In Vivo Monitoring of Inflammatory Cell Mobilization to Heart Following Myocardial Infarction

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Introduction Ultrasmall Superparamagnetic Iron Oxide (USPIO) contrast agents have recently been used in conjunction with MRI to qualitatively assess atherosclerotic plaque inflammation (1), to track mesenchymal stem cells (2-3), and to indicate organ rejection by labeled T-cells (4). Due to its T_2* effect and relatively long half-life in cells, USPIO contrast agents are ideal for assessing inflammatory or stem cell mobilization and "homing". Following administration, most USPIO agents accumulate in liver, spleen, lymph, and bone marrow. Therefore in this study, we examined the potential for inflammatory cells, pre-labeled with a USPIO agent, to home to the myocardial injury site following myocardial infarction (MI). The temporal response of USPIO deposition and simultaneous left-ventricular function following MI was assessed in rats. This study was designed to provide insight into the temporal relationship between inflammation development/resolution and cardiac function following myocardial injury.

Methods Rats (Lewis, 6-8 weeks old) were pre-labeled with a USPIO agent (ferumoxytol, Advanced Magnetics Inc.) by administering a bolus (500 µmol/kg) by tail vein administration. Ferumoxytol in the blood pool was allowed to clear for 3 days. This period was necessary to eliminate any systemic contribution of ferumoxytol labeling in the study. At day 3 post-ferumoxytol labeling, left anterior descending coronary artery (LAD) occlusion was performed. Following MI, rats were randomized into Neulasta (long term of G-CSF) treated and vehicle (saline) treated groups. MI rats were given Neulasta at a dose of 1 mg/kg or saline (2 ml/kg) by subcutaneous injection 10 min after LAD ligation.

Cardiac MRI was performed post-MI at two different time points (8 days and 2.5 months). The animals were induced and anaesthetized using a mixture of oxygen, and isoflurane (~1.5-2%) anesthesia. ECG signal was monitored and this signal was used to gate the MRI Fast Low Angle SHot (FLASH) sequence. All cardiac MRI images were acquired on a 4.7T Bruker BioSpec MRI spectrometer (Billerica, MA) using a half-birdcage surface coil.

Short-axis heart images were acquired using an ECG gated FLASH sequence. A pilot coronal image of the heart was obtained. This pilot coronal image provided a clear view of the heart apex. The short axis slices covered the entire left ventricle. A cine loop was generated for each slice with enough delays to cover the systole (ECG was triggered by R-wave, which is end-diastole). The imaging parameters were as follows: matrix dimensions, 128x128; TE/TR, 3.7/20 ms; slice thickness, 2.0 mm; FOV, 5.0 cm; 4 averages; cine loop, 10 images. All images were gated to be acquired directly after the ECG R wave at end-diastole. Image analysis was performed using ANALYZE software (AnalyzeDirect, KS), the ROI tools were used to select the areas of interest (septum and left ventricular free wall) and signal intensity values were recorded. Left-ventricular functional data was also analyzed.

Results ECG gated cardiac MRI provided high quality images for left-ventricular function determination as shown in Figure 1(A) and Table 1. Cell mobilization and localization could be clearly observed post-MI in regions where signal attenuation results from ferumoxytol labeled bone marrow derived cell recruitment. This *in vivo* data correlated with *ex vivo* high resolution MRI reflecting ferumoxytol deposition within the MI zone (Figure 1(B)). Additionally, absolute iron deposition within the MI zone was assessed using ICP-MS. Histological staining was performed in an effort to determine the ferumoxytol co-localized cell types. Figure 1(C), (D) show the corresponding Perls and ED-1 histological stains, respectively. The majority of iron-stained cells were macrophages as determined by co-localization of histological stains. However, there was a population of positive iron-stained cells that have not immediately been identified (additional arrows pointing in Figure 1(C)). The iron-labeled macrophages were localized to sites of MI (Figure 2) for a period of at least 6 months (Figure 1(A) shows heart at 2.5 months post-MI; earlier time points not shown). Hearts with MI injury clearly had an enlarged left ventricle in both vehicle and G-CSF treated groups (Table 1). However, G-CSF treated animals maintained left ventricular ejection fraction versus the vehicle treated animals during the 2.5 months period (40 versus 25%, respectively; Table 1).

Conclusions This study demonstrates that inflammatory cells pre-labeled *in vivo* with ferumoxytol (possibly of bone marrow origin) home and engraft the site of cardiac injury following myocardial infarction in rat and the spatial and temporal features of this cellular infiltration may be monitored by non-invasive MRI. The localization and the persistent nature of the labeled cells in the infarcted region, suggest that the technique described may be useful for monitoring chronic inflammatory processes in the heart. Conversely, the cellular events mediated by G-CSF may reflect cardiac reparation by homing and engraftment of progenitor cells. Further studies are ongoing to address these possibilities.

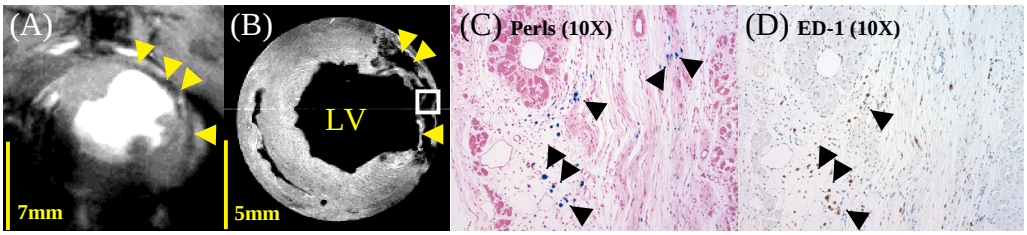

Figure 1. Examples of short-axis T_2-weighted MI rat heart images (A) *in vivo* 2.5 months post-MI, (B) *ex vivo*, (C) Perls histological stain from B for iron, and (D) ED-1 histological stain for macrophages. Arrows show where iron-labeled cells are localized at MI site.

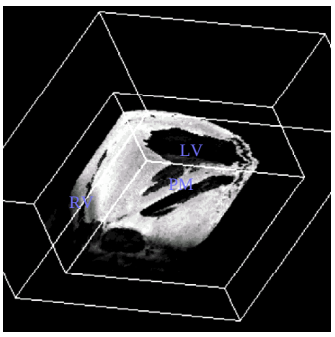

Figure 2. 3D volume rendered *ex vivo* high-resolution rat heart depicting iron deposition in MI zone.

Table 1 Heart rate (HR), Body weight (BW), LV-End diastolic volume (EDV), left ventricular systolic volume (ESV) and ejection fraction (EF) from vehicle and G-CSF treated rats. Values are expressed in mean±SD. * P<0.01

	post-MI	HR, bpm	BW, g	EDV, mm^3	ESV, mm^3	EF	LV Mass, mg
MI+Vehicle	1 week (n=3)	310 ± 11	227 ± 14	565.8 ± 116.3	322.2 ± 96.4	43.9 ± 6.2	707 ± 59
	2.5 months (n=1)	283	425	1296.7	972.0	25.0	639 ± 67
MI+GCSF	1 week (n=5)	297 ± 8	250 ± 17	593.8 ± 94.5	313.4 ± 89.6	48.2 ± 9.1	1179
	2.5 months (n=5)	292 ± 16	439 ± 13	773.3 ± 153.5	470.9 ± 161.0	40.1 ± 14.4	914 ± 108

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