Assessment of Myocardial Ca^{2+} dysregulation using Mn enhanced MRI

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Introduction: Accumulation of divalent Manganese increases tissue R1, particularly in liver, kidney and heart. Increased R1 of myocardial tissue, and therefore signal intensity is proportional to the accumulated Mn^{2+} within it. Current use of Mn^{2+} based cardiac MRI is limited to the *in vivo* assessment of myocardial viability in rodents which relies on uptake/retention of Mn^{2+} in viable tissue. In this study we assessed the feasibility of Mn^{2+} enhanced MRI to detect myocardial Ca^{2+} dysregulation. In the normal heart, extracellular free Mn^{2+} is taken up by cardiac cells predominantly via the voltage operated calcium channels. It is plausible that functional impairment of calcium channels may lead to alterations in Mn^{2+} uptake by the myocardial tissue. Here we used a delta-sarcoglycan null (*Scgd*$^{-/-}$) mouse that displays severe muscular dystrophy[1, 2] to test this hypothesis. Mutations that cause muscular dystrophy usually result in defects in structural attachment proteins that provide rigidity to the muscle cell membrane[3]. Destabilization of this attachment framework leads to intermittent tears in the sarcolemma, permitting influx of Ca^{2+} and other ions that then function as primary inducers of cellular degeneration and necrosis [3, 4].

Methods: Eight months old wild type (*WT*) (n=4), and *Scgd*$^{-/-}$ (n=3) mice were studied by Mn-enhanced MRI. Imaging was performed on a 7Tesla Bruker MR scanner using a custom made single-turn-solenoid RF coil. Mice were anesthetized with 2% (vol.) Isoflurane. Physiological monitoring was done by an MR compatible monitoring and gating system (Model 1025, SA Instruments, Inc. NY, USA). Mice were infused with highly purified 20mM, $MnCl_2.4H_2O$ (product number 529680, Sigma-Aldrich Corp) injected intraperitonealy at a rate of 0.6 ml/hr for 40 minutes. Starting simultaneously, a series Images were acquired every 1.2 minutes for 60 minutes in the mid-ventricular short axis plane using a TrueFISP sequence: TE/TR=2/4 ms, slice thickness=1.5 mm, NEX=8, flip=50°, matrix=256x256, FOV=3.4x3.4cm^2. Signal-to-noise ratio (SNR) was measured from ROIs drawn in the left ventricle and in the noise region in each image. The portion of the normalized SNR vs. time curve corresponding to $MnCl_2$ enhancement was isolated and the slope of the linear fit to that data was used as the rate of enhancement (R_{Mn}).

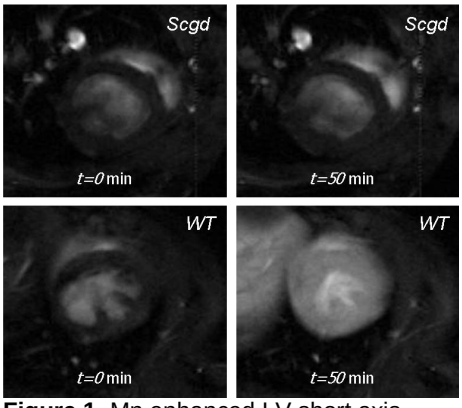

Figure 1. Mn enhanced LV short axis images of Scgd$^{-/-}$ (top) and WT (bottom) mice at start of Mn infusion (t=0 min) and at t=50 min.

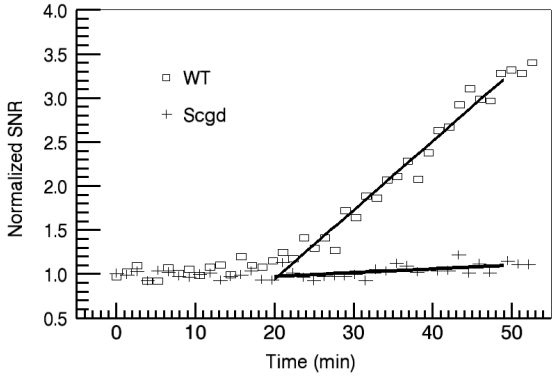

Figure 2. Rate of signal enhancement

Results: Myocardial enhancement was seen after 20 minutes of infusion in *WT* mice but barely notable in *Scgd*$^{-/-}$ mice (figure 1). The rate of enhancement for WT, R_{Mn}=*0.049±0.020* was significantly higher (*p=0.02*) than that for *Scgd*$^{-/-}$ mice (*0.006±0.003*) (figure 2).

Conclusion: This study demonstrates for the first time that Mn-enhanced MRI may be used to probe *in vivo* Ca^{2+} dysregulation in mice. The slow rate of enhancement points to the slow accumulation of Mn^{2+} in *Scgd*$^{-/-}$ myocytes compared to the *WT*. It is widely accepted that dystrophic cardiomyocytes, show altered Ca^{2+} entry and handling upon stretching and mechanical deformation compared with non-dystrophic cells[5]. Increased [Ca^{2+}] cause decreased Mn^{2+} uptake by the heart[6]. Therefore increased base level [Ca^{2+}] could have slowed Mn^{2+} uptake by the in *Scgd*$^{-/-}$ mice compared to wild type, fact that remains to be proven.

References:

1.M. Durbeej and K. P. Campbell. Curr Opin Genet Dev 2002;12(3):349.
2.R. Coral-Vazquez, R. D. Cohn, et al. Cell 1999;98(4):465.
3.K. A. Lapidos, R. Kakkar, et al. Circ Res 2004;94(8):1023.
4.N. P. Whitehead, E. W. Yeung, et al. Clin Exp Pharmacol Physiol 2006;33(7):657.
5.D. G. Allen, N. P. Whitehead, et al. J Physiol 2005;567(Pt 3):723.
6.D. R. Hunter, R. A. Haworth, et al. J Mol Cell Cardiol 1981;13(9):823.