 ms, TE = 10 ms) of the femur at 50 µm isotropic resolution at 4.0 T, using a homebuilt solenoid coil. At various time points, animals were sacrificed and bone marrow was aspirated from both femurs. Total bone marrow was stained for CD45 (cells of hematopoietic origin) or CD11b (specific for monocytes/macrophages). Flow cytometry was performed at 2 and 7 days post injection to measure the total number of bone marrow cells double positive for green fluorescent MPIOs and red fluorescent cells of interest. For some animals, femurs were dissected, decalcified and cryosectioned for histology.

RESULTS: *MRI:* MRI of femurs as early as 1 hour post injection demonstrated widespread uptake of MPIOs throughout the bone marrow, as evidenced by dark contrast in T_2* weighted gradient echo MRI (Fig 1). The degree of bone marrow contrast was dependent on amount of particles injected, with 30 ul injections producing ~ 50% overall drop on bone marrow signal intensity and 100 ul and higher nearly depleting signal intensity in the marrow. Contrast in the marrow continued to increase, even up to four days post injection (Fig 2). A further critical finding was that while at 1 hour post injection bone marrow was full of MPIOs, there was also widespread dark, punctate contrast throughout the tissue (Fig 1). During the next seven days, the dark, punctate contrast in the tissue lessened almost to control levels. Previous data on clearance rate for injected MPIOs revealed that MPIOs clear from the circulation within 5 minutes. This is confirmed by MRI as the bright streak in the center of the femur is a blood vessel visible even at 1 hour post injection, because MPIOs have cleared.

Histology and flow cytometry: Fluorescence and optical microscopy on decalcified tissue sections indeed showed the presence of MPIOs within the marrow space (Fig 3). Fluorescence microscopic analysis of bone marrow aspirates clearly showed that MPIOs were in the aspirate, but in no instance was an MPIO found within a cell. Flow cytometry for CD45 and CD11b positive cells consistently demonstrated that >95% of bone marrow cells were CD45+ and that ~ 70% were CD11b+. However, flow cytometry detected barely any double positive cells (<0.5%), that is, virtually no bone marrow cells contained MPIOs (Fig 4).

DISCUSSION: Due to their high iron content, MPIOs are an attractive alternative to nanoparticles for MRI-based cell tracking of immune cell infiltration in disease. Despite convincing MRI and pathological evidence that MPIOs arrive in bone marrow quickly following injection, and remain there for at least seven days, flow cytometry and immunohistochemistry both reveal that MPIOs are not internalized by bone marrow cells.

In light of reports of successful use of i.v. administered MPIOs to track native immune cell infiltration (3,4), a different mechanism of action is proposed. As MPIOs do not label monocytes in marrow, it is unlikely that MPIOs are carried by labeled monocytes from marrow to an injury site. Instead, we hypothesize that free MPIOs which become trapped in tissue, likely in small blood vessels, can re-enter peripheral circulation whereupon they are endocytosed by a monocyte that has already homed to disease or damage, and differentiated into a mature macrophage. This hypothesis is supported by the data showing that the punctate contrast in the tissue slowly subsides over the course of seven days and that MPIOs continue to build up in the marrow for as long as four days.

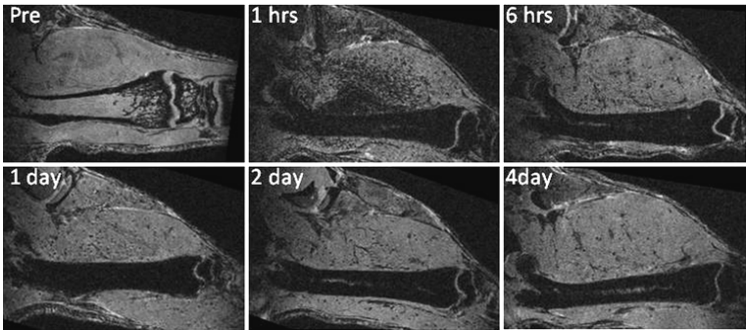

Figure 1: MRI of mouse femur. Images are pre-injection as well as various times as indicated. Note the bright blood vessel in the middle of the femur, even at 1 hr post injection, indicating clearance of the MPIOs from blood. Also, punctate dark contrast spots visibly decrease over the entire 7 day process (4 days shown).

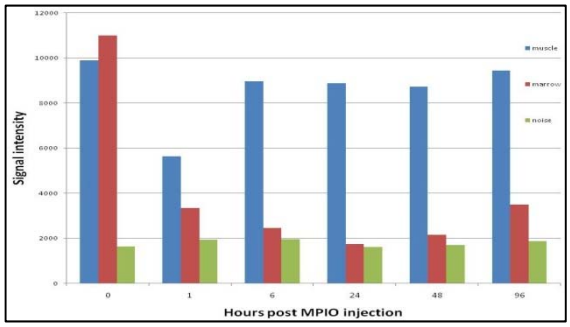

Figure 2: Signal intensity measurements of muscle (blue), bone marrow (red) and noise (green) as a function of time post-injection. Bone marrow signal continues to decrease as long as 48 hours post injection.

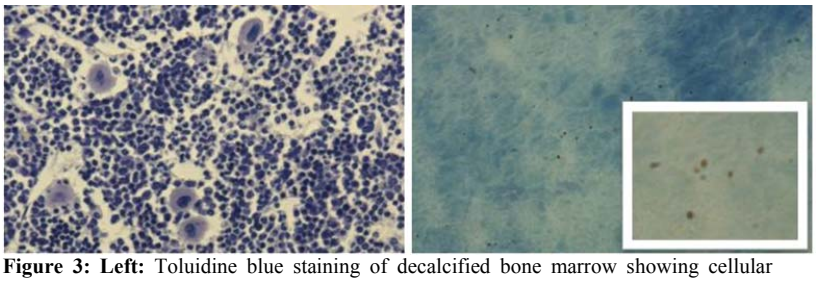

Figure 3: Left: Toluidine blue staining of decalcified bone marrow showing cellular composition. **Right:** Unstained bone section showing isolated MPIOs distributed throughout the marrow. Inset shows zoomed magnification.

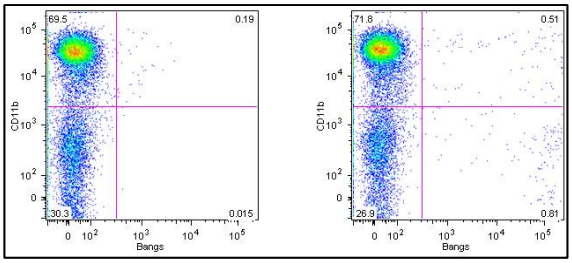

Figure 4: Flow cytometry plotting CD11b (monocytes) vs MPIOs. **Left:** control animals without MPIO injections. **Right:** animal injected with MPIOs 2 days prior.

References: 1) Jander, et al, Stroke, 38, 642-5 (2007); 2) Wu, et al, PNAS, 103, 1852-7 (2006); 3) Hitchens, et al, J Neurotrauma, 26, 1509-19 (2009).