Evaluation of Right Ventricle by Speckle Tracking and Conventional Echocardiography in Rats With Right Ventricular Heart Failure

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Summary

Speckle tracking echocardiography (STE) has been reported to be a promising technique for evaluating right ventricular (RV) function in the clinical setting. On the other hand, the usefulness of STE for RV evaluation in small animal models has not been clarified, although the rat model is among the most commonly used animal models to develop novel effective treatments against pulmonary hypertension and RV heart failure (HF).

We validated the use of STE and conventional echocardiographic variables for evaluating RV functions in a rat model by comparing the echocardiographic values of RVHF rats (n = 12) induced by monocrotaline injection with those of control rats (n = 12).

Most conventional echocardiographic variables demonstrated that RVHF rats have significant RV dysfunction. The area under the curve (AUC) values to distinguish RV dysfunction in RVHF rats from normal RV function in control rats using fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE), RV myocardial performance index (MPI), peak tissue Doppler tricuspid annular velocities at systole (Sa), and at early diastole (Ea) were 0.71, 0.98, 0.79, 0.92, and 0.91, respectively. However, using STE analysis for RV evaluation, limited reproducibility was observed (variability 19–37 %, ICC 0.74–0.88) and the only circumferential strain showed significantly lower absolute values (P = 0.039, AUC = 0.76).

To evaluate RV function in rat models, circumferential strain may be useful, however, the reproducibility and diagnostic utility were limited. Conventional echocardiographic variables such as TAPSE, tissue Doppler Sa, and Ea have superior diagnostic utility. (Int Heart J 2015; 56: 000-000)

Key words: Small animal, Rodent, Tissue Doppler, Myocardial performance index

Pulmonary hypertension (PH) with right ventricular (RV) heart failure (HF) is associated with poor prognosis, and current therapies are still unsatisfactory for treating this condition. To elucidate its pathophysiology and develop effective treatments, experiments using appropriate animal models are needed. The rat model with PH and RVHF is among the most commonly used in cardiovascular research to explore new treatments. However, currently available techniques, particularly for quantitative evaluation of the right heart in small animal models, are very limited.

In recent years, not only conventional echocardiographic imaging but also new imaging has become widely used even in RV evaluation. In particular, two-dimensional speckle tracking echocardiography (STE) has been reported to be a promising technique that quantifies myocardial deformation by tracking the ultrasonographic speckle patterns throughout the cardiac cycle in the clinical setting. However, in past animal experiments, STE has been used to evaluate left ventricular function but rarely to evaluate RV function. To date, the usefulness of STE for RV evaluation in small animal models has not yet been clarified. The present study was designed to validate and compare the use of STE and conventional echocardiographic variables for evaluating RF functions in a rat model with RVHF.

Methods

Experimental animals: Animal experiments were carried out in a humane manner, and the study protocols complied with...
the institutional guidelines of the Animal Experiment Committee of the Faculty of Medicine, The University of Tokyo.

RVHF was induced in Sprague-Dawley male rats (Charles River Laboratories Japan, Inc., Kanagawa) using monocrotaline (MCT) as previously described. A total of 26 rats aged 10-weeks weighing 310 – 360 g were randomly assigned to two groups. RVHF rats (n = 14) received a 60 mg/kg subcutaneous injection of MCT and the control rats (n = 12) a subcutaneous injection of vehicle. Each rat was housed in an air-conditioned room at 20 – 25°C with an automatic 12-hour light-dark cycle and free access to chow.

**Hemodynamic and echocardiographic studies:** Five weeks after the injection, the rats were first anesthetized using isoflurane at a concentration of 3% to 4% in a knockdown box. The concentration of isoflurane was then changed to 1.5% to 2.0% and adjusted to a dose adequate to maintain the heart rate at 300 to 350 beats per minute. Blood pressure was measured using a tail cuff system BP-98A (Softron, Tokyo). Rats with MCT injection underwent pressure measurements of the RV using a 1.2 Fr Miller-tip pressure catheter (Millar, Inc. TX, USA) through the right jugular vein. After hemodynamic measurements, echocardiographic images were acquired (Figure 1, left panel) using a Vivid7 digital ultrasound system with an 11.5 MHz 10S sector transducer (GE Healthcare, WI, USA). Images of the parasternal short-axis view at the papillary muscle level and the apical 4-chamber view were recorded with careful attention to the minimal ultrasonic scanning depth to obtain a higher frame rate (242 to 342 frames per second) with higher imaging qualities. Echocardiography was evaluated in accordance with the guideline of the American Society of Echocardiography. Echocardiographic variables for RV function evaluated were longitudinal dimension, mid dimension, basal dimension, free wall thickness, fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE), and RV myocardial performance index (MPI). Peak Doppler tricuspid annular velocity at early diastole (Ea), and peak tissue Doppler tricuspid annular velocities at systole (Sa) and at early diastole (Ea) in the apical 4-chamber view were also evaluated. Data were measured over 5 heart beats and analyzed by blinded investigators using speckle tracking software (EchoPac, GE Healthcare). Strain values of 6 segments of the LV and the strain value of one manually-adjusted segment of the RV free wall (Figure 1, right panels) were measured. The tracking qualities of each segment were automatically judged by the EchoPac software. Segments judged with poor tracking were confirmed by the investigator and manual adjustments of the myocardial edges in each frame were made as appropriate. Strain values were measured twice by a single investigator at separate times to estimate intra-observer variability, and another trained investigator repeated strain measurements at a separate time to obtain inter-observer variability. The reproducibility of strain measurements was assessed as the mean absolute percentage error, absolute difference divided by the mean of the two investigators, and the intra-class correlation coefficients (ICC).

**Statistical analysis:** Data are expressed as the mean ± SD. Average comparisons were conducted using paired t-tests. Receiver-operating characteristic (ROC) curve analysis was performed in RV variables and the area-under the curve (AUC) was calculated for identifying RV dysfunction in RVHF rats from normal RV function in control rats. P values were considered significant at less than 0.05 using SPSS Statistics 20 software (IBM, NY).

**RESULTS**

Two RVHF rats died and were excluded from the study; one at 31 days after the administration of MCT and another immediately after the induction of anesthesia for hemodynamic evaluation 35 days after the administration.

**Hemodynamic studies:** There was no significant difference between control rats (n = 12) and RVHF rats (n = 12) with respect to systolic blood pressure (136 ± 20 mmHg versus 126 ± 14 mmHg, P = NS) and heart rate (338 ± 28 versus 321 ± 55, P = NS). Systolic RV pressure in RVHF rats was 95 ± 26 mmHg, which was recognized as PH, although the range was wide (minimum value of 55 mmHg to maximum value of 139 mmHg). The ratio of RV to the whole heart weight in RVHF rats (0.30 ± 0.05) was significantly larger than that of control rats (0.23 ± 0.03, P = 0.001).

**Evaluation by conventional echocardiography:** Conventional LV variables (Table I) revealed that RVHF rats have smaller D-shaped LV on the LV short-axis view. RVHF rats showed significantly lower transmitral flow velocities. Conventional RV variables (Table II) revealed that RVHF rats have a higher RV pressure and significant RV hypertrophy.
Echocardiography in rats with RV heart failure

AUC values to distinguish RV dysfunction for FAC, TAPSE, and MPI were 0.71, 0.98, and 0.79, respectively. Variables from tissue Doppler imaging were also significantly decreased in RVHF rats. AUC values for tricuspid annular peak velocities at systole (Sa) and diastole (Ea) were 0.92 and 0.91, respectively.

**Evaluation by speckle tracking echocardiography:** At the beginning of STE analysis, the accuracy and reproducibility of the STE were evaluated. Accurate tracking rates judged automatically by EchoPac software were 100% for LV segments in the short-axis view, 95% for LV segments in the apical 4-chamber view, 92% for the RV free wall in the short-axis view, and 81% for the RV free wall in the apical 4-chamber view.

The reproducibility of rat STE (Table III) for LV segments showed that CS, RS and LS had similar variability while TS had higher variability. The reproducibility for the RV segment showed that CS and LS have similar but limited variability while RS and TS have higher variability.

As a result of STE analysis (Table IV), only CS in RV had significantly lower absolute values in RVHF rats than in control rats ($P = 0.039$). A cut-off value of CS -5.6% can distinguish RV dysfunction in RVHF rats from normal RV function in control rats at the sensitivity of 0.67 and specificity of 0.92, and the AUC was 0.76 (Figure 2).
DISCUSSION

The present study obtained the following findings. All rats with MCT injection (RVHF rats) showed PH with structural RV hypertrophy as previously reported.\textsuperscript{9,12} Significant RV dysfunction in RVHF rats was confirmed using conventional echocardiographic variables such as FAC, TAPSE, and MPI. The RV tissue Doppler absolute values, Sa and Ea, were also significantly decreased in RVHF rats. Using STE, however, only CS showed a significant difference ($P = 0.039$) for RV evaluation and the diagnostic utility was limited (AUC 0.76).

A rat model with RVHF induced by MCT administration has been used widely in animal experiments. Injection of MCT causes PH, RV dilatation, hypertrophy, increased oxidative stress,\textsuperscript{8,10} and RV dysfunction confirmed by echocardiography, magnetic resonance imaging, catheter hemodynamic assessment, and autopsies.\textsuperscript{8,11,12} Conventional echocardiographic variables such as TAPSE,\textsuperscript{9,13,14} have been used to evaluate rats with RVHF. However, in the dilated ventricle, measurement of single M-mode distance may underestimate RV dysfunction and angle-independent STE would be a better choice in this condition.

Applying STE to a rat model is challenging because rats have small echocardiographic windows and a rapid heart rate. In 2013, Koshizuka, et al.\textsuperscript{15} validated the utility of STE in a rat model with LV heart failure with preserved ejection fraction. Their inter-observer variability of STE evaluation was $8.4 - 19.2\%$ and the ICC was $0.81 - 0.91$. Our corresponding results were $21 - 30\%$ and $0.79 - 0.88$, respectively, which were similar although the reproducibility of our data was inferior. These differences may have occurred because their data did not include the results of TS, a variable known for its poor feasibility. Differences in the ultrasound system used in the studies may be another factor. They used a Vevo 2100 ultrasound device with a linear transducer (VisualSonic Inc., ON, Canada), which is specially designed for small animal experiments. However, we used clinical devices, consisting of a Vivid 7 ultrasound system and a sector transducer for pediatric use. Although the EchoPac software we used is a major and well-validated software, we included strain data that were judged automatically as being poor tracking in order to avoid arbitrary analysis. These issues may have affected our results.

Apart from STE, the utility of conventional variables such as M-mode measurements and Doppler imaging in rat models has been validated by Bjorneheim, et al.\textsuperscript{15} The inter-observer variability values of M-mode and Doppler imaging were $1.4 - 8.4\%$ and $3.2 - 12.4\%$, respectively. The better reproducibility compared to STE may encourage the use of conventional variables rather than STE when evaluating rat heart. Indeed, our results of AUC for conventional variables were $0.71 - 0.98$, while that for CS of STE was $0.76$. Of note, TAPSE showed the highest utility (AUC 0.98). It might suggest that simple M-mode measurements could be robust in assessing RV function under the circumstances of rapid heart rate with limited image quality. Tissue Doppler imaging also appears to be useful (AUC 0.91 – 0.92).

In clinical practice, many studies have reported the usefulness of STE for quantitative evaluation of RV. Pirat, et al.\textsuperscript{16} evaluated 42 patients with PH and 31 healthy controls using STE and concluded that STE can identify impaired RV function. Hardegree, et al.\textsuperscript{17} assessed 50 patients with PH before and after the initiation of therapy. They found that STE can independently predict clinical deterioration and mortality after the institution of medical therapy. Haeck, et al.\textsuperscript{18} assessed 150 patients with PH and concluded that LS in RV is a significant determinant of all-cause mortality. Based on these reports, in clinical RV evaluation, measurement of LS has been widely used and LS is currently recognized as the most useful strain variable in RV evaluation.\textsuperscript{14,17,19} However, in terms of RV evaluation in the rat model, our results showed that only CS, but not LS, has significant differences although the reproducibility of LS and CS was similar. This result goes against our expectation. Rats have relatively short long-axis length compared to that of the human heart. In the spherically-shaped heart, circumferential deformation may be a more important contribution for achieving adequate ejection fraction and cardiac output. We speculated that the relatively shorter longitudinal distance of RV may diminish the usefulness of LS in rat models. Unfortunately, our challenge to apply STE for evaluating RV function in our rat model showed suboptimal results. However, to the best of our knowledge, the use of STE for RV function in small animals has not been evaluated previously.

The present study has several limitations. Although we confirmed a significant change in RV weight and compared echocardiographic variables with those of control rats, a current gold standard for RV dysfunction is lacking. In addition, we categorized strain values from the interventricular septum into a part of the left ventricle and were omitted. RV dysfunction with PH might affect deformation of the septum and the data from the septum may have affected the global strain values in our results. We did not perform serial evaluations, and the evaluation timing after MCT administration may have affected our results. Again, we should note that the current result can be applied to rat models in cardiovascular research but not for clinical use. Further study regarding serial evaluation of changes in RV function and behavior of the septum with RV-LV interaction is necessary.

Conclusion: To evaluate RV function in rat models, circumferential strain may be useful although the reproducibility and diagnostic utility were limited. Conventional echocardiographic variables such as TAPSE, tissue Doppler Sa, and Ea may have superior diagnostic utility.

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