High-Resolution Echocardiographic Assessment of Infarct Size and Cardiac Function in Mice with Myocardial Infarction

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Background: The aim of this study was to develop a simple and reasonably precise echocardiographic method for the assessment of infarct size (IS) and cardiac dysfunction in mice after myocardial infarction.

Methods: In vivo experiments were performed in C57BL/6J wild-type mice (n = 18) before and 48 hours after left anterior descending coronary artery ligation. Endocardial length-based echocardiographic IS was validated with that by three different histologic measurements. Left ventricular function was evaluated.

Results: Excellent agreement was found between endocardial length-based echocardiographic measurement and angle-based histologic measurement of IS (r = 0.82, P < .001), and both methods strongly correlated with Tei index (r = 0.82, P < .001, and r = 0.74, P < .01) and fractional area change (r = −0.61, P < .05, and r = −0.81, P < .001).

Conclusions: Endocardial length-based echocardiographic measurement proved to be a useful method for assessing myocardial IS and is applicable for biomedical and imaging research, and appears particularly promising in studies of left ventricular remodeling after myocardial infarction. (J Am Soc Echocardiogr 2011;24:219-26.)

Keywords: Myocardial infarction, Cardiac function, Echocardiography, TTC staining, Mouse

Heart failure secondary to myocardial infarction (MI) remains the leading cause of death in Western countries. Experimental and human MI studies have proven that progressive development of left ventricular (LV) remodeling is an important prognostic factor for patient outcomes and is directly related to infarct size (IS) and cardiac function after MI.[1,2]

Mouse infarct models have been increasingly used to explore pathophysiologic and molecular mechanisms of LV remodeling and dysfunction after MI and to evaluate novel pharmaceutical and cardiac stem cell therapies for reducing IS and improving cardiac function.[3-5] In experiments involving an acute MI setting, IS is typically based on histologic measurement of the area of the infarcted region in tissue sections of the left ventricle,[6] which necessitates sacrifice and makes this method impractical for serial studies. Accordingly, noninvasive imaging techniques are needed. However, noninvasive assessment of cardiac structure and function in mice is technically challenging because of the small heart size (about 5 mm in cross section) and rapid heart rate (about 600 beats/min).[7] Cardiac magnetic resonance is currently the gold standard for the noninvasive determination of IS and for the measurement of myocardial function, with excellent reproducibility both in humans and in mice. However, cardiac magnetic resonance is relatively time consuming, costly, and not yet widely available, therefore the technique is not ideal for high-throughput studies in mice.[7-10] In contrast, echocardiography has been the predominant modality in diagnostic cardiology because of its portability, widespread availability, and real-time characteristics.[11,12] It provides an excellent noninvasive tool for longitudinally monitoring changes in LV geometry and function in mice.[13,14] However, several problems remain with the current use of echocardiography in mouse infarct model evaluation. First, many studies have simply used a single LV short-axis view to assess LV function either by M-mode or two-dimensional (2D) echocardiography. These methods ignore important geometric shape changes after infarction when calculating LV mass and systolic function.[11] Second, large variations exist between echocardiography-derived IS and histologic measurements of IS and also between IS and the extent of LV systolic and diastolic dysfunction after MI.[11-15] Finally, although three-dimensional (3D) reconstructions of echocardiography-derived LV geometry and quantitative stress echocardiography can assess regional wall motion and viability, the techniques require a tremendous amount of work and are technically challenging.[11,16]

Thus, more efficient noninvasive imaging approaches are needed for the assessment of novel therapies for heart failure secondary to

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0894-7317/$36.00

doi:10.1016/j.echo.2010.11.001

Abbreviations

EF = Ejection fraction

EKV = ECG-Based Kilohertz Visualization

FAC = Fractional area change

IS = Infarct size

IVRT = Isovolumic relaxation time

LV = Left ventricular

MI = Myocardial infarction

3D = Three-dimensional

TTC = 2,3,5-triphenyltetrazolium chloride

2D = Two-dimensional

VTI_t = Total velocity-time integral

acute MI. To address this problem, we developed a simple, rapid, and reasonably precise echocardiographic method for the contemporaneous assessment of IS and cardiac dysfunction in an acute mouse infarct model using a newly developed ultra-high-frequency small animal ultrasound system.

METHODS

Animals

Eighteen C57BL/6 mice (aged 16 weeks; 10 males; weight, 22–28 g) were used. This study adhered to the American Physiological Society's Guiding Principles in the Care and Use of Animals. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Mouse MI Model

Mice were anesthetized with a single dose of tribromoethanol (2.5%, 0.4 mL/25 g). The trachea was exposed through a midline incision and intubated with a 20-gauge intravenous catheter through the oral cavity and connected to a mouse ventilator (MiniVent Type 845; Harvard Apparatus, Holliston, MA). Respiration was controlled with a tidal volume of 350 μL at a rate of 110 strokes/min. The heart was exposed via a left thoracotomy, and the left anterior descending coronary artery was ligated immediately distal to the original of the first diagonal branch over a piece of PE-10 tube using an 8-0 silk suture. Occlusion of the left anterior descending coronary artery was visually confirmed by rapid myocardial blanching, as well as by ST-segment elevation on electrocardiography. The chest and skin incisions were closed in two layers with 4-0 suture. Mice remained on ventilation for a period of 30 to 45 min. The endotracheal tube was removed once spontaneous breathing resumed and the heart rate reached >500 beats/min on electrocardiography. Mice recovered from the operation for 48 hours before echocardiographic and histologic studies.

Echocardiography

Transthoracic echocardiography was performed twice: one day before MI and 48 hours after MI. We developed a simple protocol for rapid and comprehensive echocardiographic assessment of cardiac structure and function after MI and refined it on the basis of previous reports.[12,17,18] Image analysis was performed by a single observer blinded to the experimental conditions and results of the histology-defined IS measurements.

Instrument. We used a high-resolution Vevo 770 ultrasound system (VisualSonics Inc., Toronto, ON, Canada) equipped with a 30-MHz probe (RMV-707B), with an attached Integrated Rail System III for imaging acquisition. The ECG-Based Kilohertz Visualization (EKV) acquisition mode was used for 2D cine loop recordings. EKV reconstruction is a technique that synthesizes 2D images from a series of cardiac cycles and reconstructs one representative heart cycle that is spatially precise and synchronized to the animal's electrocardiogram.[17]

Animal Preparation. Animal preparation was similar to that in previous studies.[12,17,18] Briefly, before and during the ultrasound scanning, the mouse was lightly anesthetized with a mixture of 1% to 2% isoflurane gas and 100% oxygen. The mouse's body temperature was maintained at 37 ± 0.5°C to minimize heart rate variation throughout the procedure. The electrocardiographic signal was obtained from the electrode pads on the mouse platform. The ultrasound probe was placed on the mouse's shaved chest using warm ultrasound gel (Aquasonic 100; Parker Laboratories, Fairfield, NJ) as a coupling medium.

Image Acquisition and Measurements. ECHOCARDIOGRAPHIC DETERMINATION OF IS. Our preliminary data showed that the wall begins to thin 48 hours after MI (not shown). Thus, we adopted an endocardial length-based measurement to assess IS[6] and validate it with the histology-determined IS.

In this method, four equally spaced serial LV short-axis views were acquired. In detail, the distance from the mitral valve plane to the apex was measured by moving the transducer with the aid of the rail system and built-in ruler (micrometer) on the mouse platform. The transducer was moved in steps of one fifth of the distance (about 1 mm apart), and four equally spaced serial LV short-axis views were obtained. We identified the infarct region (the akinetic segment) from each of the four short-axis views from visual assessment of wall thickening and wall motion. Once the infarct segment was determined, infarct length was manually traced along the endocardial border at end-diastole. The IS by echocardiography was expressed as the average of the percentage of the total infarct length to the total LV endocardial circumferential length in all four short-axis views.

ECHOCARDIOGRAPHIC DETERMINATION OF LV AND LEFT ATRIAL DIMENSIONS. The maximal diastolic and systolic LV long-axis 2D lengths (Simpson length, d; Simpson length, s) were measured from the parasternal long-axis view and used in the modified Simpson's method for computing LV volumes and ejection fraction (EF). The maximal left atrial anterior-posterior dimension was also measured in this view. By rotating the transducer 90° clockwise from the above 2D view, the LV short-axis view at the papillary muscle level was obtained, and the LV areas at end-diastole and end-systole were traced and recorded. LV maximal anterior-posterior dimensions during diastole and systole at the papillary muscle level were measured by M-mode echocardiography.

ECHOCARDIOGRAPHIC DETERMINATION OF GLOBAL LV SYSTOLIC FUNCTION USING MODIFIED SIMPSON'S METHOD. We obtained the LV cross-sectional areas by tracing the diastolic and systolic endocardial borders in each of the four equally spaced LV short-axis slices before and after MI. On the basis of these measurements and the Simpson length (d, s), the fractional area change (FAC; percentage), EF (percentage), and stroke volume (microliters) were computed using the Vevo 770 Standard Measurement Package. Note that this measurement represented the FAC of the short-axis view that most closely approximated the midpapillary level.

DETERMINATION OF LV DIASTOLIC FUNCTION BY PULSED DOPPLER. LV diastolic function was evaluated by transmitral and pulmonary venous pulsed Doppler echocardiography.

For transmitral Doppler, the peak flow velocities during early diastole (E wave) and during atrial contraction (A wave) were measured across the mitral valve. Because merging of the E and A peaks occurred in a majority of the mice because of rapid heart rates