Studying Indirect Ca^{2+} Alterations Following Myocardial Infarction in a Murine Model Using T_1-Mapping Manganese-Enhanced MRI

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Introduction

Calcium (Ca^{2+}) is an important regulator of cardiac contractile function. Efflux mechanisms controlling intracellular Ca^{2+} concentration are regulated by the sodium-calcium exchanger (NCX) and plasma membrane Ca^{2+}-ATPase. During myocardial ischemia, the reverse mode of the NCX causes intracellular Ca^{2+} concentration overload, which exacerbates tissue injury. Although it is established that diminished cardiac performance in heart failure is due to abnormal intracellular Ca^{2+} handling, limited techniques exist to monitor *in vivo* intracellular Ca^{2+} fluctuations across the plasma membrane (1). One potential technique for indirect monitoring of intracellular Ca^{2+} movement *in vivo* is to use a surrogate marker such as manganese (Mn^{2+}) as a molecular contrast agent. Mn^{2+} has a comparable ionic radius and chemical properties to Ca^{2+}, plus shortens the T_1 relaxation time (2). Therefore, Mn^{2+} would be an ideal MR contrast agent for indirectly studying alterations in Ca^{2+} influx and efflux (3).

Ex vivo studies observe an increase in intracellular Ca^{2+} following myocardial infarction, but to the best of our knowledge this increase has not been detected using an *in vivo* technique. The goal of this study is to focus on the use of MEMRI to quantify Mn^{2+} efflux in a mouse myocardial infarction model. Producing T_1-maps at multiple time points post-Mn^{2+} infusion allows regional washout curves to be determined and examined. This technique could be used as a diagnostic tool, providing an *in vivo* means of monitoring Mn^{2+} efflux, potentially through Ca^{2+} pathways.

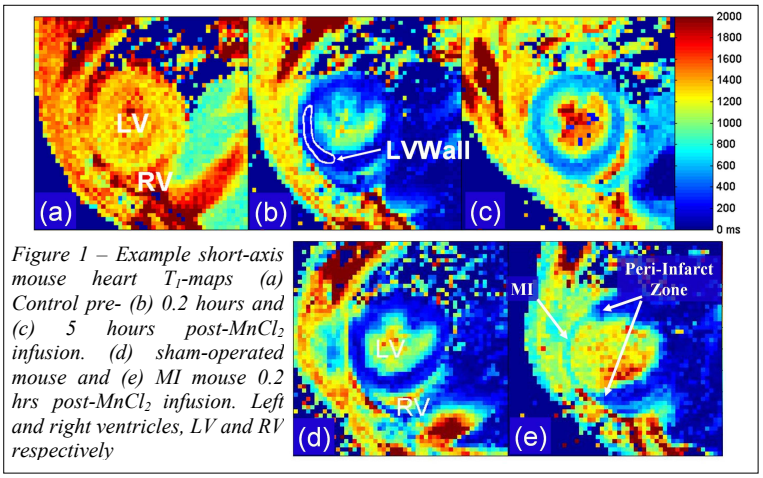

Figure 1 – Example short-axis mouse heart T_1-maps (a) Control pre- (b) 0.2 hours and (c) 5 hours post-$MnCl_2$ infusion. (d) sham-operated mouse and (e) MI mouse 0.2 hrs post-$MnCl_2$ infusion. Left and right ventricles, LV and RV respectively

Methods

The experiment results used to study myocardial Mn^{2+} efflux were acquired using T_1-mapping manganese-enhanced MRI (MEMRI) experiments in adult male C57Bl/6 mice (7-13 weeks old, 24.7±2.1g, n = 31 mice total, n_{maps} = 91 T_1 maps). Sample T_1 maps pre- and post-$MnCl_2$ infusion are shown in Figure 1.

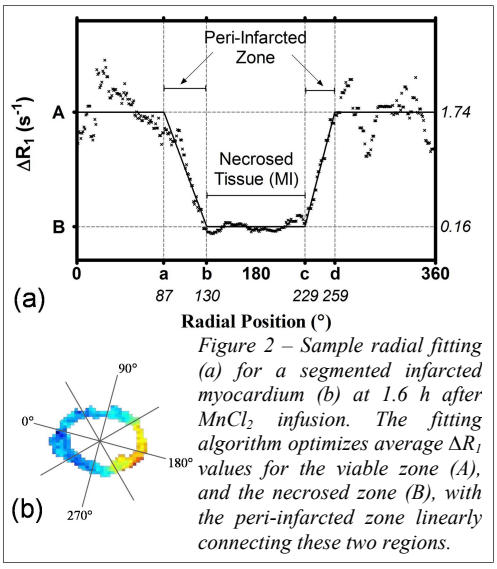

Figure 2 – Sample radial fitting (a) for a segmented infarcted myocardium (b) at 1.6 h after $MnCl_2$ infusion. The fitting algorithm optimizes average ΔR_1 values for the viable zone (A), and the necrosed zone (B), with the peri-infarcted zone linearly connecting these two regions.

Changes in left ventricular free wall (LV Wall) relaxation rate (ΔR_1) washout curves were obtained for three groups: Control (no surgical intervention, n_{maps} = 36), Sham operated (thoracotomy only, n_{maps} = 25) and Myocardial Infarction (MI, left anterior descending coronary artery ligation, n_{maps} = 30). All groups were infused with 190 nmoles/g total body weight (BW) $MnCl_2$, with infusions occurring at a rate of 0.6 ml/hr via the tail vein.

In-house software was used on the T_1-maps in order to isolate and segment the myocardium into 360 × 1° segments. Average T_1 values around the myocardium were used for the Control and Sham groups. A fitting algorithm was used for the MI mice, delineating the viable (healthy), necrosed (infarcted) and peri-infarcted (ischemic) tissue (Figure 2a). This provided an unbiased analysis of the regional ΔR_1 values (ΔR_1 = $1/T_1$ post – $1/T_1$ pre-$MnCl_2$ infusion). The average regional LV Wall T_1 values and ΔR_1 values were calculated as a function of time post-$MnCl_2$ infusion, where $\Delta R_1(t)$ = R_1(t hours post-$MnCl_2$ infusion) – R_1(pre-$MnCl_2$ infusion). First-order exponential fits were applied to the *in vivo* efflux data.

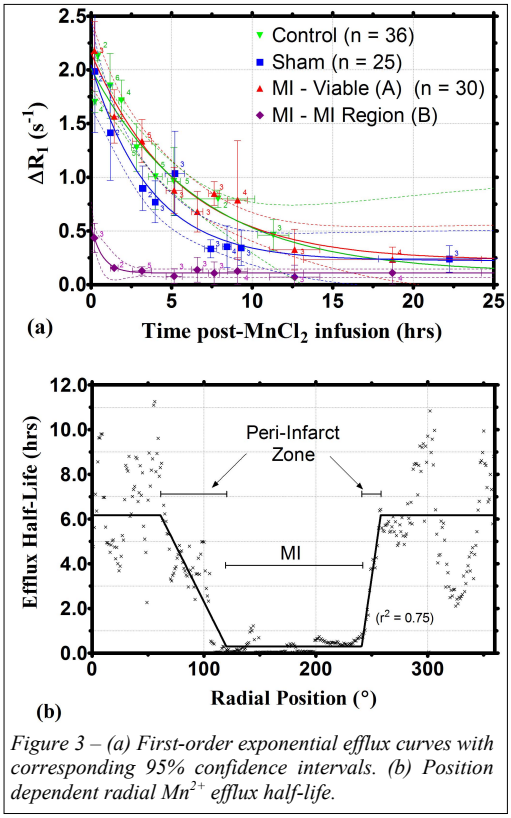

Figure 3 – (a) First-order exponential efflux curves with corresponding 95% confidence intervals. (b) Position dependent radial Mn^{2+} efflux half-life.

Results

Mn^{2+} efflux rates in the remote, viable regions of the MI myocardium were not significantly different than the Control and Sham groups (Figure 3a). However, the infarcted site had a significantly reduced efflux half-life ($p < 0.05$). Studying the regional variations in exponential half-life within the MI model (Figure 3b), regional variations in efflux half-life across both the infarcted and adjacent zones were noted. The rate of Mn^{2+} efflux in the infarcted and ischemic tissue corresponded to myocardial Mn^{2+} efflux rates in healthy mice following larger $MnCl_2$ infusion doses, approximately 280 nmoles/g (data not shown).

Conclusions

Results from this study demonstrated the sensitivity of the T_1-mapping MEMRI technique for detecting and measuring Mn^{2+} efflux in the myocardium of both healthy and post-MI mice. This study demonstrated a novel technique for identifying and quantifying specific regional differences in tissue characteristics that occur after a MI in the murine model. Regional alterations in Mn^{2+} efflux were detected, and suggested alterations in post-MI Ca^{2+} handling indicatice of altered NCX activity or increased Mn^{2+} content in viable but ischemic tissue, consistent with changes in Ca^{2+} handling post-MI. This technique could potentially be developed to indirectly provide *in vivo* assessment of Ca^{2+} handling alterations.

References

1. Zhang XQ, et al. *J Appl Physiol* 1999; **86**; 3: 943-950. **2.** Mendonca-Dias MH, et al. *Semin Nucl Med* 1983; **13**; 4: 364-376. **3.** Anderson M. *J Gen Physiol* 1983; **81**; 6: 805-827.