Monitoring Dynamic Calcium Homeostasis Alterations by Cardiac Manganese-Enhanced MRI (MEMRI) with T_1 Mapping in a Murine Myocardial Infarction Model

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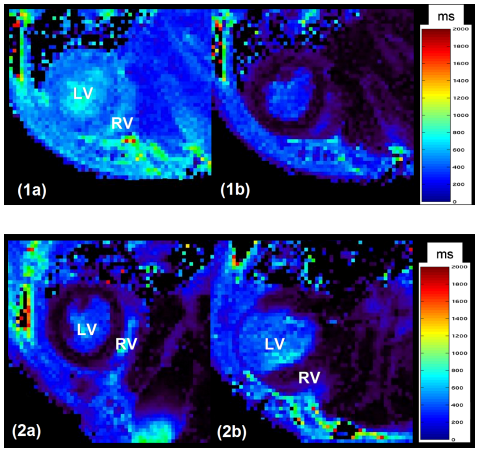
Introduction

Manganese ions (Mn^{2+}) enter viable myocardial cells via voltage gated calcium channels (1). Due to their T_1 shortening effect Mn^{2+} has become a useful molecular MRI contrast agent as a calcium surrogate marker in the study of potential calcium flux (2). In spite of the established importance of calcium regulation in the heart both prior to, and following, myocardial injury, strategies to assess calcium homeostasis in affected cardiac tissue are extremely limited. There is potentially a large range of calcium concentrations in the left ventricular free wall (LVWall) surrounding the injury site of injured myocardium (3). In this study, we have implemented a T_1-mapping protocol to enable the *in-vivo* quantification of the absolute manganese content in the mouse heart. Using this technique we propose studying a myocardial infarction mouse model to determine the extent of Mn uptake in various regions of the myocardium. This non-invasive imaging implementation may provide a potential method to examine salvageable myocardium for future therapeutic treatment or early diagnosis/prevention of cardiovascular disease.

Methods

Manganese-Enhanced MRI (MEMRI) experiments were performed in adult male C57Bl/6 mice (5-13 weeks old, 22.62±2.19g). The animals were anesthetized with a mixture of medical air, oxygen (1:1) and isoflurane and maintained at 1.0-2.7% isoflurane. $MnCl_2$ was infused into the tail vein at concentrations ranging from 25 to 300 nmoles/g total body weight (BW) at a constant rate of 0.6 ml/hr. Images were acquired on a 7.0-T, 20-cm horizontal bore BioSpec MRI spectrometer (Bruker Instruments, Billerica, MA) equipped with a micro imaging gradient insert (950 mT/m). Animal setup procedures followed those previously described (2). A 35 mm inner diameter volume coil was used to transmit and receive at ^1H frequency. Mn^{2+} signal enhancement was monitored with a T_1-weighted ECG gated Gradient Echo Flow Compensated (GEFC) pulse sequence; matrix = 128 x128; TE = 3.5 ms; TR = 35ms; slice thickness = 1.0 mm; FOV = 3.0 x 3.0cm; and NA = 6. Both pre-Mn^{2+} and post-Mn^{2+} T_1-map short axis heart images were acquired with an ECG-gated, flow-compensated Look-Locker MRI pulse sequence as previously described (4). The T_1-mapping parameters were as follows: matrix = 128 x 128; Inter-TE/TR = 2.5 ms/10 sec; slice thickness = 1.0 mm; FOV = 3.0 x 3.0 cm; NA = 2; inversion time/interval = 9/150 ms; echo images = 50. The total imaging time per T_1-map was approximately 43 minutes. The T_1 value of each pixel was calculated in two steps using a custom written C^{++} program (4). Regions of Interest (ROI) analysis on the calculated T_1 maps included the LVWall, septum, and liver, and was performed using AMIDE (5). The average regional T_1 value and ΔR_1 values were calculated, where ΔR_1 = (post-Mn^{2+} infusion R_1) – (pre-Mn^{2+} infusion R_1).



Figures 1 and 2 – *Example short-axis mouse heart T_1-maps. (1) Control pre-Mn^{2+} (a) and post-Mn^{2+} (b). (2) Post-Mn^{2+} sham operated (a) and MI (b).*

Absolute Mn content for heart and blood samples were measured by inductively coupled plasma-mass spectrometry (ICP-MS) analysis (CANTEST Ltd.). *In-vivo* absolute Mn concentration maps were then produced based on a relationship between the observed ΔR_1 values in the LVWall and interventricular septum post-infusion, and the absolute Mn elemental analysis results.

For the myocardial infarction studies, the mice were divided into control, sham-operated (open chest without myocardial injury) and myocardial infarction (open chest with left anterior descending (LAD) coronary artery ligation; MI) groups. All three groups were infused with 282.50±4.00 nmoles/g BW $MnCl_2$, with ΔR_1 values calculated from the acquired T_1-maps as above. ROI data for the MI was taken directly at the injury site (MI) and in the LV wall surrounding this region (LVWall).

Results

Sample control cardiac short axis T_1-maps, both pre- and post- Mn^{2+} infusion, are shown in Figures 1a and b, with post-Mn T_1-maps for sham operated and MI mice shown in Figures 2a and b. The effect of altering the concentration of infused Mn^{2+} on the LVWall relaxivity change, ΔR_1, is shown in Figure 3. After an initial increase of ΔR_1 with infused Mn^{2+} concentration (region A) a plateau region occurs above 197 nmoles/g BW (B). ICP-MS data showed a linear relationship between the infused Mn^{2+} concentration, X (nmoles/g BW), and the absolute heart Mn concentration, Y (µg/g dry weight), with Y =0.70X-25.9 (r^2=0.94). This data is shown alongside the LVWall ΔR_1 data in Figure 3. A relaxivity of 5.27 $mM^{-1}s^{-1}$ was calculated, enabling Mn concentration maps to be produced from the acquired T_1-maps. Figure 4 shows the effect of myocardial injury on the uptake of Mn^{2+}. Comparing the LVWall ΔR_1 values across all three groups, however, shows that there is a significant difference in relaxivity (p=0.03, ANOVA, single factor). Cross comparing all of the groups with a Bonferroni test demonstrates that both the MI LVWall and injury site ΔR_1 values are significantly less than the control and sham-operated groups (p<0.05).

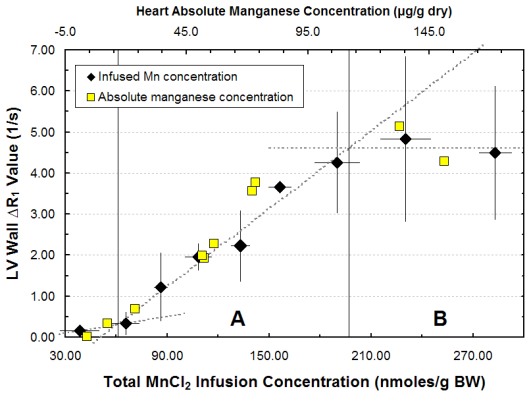


Figure 3. *Effects of both the infused Mn^{2+} concentration and absolute heart Mn concentration on the LVWall ΔR_1. Two different uptake regimes (A and B) are shown.*

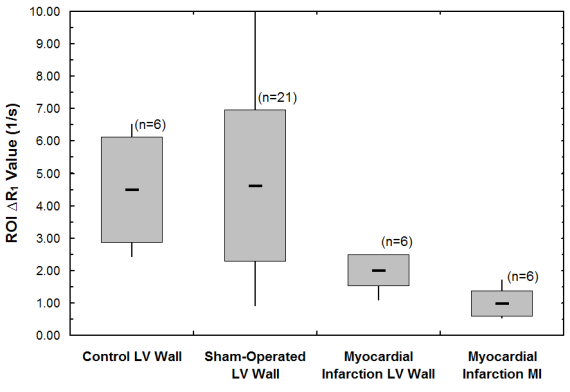


Figure 4. *Effect of myocardial injury on the uptake of Mn^{2+}. Average ROI ΔR_1 (horizontal line) ± SD (shaded box), with the data range shown (vertical line).*

Conclusions

This study demonstrates that T_1-mapping of cardiac Manganese-Enhanced MRI can be used to quantify the *in-vivo* manganese content. Applying this to a myocardial infarction model demonstrates the sensitivity of the technique to delineate between regions of the heart with altered Mn uptake. This information can potentially be used to estimate salvageable myocardium from the Mn content calculation in a pre-clinical myocardial infarction mouse model.

References

1. Narita K, Kawasaki F, Kita H., Brain research 1990;510(2):289-295. **2.** Hu TC, et al., Magn Reson Med 2001;46(5):884-890. **3**. Hu TC, et al., NMR in biomedicine 2004;17(8):620-626. **4**. Chuang KH, Koretsky A., Magn Reson Med 2006;55(3):604-611. **5.** Loening AM, Gambhir SS., Mol Imaging 2003;2(3):131-137.