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Relation between Blood and Cardiac Manganese during Manganese Enhanced Magnetic Resonance Imaging (MEMRI) with T₁ Mapping in the Rodent

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Abstract

The Manganese ion (Mn²⁺) enters viable myocardial cells via voltage gated calcium channels. Because of its shortening of T₁ and its relatively long half-life in cells, Mn²⁺ can serve as an intracellular, molecular contrast agent to study calcium influx in the myocardium. One major concern for using Mn²⁺ is its sensitivity over a limited range of concentrations using T₁-weighted images for visualization, which limits its potential in quantitative techniques. Therefore, in this study, a T₁ mapping method has been implemented for cardiac manganese-enhanced MRI (MEMRI) to enable a quantitative estimate of influx over a wide range of concentrations. This method was used to compare the relation between T₁ changes in the heart as a function of cardiac and blood Mn²⁺ levels. We observed a non-linear relationship between the blood Mn²⁺ infused and LV wall R₁. At low levels of blood Mn²⁺ there was less cardiac enhancement than at higher levels of blood Mn²⁺. However both myocardium and blood level of Mn²⁺ were not linearly proportional to the total Mn²⁺ infused, most likely due to liver or kidney clearance.

Keywords

T₁ mapping; cardiac MRI; rat; heart; manganese

INTRODUCTION

Manganese ions (Mn²⁺) can enter viable myocardial cells via voltage-gated calcium channels (1, 2). Due to its T₁-shortening effect and relatively long half-life in cells, Mn²⁺ has become a very useful molecular MRI contrast agent to study perfusion or viability (3). Recently, manganese-dipyridoxyl diphosphate (MnDPDP), a phase III approved contrast agent used for liver MRI, demonstrated slow release of Mn²⁺ (4, 5) and was found to be retained in non-ischemic myocardial tissue as manganese ions (6). These studies suggest that

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not only can myocardial infarction size be quantified, but also that Ca^{2+} homeostasis may be assessed by using free Mn^{2+} ions.

Intracellular calcium is a central regulator of cardiac contractility, but little is known about how Ca^{2+} homeostasis is affected in murine ischemic myocardial tissue. Despite the importance of Ca^{2+} regulation in the heart, there are currently no established radiological imaging techniques for visualizing Ca^{2+} channel activity. *In vivo* studies show that Ca^{2+} channel blockers inhibit Mn^{2+} -induced MRI signal enhancement and that positive inotropes that increase Ca^{2+} influx further increase Mn^{2+} -induced MRI signal enhancement (7). Therefore, MEMRI represents a novel means to characterize voltage-gated Ca^{2+} channel activity quantitatively in homogeneous, well perfused heart without impaired tissue integrity. Additionally, in the infarcted heart, MEMRI may be used to measure the myocardial infarct size as defined by cellular functionality and to characterize simultaneously the efficiency of anatomical function for the left ventricle at enhanced spatial resolution (8).

An issue with MEMRI studies of heart remains the quantitative relation between infused manganese, blood Mn^{2+} levels, cardiac Mn^{2+} levels, and T_1 changes measured. Without a clear understanding of the intricate relationships between the total infused manganese and its time-dependent concentration in different tissues, changes in signal intensity could potentially be mis-interpreted biologically and diagnostically. To begin to address this issue we have measured the relations between infused Mn^{2+} , blood Mn^{2+} , cardiac Mn^{2+} , and cardiac T_1 . Interestingly but not unexpectedly, we found that the relation between the blood Mn^{2+} level, cardiac Mn^{2+} levels, and T_1 is non-linear. At lower total doses, smaller changes in cardiac Mn^{2+} and T_1 were observed than at the higher doses of infused MnCl_2 . The apparent relaxivity of Mn^{2+} as quantified using an improved Look-Locker (9) mapping technique is similar in blood and heart. These results indicate that the relation between blood Mn^{2+} and cardiac Mn^{2+} is complicated and should be considered when interpreting quantitative results for the cardiac MEMRI.

MATERIALS AND METHODS

Animal Preparation

All animal work was performed following the guidelines of the Animal Care and Use Committee and the Animal Health and Care Section of the National Institute of Neurological Disorders and Stroke, National Institutes of Health (Bethesda, MD, USA). Adult male Sprague-Dawley rats (body weights 147–369 g) were used in this study. The animal was initially anesthetized by 5% isoflurane (in a 1:1:1 air:nitrogen:oxygen mixture), positioned prone upon animal cradle, and maintained at 1.5–2.5% isoflurane during the MRI session.

Blood samples were collected from a sub-set of rats ($n = 7$). Immediately after infusing with 0.0 – 4.0 nmoles/min/g body weight of MnCl_2 , ~0.5 mL of blood was taken from the tail or femoral vein.

MRI

Images were acquired on a horizontal 7.0 T BioSpec MRI spectrometer (Bruker Instruments, Billerica, MA) equipped with a 12.0-cm micro imaging gradient insert (100 gauss/cm). The animal setup procedure followed the methodology described in a previous study (7). A standard volume coil was used for transmit and an actively decoupled surface coil at ^1H frequency for receive (300 MHz; Bruker Instruments, Billerica, MA). ECG and respiratory signals were monitored by a physiological monitoring system (SA Instruments, Inc., Stony Brook, NY) and the ECG signal was used to gate the MRI Fast Low Angle SHot (FLASH) and the Look-Locker sequences. The ECG gating window was set immediately after the R-wave.

A range of MnCl_2 doses was used: 0.0–4.0 nmoles/min/g body weight (BW), which was previously shown to provide adequate T_1 shortening without eliciting a cardiac depressive effect (7). The Mn^{2+} was infused either into the rat tail or femoral vein at 1.0 or 3.0 ml/hr for approximate 15–20 minutes, depending on the rat body weight. Due to the slow infusion rates and volume of the saline solution in the i.v. line, a slight delay was observed between the beginning of infusion and the rise in signal intensity in the heart.

Short-axis heart images were acquired using an ECG-gated FLASH sequence. A coronal image of the heart was obtained to provide a clear view of the heart apex and to assist in the prescription of the short axis image. The number of image slices in the short axis series ($N=6-8$) allowed for full coverage of the entire left ventricle. The imaging parameters were as follows: matrix dimensions, 128×128 ; TE/TR, 3.0/50.0 ms; slice thickness, 1.5 mm; FOV, 4.0 cm; 4 averages. The total imaging time for a single slice, 10 frame cine-loop was ~2.5 minutes. All images were gated to be acquired directly after the ECG R-wave at end-diastole.

To minimize motion and inflow artifacts, the T_1 maps were acquired by an ECG-gated flow-compensated version of the Look-Locker sequence described in a previous study (10) using the following parameters: matrix, 128×68 ; TE/TR, 2.79 ms/10 sec; flip-angle, $7^\circ-10^\circ$; Slice thickness, 1.5 mm; FOV, 4.0 cm; 2 averages; inversion time/interval, 8.58/185 ms; echo images, 50. The total imaging time for a T_1 map was ~23 minutes.

Data Analysis

T_1 Map—The T_1 value for each pixel was calculated in two steps using a custom-written C++ program as previously described (10). First, the signal recovery of each pixel was fitted by the Levenburg-Marquardt non-linear three-parameter curve-fitting algorithm. Then, the obtained parameters were substituted to solve for the actual T_1 relaxation time. A region-of-interest (ROI) analysis was performed using AMIDE (11) for the average regional T_1 value. The ROI tools were used to select the areas of interest (septum, left ventricular free wall, and chest wall).

Statistics—Relaxation rate data are presented as mean $\pm$ SEM (standard error of the mean). The change in the relaxivity, R_1 , was determined by taking the difference between

pre- and post-manganese infusion $1/T_1$ values. Unless otherwise stated, the correlation factors were determined using linear regression. Significance was set to $P < 0.05$.

Analytical Procedures—Absolute manganese content for left-ventricular myocardium (sectioned according to in-vivo MRI location) and blood samples were measured by inductively coupled plasma-mass spectrometry (ICP-MS) analysis (West Coast Analytical Service, Santa Fe Springs, CA). These values were then correlated to the in-vivo R_1 values.

RESULTS

ECG gated cardiac MRI provided good images (Figure 1). Figure 1a, b show the T_1 -weighted short-axis heart images of a Sprague-Dawley rat before and after infused with $MnCl_2$ solution. At this particular manganese concentration, 4.0 nmoles/min/g BW, the signal enhancement in the myocardium is clearly seen. The corresponding T_1 maps using the Look-Locker sequence are shown in Figure 1c, d.

The T_1 values in the interventricular septum (S), left ventricular free wall (LV Wall), and chest wall (CW) were calculated. The infusion of Mn^{2+} in rodent hearts leads to a shortening of T_1 decrease even under relatively low Mn^{2+} infusion concentration (Table 1). As a function of concentration, two different concentration regimes were noted in which the T_1 values decreased with the introduction of Mn^{2+} . Figure 2 shows a plot of total $MnCl_2$ infused versus R_1 of left ventricular wall. This data can be separated into two regimes. In the first regime, the range of T_1 values decreased from 1456 ± 173 to 390 ± 24 ms as the manganese concentration increased from baseline to 148.52 ± 0.15 nmoles/g BW (Table 1). In the second concentration regime, in which the concentration levels were higher than the first regime (148.52 ± 0.15 to 240.28 ± 2.49 nmoles/g BW), the T_1 values changed from 390 ± 24 to 123 ± 34 ms. The change in relaxation rate R_1 as a function of the Mn^{2+} concentration demonstrates the change in R_1 with Mn^{2+} is non-linear, with R_1 increasing more slowly at low Mn^{2+} concentration, and with R_1 changing faster at higher Mn^{2+} doses.

As noted previously, Mn^{2+} infusion leads to bi-phasic behavior in relaxivity as a function of concentration, with the ranges for each phase approximately covering 0.0–2.5 and 2.5–4.0 nmol/min/g BW, respectively. Assuming that the bi-phasic behavior in relaxivity can be modeled as two different linear functions that intersect at a particular infused dose, data were fit using unweighted least-squares as the intersection point was varied between 120 and 200 nmoles/g BW. An intersection of 165.5 nmoles/g, indicating the infused dose at which the transition occurs, provided the best fit ($Y_{low} = 0.015X - 0.025$; $Y_{high} = 0.080X - 10.724$, where $X = MnCl_2$ concentration). The multiple correlation coefficient for this fit was determined to be $r^2 = 0.97$ ($P < 0.01$), accounting for more variance as expected than a simple linear fit over the full concentration range ($r^2 = 0.83$).

The first phase of the concentration dependent curve due to Mn^{2+} infusion between baseline and 165.5 nmoles/g BW has R_1 of 0.00 to $1.87 \pm 0.07 \text{ s}^{-1}$. Interestingly, the second phase of the concentration dependent curve due to Mn^{2+} infusion with concentrations between 165.5 and 240.28 ± 2.49 nmoles/g BW exhibits R_1 that range from 1.87 ± 0.07 to $7.94 \pm 2.30 \text{ s}^{-1}$. The magnitude of change for R_1 in the second phase was ~ 3.25 times the change

displayed in the first phase, accompanied by a concentration increase of only 62% additional Mn^{2+} . To assess whether this non-linear dependence of R_1 and infused Mn^{2+} was due to uptake into heart or effects on blood, Mn^{2+} levels were measured from heart and blood. Since this bi-phasic observation may be due to various physiological effects from the expected linear R_1 concentration dependent curve, we sent out blood and myocardium tissue samples for absolute manganese quantification to characterize the potential uptake and binding effects in both the myocardium and blood-pool.

The effect of altering the total infused Mn^{2+} concentration on absolute left ventricular wall Mn^{2+} content which is shown in Figure 3(a). Mn^{2+} infusion leads to an accumulation of manganese within the left-ventricular wall that is also bi-phasic. The relation between cardiac Mn^{2+} concentration and R_1 is shown in Figure 3(b). Manganese accumulation leads to a near linear response in R_1 value. The R_1 may saturate at the highest concentration of Mn^{2+} but higher doses will be repeated to determine if this is the case. The apparent relaxivity as determined from the best fit slope to this data is $41.6 \text{ g dry weight} \cdot \mu\text{g}^{-1} \cdot \text{sec}^{-1}$. Even if R_1 saturates at high concentrations, the saturation point occurs at concentrations induced by doses well beyond those at the breakpoint in the bi-phasic response. For example, Figure 3(b) suggests that relaxivity saturates at $\sim 160 \mu\text{g/g}$ concentration, which would occur at a total infusion of $\sim 240 \text{ nmoles/g BW}$ according to Figure 3(a). Considering this observation, the bi-phasic relation between total Mn^{2+} infused and R_1 (for cardiac Mn^{2+}) seen in Figure 2 is not due to a change in apparent relaxivity.

The effect of altering the total infused Mn^{2+} on absolute blood sample manganese concentration in $\mu\text{g/cc}$ of sample is shown in Figure 4(a). The R_1 values are determined by *ex vivo* T_1 maps of blood samples. Mn^{2+} infusion also leads to a bi-phasic relation between infused Mn^{2+} and blood-pool concentration of Mn^{2+} . The transition occurs at a similar concentration ($\sim 165 \text{ nmoles/g BW}$) to the transition in Figure 2.

Figure 4(b) shows the relation between blood Mn^{2+} and R_1 measured from blood. Similar to the heart, there is a lower change in R_1 which saturates at the highest dose of Mn^{2+} . Thus the non-linear relation between total infused Mn^{2+} and R_1 in the heart is potentially a consequence of the non-linear relation between total infused Mn^{2+} and blood levels of Mn^{2+} .

DISCUSSION

The present study demonstrates that T_1 mapping of cardiac Manganese-Enhanced MRI has the sensitivity to provide quantitative information over a range of Mn^{2+} concentrations that extend much lower than previously reported. The homogeneous enhancement can be observed over time in the non-injured myocardium, consistent with Mn^{2+} retention within viable tissue in agreement with the previous observations (6, 12–17). This method provides a useful way to quantify and optimize Mn^{2+} infusion protocols by knowing the dynamic range of relaxation changes over a range of the Mn^{2+} concentrations. Additionally, the quantitative characterization of Mn^{2+} concentrations determined using T_1 -mapping could provide a potential technique for monitoring *in vivo* Ca^{2+} levels non-invasively.

An interesting observation was the relation between total infused Mn^{2+} and R_1 of the heart was non-linear, with the R_1 increasing more rapidly for changes in Mn^{2+} infused at higher concentrations. While there are many potential explanations of this non-linear behavior, the fact that the blood Mn^{2+} concentration was also non-linear with infused Mn^{2+} and showed functional changes at similar concentration levels to the heart suggests that this effect may be driving the effect in R_1 in the heart. The faster rise in R_1 at higher infused Mn^{2+} was likely due to a faster rise in blood Mn^{2+} at these higher doses. The fact that less infused Mn^{2+} leads to larger blood levels of Mn^{2+} is probably due to saturation of Mn^{2+} excretion by the liver and kidney at these higher doses causing blood levels to rise. Interestingly, the changes in T_1 in heart and blood were linear with total Mn^{2+} concentration. The apparent relaxations were $9.64 \text{ mM}^{-1} \cdot \text{sec}^{-1}$ for heart and $11.24 \text{ mM}^{-1} \cdot \text{sec}^{-1}$ for blood. At the highest concentrations of Mn^{2+} some saturation in R_1 was found to occur and this observation was consistent in blood. At these high doses of Mn^{2+} significant changes in T_2 and T_2^* effects can get large. It may be that these effects caused error in T_1 measured by the Look-Locker sequence.

In conclusion, T_1 mapping with the Look-Locker sequence enabled quantification of T_1 in the rat heart over a range of infused Mn^{2+} concentrations. This method allowed the relation between infused Mn^{2+} and R_1 to be predicted. The clear R_1 variation from the T_1 map has shown a large dynamic range comparing to T_1 -weighted sequences. This is $\sim 2\text{--}3$ folds less of manganese concentration comparing to concentration dependent result for mice ($\sim 45\%$ signal intensity enhancement just over $75.00 \text{ nmoles/g BW}$). Myocardium and blood-pool R_1 was linear with Mn concentration. However, myocardium and blood level of Mn^{2+} was not linear with the total Mn^{2+} infused, most likely due to liver or kidney clearance. This non-linear relation was evident from our studies, and this effect could be explained by a non-linear relationship between infused Mn^{2+} and blood Mn^{2+} . This cautionary result strongly suggests that one should be careful when quantitatively analyzing MEMRI of the heart, given the relation between blood Mn^{2+} and infused Mn^{2+} is not linear. It is expected the changes in clearance of Mn^{2+} by liver and kidney could further specify the dynamics relating blood-pool concentration, myocardium concentration, and infusion dosage as measured using T_1 -mapping.

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Figure 1.

Examples of short-axis rodent heart images with T_1 mapping at the particular manganese concentration, 4.0 nmoles/min/gm BW. (a) T_1 -weighted pre-Mn²⁺ infusion image; (b) T_1 -weighted post-Mn²⁺ infusion image; (c) pre-Mn²⁺ T_1 map; (d) post-Mn²⁺ T_1 map. The infusion of Mn²⁺ in rodent hearts clearly exhibited a large range of T_1 values (baseline ~ 1456 ms; 240 nmoles/g BW ~ 123 ms) even under relatively low Mn²⁺ infusion concentration.

Figure 2.

Effect of altering the concentration of infused Mn²⁺ on relaxivity of left ventricular wall.

The x-axis shows the total concentration of onset infused Mn²⁺ normalized to rat BW. The y-axis shows the difference of relaxivity pre- and post-infusion (error bars = 1 SD). As a function of concentration, two different concentration regimes were noted in which the T₁ values increased with the introduction of Mn²⁺. In the first regime, the range T₁ values decreased from 1456±173 to 390±24 ms as the manganese concentration increased from baseline to 148.52±0.15 nmol/g body weight. In the second concentration regime, 240.28±2.49 nmol/g body weight, the T₁ values changed from 390±24 to 123±34 ms.

Figure 3.

(a) Effect of total manganese concentration determined by ICP-MS on relaxivity of LV wall. The x-axis shows the total concentration of Mn^{2+} infused. The y-axis shows the absolute left-ventricular wall concentration in mg/g dry weight. Similar to the concentration dependent curve, myocardium Mn concentration due to $MnCl_2$ infusion is also non-linear. (b) The Mn concentration effect on R_1 values in 1/s. This plot can be used to estimate the *in vivo* Mn concentration.

Figure 4.

(a) Effect of total manganese concentration determined by ICP-MS on relaxivity of blood pool samples. The x-axis shows the total concentration of Mn^{2+} infused. The y-axis shows the absolute blood sample concentration in $\mu g/cc$ blood volume. Similar to the concentration dependent curve, myocardium Mn concentration due to $MnCl_2$ infusion is also non-linear. (b) The Mn concentration effect on R_1 values in units of 1/s. This plot shows the potential relationship between Mn^{2+} concentration responsible for R_1 changes.

Effect of altering the doses of infused Mn^{2+} on relaxivity of left ventricular wall. A range of $MnCl_2$ infusion rate was used: 0.0–4.0 nmoles/min/g body weight(BW) which resulting up to a total Mn^{2+} infusion dose of 240 nmoles/g BW. The resulting T_1 (ms), R_1 (1/sec), corresponding Mn content($\mu g/g$ dry weight) by elemental analysis, and calculated *in vivo* Mn content($\mu g/g$ dry weight) were presented for the LV Wall. The *in vivo* Mn content was calculated from the calibration curve of total Mn content determined by ICP-MS on relaxivity of LV wall.

Table 1

Mn^{2+} Infusion Dose (nmoles/g BW)	T_1 LV Wall (ms)	R_1 LV Wall (1/sec)	Elemental Analysis LV Mn($\mu g/g$ dry weight)	In Vivo Mn Content ($\mu g/g$ dry weight)
0.00 (n=15)	1456 $\pm$ 173	0.00	2.22 (n=1)	---
13.88 (n=1)	1237	0.06	4.96 (n=1)	2.47 (n=1)
63.33 $\pm$ 7.47 (n=4)	720 $\pm$ 174	0.82 $\pm$ 0.33	8.59 (n=1)	6.85 (n=1)
107.37 $\pm$ 12.61 (n=4)	373 $\pm$ 87	2.13 $\pm$ 0.60	---	---
148.52 $\pm$ 0.15 (n=2)	390 $\pm$ 24	1.87 $\pm$ 0.07	36.4 (n=1)	---
176.97 $\pm$ 7.63 (n=2)	309 $\pm$ 6	2.65 $\pm$ 0.15	60.2 (n=1)	---
193.69 $\pm$ 0.15 (n=3)	171 $\pm$ 16	5.21 $\pm$ 0.53	201 $\pm$ 83 (n=2)	130 $\pm$ 7 (n=3)
208.01 $\pm$ 2.17 (n=2)	136 $\pm$ 12	6.57 $\pm$ 0.70	163 (n=1)	158 $\pm$ 7 (n=2)
240.28 $\pm$ 2.49 (n=3)	123 $\pm$ 34	7.94 $\pm$ 2.30	180 (n=1)	354 (n=1)

Values are expressed as mean $\pm$ SD.