T1 mapping of the heart with cardio-respiratory-gated Look-Locker MRI quantifies T1 shortening due to Gd-labeled macrophage infiltration after acute myocardial infarction

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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 cell signaling. New therapies against post-infarct remodeling and dysfunction may target macrophage activity. Previously, post-MI macrophage infiltration in mice has been imaged by labeling macrophages *in vivo* with iron oxide particles and using T2*-weighted imaging, or with liposomes containing gadolinium (Gd-liposomes) and employing heavily T1-weighted inversion-recovery imaging (1). The purpose of the present study was to quantify the degree of T1 shortening achieved by intravenous injection of Gd-liposomes in a mouse model of reperfused MI.

Methods: MI was induced in mice by 1-hour occlusion of the left anterior descending coronary artery followed by reperfusion. Gd-liposomes were prepared by a slightly modified standard reverse-phase evaporation procedure (1,2). Two days post-MI, when macrophage infiltration of the infarct zone is known to intensify (1), Gd-liposomes were injected intravenously into 5 mice. Mice received injections of either 100μL (n=3) or 200μL (n=2) Gd-liposomes. Control data (n =3) were acquired from post-MI mice that did not receive Gd-liposomes. All mice underwent MRI on a 7T ClinScan system (Bruker, Ettlingen, Germany) 5 days post-MI, at the peak of macrophage infiltration into the infarct zone (1). 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). Multi-slice short-axis cine MRI was initially performed to locate a mid-ventricular slice containing a large region of infarcted myocardium. Next, a recently-developed cardio-respiratory gated (CRG) spiral Look-Locker pulse sequence (3) was used for T1 mapping of the selected slice. This sequence accurately measures myocardial T1 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 T1, which employed Bloch-equation modeling and parameter (T1) optimization. Maps of myocardial T1 relaxation were filtered using a median crescent filter with a kernel of 2 pixels in the radial direction and 7 pixels in the circumferential direction (3).

Results: Figure 1 shows 4 typical images of T1-relaxation acquired using the CRG Look-Locker pulse sequence. These images were acquired at a range of inversion times from a mouse injected with 200 μL Gd-liposomes. The large region of infarction with macrophage infiltration (anterior, lateral, and inferior walls) demonstrates noticeable T1 shortening (white arrows) compared to the non-infarcted septum (Fig. 1C). Figure 2 compares sample T1 maps from CRG Look-Locker images acquired 5 days post-infarction in a control mouse (Fig. 2A) and in a mouse given 200 μL Gd-liposomes on day 2 post-MI (Fig. 2B). Substantial T1 shortening of the infarct zone was seen (white arrows), with T1 decreasing from 1790 ms in the control mouse to 820 ms in the mouse receiving 200μL Gd-liposomes. Interestingly, a moderate decrease in T1 was also observed in the non-infarcted zone (purple arrow, from 1570ms in the control mouse to 1310ms in the mouse injected with 200μL Gd-liposomes), possibly representing either a low concentration of macrophages in that region, or nonspecific labeling of other cells. Figure 3 summarizes the mean T1 values within infarcted myocardium in control mice, as well as mice receiving injections of either 100μL or 200μL Gd-liposomes. Infarct zone T1 decreased significantly in mice injected with 100μL Gd-liposomes ($p<0.05$ vs. control), and decreased further in mice injected with 200 μL Gd-liposome ($p<0.05$. vs. 100μL).

Conclusions: While T2*-shortening agents are generally considered to provide high sensitivity for cell labeling, the present data demonstrate significant T1 shortening of the myocardium after labeling macrophages with Gd-liposomes post-MI. Specifically, our results demonstrate a dose-dependent T1 shortening of the infarct region 5 days after MI following *in vivo* labeling of macrophages using intravenous Gd-liposomes injected 2 days post-MI. A novel pulse sequence designed specifically for accurate T1 mapping of the mouse heart, even during the irregular respiratory and cardiac rhythms that occur after acute MI, provided highly reproducible measurements of myocardial T1. Macrophage labeling with Gd-liposomes and T1-mapping with CRG Look-Locker imaging may prove useful for quantitative MRI of post-MI macrophage infiltration in preclinical murine studies.

References: (1) Beyers RJ, et al., Serial MRI Assessment of Macrophage Activity in the Murine Heart after Myocardial Infarction using Gadolinium-labeled Liposomes as a Positive Contrast Agent. Circulation 2007; 116:S759 (Abstract).
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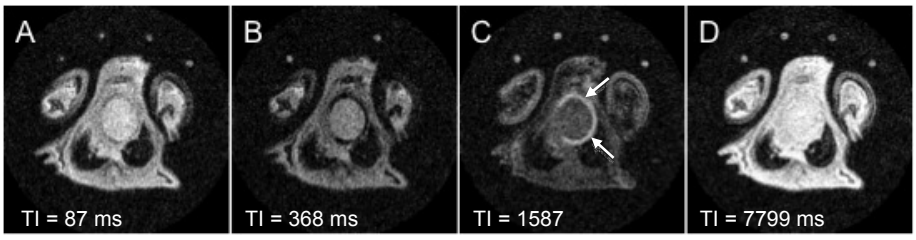

Figure 1: *In vivo* Look-Locker images at different inversion times during T1 relaxation of the myocardium acquired on day 5 post-MI in a mouse injected with 200 μL Gd-liposomes on day 2 post-MI. A: Image immediately after inversion. B: Image at null point of infarct zone myocardium. C: Enhancement of the infarcted myocardium with labeled macrophages (white arrows). D: Image at thermal equilibrium.

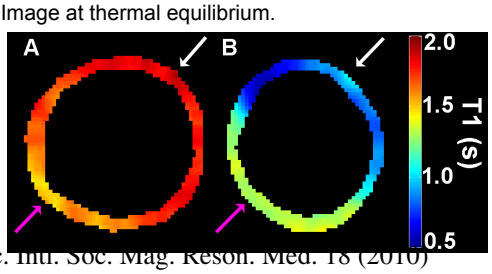

Figure 2: Illustrative day 5 T1 maps from a control mouse (A) and a mouse injected with 200 μL Gd-DTPA liposomes at day 2 post-MI (B). A large decrease in regional T1 is evident in the infarct zone (white arrows), and a smaller decrease in regional T1 is seen in the non-infarcted zone (purple arrows).

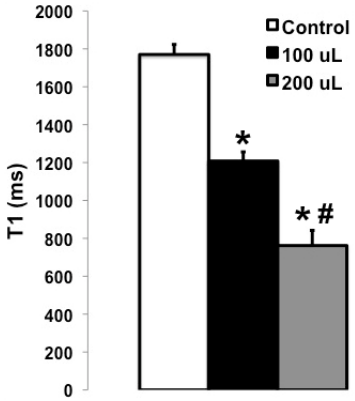

Figure 3: T1 of infarct zone myocardium obtained from CRG Look-Locker MRI for control mice (n=3), mice injected with 100 μL Gd-liposomes (n=3), and mice injected with 200 μL Gd-liposomes (n=2). (*$p<0.05$ vs. control, #$p<0.05$ vs. 100 μL)