Modeling Cardiac Manganese Efflux Using T_1-Mapping Manganese-Enhanced MRI in a Murine Model

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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 in part by the sodium-calcium exchanger (NCX). 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). Furthermore, Mn^{2+} enters viable myocardial tissue via the L-type voltage-gated Ca^{2+} channels (3) and can be modulated by various inotropic agents (4).

Our strategy is to use these Mn^{2+} properties with cardiac manganese-enhanced MRI T_1-mapping methods to assess Mn^{2+} efflux rates. Producing T_1-maps at multiple time points post-Mn^{2+} infusion allows *in vivo* washout curves to be determined and examined. This study attempts to model the rates of myocardial Mn^{2+} influx and efflux following infusion of varying doses of $MnCl_2$. A two compartment model was used to model the temporal change in myocardial Mn content post-$MnCl_2$ infusion. This technique could be applied to a myocardial infarction model and used as a diagnostic tool, providing an *in vivo* means of quantitatively monitoring Mn^{2+} efflux, potentially through Ca^{2+} pathways.

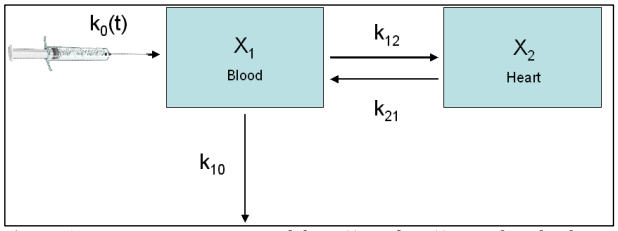

Figure 1. *Two compartment model. $X_1(t)$ and $X_2(t)$ are the absolute Mn content [nmoles] for the for the blood and heart compartments respectively. k_{ab} [hr^{-1}] are first order rate constants from compartment a to b, where k_{10} is defined as the rate constant for Mn^{2+} efflux from the blood to organs other than the heart.*

Methods

The experiment results used for the modeling were acquired using T_1-mapping manganese-enhanced MRI (MEMRI) experiments in healthy adult male C57Bl/6 mice (7-13 weeks old, 24.7±2.1g, n = 31 mice, n = 112 T_1 maps), as previously described (5). Changes in left ventricular free wall (LV Wall) relaxation rate (ΔR_1) washout curves were obtained for four groups infused with 65, 120, 190 or 280 nmoles/g total body weight (BW) $MnCl_2$, with infusions occurring at a rate of 0.6 ml/hr via the tail vein. 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). Using elemental analysis data from select heart and blood samples the absolute Mn content for the myocardium and blood was estimated from the *in vivo* ΔR_1 values. The $MnCl_2$ pharmacokinetic properties were modeled using a two compartment analysis, containing components for the blood and myocardium. Absolute Mn content was given by $X_1(t)$ and $X_2(t)$ [nmoles] for the blood and heart compartments respectively. $MnCl_2$ was infused into the blood compartment at a rate of $k_0(t)$ [nmoles·hr^{-1}], modeled with a step function where $k_0(t) = k_0\ (0 < t < T)$, and $k_0(t) = 0\ (t > T)$ (T [hrs] is the infusion time, dependent on the $MnCl_2$ infusion dose and mouse weight). First order rate constants, k_{ab} [hr^{-1}], were used to describe the rate of transfer from compartment a to b, where k_{10} is defined as the rate constant for Mn^{2+} efflux from the blood to organs other than the heart (e.g. liver, kidney and other major organs). A system of ordinary differential equations (ODEs) was setup for the model with dependent variables X_1 and X_2, and independent variable t [hrs]. Conversion from *in vivo* ΔR_1 values to absolute Mn content and initial conditions, $X_1(0)$ and $X_2(0)$, were obtained from heart and blood samples acquired at various times post-infusion, measured by inductively coupled plasma-mass spectrometry (ICP-MS) analysis (CANTEST Ltd., North Vancouver, BC, Canada). A least square fitting method was applied to fit solutions of the ODEs to the *in vivo* data by minimizing the sum of the error squared. Both *in vivo* cardiac data and *ex vivo* blood sample data was fit simultaneously to acquire least square fits for the first order rate constants, k_{ab}, for each dose group.

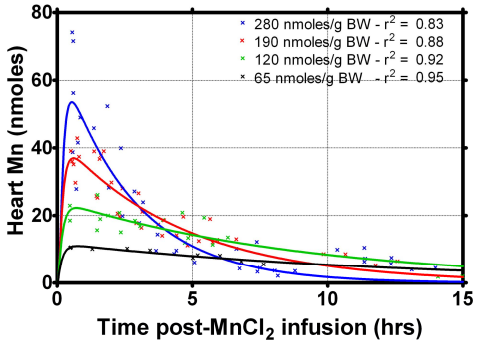

Figure 2. *Two compartment cardiac model (solid lines) overlaid on in vivo temporal Mn^{2+} efflux data (crosses).*

Results

Figure 1 shows a schematic diagram of the two compartment pharmacokinetic model. *In vivo* Mn^{2+} efflux data for each group is shown in Figure 2, with the least square fit model data and associated r^2 values shown. The model produced an average r^2 value of 0.89±0.05 for the four groups, an increase versus first order exponential fits with r^2 = 0.86±0.08. Dose dependent least square values for rate constants k_{ab} are shown in Figure 3. There is a trend for the transfer rate constant to increase with increasing $MnCl_2$ dose, as would be expected physiologically. Most notably the transfer rate for myocardial efflux, k_{21}, is more that four times larger for a $MnCl_2$ dose of 280 nmoles/g BW compared to a dose of 65 nmoles/g.

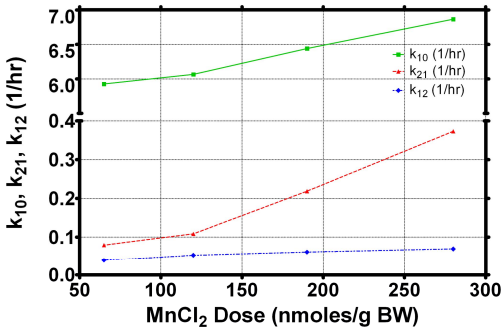

Figure 3. *Least square best fit Mn^{2+} transfer rate constants as predicted by the two compartment model.*

Conclusions

The two compartment model appears to provide a good approximation to the complex dynamics of cardiac Mn^{2+} fluxes. The model has been able to predict transfer rate constants, which represent physiological variables such as the L-type voltage-gated Ca^{2+} channels (k_{12}) and the NCX (k_{21}). Increasing the $MnCl_2$ infusion doses increase the rate of Mn^{2+} uptake in the heart (k_{12}), which results in an increased myocardial Mn^{2+} concentration. This in turn increases the rate of Mn^{2+} efflux (k_{21}). It is expected that the primary Mn^{2+} efflux mechanism is via the NCX, although further work is warranted to study this phenomenon. Although the inferred analogue of Mn^{2+} efflux to that of Ca^{2+} efflux needs to be further investigated and is expected to be a complicated relationship, it is expected that changes in rates of Mn^{2+} flux will contain information regarding Ca^{2+} flux. This technique provides a method to monitor potential myocardial insult or injury models, where information such as the transfer rates, particularly cardiac efflux (k_{21}), could provide an early diagnostic tool.

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. **4.** Hu TC, et al. *Magn Reson Med* 2001; **46**; 5: 884-890. **5.** Waghorn BJ, et al. *NMR Biomed* 2008; In Press.