Serial Quantitative MRI of Post-Infarct Macrophage Infiltration of the Mouse Heart Using Gd-Liposomes and R1-Mapping

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Introduction: Acute myocardial infarction (MI) is followed by a multi-step wound healing response that is initiated by the immune system. Among the immune cells that participate in this process, macrophages play critical roles in clearing the necrotic debris and in modulating angiogenesis and infarct healing. Previously, *in vivo* MRI of post-MI macrophage infiltration used iron oxide particles and T_2*-weighted imaging (1-3). However, these methods produce negative contrast, which persists after macrophage departure and pose challenges to quantitation. We previously demonstrated that post-MI macrophage infiltration could be detected by labeling macrophages with a T_1-shortening contrast agent and performing T_1-weighted imaging (4). We hypothesized that R_1 (R_1=1/T_1) mapping after labeling macrophages with a T_1-shortening contrast agent would enable the measurement of macrophage arrival to and departure from the infarct zone.

Methods: Liposomes containing gadolinium (Gd-liposomes) were prepared by a slightly modified standard reverse-phase evaporation procedure (4,5). Two days before MI (day -2), Gd-liposomes were injected intravenously into 5 mice. Mice received injections of either 50 µL (n=3) or 100 µL (n=2) Gd-liposomes. MI was induced in mice by a 1-hour occlusion of the left anterior descending coronary artery followed by reperfusion on day 0. Because infarct-related edema and, consequently, R_1 change with time after MI (6), we imaged the temporal course of R_1 in post-MI control mice that were not injected with Gd-liposomes. MRI R_1-mapping of the heart and spleen was performed at days -3 (baseline) and -1 (1 day post-injection of Gd-liposomes) before MI, and at days 1, 4 and 7 post-MI (Figure 1). All mice underwent MRI on a 7T ClinScan system (Bruker, Ettlingen, Germany). Mice were positioned prone within the scanner, body temperature was maintained at 36 ± 0.5° C using thermostated circulating water, and anesthesia was maintained using 1.25% isoflurane in O_2. During imaging, physiological monitoring and gating of the ECG and respiration was performed using an MRI-compatible system (SAII, Stony Brook, NY). For each imaging study, multi-slice short-axis cine MRI was performed to locate a mid-ventricular slice containing a large region of infarcted myocardium. Next, a cardio-respiratory gated (CRG) spiral Look-Locker pulse sequence (7) was used for R_1 mapping of the selected slice. This sequence accurately measures myocardial R_1 in mice, even when respiration and the ECG are erratic, such as after acute MI. Specific CRG Look-Locker parameters were: time between inversion pulses = 7000-8000 ms, number of inversion times sampled = 65 - 80, flip angle = 3°, TE = 0.67 ms, number of spiral interleaves = 87, and number of averages = 3. After MRI, Look-Locker images were exported to a workstation for calculation of myocardial R_1 using a least squares fit to a Bloch equation inversion-recovery curve. Maps of myocardial R_1 were filtered using a median crescent filter with a kernel of 2 pixels in the radial direction and 7 pixels in the circumferential direction (7). Cine DENSE MRI assessed myocardial strain at a matched location.

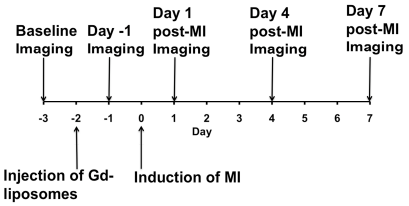

Figure 1: Timeline of the experimental design.

Results: R_1 mapping after monocyte labeling detected macrophage infiltration of the infarcted anterolateral wall post-MI. An example at day 4 post-MI is shown in Figure 2 (arrows point to the region of macrophage infiltration). Spatially, R_1 lengthening was confined to the dysfunctional infarct zone as defined by abnormal myocardial strain (green region on Figure 2B). In labeled mice, the R_1 of the infarcted anterolateral wall was normal at days -3, -1 and was significantly increased at day 4 post-MI (Figure 3). In unlabeled control mice, the R_1 of the infarcted myocardium decreased significantly post-MI, likely due to infarct-related edema (Figure 3). Thus, ΔR_1, calculated as the difference of R_1 between the labeled and the control mice, increased significantly on days 1, 4, and 7 post-MI in the infarct zone, with a maximum increase on day 4 (Figure 4). The time-course of R_1 in the infarcted myocardium of labeled mice, without being referenced to the R_1 of control mice, did not demonstrate the T_1 shortening effect of Gd-liposomes on days 1 and 7 post-MI since it is nullified by the shortening of R_1 due to edema. A small increase in ΔR_1, though not statistically significant, was also detected in the remote zone of the myocardium on days 1 and 4 post-MI, possibly indicating the presence of some macrophages in that region (Figure 4). R_1 mapping of the spleen showed that monocytes remain labeled with Gd-liposomes through day 7 post-MI.

Conclusions: R_1-mapping after labeling monocytes with Gd-liposomes enables the quantitative measurement of macrophage infiltration of the infarct zone after MI. The spatiotemporal macrophage kinetics measured *in vivo* by these methods agrees with prior *in vitro* histological studies (8,9).

References: (1) Sosnovik et al., *Circulation* 2007; 20;115(11):1384-91. (2) Yang et al., *MRM* 2010;63(1):33-40. (3) Montet-Abou et al., *European Heart Journal* 2010, 31(11): 1410-20. (4) Beyers et al., *Circulation* 2007; 116:S759 (Abstract). (5) Szoka et al., *PNAS* 1978; 75:4194-4198. (6) Naresh et al., Proc ISMRM. 2010. Page :1926 (7) Vandsburger et al., *MRM* 2010 63:648–657. (8) Vandervelde et al., *Cardiovascular Pathology* 2006; 15:83-90. (9) Yang et al. *Exp Physiol* 2002, 87(5): 547-55.

Acknowledgements: This work was funded in part by NIH R01 EB001763 and US-Israel Binational Science Foundation grant 2007290.

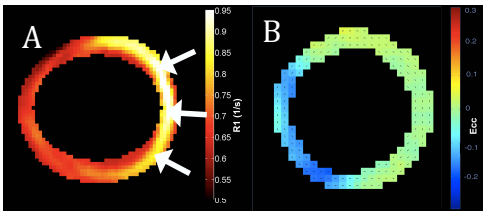

Figure 2: (A): Illustrative R_1 map from a day 4 post-MI mouse injected with 100 µL Gd-liposomes on day -2. R_1 significantly increased in the infarcted myocardium (white arrows) while R_1 is normal in the remote myocardium. The region with increased R_1 is co-localized with the region of reduced function as measured by circumferential strain (B, green zone).

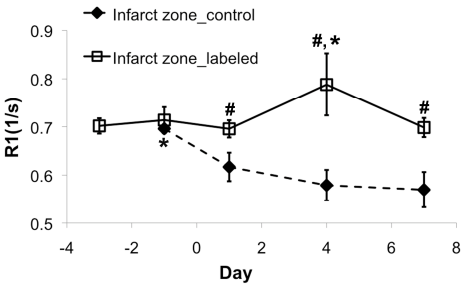

Figure 3: R_1 measurements in the infarct zone of the control (diamonds) and the labeled mice (squares). R_1 increased significantly on day 4 post-MI in the labeled mice (*p<0.05 vs. all days). R_1 decreased significantly post-MI in the control mice (*p<0.05 vs. all days). R_1 significantly increased post-MI in the labeled mice as compared to the control (#p<0.05 vs. same day control).

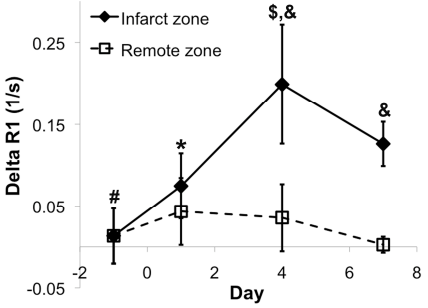

Figure 4: Delta R_1 measurements in the infarct zone (diamonds) and the remote zone (squares). Delta R_1 increased significantly post-injection in the infarct zone with maximum increase on day 4 post-MI (#p<0.05 vs. all days, *p<0.05 vs. day 4 and day 7 post-MI, $p<0.05 vs. day 7 post-MI). Delta R_1 significantly increased on day 4 and day 7 post-MI in the infarct zone as compared to the remote zone (&p<0.05 vs. same day remote zone).