2.12. Manganese Enhanced MRI (MEMRI)

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A. OBJECTIVE

Manganese is a divalent ion which can act as a marker for calcium, entering cells through the same routes. It is also an enhancement agent on T1 weighted MRI images, and can be quantified. MEMRI can therefore be used to measure myocardial and skeletal muscle calcium influx *in vivo* in mice.

B. CAUTIONS

- Manganese enhancement is affected by changes in the heart rate. Therefore, it is important to ensure mice are imaged at comparable temperatures and that there is no significant cooling of the animal before transfer to the scanner.
- Strain differences in the heart rate or changes in the heart rate due to treatment complicate interpretation of MEMRI data and should be taken into consideration.
- All the mice in one study should be scanned as close in time as possible as there is natural drift of values obtained from the magnet- use of a phantom should be considered for studies which will span a long time period.
- The heart rate, respiratory rate and temperature should be noted at regular intervals throughout the experiment.

C. MATERIALS AND EQUIPMENT

- Manganese Chloride in saline solution (60mM, Sigma-Aldrich 244589)
- Tubing (BPE-T10 for cannula and BPE-T50 for infusion line)
- 1 ml syringe without needle x2
- Yellow 30G needle
- Blue 23G needle
- 39 mm diameter quadrature birdcage volume coil (Rapid Biomedical GmbH). *A smaller coil (32 mm diameter) may be used for mice up to approximately 25 g.*
- Sled (Dazai Research Instruments, Toronto, Canada).
- 7 Tesla horizontal bore Varian microimaging systems equipped with a 12-cm microimaging gradient insert (maximum gradient 40 gauss/cm), (Varian Inc., Palo Alto, CA, USA).
- Cannula (made from 5 cm BPE-T10 tubing with a 30G broken-off needle inserted in the end)
- Infusion pump
- Infrared lamp
- 70% ethanol
- Tissue Tek glue
- Veet Depilatory cream
- Scales to weigh mouse
- Heat mat

D. METHODS

1. BPE-T50 tubing sufficiently long enough to reach from the infusion pump to the scanner is filled with manganese chloride using a 5 ml syringe and attached to a 1ml syringe with a Blue 23G needle. CARE MUST BE TAKEN TO ENSURE THE INFUSION LINE IS FREE OF AIR BUBBLES.

2. The manganese syringe is placed in the infusion pump and the pump is allowed to run for a short time until manganese is flowing out.

3. The mouse is weighed and anesthetized in 5% isofluorane in oxygen at a flow rate of 0.5 litres per minute in a chamber, shaved and de-haired (using depilatory cream) for the ECG contacts, then transferred to a nose cone and maintained at 2-3% isofluorane for tail vein cannulation. CARE MUST BE TAKEN TO AVOID COOLING OF THE MOUSE DURING THIS PROCESS- MICE SHOULD BE LAID ON A HEATED BLANKET.

4. The tail is wiped with ethanol and warmed using an infrared lamp to dilate the veins. The tail vein is cannulated and correct localization of the needle in the vein is confirmed by injection of a small volume of saline. The needle is secured with tissue glue.

5. The mouse is transferred to the scanner and laid prone on a cradle which allows monitoring of external body temperature, respiratory rate and the heart rate.

6. The saline syringe is removed from the cannula and the manganese line attached. CARE MUST BE TAKEN TO AVOID BLEEDING INTO THE CANNULA WHICH MAY CLOT AND BLOCK THE INFUSION OF MANGANESE.

7. The mouse is inserted into the coil and taped into place.

8. In the scanner the mouse is maintained at 1-1.8% isofluorane (0.5L oxygen/minute throughout) through a nose cone and body temperature is maintained using a warm air blower system. *Alternative: some MRI scanners have a hot water or heat blanket heating system.*

9. Following power calibration and global shimming, a series of four pilot transverse images are acquired over the heart. Single slice coronal and sagittal images are then obtained in order to view the apex and mitral valve planes. These images are used to plan for the true short axis plane.

10. Single slice gradient echo short axis images at the level of the papillary muscles (T_1 weighted parameters: TR = 35 ms, TE = 3.5 ms, flip angle 60°, FOV 30 mm×30 mm, data matrix 128×128, 1 mm slice thickness, 6 averages) are taken.

11. Prior to manganese infusion, four baseline images are acquired in order to average any variations due to changes in TR as a result of fluctuations in the heart rate.

12. Manganese chloride is given by intravenous infusion through the tail vein cannula at a flow-rate of 0.6 ml/hour. Flow time is adjusted according to weight to give a total dose of 190 nmol/g body weight (for example, for a 30 g mouse, this would result in a 9.5 minute infusion). THE LENGTH OF THE CANNULA CONTAINING SALINE MUST BE FACTORED INTO THE INFUSION TIME.

13. Images are taken at 2.5 mins, 5 mins and then at 5 minute intervals thereafter, for a total of 30 minutes. A relative increase in T_1 weighted contrast indicates increased manganese uptake.

14. The mouse is recovered on a heat mat and returned to its cage.

E. EVALUATION AND INTERPRETATION OF RESULTS

- The time course stack of images is analyzed in ImageJ (http://rsb.info.nih.gov/ij).
- An area of interest is drawn to fit inside the myocardium (and the triceps and chest wall if required) and average signal intensity is measured.
- If necessary, minor adjustments of these regions are made for subsequent images in the stack and the increase in myocardial contrast enhancement is expressed as a percentage increase from the average of the four baseline images (which show little or no variation).
- MEMRI data is expressed as an enhancement ratio over the baseline.

F. REFERENCES

Blain A, Greally E, Laval S, Blamire A, Straub V, et al. (2013) Beta-Blockers, Left and Right Ventricular Function, and *In-Vivo* Calcium Influx in Muscular Dystrophy Cardiomyopathy. PLoS ONE 8(2): e57260.

Greally E, Davison BJ, Blain A, Laval S, Blamire A, Straub V, MacGowan GA (2013) Heterogeneous abnormalities of in-vivo left ventricular calcium influx and function in mouse models of muscular dystrophy cardiomyopathy. J Cardiovasc Magn Reson. Jan 16;15(1):4.