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Noninvasive Quantification of Regional Ventricular Function in Rats:

Assessment of Serial Change and Spatial Distribution Using

Ultrasound Strain Analysis

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ABSTRACT

Background: The optimal method for quantitative assessment of regional ventricular function in rats remains unclear. The goal of this study was to investigate the utility of ultrasonic strain rate (SR) and strain analysis in evaluating the serial change and spatial distribution of regional contractile function in rats.

Methods: Twenty-two anesthetized rats underwent incremental dobutamine infusion (protocol 1) for assessment of serial change or underwent coronary ligation (protocol 2) for assessment of spatial distribution. For protocol 1, the serial change of systolic SR and strain during dobutamine was measured in the posterior myocardium on the short-axis view, and the systolic strain was compared with the percent change in wall thickening (%WT). For protocol 2, the spatial distribution of strain profile was analyzed in normal, peripheral ischemic, and central ischemic regions that were identified by myocardial contrast echocardiography.

Results: In protocol 1, the incremental dobutamine infusion resulted in a gradual increase in peak systolic SR. In contrast, peak systolic strain increased with low-dose dobutamine but tended to decrease for higher doses of dobutamine. Further, the serial change of peak systolic strain corresponded to changes in %WT, but the strain values were always lower than %WT. In protocol 2, the strain profile indicated postsystolic thickening in the peripheral ischemic region and indicated systolic wall thinning in the central ischemic region.

Conclusions: Ultrasonic determination of SR and strain is an accurate and noninvasive method of quantitation of the serial change and spatial distribution of regional contractile function in rats.

Experimentation with various rat models has greatly advanced our understanding of cardiovascular disease. However, the small size of the rat ventricle has hampered accurate noninvasive determination of ventricular function, thereby limiting some avenues of investigative research. While the recent development of ultrasound transducer technology has permitted noninvasive estimation of global ventricular function in rat models (1-4), quantitative assessment of regional ventricular function remains technically challenging.

New commercially-available ultrasound systems hold promise for the noninvasive determination of regional myocardial strain rate (SR) and strain. While these techniques allow quantification of regional contractile function with high temporal and spatial resolution, resulting in objective assessment of ventricular wall motion (5-7), their use has not been tested in the rat heart. The goal of this study was to investigate the utility of ultrasonic strain rate (SR) and strain analysis in evaluating the serial change and spatial distribution of regional contractile function in rats.

METHODS

Animal Preparation

All animal studies were performed in accordance with guidelines for the care and use of laboratory animals at Osaka University Medical School, Osaka, Japan. In all, 22 male Sprague-Dawley rats were anesthetized with pentobarbital (50 µg/g intraperitoneally), placed supine on a Styrofoam board, intubated, and ventilated with room air using a small-animal respirator pump (Model SN-480-7, Shinano, Tokyo, Japan). Anesthesia was maintained throughout the experiment with additional use of anesthetic as required, and electrocardiography (ECG) was continuously monitored from limb leads. The anterior chest and left inguinal regions were shaved, and a 1-mm polyethylene catheter was placed in the left femoral vein for administration of drugs and contrast microbubbles.

Normal closed-chest rats were used to determine the feasibility of the serial change analysis in SR and strain by a normal dobutamine stress model (protocol 1), while acute ischemic rats were employed to characterize the spatial distribution analysis in strain (protocol 2). In the acute ischemic model, left thoracotomy was performed along the fourth or fifth intercostal space, followed by exposure of the left ventricle and ligation of the left anterior descending artery (LAD) with 6-0 surgical suture.

Ultrasound Data Acquisition

Echocardiographic studies were performed using the Vivid 7 system (GE Medical Systems, Milwaukee, Wis) and a 10 S transducer (11.5 MHz) with high temporal and spatial resolution. The Styrofoam board was tilted to maintain the rat in the left lateral decubitus position during image acquisition. The transducer was placed directly on the chest wall and then fixed at the parasternal short-axis view using a mechanical arm. For the opened chest acute ischemic model, the chest was not subsequently closed, but the transducer was placed against the intact portion of the chest wall to avoid direct transducer compression of the exposed heart. Further, the open wound was sealed with saline and acoustic coupling gel to avoid lateral image deterioration.

Using a zoomed image window, color Doppler myocardial velocity data were acquired at a frame rate of 203 frames per second, a sector angle of 30º, and an image depth of 15 mm. Beam focus was set at 10 mm. Digital data of ten consecutive heart cycles were recorded and transferred to a PC workstation for off-line analysis.

Myocardial Contrast Echocardiography

Myocardial contrast echocardiography (MCE) is an established method for the assessment of a risk area during ischemia, and this method is suitable for application in small animals, including

the mouse (8). To estimate the risk area in the acute ischemic model, MCE was performed using a 10

S transducer. Transmitting and receiving frequencies were modified to 5.0 and 9.5 MHz for

harmonic mode, respectively, and mechanical index was set at 1.2. Contrast was produced by a bolus

injection (0.2 mL) of 5% Definity (Bristol-Myers Squibb Medical Imaging, Billerica, Mass) solution

via the femoral catheter. Intermittent end-systolic images were acquired at every twelfth cardiac

cycle after contrast injection. Contrast injection was performed at least twice for assessment of

reproducibility.

Experimental Protocol 1

The purpose of protocol 1 was to evaluate the feasibility of quantitative assessment of

ultrasonic SR and strain and to analyze the serial change of regional contractile function in a normal

rat dobutamine stress model. This portion of the study involved thirteen rats (body weight, 288 ± 32

g) that underwent an incremental dobutamine infusion protocol (3, 5, 10, and 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$).

Color Doppler velocity and two-dimensional (2D) data were acquired at baseline, during infusion,

and at recovery. Each dose of dobutamine (with the exception of the first dose) was given over the

15 min before the data acquisition. After the first infusion, data were collected at the 20 min time

point, because of the delay for the catheter filling time. Recovery data were collected 30 min after

the infusion.

Experimental Protocol 2

The purpose of protocol 2 was to evaluate whether spatial distribution analysis of

ultrasonic strain could be applied to an acute ischemic rat model. This portion of the study involved

nine rats (body weight, 349 ± 8 g). Color Doppler velocity and 2D data were acquired before LAD

ligation. MCE was then performed at 5 to 10 min after the ligation, and color Doppler velocity and

2D data were again collected after disappearance of the contrast microbubbles.

Data analysis

A detailed description of the data processing and the theoretical background of ultrasound SR and strain analysis in a human heart is provided by Heimdal et al (5). Radial myocardial SR and strain were calculated from color Doppler velocity data using EchoPac software (GE Medical Systems, Milwaukee, Wis). For the assessment of the rat heart, a minimal calculation distance of 2 mm was used for SR.

For protocol 1, SR and strain profiles were analyzed on the posterior myocardium using an elliptic region of interest (ROI) measuring 1 mm in longitudinal diameter and 2 mm in transverse diameter. ROIs were manually tracked at the center of the myocardial wall, and strain profile was obtained by integrating the mean SR values over time using end-diastole as the reference point. Obtained SR and strain profiles were handled without smoothing. Peak systolic SR and strain values were measured in each of the ten heart cycles. The maximal and minimal values were discarded, and the remaining eight values were averaged. The serial change of peak systolic SR and strain before, during, and after the dobutamine infusion was then evaluated. In addition, the serial strain change was compared to the percent change in wall thickening (%WT). Another observer, who blinded to the strain values, measured the end-diastolic and end-systolic myocardial thickness (EDT and EST, respectively) in the posterior wall using anatomical M-mode tracing derived from underlying 2D images. %WT was calculated as [(EST - EDT) / EDT] × 100.

In protocol 2, the risk area was visually assessed by MCE images. For the spatial distribution analysis of ultrasonic strain, five circular ROIs, measuring 1 mm in diameter, were positioned on the anterior and posterior myocardium as illustrated in Figure 1. One ROI was set on the normal posterior wall (segment A), while the other four ROIs were set on anterior wall (segments

B to E). Segments C and D were placed at the top of anterior wall, and segments B and E were placed lateral to segments C and D, respectively. Peak systolic strain value in each ROI was calculated as described in protocol 1. Because peak systolic strain values varied with different Doppler angles, even among the normal segments, the corrected values were used as the ratio of peak systolic strain during ischemia to peak systolic strain in preischemia (ε ratio).

Interobserver and Intraobserver Variability

Ten color Doppler velocity and 2D data sets were randomly selected from baseline and each dobutamine stress stage. To determine the interobserver variability for SR and strain values, data analysis was repeated by a second observer who was blinded to the values obtained by the first observer. To assess intraobserver variability, the analysis of the data was repeated two weeks later by the same observer.

Statistical Analysis

All values are expressed as mean $\pm$ SD. Multiple comparisons were performed by ANOVA with post hoc Scheffe's test. The interobserver and intraobserver variability were expressed as the percent difference from the mean of the two readings. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

Protocol 1 (Assessment of Serial Change)

Baseline and maximal heart rates during the dobutamine infusion were 419 ± 40 and 488 ± 32 bpm, respectively. For all stress stages, myocardial SR and strain imaging was achieved in the short-axis view in all rats. The SR and strain profiles in baseline posterior wall are illustrated in

Figure 2. Although the diastolic SR profile did not show a biphasic shape due to the high heart rate, SR and strain profiles were relatively readable without the need for smoothing transformation of the data.

Baseline peak systolic SR and strain in the posterior wall was 7.6 ± 2.2 s^{-1} and $27 \pm 12\%$, respectively. The incremental dobutamine infusion resulted in a gradual increase in peak systolic SR (15.4 ± 4.6 s^{-1} at 3 µg • kg^{-1} • min^{-1}, $P < 0.001$ vs. baseline; 21.3 ± 3.1 s^{-1} at 20 µg • kg^{-1} • min^{-1}, $P < 0.001$ vs. 3 µg • kg^{-1} • min^{-1}; Figure 3). In contrast, peak systolic strain increased with low-dose dobutamine ($53 \pm 17\%$ at 5 µg • kg^{-1} • min^{-1}, $P < 0.05$ vs. baseline) and tended to decrease with higher doses of dobutamine ($51 \pm 13\%$ at 20µg • kg^{-1} • min^{-1}, $P = 0.13$ vs. baseline, Figure 3). The serial change of peak systolic strain during the dobutamine infusion corresponded with the serial change in %WT, but the strain values were always lower than the %WT (Figure 3).

The interobserver variability for peak systolic SR and peak systolic strain was 6.4% and 3.2%, respectively. The intraobserver variability for peak systolic SR and strain was 5.1% and 4.9%, respectively.

Protocol 2 (Assessment of Spatial Distribution)

Heart rates before and after the LAD ligation were 440 ± 63 and 387 ± 88 bpm, respectively. In the ligation model, apparent contrast defects during MCE imaging were consistently present in the antero-lateral wall (e.g. from 12 to 3 o'clock, Figure 4). Thus, segment E in Figure 1 was always present within the ischemic region, while segment B was always present within the normal myocardium. Further, the borderline between normal and ischemic regions was located in segment C or between segments C and D. In all rats, the strain profiles demonstrated a postsystolic thickening pattern in the borderline segment (segment C, Figure 4) and an inverted pattern in the ischemic segment (segment E, Figure 4). In the normal posterior wall (segment A), the peak systolic

strain values before ischemia were similar to those during ischemia (Figure 5). However, as the

segments in the anterior wall (segments B to E) shifted toward the center of the risk area, the ε ratio

gradually decreased (Figure 5).

DISCUSSION

Many studies have utilized M-mode and 2D echocardiography for the assessment of

ventricular function in rats. While this technique is adequate in the absence of regional wall motion

abnormalities, the small size of the rat heart and the relatively fast heart rate preclude accurate

measurements of left ventricular function in the presence of regional wall motion abnormalities, such

as in the rat cardiac ischemic model. Derumeaux et al. recently reported that the myocardial velocity

gradient (MVG) derived from tissue Doppler imaging was impaired in rats with pressure

overload-induced left ventricular hypertrophy (9). However, it is not clear whether MVG can be

used for assessment of the spatial distribution in regional ventricular function, because the authors

studied only the posterior myocardium on an M-mode line. Thus, the present study used a

dobutamine stress model as well as a LAD ligation model, which yields different spatial distribution

in regional function, and demonstrated the feasibility of quantitative assessment of systolic SR and

strain and the serial change and spatial distribution of systolic strain in rats through the use of a high

temporal and spatial resolution ultrasound system.

Assessment of Serial Change in SR and Strain

Measurements of ultrasonic SR and strain have been validated in large animal and human

studies (6, 7). Under normal heart conditions, incremental dobutamine administration induced a

gradual increase in systolic SR. In contrast, systolic strain increased with low-dose dobutamine

infusions, but remained stable or tended to decrease with higher concentrations of dobutamine (10,

11). These data are consistent with results from the present study using the rat model. Moreover, the

serial change of peak systolic strain corresponded to changes in %WT, suggesting that the percent

change in SR and strain can be accurately measured, even in the rat heart.

The present study also demonstrated that absolute peak systolic strain values were

consistently 20% lower than the %WT. This difference may result from the calculation distance of

SR employed our protocol; although the minimal distance was set at 2 mm, this value may be too

large for the rat myocardium. Thus, future study to measure absolute SR and strain values would be

of benefit. Despite this result, we believe that the assessment of percent change in SR and strain by

the present ultrasound system remains useful, as laboratory animal studies can typically employ

serial measurements to assess relative values of SR and strain.

Assessment of Spatial Distribution in Strain during Ischemia

Ultrasonic SR and strain have been also investigated in large animals and human with

acute and chronic ischemia (5-7, 10, 12-17). The analysis of the deformation parameters permits

quantitative assessment of regional ventricular function in ischemic or postischemic conditions.

Further, the spatial distribution analysis of these parameters is useful for objectively detecting

ischemic myocardium (12, 13). In the present study, postsystolic thickening was present in the

peripheral ischemic region, and systolic wall thinning was demonstrated in the central ischemic

region. This result is similar to findings from previous studies using large animals (18, 19) and

suggests that spatial distribution assessment can be performed accurately in rats with acute ischemia

through the use of ultrasonic strain.

Because ultrasonic SR and strain are calculated using myocardial Doppler velocities, these

parameters are essentially influenced by the Doppler beam angle (20), and the angle dependency

should be considered when conducting spatial distribution assessment of these parameters. In our rat

data, the systolic strain value in each segment before the LAD ligation varied secondary to angle

dependency. Thus, the peak systolic strain value in each segment after the ligation was compared

with that before the ligation (ε ratio) to correct the angle dependency. Because the ε ratio in each

segment gradually decreased as the segment moved toward the center of risk area derived by MCE,

this corrected parameter may be of utility in the spatial distribution assessment of regional

contraction in rats.

Study Limitations

Because ultrasonic SR and strain in rats were not compared assessment by a gold standard

technique, such as MRI or sonomicrometry, these deformation parameters could not be validated in

the present study. The comparison between strain and %WT may not be sufficient for the validation.

Thus, it is not clear whether the absolute values of these ultrasonic parameters can be accurately

determined using the commercially-available ultrasound system.

The averaged heart rate in our rats was greater than 400 bpm, even under anesthetic

conditions. Although the frame rate was set at 203, it is not clear whether this high frame rate was

sufficient. While a higher frame rate setting is possible in this system, the spatial resolution would

suffer. Thus, the above-mentioned frame rate was chosen to balance the requirements for temporal

and spatial resolution. The resulting favorable interobserver and intraobserver variability may

support use of these settings.

The position of ROI for analyzing SR and strain was manually tracked carefully frame by

frame. Nevertheless, velocity from the same point in the myocardium during cardiac cycle could not

be recorded, because the heart on the short-axis projection is swinging in the azimuthal plane. In

clinical settings, longitudinal SR and strain analysis on apical views are usually used to avoid the

influence of swinging. We did not use the apical approach because of the difficulty in acquiring good

images.

CONCLUSIONS

Ultrasonic determination of SR and strain is an accurate and noninvasive method of

quantitation of the serial change and spatial distribution of regional contractile function in rats.

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FIGURE LEGENDS

Figure 1:   Schematic illustration of the position of the regions of interest (ROIs) in an acute

ischemic model of protocol 2. One ROI was set on the normal posterior wall (segment A), and the

other four ROIs were set on the anterior wall (segments B to E). See text for details.

Figure 2:   Strain rate (SR) and strain profiles in the posterior wall at baseline. The fast heart rate

precludes expression of a two-peak shape in the SR profile during diastole.

Figure 3:   Peak systolic strain rate (SR), peak systolic strain, and percent change in wall thickening

(%WT) in the posterior wall during the dobutamine infusion. $*P < 0.001$ vs. baseline; $†P < 0.001$ vs.

$3 \ \mu g \bullet kg^{-1} \bullet min^{-1}$; $‡P < 0.05$ vs. baseline.

Figure 4:   Myocardial contrast echocardiography (MCE) in an acute ischemic model (left) and the

strain profile in each segment (right). The clear contrast defect represents the risk area. Strain

profiles show a postsystolic thickening pattern in the ischemic border-zone (segment C) and an

inverted pattern in the center of ischemia (segment E).

Figure 5:   Ratio of peak systolic strain during ischemia to peak systolic strain during preischemia (ε

ratio) in each segment. The ε ratio gradually decreases as the segment shifts toward the center of the

risk area. $*P < 0.05$; $†P < 0.01$.