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Non-invasive echocardiographic assessment of cardiac function in the *mdx* mouse model

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TABLE OF CONTENTS

1. OBJECTIVE

The objective of this standard operating procedure (SOP) is to describe the technique of non-invasive echocardiography in the mouse model of Duchenne muscular dystrophy (DMD). Cardiomyopathy is an increasingly important aspect in the treatment of DMD and cardiac evaluation must be an integral part of all pre-clinical drug trials. These techniques provide the ability for longitudinal measurements and results are comparable to human clinical echocardiography measurements, an important strength for helping to move drugs into clinical trials.

In brief, mice are anesthetized with inhaled isoflorane and placed on a heated imaging platform. The temperature and heart rate of the mouse is constantly monitored to minimize physiological variation. Mice are imaged using a high frequency echocardiography machine (Vevo 770, VisualSonics, Inc., Toronto, Canada) and a standardized protocol is followed to systematically evaluate cardiac size and function. Once completed, the mice are easily woken up after breathing oxygen for a short time and are returned to their cage.

The aim of this SOP is to describe the methods for high frequency echocardiography in mice and discuss the advantages and disadvantages of the protocol.

2. SCOPE AND APPLICABILITY

This SOP will describe the protocol for high frequency echocardiography in mice. This SOP ill not include an extensive discussion regarding the use of high (30 MHz) versus low (15 MHz) frequency echocardiography or type/ dose of anesthesia used in the procedure. The use of high frequency echocardiography requires a technician trained in small animal echocardiography under the guidance of a cardiologist.

3. CAUTIONS

Advantages: Echocardiography is non-invasive and is also ideal for longitudinal studies of cardiac function. Its use may in turn reduce the total number of animals required for a study, thereby saving time and resources. High-frequency echocardiography has improved resolution that increases the technique's power, and a statistical analysis has shown that only 3 to 5 animals are needed to obtain statistical significance for a 20% difference in the measurement of shortening fraction. High-frequency echocardiography also facilitates expanded analysis of cardiac structure and function, including measurements and calculations that cannot be performed on clinical platform ultrasound machines.

Disadvantages: The cost of a new high-frequency ultrasound system is approximately $200,000.00 in US dollars. It takes approximately 30 min to scan an individual mouse, once the operator has been trained in using high-frequency echocardiography. Since cardiac function relies on the overall physiology of the mouse, poor maintenance of experimental conditions will significantly affect results. Too much anesthesia and lower body temperature are two main factors which will lead to decreased heart rates and cardiac function, no matter what type of pathology is present. Consistent experimental conditions are extremely important within individual studies, during longitudinal studies, and also across laboratories to ensure comparable results. The required echocardiography system is expensive and should only be used by trained personnel. Data analysis must be performed by a trained technician, and data acquisition needs to be carefully adapted to take into account the mouse strain, age, sex, and genetic mutation involved.

4. MATERIALS

- Vevo 660 or 770 (VisualSonics, Toronto, Canada) with the RMV 707 scanhead
- Heated animal platform, THM 100 (Indus Instruments, Houston, TX, USA) or a VEVO mouse handling platform with a Physiological Controller Unit
- Isoflurane
- Oxygen
- Anesthesia (isoflurane) blender and tubing with anesthesia scavenging system (activated charcoal absorption filter– (VaporGuard, VetEquip, Pleasanton, Ca, USA)
- Optional for non-invasive BP measurement: SC1000 (Hatteras Instruments, Cary, NC, USA)
- Depilatory cream
- Ultrasound gel
- Ophthalmic ointment
- Electrode gel
- Gauze
- Cotton tip applicators
- Tape
- Heating lamp

5. METHODS

Preparation of the mouse

1. Place the mouse in an induction chamber with constant inflow of 5% isoflurane mixed with 100% oxygen.
2. Once the mouse is asleep, remove it from the induction chamber, weigh it, and place it on a heating platform with electrocardiogram contact pads (THM 100, Indus Instruments, Houston, TX, or VEVO mouse handling platform).

3. Place the nose into a nose cone with 1-2% isoflurane in 100% oxygen. Passively evacuate excess gases using an activated charcoal absorption filter (VaporGuard, VetEquip, Pleasanton, Ca).
4. Cover the eyes with a petroleum-based ophthalmic ointment.
5. Place electrode gel on the paws and tape the paws over the electrocardiogram contact pads on the heating platform.
6. Lubricate a rectal probe with gel, place it in the rectum, and tape it to the platform. Maintain the temperature at 36.5 to 37.5 °C.
7. Place a blood pressure (BP) cuff around the tail and place the tail in the sensor assembly for non-invasive blood pressure monitoring.
8. Continuously monitor the temperature, heart rate (HR), and BP during the scanning.
9. Apply depilatory cream to the chest of the mouse using a cotton applicator tip and remove the cream after 2 min with a gentle rolling motion of the cotton tips, then clean the chest with distilled water.
10. Place ultrasound gel on the chest of the anesthetized mouse.
11. Place the ultrasound probe in contact with the ultrasound gel and perform the scan.

Scanning procedure

Obtain the following views as described below:

1. Modified parasternal long axis: Orient the scanhead directly in the longitudinal plane, with the notch at approximately 1 o'clock. Locate the ascending aorta and obtain a 2-D cine loop.
2. Modified parasternal long axis: Adjust the mouse platform so that the pulmonary valve and pulmonary artery are imaged. Obtain a 2-D cine loop.
3. Modified parasternal long axis: Change to pulse wave Doppler mode. Place the pulse width gate in the outflow jet of the pulmonary valve. Obtain a spectral Doppler image.
4. Modified parasternal long axis: Obtain a long axis image of the left ventricle with the plane of the mitral valve. Acquire a 2-D cine loop.
5. Modified parasternal long axis: Obtain an EKV image of the left ventricle.
6. Turn the scanhead 90 degrees so that the notch is at 3 o'clock.
7. Modified parasternal short axis: Obtain an image of the left ventricle at the level of the papillary muscles, and obtain a 2-D cine loop.
8. Modified parasternal short axis: Obtain an EKV image of the left ventricle at the level of the papillary muscles.
9. Modified parasternal short axis: Change to M-mode imaging and obtain an image of the left ventricle, ensuring good endocardial border delineation.
10. Modified parasternal short axis: Adjust the animal platform to image the tricuspid valve. Obtain a 2-D image of the tricuspid valve.

11. Modified parasternal short axis: Change to pulse wave Doppler mode and place the pulse gate just distal to the tricuspid valve, paralleling the inflow jet as closely as possible. Correct the angle as needed, by no greater than 10°. Obtain a spectral Doppler image.
12. Modified suprasternal notch view: Adjust the animal platform so that the scanhead is directed caudally at a 45° angle at the level of the upper sternum. Locate the ascending aorta.
13. Modified suprasternal notch view: Change to pulse Doppler mode. Place the pulse width gate in the ascending aorta, paralleling the aortic outflow stream as closely as possible. Correct the angle as needed, by no greater than 10°. Obtain a spectral Doppler image of the aortic outflow.
14. Rotate the scanhead approximately 90 degrees leftward and move the mouse platform cranially. Align the scanhead with the lower left chest near the end of the ribcage.
15. Modified apical four-chamber view: Locate the left ventricle at the level of the mitral valve.
16. Modified apical four-chamber view: Obtain a 2-D image of the mitral valve.
17. Modified apical four-chamber view: Change to spectral Doppler mode. Place the pulse width gate in the mitral inflow stream, as close to parallel to the flow as possible. Correct the angle as needed, by no greater than 20°. Obtain an image of the mitral inflow.
18. Once the imaging is completed, remove all probes and monitors from the mouse.
19. Clean the mouse with water and allow it to recover on the heated platform or in warmed cage. Once the mouse is awake, return it to its cage.

6. EVALUATION AND INTERPRETATION OF RESULTS

Qualitative and quantitative measurements are made offline using analytic software (VisualSonics, Toronto, Canada). Electrocardiogram kilohertz-based visualization (EKV™) software analysis produces offline reconstructions for simulated 250 to 1,000 Hz static and cine loop images. All measurements are made using the leading edge to leading edge technique:

1. Aortic diameter: Measure at the sinotubular junction in the modified parasternal long axis.
2. Pulmonic valve diameter: Measure at the level of the pulmonic valve in the modified parasternal long axis.
3. Pulmonic outflow peak velocity and velocity time integral: Measure the peak velocity and trace the pulmonic outflow Doppler envelope.

4. Left ventricle end-diastolic volume using Simpson's method: Using EKV imaging in the modified parasternal long axis, measure the internal volume of the left ventricle using four Simpson measurement areas at the end of diastole.
5. Left ventricle major axis (diastole): Measure from the apex of the LV endocardium to the level of the mitral valve at end diastole.
6. Left ventricle end-systolic volume using Simpson's method: Using EKV imaging in the modified parasternal long axis, measure the internal volume of the left ventricle using four Simpson measurement areas at the end of systole.
7. Left ventricle major axis (systole): Measure from the apex of the LV endocardium to the level of the mitral valve at end systole.
8. M-mode measurement: Using the program calipers, measure the IVS thickness (d), LVID(d), LVPW(d), IVS(sys), LVID(sys), and LVPW(sys).
9. LV mass: Outline the endocardial border and epicardial border of the modified parasternal short axis EKV image.
10. Ascending aorta peak velocity and velocity time integral: Measure the peak velocity and trace the aortic outflow Doppler envelope.
11. Tricuspid valve E wave, A wave, E at A wave: Measure the peak velocity of the E and A waves of the tricuspid inflow Doppler envelope. At heart rates of around 450 to 500 bpm, the E and A waves fuse, and only one measurement is made, the E at A wave measurement.
12. Mitral valve E wave, A wave, E at A wave: Measure the peak velocity of the E and A waves of the mitral inflow Doppler envelope. At heart rates of around 450 to 500 bpm, the E and A waves fuse, and only one measurement is made, the E at A wave measurement.
13. Percent shortening fraction: Calculate from the M-mode measurements using the leading edge to leading edge method, based on the formula %Shortening Fraction (%SF) = left ventricular internal diameter (diastole) [LVID(d)] – left ventricular internal diameter (systole) [LVID(s)] / LVID(d).
14. Stroke volume [SV (calc)]: Use the formula SV= {[π (Aortic diameter)^2/4]*[Aortic velocity time integral].
15. Cardiac output [CO (calc)]: Use the formula CO = SV(calc)*heart rate (HR).
16. Velocity of circumferential fiber shortening corrected for heart rate (VCFc): Use the formula {[LVID(d)] –[LVID(s)] / LVID(d)}/ Ejection time/square root of the preceding RR interval.
17. Left ventricular meridional wall stress (WS^m): Use the formula 0.334[systolic blood pressure (SBP) * LVID(s)] / {left ventricular posterior wall thickness in systole (LVPWs)[1+(LVPWs/ LVIDs)]}.
18. Myocardial performance index (MPI): Use the formula (isovolumic contraction time + isovolumic relaxation time/ ejection time).
19. Left ventricular mass (LVM): Calculate using the area-length method (LVM (g) = 1.05 {[5/6 * LV epicardial area (LV long axis in diastole + myocardial thickness)] – [5/6* LV endocardial area *LV long axis in diastole)]}.

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8. APPENDIX

The following article describes the methods used and provides tables of echocardiographic data:
Spurney, CF, Knoblach, S., Pistilli, EE, Nagaraju, K, Martin, GR, Hoffman, EP (2008) Neuromuscular Disorders 18, 371–381.