Micrometer-sized Particles of Iron Oxide (MPIOs) Enhanced Cardiac Imaging to Potentially Monitor Inflammatory Cells Infiltration in a Murine Model of Myocardial Infarction

Y. Yang[1,2], B. Waghorn[1,2], Y. Yang[1], B. Klein[1], N. Yanasak[1], and T. C-C. Hu[1,2]

[1]Small Animal Imaging, Department of Radiology, Medical College of Georgia, Augusta, GA, United States, [2]Nuclear and Radiological Engineering / Medical Physics Program, Georgia Institute of Technology, Atlanta, GA, United States

Introduction

Micrometer-sized Particles of Iron Oxide (MPIOs) have become a very popular contrast agent for Magnetic Resonance (MR) cellular imaging and cell tracking (1). MPIOs allow more sensitive detection of single cells relative to ultra-small superparamagnetic iron oxide particles (USPIOs) with similar iron content (2). The large size of MPIOs allows the MRI detection of single cells (3) or even single particles (4). It is known that MPIOs can be readily taken up by inflammatory cells such as macrophages (5). We hypothesize that the labeled inflammatory cells (e.g., macrophages) will be visible in MR images after infiltrating and engrafting to the site of injury. In this study, we examined the signal intensity attenuation potentially due to MPIO labeled inflammatory cell infiltration into the infarction site following myocardial injury, suggesting the accumulation of macrophages labeled with MPIO particles (Bangs particles), in both T_2^* and T_2 weighted images. Cardiac T_2 mapping was also performed. The MRI findings were compared with *ex vivo* fluorescent imaging and histological analysis to confirm that signal intensity changes were a result of the presence of labeled cells.

Methods

Adult male C57 BL/6 mice (body weights 23.6 $\pm$ 1 g) were used in this study. The animal was anesthetized by 1-2% isoflurane during injection (0.04 mg Fe/g BW) of Bangs particles (Bangs Laboratories, Inc.). These particles are styrene/divinyl benzene-coated magnetic microspheres with a mean diameter of 1.63 μm, encoded with suncoast yellow fluorescent label.

Myocardial infarction (MI; n=6; open chest with Left Anterior Descending (LAD) coronary artery ligation) and sham (n=4; open chest without myocardial injury) groups underwent surgery 7 days following Bangs particle injection. This period allows the Bangs particles to be cleared from the blood pool, minimizing potential signal attenuation contribution from the blood pool.

MRI was performed 1 day before surgery as baseline, 3 days, 7 days and 14 days post-MI. Images were acquired on a horizontal 7.0-T, 20-cm MRI spectrometer (Bruker Instruments, Billerica, MA) equipped with a micro imaging gradient insert (950 mT/m). A 35mm inner diameter volume coil was used to transmit and receive at ^{1}H frequency. Animal setup procedure followed those previously described (6). ECG and respiratory signals were monitored and used for gating (SA Instruments, Inc., Stony Brook, NY). Short-axis heart images were acquired using a cardiorespiratory-gated Gradient Echo sequence with Flow Compensation (GEFC; TR = 120 ms, TE = 4 ms, FA = 30^o , FOV = 30 x 30 mm, Matrix Size = 256 x 256) and Multiple Slices Multiple Echoes (MSME; TR = 1000 ms, Average TE = 20 ms, FOV = 30 x 30 mm, Matrix Size = 256 x 256) T_2 Mapping sequence. A GEFC cine loop (TR = 16 ms, TE = 4 ms, FA = 25^o , FOV = 30 x 30 mm, Matrix Size = 256 x 256, Movie Frames/Cycle = 8) was also performed to acquire cardiac function parameters. Region-of-interest (ROI) analysis was performed on Paravision 4.0 software (Bruker Instruments, Billerica, MA). The ROI tools were used to select the areas of interest (MI site, left ventricle wall reference, septum, liver and background of noise). A contrast-to-noise ratio (CNR) showing the difference between the MI site and left ventricle wall reference was calculated.

The heart was removed after the terminal MRI and fluorescent imaging was performed on a 2-D IVIS 100 Imaging System (Xenogen Corporation, CA). Fluorescent imaging of the whole body of mice positioned supine was also performed immediately after injection of Bangs particles to make sure the infusion was successfully accomplished. The heart was cut into sections for staining and histological analysis once MRI and fluorescent imaging were finished. H&E staining, macrophage and iron staining were performed.

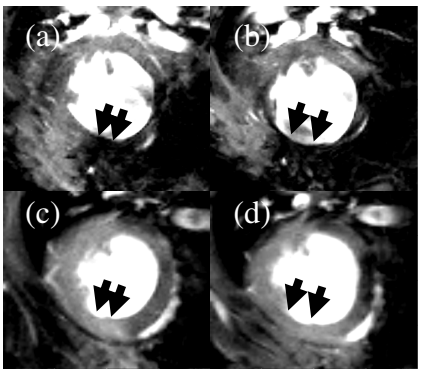

Figure 1. GEFC T2 * weighted short-axis heart images 3 days (a), 7 days (b) after MI surgery; 3 days (c) and 7 days (d) after sham surgery. Black arrows point to MI site (an assumed MI site was used for comparison in sham group).

Results

Signal intensity attenuation of the MI site was observed in the MI group, but it was not observed in the Sham group (**Figure 1**). A hypointense area in the MI site was observed in GEFC T_2* weighted images, and dark spots with sharp edge were observed in MSME T_2 weighted images and T_2 mapping (images not shown). The CNR of MI site was respectively 0.39, -7.19, -8.95, and -9.72 at the time points of baseline, 3 days, 7 days, and 14 days post-MI. The CNR of assumed MI site in Sham group was -0.57, -1.57, 0.49 and -0.09, respectively. In the *ex vivo* fluorescent imaging, signal was observed in the MI area indicative of localized Bangs particles, while the Sham group does not show enhanced signal intensity (**Figure 2**). The findings need further confirmation by histological analysis.

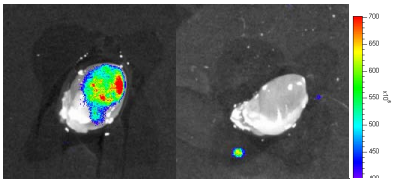

Figure 2. Fluorescent imaging of removed heart 14 days after MI (a) and sham surgery (b). Enhanced signal intensity indicates localized Bangs particles in the MI site.

Conclusions

This study demonstrates that inflammatory cells mobilized due to myocardial injury can be potentially tracked with Bangs particles labeled *de novo*. The pronounced reduction of signal intensity at the myocardial infarction site can be observed. Both T_2* and T_2 relaxation times were largely shortened due to accumulation of iron from the Bangs particles. These Bangs particles were taken up by macrophages. This provides a potential technique for monitoring the migration of macrophages into the MI area. Furthermore, the signal intensity attenuation in T_2 weighted images might provide additional insight and quantitative information regarding the disease progression following myocardial infarction.

References

1. Michel Modo, Mathias Hoehn, Jeff W.M. Bulte. Molecular Imaging. 2005;4(3):143-164.
2. John B. Williams, Qing Ye, et al. J. of Magn. Reson. Imag. 2007;25:1210-1218.
3. Hinds KA, Hill JM, et al. Blood. 2003; 102:867-872.
4. Shapiro EM, Skrtic S, Sharer K, et al. Proc. Natl. Acad. Sci. USA. 2004;101:10901-10906.
5. Shapiro EM, Skrtic S, Koretsky AP. Magn. Reson. Med. 2005;53:329-338.
6. Hu TC-C, Pautler RG, MacGowan GA, Koretsky AP. Magn. Reson. Med. 2001;46(5):884-890.