ONLINE SUPPLEMENT

EXPANDED METHODS

Experimental Myocardial Infarction

Left anterior descending (LAD) coronary artery ligation was performed as previously described.[10] In brief, animals were anesthetized using pentobarbital (65mg/kg) and the onset of deep anesthesia was confirmed by the absence of hind-limb pain reflexes. Mice were intubated using a fine polyethylene cannula connected to a standard small rodent ventilator (Harvard 680, South Natick, MA) set to a volume of 160 ul at 140 strokes per minute. Each mouse was then positioned on its right side to expose the left chest. The skin was incised from the sternum to the left anterior axillary line at the level of the 5th intercostal space. The pectoralis muscles were incised and the chest opened at the 5th intercostal space. A small rib spreader was introduced to open the operation field and the pericardium was bluntly removed. Experimental MI was induced by permanent ligation of the LAD artery 2 mm from its origin between the pulmonary outflow tract and the edge of the left atrium, using a 6-0 prolene suture. Induction of acute MI was considered successful when the anterior LV wall turned pale and ST-segment elevation was observed on simultaneous electrocardiography. The chest was subsequently closed and the lungs inflated. Sham-operated animals underwent an identical procedure without coronary artery ligation. To maintain body temperature throughout the procedure, each mouse was positioned on a heating pad while anesthetized. Following chest closure, animals were extubated upon spontaneous recovery from anesthesia and resumption of spontaneous breathing. Buprenorphine (0.03-0.06 mg/kg) was then administered twice daily for 3 days to prevent post-operative discomfort. All procedures were approved by the Institutional Animal Care and Use Committee.

Echocardiography

For *in vivo* echocardiography, mice were lightly anesthetized using 1% isoflurane in oxygen and then fixed in the supine position on a heated platform. Electrode gel was applied to limb leads to obtain concurrent electrocardiographic recording during the exam. Isoflurane concentration was lowered as needed to allow heart rates to return to physiologic range (>500 bpm). Echocardiography was performed using an 18-38MHz linear-array transducer with a digital ultrasound system (Vevo 2100 Imaging System, VisualSonics, Toronto, Canada). Image acquisition was initiated with the transducer probe placed along the left sternal border to obtain the parasternal long axis view, which displays both the apex and the outflow tract of the left ventricle. Following acquisition of long-axis images, the probe was rotated 90degrees to obtain and record short-axis images at the level of the mid-papillary muscles. An M-mode gate was placed through the center of the papillary level short-axis view to obtain standard M-mode recordings.

To ensure good quality images for speckle-tracking based strain analyses, all image acquisition was performed at a high frame rate (>200 frames per second), which provides optimal temporal resolution and reduces image analysis artifacts. Thus, frame rate was increased as needed for each echocardiographic view by decreasing the depth and/or narrowing the imaging sector width. Adjusting image sector size was performed also to improve spatial resolution and to minimize image dropout of various wall segments (particularly the basal and apical wall segments in the long-axis view, and the lateral wall segments in the short-axis view), while also ensuring that the endocardial and epicardial borders of the LV would be captured. Care was taken to record images (≥3 cardiac cycles per loop) where translational

motion or breathing artifacts were absent or minimized. Since speckle-tracking based strain analysis is susceptible to even small variations in how echocardiographic views are obtained, in addition to image tracing technique, all images were acquired and analyzed by the same blinded investigator (MB).

Conventional Echocardiographic Measurements

Conventional echocardiographic measurements were obtained from grayscale M-mode images acquired in the parasternal short-axis view at the mid-papillary (midwall) level of the LV, and also from 2D images acquired from the parasternal long-axis and short axis views. For measures of LV diameter and wall thickness, measurements were made from short-axis M-mode images for 3 consecutive cardiac cycles and then averaged. M-mode based measurements included LV end-diastolic diameter, LV end-systolic diameter, anterior wall [AW] and posterior wall [PW] thicknesses, LV fractional shortening ([LV end-diastolic diameter − LV end-systolic diameter]/LV end-diastolic diameter × 100), and wall thickening ([systolic wall thickness − diastolic wall thickness]/systolic wall thickness × 100). To obtain fractional area change (FAC), left ventricular endocardial area was traced from both short- and long- axis B-mode loops at end-diastole and at end-systole. LV end-systolic and end-diastolic volumes and LV ejection fraction (EF) were measured from 2-dimensional parasternal long-axis views. LV mass was calculated using end-diastolic epicardial and endocardial area according to the following formula: LV mass=1.05 x (5/6 x epicardial area x epicardial major axis + T) - (5/6 x endocardial area x endocardial major axis), where T = sqrt(epicardial area)/ π) – sqrt (endocardial area/π). At 7 weeks, the total epicardial and endocardial border lengths were measured from the parasternal long axis view, in addition to the lengths of epicardial and endocardial border affected by scar. Infarct size was calculated as a percentage, based on the mean of the epicardial and endocardial ratios of infarct-to-total length x 100.

Pathologic Assessment of Cardiac Remodeling

Following echocardiography at the 7-week time point, each animal was sacrificed. Hearts were fixed using a defined end diastolic pressure of 5mmHg. Briefly hearts were perfused using a Langendorff apparatus, a balloon was inserted into the left ventricle through the mitral valve. The pressure in the balloon was adjusted to reach an end-diastolic pressure of 5 mmHg. Hearts were arrested in diastole using KCl and further perfused for 15 minutes using 10% formaldehyde. Hearts were unmounted and stored in 10% formaldehyde overnight with the pressured balloon still in place. The balloon was removed and the hearts weighted. For histological assessment of infarct size, hearts were cut into 3 transverse slices (basal, middle, and apical) parallel to the atrioventricular groove, embedded in paraffin, and stained using Masson trichrome stain. Images of the whole heart were recorded and infarct size was calculated as the mean percentage of epicardial and endocardial circumference occupied by scar tissue, as measured from and averaged over 3 serial cross sections of the LV.

Statistical Analysis

All continuous data are presented as mean ± standard error (SE). After testing for inequality of variances, the difference between echocardiographic measurements before and after surgery was tested using one-way analysis of variance (ANOVA) for repeated measurements. If the results of analysis of variance were significant, paired Student's *t* tests were used. Comparison of echocardiographic parameters between groups of mice and groups of segments was analyzed using one-way ANOVA for repeated measurements. If the interaction of time and group was significant, unpaired Student's *t* tests were used to compare

echocardiographic parameters between groups at the same time point. Two-way ANOVA was used to assess for the possible interaction of treatment group on time from baseline to each of the LV measures. Spearman correlations were used to assess the association of early (3 week) measures of longitudinal strain and strain rate with later (7 week) measures of LV remodeling, as represented by percent change in LVEDD (from week 3 to 7). A 2-tailed P value of <0.05 was considered statistically significant. Statistical analyses were performed using R version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria).

PROTOCOL FOR SPECKLE TRACKING BASED STRAIN ANALYSIS IN MICE

Image Acquisition

1. Perform 2D B-mode echocardiography on lightly anesthetized mouse in standard fashion.
2. Image parasternal long axis view in 2D. Optimize frame rate to >200 fps by narrowing imaging sector width and decreasing depth as needed.
3. Align focus depth with the LV posterior wall epicardium. Adjust both gain and dynamic range to optimize contrast.
4. Acquire cine loops in the parasternal long axis view that contains at least 3 consecutive cardiac cycles where there is complete and optimal visualization of both endocardial and epicardial borders, and where image artifacts (e.g. near field artifacts, breathing/translational motion artifacts, etc.) are avoided or minimized.
5. Image parasternal short axis view in 2D at the level of the mid-ventricle (papillary muscle level). Optimize frame rate to >200 fps by narrowing imaging sector width and decreasing depth as needed.
6. Acquire cine loops in the parasternal short axis view that contains at least 3 consecutive cardiac cycles where there is complete and optimal visualization of both endocardial and epicardial borders, and where image artifacts (e.g. near field artifacts, breathing/translational motion artifacts, etc.) are avoided or minimized.

Image Analysis

1. Review all acquired loops for quality. Select the best quality loop based on presence of the following factors: optimal frame rate, absence of artifacts, myocardial visualization, and contrast of endocardial and epicardial borders. If any of these factors is absent and/or if there is dropout of 2 or more myocardial segments in all acquired loops (where no 2 consecutive loops are deemed adequate in image quality), then exclude entire echocardiographic view from analysis. If at least 2 consecutive acquired loops are deemed to have adequate quality for analysis, open these select loops using the VevoStrain package (which will convert and import the loops into the strain analysis application).
2. For each good quality image, play the cine loop within the strain analysis application interface to assess location and relative motion of the endocardial border during systole and diastole.
3. Place an M-mode gate through the B-mode loop shown.
4. Specify the start and end of each cardiac cycle using the widest diameter of the endocardial border (as also indicated by M-mode tracings) and onset/upslope of the electrocardiographic R wave as reference points.
5. Narrow the selection to the 3 cardiac cycles with the best visualization of endocardial and epicardial borders and no breathing artifacts (2 cardiac cycles if only 2 loops were available for import). Once the selection has been made, advance to the analytical functions.
6. At end-diastolic frame of the 1st selected cardiac cycle, trace just within the endocardial border. Use 8-12 points total to trace the endocardium in its entirety (base to apex to base in the long-axis view; anterior to lateral to inferior to septal in the short-axis view). Papillary muscle or trabeculae should be excluded during endocardial border tracing. Place tracing points closer together along more curved segments and farther apart along straighter segments. In cases where the endocardial border is not optimally visualized at end-diastole, select a frame at an alternate time point in the cardiac cycle when the endocardial border is more optimally traceable, while favoring time points that are closer to end-diastole.

7. Process endocardial tracing. Inspect tracking of the processed tracings and, if tracking is poor (either endocardial border motion lags tracing, or tracing lags endocardial border motion), then re-trace as needed to optimize endocardial tracking.
8. After satisfactory endocardial tracking has been achieved, select the edit trace option and activate automated appearance of epicardial tracing. Adjust overall width between endocardial and epicardial tracings as needed. Next, adjust the epicardial tracing such that the tracing is just within the epicardial border.
9. In cases where sufficient quality tracings cannot be achieved for all 3 cardiac cycles (if 3 loops total were imported), alternate cycles may be selected (or the total number of cycles may be reduced to 2 cardiac cycles).
10. Process endocardial and epicardial tracings. Check tracking of the processed tracings and, if tracking is poor (either endocardial border motion lags tracing, or tracing lags endocardial border motion), then re-trace as needed.
11. Once optimal tracking has been achieved, activate tracking analysis of cine loop to generate both longitudinal (or circumferential) and radial strain and strain rate curves. Activate peak analysis and record average peak of *strain* curves for each of the 2 axes in each view (longitudinal and radial in the long-axis view; circumferential and radial in the short-axis view). Record average peak of *strain rate* curves for each of the 2 axes in each view (longitudinal and radial in the long-axis view; circumferential and radial in the short-axis view).
12. Digitally save all image analysis data.