Myocardial Ca2+ Influx Deficits in a Mouse Model of Sandhoff's Disease Utilizing MEMRI

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Sandhoff disease is a lysosomal storage disorder inherited in an autosomal recessive manner characterized by excessive buildup of the GM2 ganglioside, caused by mutations in the HexB gene [1]. The neurodegenerative effects of this GM2 ganglioside accumulation include motor function deterioration and spasticity [1]. There have been reports of GM2 ganglioside aggregation in other organs, specifically the heart, where there is a disruption of normal cardiac activity [2]. Manganese-enhanced MRI (MEMRI) has been shown to reflect the rate of calcium influx into the heart via voltage gated channels [3]. Studies also indicate manganese is an intracellular contrast agent, and consequently, changes in signal enhancement due to manganese infusion demonstrate intracellular calcium levels [3], [4]. In the present work, these unique advantages offered by MEMRI were used to investigate the effects of cardiac GM2 ganglioside accumulation in a mouse model of Sandhoff disease.

Methods

Mating pairs of HexB -/- (knockout) mice were obtained from Jackson Labs, and a colony was established within the lab. DNA was purified from a mouse tail sample using the Puregene DNA Purification Kit; these DNA samples were then analyzed using an agarose gel electrophoresis to visualize the fragments and determine genotype. Nine twelve-week old mice were imaged: three wildtypes (HexB +/+), three heterozygotes (HexB +/-), and three knockouts (HexB -/-). All images were acquired on a 9.4 T Bruker magnet system. A 117.96 mM manganese chloride solution was prepared by dissolving solid $MnCl_2$ in stock saline. Manganese infusion was performed intravenously through the tail vein, using a syringe pump to deliver manganese chloride solution at a rate of 0.2 mL/hr for a total volume of 0.1 mL $MnCl_2$ and 0.05 mL saline. ECG electrodes were attached to the paws to gate signal acquisition with the end diastolic moment. Temperature was maintained at 37° C, and Isoflo kept at 2.5% during imaging. Images were acquired with Fast Low Angle SHot (FLASH) sequence (256x128, TR = 23.6 ms, TE = 1.4 ms, FA = 30 degrees, slice thickness = 1.0 mm, 3.0 cm FOV) for 80 repetitions over an experimental time course of approximately 90 minutes. Image analysis was carried out in Paravision v. 3.0.2. Signal intensities were calculated from a region of interest defined in the Left Ventricular Wall and normalized to an external water phantom. Signal intensities were then averaged for both pre-manganese and post-manganese steady states in each mouse, and the percent enhancement was calculated. Statistics were done in Prism v 4.0, and the difference between means was compared using an unpaired, two sample t-test.

Results

For all three genotypes, the difference in signal intensity between the pre and post manganese steady states was significant ($p < 0.05$). However, the signal enhancement was noticeably distinct in the HexB +/+ and +/- genotypes when compared to the HexB -/- genotype (Fig. 1). The average percent signal enhancement in the wildtype mice was approximately twice that in the knockout mice. The average percent enhancements of the three groups were compared (Fig. 2). The difference in percent enhancement between the wildtype and knockout samples was significant ($p = 0.02$) while the percent enhancement difference between the heterozygotes and knockouts was also noticeable. Also, significant hypertrophy of the knockout hearts was apparent from the images obtained. There was no significant difference in percent enhancement between the wildtype and heterozygote samples.

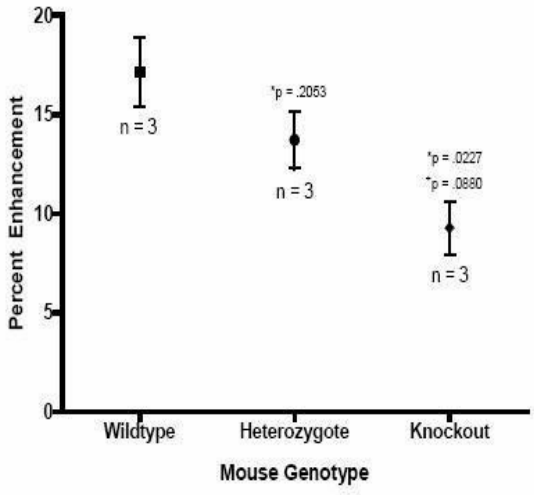

Fig. 2
Percent Enhancement between groups compared using unpaired, two sample t-test.
* denotes comparison with Wildtype.
* denotes comparison with Heterozygote.

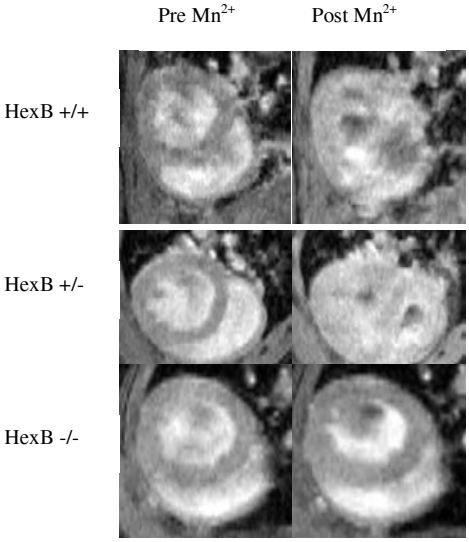

Fig. 1: Selected pre and post manganese infusion images for all HexB genotypes. Note hypertrophy of knockout hearts

Discussion and Conclusions

The decreased signal enhancement due to manganese infusion observed in the HexB knockout mice is likely a result of deficits in calcium influx and intracellular calcium levels in the hearts of these mice. The reason for such deficiencies is unclear. It is possible the GM2 ganglioside accumulation itself is directly responsible for the observed reduction in calcium influx and intracellular concentration, perhaps by inhibiting calcium influx through voltage gated channels. There may be a similar mechanism of GM2 ganglioside aggregation disrupting calcium flow in the excitable cells of the nervous system, potentially accounting for the neurodegenerative symptoms more commonly observed in Sandhoff disease. Indeed, it has been observed that neurons cultured from HexB -/- mice have reduced calcium uptake via the sarco/endoplasmic reticulum, which is analogous to the abnormal calcium handling of the sarcoplasmic reticulum detected in metabolic cardiomyopathies [2], [5]. These studies further validate a model of ganglioside accumulation disrupting normal cardiac and neural function by disturbing calcium handling. The ability of MEMRI to quantitatively assess these variations in calcium influx and intracellular calcium concentrations *in vivo* establishes the technique as a useful tool for noninvasively tracking calcium flow in a variety of systems.

References

[1] Tifft, C. J., Proia, R.L. *Annals of Medicine*, 29: 557-561, 1997.
[2] Guertl, B., et al. *Int. J. Exp. Pathol*, 81(6):349-72, Dec 2000.
[3] Hu, T C.-C., et al. *MRM*, 46: 884-890, 2001.
[4] Silva, A.C., et al. *NMR Biomed,* 17: 532-543, 2004.
[5] Pelled, D., et al. *J. Biol. Chem*., Vol. 278, Issue 32, Aug 2003.