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Assessment of Left Ventricular Systolic Function Using Contrast Two-Dimensional Echocardiography With a High-Frequency Transducer in the Awake Murine Model of Myocardial Infarction

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The estimation of global left ventricular function using M-mode echocardiography has technical limitations in the murine model of myocardial infarction (MI), but the recent improvements in 2-dimensional (2-D) echocardiography using a high-frequency transducer provide more accessible images. Furthermore, intravenous injection of contrast agent has the additional benefit of enhancing the endocardial border in the murine heart. The present study was designed to evaluate the value of 2-D echocardiography with intravenous injection of contrast agent in the assessment of global systolic function of the murine heart with MI. Two-dimensional and M-mode echocardiography without and with intravenous injection of contrast agent (Optison, 0.1–0.15 ml) were performed in 76 awake mice 2 days before and 2 days after left coronary artery ligation. Fractional shortening (FS) was calculated from the end-diastolic and end-systolic diameters on M-mode echocardiography, and fractional area change (FAC) from the end-diastolic and end-systolic areas on 2-D echocardiography. Both FS and FAC were compared with the areas of hypoperfusion observed in the pathological samples. The use of contrast agent improved the number of hearts that could be evaluated by both the M-mode and 2-D method (M-mode: non-contrast 87% vs contrast 99%, $p<0.01$; 2-D: non-contrast 26% vs contrast 89%, $p<0.001$). FAC from the 2-D method correlated better with the region of hypoperfusion in the pathological samples than did FS from the M-mode method (FAC: $r=0.84$ vs FS: $r=0.51$). In conclusion, FAC obtained from 2-D contrast echocardiography is useful for noninvasive assessment of global systolic function in infarcted murine hearts and can be used to serially assess systolic function in various models of the murine heart. (*Jpn Circ J* 2001; **65:** 979–983)

Key Words: Echocardiography; Left ventricular function; Mouse; Myocardial infarction

Left coronary artery ligation is one of the most common methods of creating a model of myocardial infarction (MI) in the murine heart and M-mode echocardiography has been used to assess cardiac function because of its higher sampling frequency, which is particularly useful when assessing a heart rate (HR) of 600 beats/min or greater[1–9] However, the estimation of global left ventricular (LV) function using M-mode echocardiography has limitations when it is applied to an infarcted heart[10] and 2-dimensional (2-D) echocardiography is more suitable. In murine hearts with their small size and high heart rate, however, even this method has been limited in its application for assessment of cardiac function because of the difficulty in obtaining adequate 2-D images.

The recent improvements in 2-D echocardiography of a higher resolution and higher frame rate using a high-frequency transducer provide more accessible images for assessment of cardiac function in the murine model[2,11–13] and, furthermore, intravenous injection of contrast agent has the additional benefit of enhancing the endocardial border in the murine heart[11] The purpose of this study was to evaluate the value of 2-D echocardiography using a high-frequency transducer plus intravenous contrast injection in the assessment of cardiac function in a murine model of MI.

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Methods

All mice used in this study were maintained in an animal facility in accordance with the American Association for Accreditation of Laboratory Animal Care. The mice were housed no more than 5 per cage with 12:12 h light:dark cycles, and were given free access to rodent chow and water. The protocol was approved by the Institutional Animal Care and Use Committee of Columbia University.

Surgical Procedures

A total of 76 male Swiss-Webster mice (Taconic, Germantown, NY, USA), ranging in age from 8 to 24 weeks and weighing 27.4–46.7 g (mean, 36.1±5.4 g), were used in this study. Following pre-operative echocardiography, which was performed 2 days prior to the surgery in an

awake condition (as described later), mice were anesthetized with a mixture of intraperitoneal ketamine (50 mg/kg) and xylazine (6 mg/kg). Endothoracheal intubation was performed (22 gauge Angiocath, Beckton-Dickinson, Sandy, UT, USA) and the animals were then ventilated with room air (Harvard Apparatus; tidal volume = 1.0 ml, rate = 150 breath/min, PEEP = 5 cmH_2O).

Myocardial infarction was produced according to the method described by Patten et al! Briefly, the fourth intercostal space was entered and the left coronary artery ligated at approximately its midpoint using an 8-0 polypropilen suture under an operating microscope (Leica Microsystems Inc, Deerlake Road, IL, USA).

Two days following ligation, the mice underwent echocardiography again in an awake state (see later). Animals were then anesthetized with ketamine/xylazine (50/6 mg/kg, ip), and the right carotid artery was cannulated (24 g Angiocath, Beckton-Dickinson). Following intracoronary injection of potassium chloride in saline solution (40 mmol/L) to obtain diastolic arrest, 5% Evans-blue dye was injected. The heart was excised and sectioned transversely at the mid-papillary level, and photographs of the freshly sectioned hearts were taken.

Echocardiography in Awake Mice

Transthoracic echocardiography was performed 2 days prior to and 2 days following surgery using an Acuson cardiac ultrasound machine (Sequoia, Acuson, Mountain View, CA, USA) with a 13 MHz transducer at high frame rate imaging (162 Hz).

Mice were shaved and picked up by the skin around the occiput, which less frequently causes suffocation than using the skin around the neck. The body of the mouse was then held gently using the thenar and the fingers. Special care must be taken to avoid excessive compression and retraction, which both cause bradycardia. In mice, the heart rate is a good index of the gentleness of handling. Coupling gel was placed on the chest and scanning was begun, again avoiding excess chest compression with the probe.

Initially, 2-D short-axis images and M-mode images were recorded without contrast agent. After obtaining these unenhanced images, 0.1–0.15 ml of echo contrast agent (Optison™, Molecular Biosynthesis, San Diego, CA, USA)[11,14] was injected intravenously through the tail vein using a 27 or 28 g needle followed by acquisition of the same images, but enhanced, in an awake state.

Assessment of Systolic Function

LV fractional shortening (FS) was calculated from M-mode measurements taken at the mid-papillary level before and after contrast injection using the following formula:

$$FS\ (\%) = (EDD - ESD)/EDD \times 100$$

where, EDD is the LV end-diastolic diameter and ESD is the LV end-systolic diameter. Fractional area change (FAC) was also calculated from 2-D contrast echocardiography LV short-axis images at the mid-papillary level before and after contrast injection using the following formula:

$$FAC\ (\%) = (EDA - ESA)/EDA \times 100$$

where EDA is the LV end-diastolic area and ESA is the LV end-systolic area, each measured at the mid-papillary level. The maximum and minimum values in 1 cardiac cycle were obtained by an experienced observer manually tracing the endocardial border of the LV.

Table 1 Number of Hearts Evaluated at the Mid-Papillary Level

	M-mode	*2-D*
C+	*75/76 (99)**	*68/76 (89)***
C–	*66/76 (87)*	*20/76 (26)†*

*Data are expressed as the number of evaluated hearts/total number of hearts (%). All assessment was performed in end-diastole. The number of evaluated hearts using the 2-D method is defined as the number of hearts in which the entire endocardial border could be delineated at the mid-papillary level. C+, with contrast; C–, without contrast. Data are expressed as mean±SD. *p<0.01, **p<0.001 compared with C–. †p<0.001 compared with M-mode.*

Pathology

Photographs of the fresh Evans-blue stained heart sectioned at the mid-papillary level were scanned with Photoshop 4.0 (Adobe Systems, Inc, Mountain View, CA, USA). When an area within a section was determined to be non-stained or lightly-stained, it was considered to be hypoperfused and the area of hypoperfusion was quantified and calculated using a software package (Image Pro Plus, Media Cybernetics, MD, USA) with the following formula:

$$\text{Hypoperfusion area}\ (\%) = \text{Area of hypoperfusion} / \text{Total area at the mid-papillary level} \times 100$$

Statistical Analysis

All values are expressed as mean ± standard deviation, unless otherwise indicated. In order to compare endocardial border delineation, M-mode and 2-D measurements, with and without the use of contrast agent (C+ and C–, respectively), unpaired Student's t-test was performed. The paired Student's t-test was used to compare pre- and post-surgical values of M-mode and 2-D measurements and cardiac function. Correlations between FAC, FS and ischemic area at the mid-papillary level were determined by linear regression analysis. For categorical variables, chi-square tests were performed. A p value of less than 0.05 was considered to be significant.

Results

Effect of Contrast Enhancement

The number of hearts evaluated at the mid-papillary level is shown in Table 1. Using the M-mode method, 87% of hearts could be assessed even without contrast, but contrast agent further improved the utility of M-mode and allowed almost all (99%) hearts to be evaluated. On the other hand, only 26% of hearts could be used for 2-D evaluation without contrast enhancement (Fig 1). However, delineation of the endocardial border was significantly improved by the use of contrast agent which increased the number of assessible hearts up to 89% (Fig 2).

Systolic Function

The average HR of the mice was 520±21 (range, 450–600) beats/min pre-operatively and 474±41 (range, 360–555) post-operatively (p<0.0001).

The changes in the measurements obtained by both methods before and after coronary ligation are summarized in Table 2. The ESD was slightly larger after left coronary ligation, although the EDD was not significantly changed. Accordingly, FS was slightly lower post coronary ligation (52.0±5.9% vs 47.6±5.5%, p<0.0001). Similarly, EDA was not changed significantly, but ESA was increased (p<

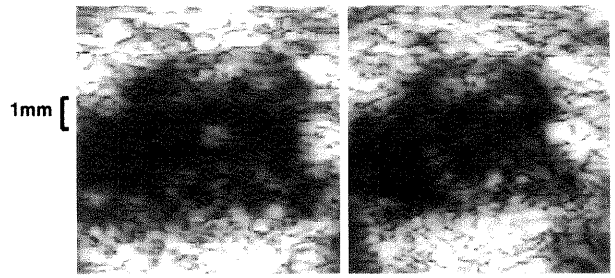

Fig 1. Two-dimensional echocardiograms without contrast agent (left: end-diastole; right: end-systole).

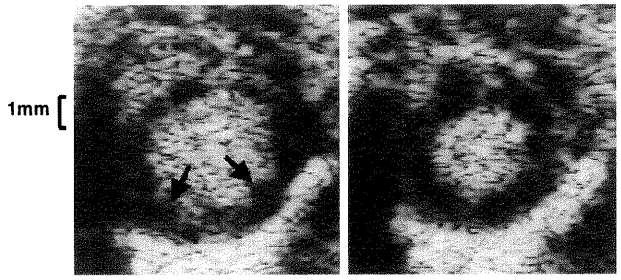

Fig 2. Two-dimensional echocardiograms with contrast agent in the same mouse in Fig 1 (left: end-diastole; right: end-systole). Arrows indicate papillary muscles.

0.0001). Therefore, the calculated FAC was decreased (65.2±6.6% vs 53.1±10.9%, p<0.0001).

FS and FAC were then correlated with the areas of hypoperfusion at the mid-papillary level (Fig 3) and it was found that FAC provided better correlation (r=0.84 vs 0.51).

Side Effects of Optison™

For human use, 0.5 ml iv is the recommended initial dose of Optison™, so for mice that weigh between 35–40 g, 1/2000 dose (ie, 2.5×10^{-4} ml) was presumed to be the proper dose. We found it technically impossible to inject such a small amount. Following venipuncture, it is necessary to ensure that the needle is properly engaged in the vessel and that the contrast flows freely into the vein, procedures which in our hands require 0.1–0.15 ml injection. Even with such a seemingly large bolus, serious complications (eg, fluid overload or pulmonary capillary embolism) were never seen. Insofar as Optison™ consists of human albumin, an allergic reaction or acute rejection was anticipated, but neither of these adverse events was observed in this series.

Discussion

In the present study, we demonstrated that the definition of the endocardial border for purposes of quantifying systolic cardiac function in a murine model of MI by 2-D analysis is improved by the use of contrast agent and that the images were superior to those using M-mode tracings.

Echocardiographic Assessment of Global Systolic Function in Murine Models in Previous Reports

In most of the previous reports, M-mode echocardiography has been used because of its higher sampling frequency, which is particularly useful when assessing HR of 600 beats/min or greater![1–9] However, M-mode method has some potential problems when used for mice. It is well known that the M-mode method has limited reliability in estimating global function in an infarcted human heart[10] and that 2-D echocardiography is more suitable. In murine hearts with their small size and high HR, however, 2-D echocardiography has had limited application because of the difficulty in obtaining adequate images. Recent improvement in 2-D echocardiography with higher resolution and higher frame rate using a high-frequency transducer provides more accessible images for cardiac function in murine model[2,11–13] Futhermore, intravenous injection of contrast agent has additional benefit of endocardial border enhancement in murine heart![11] However, there has not been a report evaluating systolic function in a murine model of MI using this method.

Present 2-D Contrast Echocardiographic Assessment of Cardiac Function in a Murine Model of MI

In the present study, delineation of the endocardial border was significantly improved by the use of contrast

Table 2 Changes in Cardiac Function Calculated From M-Mode Measurements

	Pre-ligation	*Post-ligation*
M-mode		
EDD (cm)	*0.38±0.03*	*0.37±0.03*
ESD (cm)	*0.18±0.02*	*0.19±0.02**
FS (%)	*52.0±5.9*	*47.6±5.5***
2-D		
EDA (cm^2)	*0.108±0.014*	*0.104±0.014*
ESA (cm^2)	*0.037±0.006*	*0.050±0.018***
FAC (%)	*65.2±6.6*	*53.1±10.9***

*EDD, end-diatolic diameter; ESD, end-systolic diameter; FS, fractional shortening; EDA, end-diatolic area; ESA, end-systolic area; FAC, fractional area change. Data are expressed as mean±SD. *p<0.05, **p<0.0001 compared with Pre-ligation.*

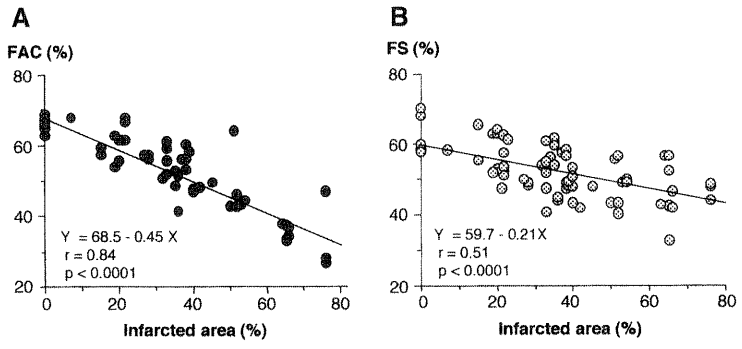

Fig 3. Correlation between total hypoperfusion area at the mid-papillary level and FAC (A) and FS (B). FAC, fractional area change; FS, fractional shortening.

agent in 89% of the hearts evaluated. This technique has 2 advantages. Firstly, it has a high success rate of tracing the endocardial border on the LV short-axis view. Secondly, it allows serial assessment of systolic function because it is not an invasive procedure and so can be repeatedly performed. Moreover, entire procedures can be carried out with the animals in an awake state. Although echocardiographic assessment of cardiac function in mice has been performed under anesthesia, this has certain effects on the heart[15]

As shown in the present study, FAC from 2-D echocardiography and FS from M-mode echocardiography, both measured at the mid-papillary level, had good correlation with area of infarction, but FAC had a significantly better correlation because the 2-D based method is more suitable for assessment of systolic function when there is a regional abnormality. Thus, FAC obtained from 2-D contrast echocardiography is a reliable index of systolic function in a murine model of MI.

Study Limitations

We did not validate parameters of systolic cardiac function in awake mice with those estimated by another established method. In human echocardiography, the estimation of global cardiac function obtained by echocardiography has been validated by other methods, such as LV angiography[10,20,21] or gated single photon emission computed tomography[20,22–25] Recently, there has been successful measurement of LV volume in mice using a miniaturized conductance catheter;[26,27] gated magnetic resonance imaging;[28,29] biplane LV angiography[30] and radionuclide ventriculography;[31] the last method being used in murine hearts with an occluded left anterior descending artery. Although each of these techniques provided excellent accuracy, they are still not widely available and are technically demanding. In addition, they require anesthesia and some invasive procedures, such as cannulation of the jugular vein, which we tried to avoid in the present study.

The recently introduced method of assessing global systolic function in infarcted mice hearts using 3-D reconstruction of 2-D cross-sectional images may be ideal;[13] but the use of the 2-D method in murine hearts is still limited by inadequate endocardial border delineation, even with high resolution echocardiography.

The systolic function of the murine heart has been assessed by %FAC from 2-D echocardiograms with or without contrast enahancement;[2,11] but neither those previous studies nor ours has shown reproducibility of the echocardiographic measurements. Thus, it is necessary to examine reproducibility in a systematic fashion in future studies.

Conclusions

The FAC obtained from 2-D contrast echocardiography is a useful noninvasive assessment of global systolic function in the infarcted murine heart and can be used to serially assess systolic function in various murine heart models.

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