# Effect of SEA0400 on Cardiac $Mn^{2+}$ efflux rates, using $T_1$-mapping Manganese-Enhanced MRI (MEMRI) in a Murine Model

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## Introduction

The $Na^+/Ca^{2+}$ exchanger (NCX) is an important mechanism for the regulation of intracellular $Ca^{2+}$ concentration (1). Protocols that selectively inhibit this exchanger can have potential therapeutic effects during myocardial ischemic-reperfusion injuries (2). Therefore, it is beneficial to monitor $Mn^{2+}$ which acts as a surrogate marker for $Ca^{2+}$ efflux from the myocardium. The reason for using $Mn^{2+}$ as a $Ca^{2+}$ tracer is due to its $T_1$ shortening effect. By implementing a $T_1$-mapping protocol absolute cardiac $T_1$ values can be calculated at multiple time points post $Mn^{2+}$ infusion, and the washout effect of the $Mn^{2+}$ relaxivity enhancement can be studied *in-vivo*. In this study, we examine the potential of this technique to probe the effects of the cardiac NCX using SEA0400 (3), a NCX inhibitor. SEA0400 was injected into a murine model and its effect on the washout of the $Mn^{2+}$ enhanced cardiac signal was studied. If this technique proves sensitive enough both SEA0400 and an inotropic agent can be used to quantitatively probe calcium fluxes *in-vivo* in different cardiac disease models.

## Methods

Manganese-enhanced MRI experiments were performed in adult male C57Bl/6 mice (7-9 weeks old, 23.2±1.5g). The animals were anesthetized with a mixture of medical air, oxygen (1:1) and isoflurane, and maintained at a heart rate of 463±31 bpm with 1.0-2.7% isoflurane. For the initial temporal washout study a single $MnCl_2$ concentration of 282.6±6.0 nmoles/g total body weight (BW) was infused into the control mice. All infusions were completed on a surgical bench at a constant rate of 0.6 ml/hr with the aid of a syringe pump. $T_1$-maps were acquired at various time points post infusion ranging from 0.2 hrs – 4 days.

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 (4). A 35 mm inner diameter volume coil was used to transmit and receive at $^1H$ frequency. ECG and respiratory signals were monitored by a physiological monitoring system (SA Instruments, INC.). 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 (5). 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 (5). Regional $T_1$ values were calculated from left ventricular free wall (LVWall) region of interest (ROI) analysis on the calculated $T_1$-maps using AMIDE (6). *In-vivo* $\Delta R_1$ values were calculated as (post-$Mn^{2+}$ infusion $R_1$) – (pre-$Mn^{2+}$ infusion $R_1$)

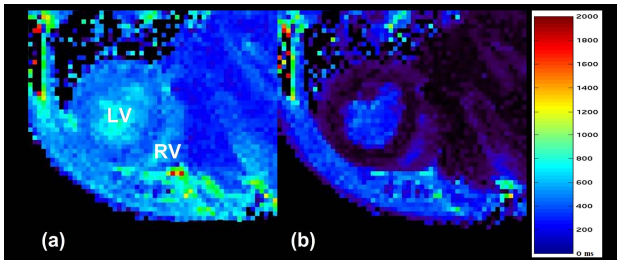


Figure 1. – *Example short-axis mouse heart $T_1$-maps (a) pre-$Mn^{2+}$ infusion and (b) post-$Mn^{2+}$ infusion. Shown are the left and right ventricles (LV and RV respectively).*

In order to further examine individual efflux mechanisms the mice were divided into 2 groups, control and SEA0400. $MnCl_2$ was infused into the tail vein at a concentration of 190.0±1.0 nmoles/g BW. This concentration has previously been shown to generate a significant signal enhancement while mediating the toxic effects. One hour post $Mn^{2+}$ infusion the SEA0400 mice were injected i.p. with 10 mg/kg SEA0400 (Taisho Pharmaceutical Co. Ltd). $T_1$-maps were acquired for both groups at times ranging from 0.2–31.9 hrs post $Mn^{2+}$ infusion. A $\Delta R_1$ washout curve was produced as above.

## Results

Sample cardiac short axis $T_1$-maps, both pre- and post- $Mn^{2+}$ infusion, are shown in Figure 1. Infusion of 282.6±6.0 nmoles/g BW $MnCl_2$ yielded $\Delta R_1$ of approximately 4.5 /sec immediately post infusion. This $T_1$ shortening effect was rapidly attenuated, with $\Delta R_1$ being reduced to 50% in only about 2.5 hours post infusion, as shown in Figure 2. A similar efflux rate was seen with an infusion concentration of 190.0±1.0 nmoles/g BW, Figures 2 and 3, with an initial $\Delta R_1$ of approximately 3.5 /sec. The linear regression over 4 hours post infusion shows that the two data groups have the same initial rate of efflux (p=0.67) with a slope of –0.48 (/sec)/hr. However, there is a visually delayed signal intensity attenuation as seen by the shift in data points for the two groups. Using the linear regression over the first 4 hours there is a significant intercept shift (p=0.001) corresponding to a 1.2 hour increase in relaxivity enhancement.

## Conclusions

This study shows that 50% of the $Mn^{2+}$ enhanced signal in the LVWall is attenuated within approximately 2.5 hours post infusion at 282.6±6.0 nmoles/g BW $MnCl_2$. Similar trends have been observed at the infusion concentration of 190.0±1.0 nmoles/g BW $MnCl_2$. Thus far, the Mn efflux curve is multi-faceted, potentially indicative of the calcium efflux mechanisms. The $T_1$-mapping protocol provides a more quantitative means of studying the $Mn^{2+}$ efflux mechanism. At this point, by inhibiting the NCX with SEA0400, we have seen a trend in $Mn^{2+}$ efflux modulation. Future work to study the actions of the NCX will be warranted by using various pharmaceutical agents. The implementation of $Mn^{2+}$ efflux could therefore provide a pre-clinical model to examine $Ca^{2+}$ efflux in various disease models.

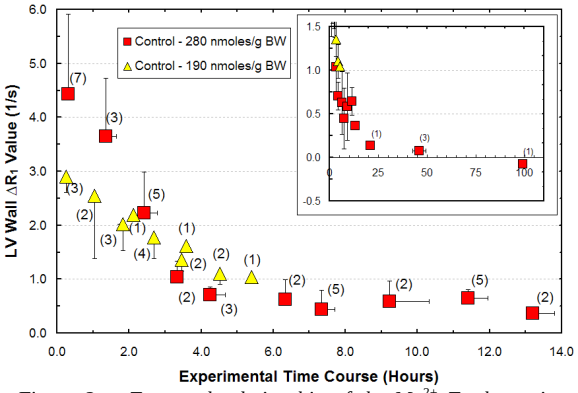


*Figure 2. – Temporal relationship of the $Mn^{2+}$ $T_1$ shortening effect. Shown is the experimental time course post- $Mn^{2+}$ infusion, against LVWall $\Delta R_1$, with sample size in parentheses.*

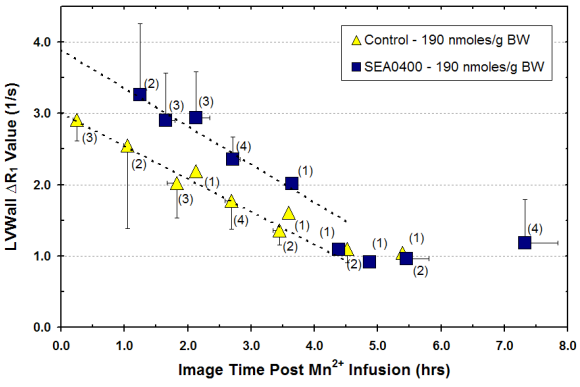


*Figure 3 – The effect of inhibiting the NCX channel with SEA0400 on the temporal $Mn^{2+}$ washout in the LVWall.*

## References

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