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Assessment of segmental wall motion abnormalities using contrast two-dimensional echocardiography in awake mice

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Suehiro, Kotaro, Shin Takuma, Carol Cardinale, Takeshi Hozumi, Juichiro Shimizu, Hideaki Yano, Marco R. Di Tullio, Jie Wang, Craig R. Smith, Daniel Burkhoff, and Shunichi Homma. Assessment of segmental wall motion abnormalities using contrast two-dimensional echocardiography in awake mice. *Am J Physiol Heart Circ Physiol* 280: H1729–H1735, 2001.—Murine models of cardiac disease are becoming an important tool for studying pathophysiological processes. Development of methods to accurately assess ventricular function are therefore important. The purpose of this study was to evaluate the feasibility of echocardiographic assessment of segmental wall motion abnormalities in a murine model of myocardial infarction. Two-dimensional contrast (C+) and noncontrast (C–) echocardiography were performed in 76 awake mice 2 days before and 2 days after left coronary ligation. The short-axis images obtained with two-dimensional echocardiography and corresponding postmortem cross-sectional histological samples stained with Evans blue dye were each divided into 16 segments, and all matched segments were examined for correlation between wall motion abnormalities and myocardial hypoperfusion. With the use of contrast enhancement, the number of visualized segments was significantly increased (base: C– 86%, C+ 98%; midpapillary: C– 57%, C+ 89%; apex: C– 30%, C+ 74%). Agreement between echocardiographically assessed regional wall motion abnormalities and pathologically determined hypoperfusion in basal, midpapillary, and apical levels were 90%, 93%, and 93%, respectively. Agreement between echocardiographically normal wall motion and pathologically normal findings in basal, midpapillary, and apical levels were 99%, 88%, and 71%, respectively. Thus echocardiographic assessment of segmental wall motion in awake mice was feasible and the accuracy was improved with the use of a contrast agent.

ischemia; ultrafonography; coronary artery disease

THE MOUSE HAS EMERGED as an important experimental model because of the availability of transgenic strains commonly used in the study of the cardiovascular system. In larger animals, echocardiography is a standard method to follow changes in global cardiac function, regional wall motion, heart size and mass, and blood

flow. However, echocardiographic assessment of murine hearts has practical limitations because of their small size and rapid heart rate (HR). Successful echocardiographic assessment of murine hearts, including the measurement of left ventricular (LV) ejection fraction, percent fractional shortening, change of LV dimension, mass, and myocardial perfusion has recently been reported. (2–5, 9, 11, 12, 15, 17). However, there have only been a few reports analyzing regional wall motion by echocardiography in murine hearts (11). Although two-dimensional echocardiography with intravenous injection of a contrast agent may be useful in the analysis of regional wall motion abnormalities in murine hearts, this technique has not been validated.

In the present study, we compared two-dimensional contrast echocardiographic analysis of regional contractile function with pathological findings, and we investigated the feasibility of segmental wall motion analysis in a murine model of myocardial infarction.

METHODS

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

Surgical procedures. A total of 108 male Swiss-Webster mice (Taconic; Germantown, NY) ranging in age from 8 to 26 wk and weighing 26.9–49.7 g (mean 37.0 ± 5.9 g) were studied. Echocardiography was performed on each awake mouse (as described below) before any surgical procedure to acquire baseline images. Two days after the baseline echocardiography, the mice were anesthetized with ketamine-xylazine (50/6 mg/kg) via an intraperitoneal injection. In the supine position, endotracheal intubation was performed, and the mice were ventilated with room air using an animal respirator (Harvard Apparatus; tidal volume, 1.0 ml; ventilation rate, 150 breaths/min; positive end-expiratory pressure, 5 cmH₂O).

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All surgical procedures were carried out with an operating microscope (Leica Microsystems; Deerlake Road, IL) at $\times 5$ to $\times 24$ magnifications. The mice were placed in the right lateral position, and a left thoracotomy was performed. The fourth intercostal space was entered using scissors and blunt dissection. An 8-0 polypropylene suture was placed at approximately the midpoint of the left coronary artery and tied as described by Patten et al. (10). Here, the left coronary artery often has a large branch, which we ligated to the main trunk. Occlusion of the artery was confirmed by observing pallor of the LV wall. The chest cavity was then closed by layers with 3-0 silk suture, and the pneumothorax was reduced using a chest tube and a syringe. The animals were gradually weaned from the respirator. Once spontaneous respiration resumed, the endotracheal tube was removed, and the animal was placed in a heated bed.

Two days after left coronary artery ligation, the mice underwent echocardiography again in an awake state (see *Echocardiography*). Animals were then anesthetized with ketamine-xylazine (50/6 mg/kg ip), and the right carotid artery was cannulated (24-gauge Angiocath, Beckton Dickinson; Sandy, UT). To achieve diastolic cardiac arrest, potassium chloride in saline solution (40 meq/l) was injected through the cannula into the coronary arteries. Immediately after cardiac arrest, 5% Evans blue dye was injected into the cannula. The heart was excised and sectioned transversely at the basal level, the midpapillary level, and the apical level. Photographs of the freshly sectioned hearts were obtained.

Echocardiography. Transthoracic echocardiography (Sequoia, Acuson; Mountain View, CA) was performed 2 days before and 2 days after surgery with the use of a 13-MHz transducer at high frame rate imaging (162 Hz). The images were digitally captured as a cine loop. Mice were held gently and the probe was applied with minimal pressure onto the chest wall. LV wall motion was recorded using M-mode echocardiography. The end-diastolic phase was determined as the timing of the maximal LV diameter. HR was calculated from the period between two consecutive end-diastolic points from M-mode echocardiography.

Initially, two-dimensional short-axis images were recorded without an echocardiographic contrast agent. After these unenhanced images were obtained, 0.1–0.15 ml of echo contrast agent (Optison, Molecular Biosynthesis; San Diego, CA) were injected intravenously through the tail vein using a 27- or 28-gauge needle followed by acquisition of the same images. The tail vein was used for this injection because it is relatively easy to access in awake mice and it can be used repeatedly. The injection was carried out over 5–10 s without flushing.

Assessment of LV wall motion abnormality. The two-dimensional echocardiographic images were divided into 16 segments as recommended by the American Society of Echocardiography (13): anteroseptal wall (AS), anterior wall (A), anterolateral wall (AL), posterolateral wall (PL), inferior wall (I), inferoseptal wall (IS), lateral wall (L), and septal wall (S) at basal and midpapillary levels, and A, L, I, and S at apical level. Each study was analyzed by one investigator unaware of the detail of surgical procedures or pathological findings. When the entire endocardial border could be delineated in a segment, the segment was considered to be visualized. All visualized segments were assessed for wall motion abnormality compared with the echocardiographic images before coronary ligation. Segments were counted as abnormal when they presented any degree of wall motion abnormality, e.g., hypokinesis, akinesis, and dyskinesis, in any part of each segment. We judged wall motion not only by endocardial movement but also by wall thickening during

systole. All visualized segments were divided according to whether they had wall motion abnormality or not. Wall motion of each segment was then correlated with pathological findings (see below).

Pathology. Photographs of the fresh Evans blue-stained heart sections were scanned (Photoshop 4.0, Adobe Systems; Mountain View, CA) into a computer (Power Macintosh G3, Apple Computer). The image of each section was divided into 16 segments in a similar fashion to the echocardiographic images according to the American Society of Echocardiography recommendation (13). Pathological findings were analyzed by an investigator unaware of the surgical procedures and echocardiographic findings. When a segment included any hypoperfused areas defined as nonstaining or an area lightly stained with Evans blue dye, the segment was scored as being a hypoperfused area.

Statistical analysis. All values are expressed as means \pm SD unless otherwise indicated. Paired Student's *t*-test was used to compare HR before and after left coronary artery ligation. For categorical variables, McNemar χ^2 tests were performed. Spearman correlation was applied to estimate the relationship between total segment number of wall motion abnormalities by echocardiography and hypoperfusion by pathology. A *P* value < 0.05 was considered significant.

RESULTS

A total of 108 mice underwent left coronary ligation, of which 76 (70.4%) survived until the second postoperative day. Twenty-eight of 108 (25.9%) expired from complications due to surgery and/or presumed myocardial infarction. Two mice (1.9%) died after induction of anesthesia. One (0.9%) died during echocardiography. Another (0.9%) died just after injection of the contrast agent.

HR in awake mice before left coronary ligation ranged between 450 and 600 (520 ± 21) beats/min. However, 2 days after surgery, HR significantly decreased to between 360 and 555 (474 ± 41 , $P < 0.0001$) beats/min.

Figure 1 illustrates a representative two-dimensional short-axis image without (A and B) and with (C and D) contrast in a normal mouse. With the intravenous administration of Optison, right ventricular (RV) opacification was enhanced within 5–15 s. During that time, the left ventricle could not be visualized because of a strong shadowing effect from the RV. Approximately 10–30 s after this initial RV opacification, LV cavity opacification was noted. At this point, visualization of the LV chamber and a more clear definition of the LV endocardium was feasible without significant RV shadowing, although the RV was still moderately enhanced with contrast. LV visualization was enhanced for ~ 3 min after the administration of Optison. It was possible to record two-dimensional images at three different levels after one injection of contrast agent (0.1–0.15 ml).

The endocardium of the heart was divided into 16 standard segments, 6 at the basal and midpapillary levels and 4 at the apical level. A comparison of the number of endocardial segments clearly visualized at each level with and without contrast is shown in Table 1. These data are derived from echocardiograms performed on 76 mice in the baseline condition, before

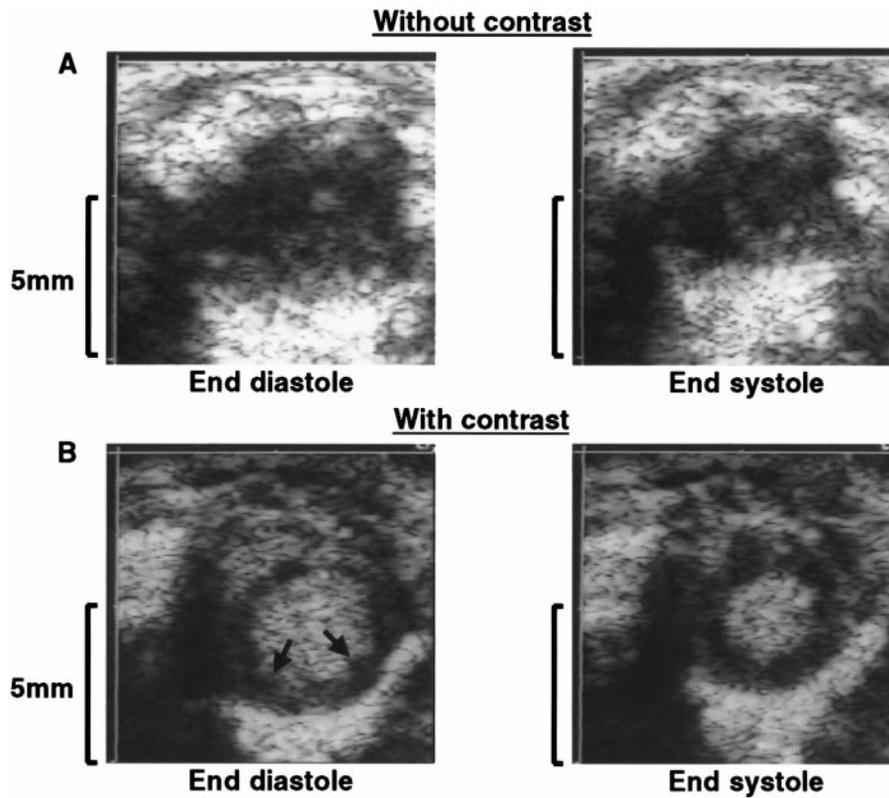


Fig. 1. Representative echocardiographic images of a normal heart in the midpapillary level without (A) and with (B) contrast enhancement. Arrows indicate papillary muscles.

myocardial infarction. Use of contrast enhancement significantly increased the number of visualized segments at all levels.

Distribution of segments of hypoperfusion due to left coronary artery ligation. Because we ligated the left coronary artery at its midpoint, the number of hypoperfused regions was relatively small in the basal level compared with both the midpapillary and apical levels. Only 43 segments of 456 basal segments (9%) showed histological evidence of myocardial hypoperfusion. In the midpapillary level, 163 segments (36%) demonstrated hypoperfusion. The anterior (A), AL, and inferolateral (IL) walls were consistently affected by left coronary ligation [A, 49/76 segments (65%); AL, 68/76 segments (90%); and IL, 61/76 segments (80%), respectively]. Interestingly, even the inferior wall (I) was moderately affected [39/76 segments (51%)]. The “border zone” between the hypoperfused and the normal area was most frequently seen in the anterior wall and the inferior wall. In the apical level, 101 of 304 segments (33%) demonstrated hypoperfusion. The an-

terior wall (A) and the lateral wall (L) were severely affected [A, 49/76 segments (65%); L, 56/76 segments (74%)], whereas the inferior wall was affected in a relatively small number of animals [16/76 segments (21%)]. The border zone was frequently seen in the anterior and the inferior wall in the apical level.

Correlation between echocardiographic diagnosis for regional abnormality. Figure 2, A and B, shows a representative two-dimensional contrast short-axis image at the midpapillary level in an infarcted mouse heart.

No animal exhibited wall motion abnormality on the baseline echocardiogram. After coronary ligation, only a small percentage of segments in the basal level (48 of 446 visualized segments, 11%) exhibited wall motion abnormalities. At the midpapillary level, 185 of 406 visualized segments (46%) exhibited wall motion abnormalities. Severe wall motion abnormalities were found in the A, AL, and IL walls. Wall motion in the inferior wall was also affected in several animals. At the apex, 128 of 222 visualized segments (58%) showed wall motion abnormalities. This was mainly confined to the anterior and lateral walls.

The agreement between echocardiographically assessed regional wall motion abnormalities and pathologically determined hypoperfusion (Evans blue dye technique served as the standard) is shown in Table 2. Agreement between echocardiographically assessed regional wall motion abnormalities and pathologically determined hypoperfusion in basal, midpapillary, and apical levels were 90%, 93%, and 93% after contrast injection. Table 3 shows the agreement between echocardiographically assessed normal wall motion and a

Table 1. Visualized segments with and without contrast enhancement

	Basal	Midpapillary	Apical	Total
C-	393/456 (86)	258/456 (57)	92/304 (30)	743/1,216 (61)
C+	446/456 (98)*	405/456 (89)*	225/304 (74)*	1,076/1,216 (88)*

Data are expressed as the number of visualized segments per the number of all the original segments. Numbers in parentheses represent agreement percentage. C-, without contrast; C+, with contrast. * $P < 0.0001$ compared with C-.

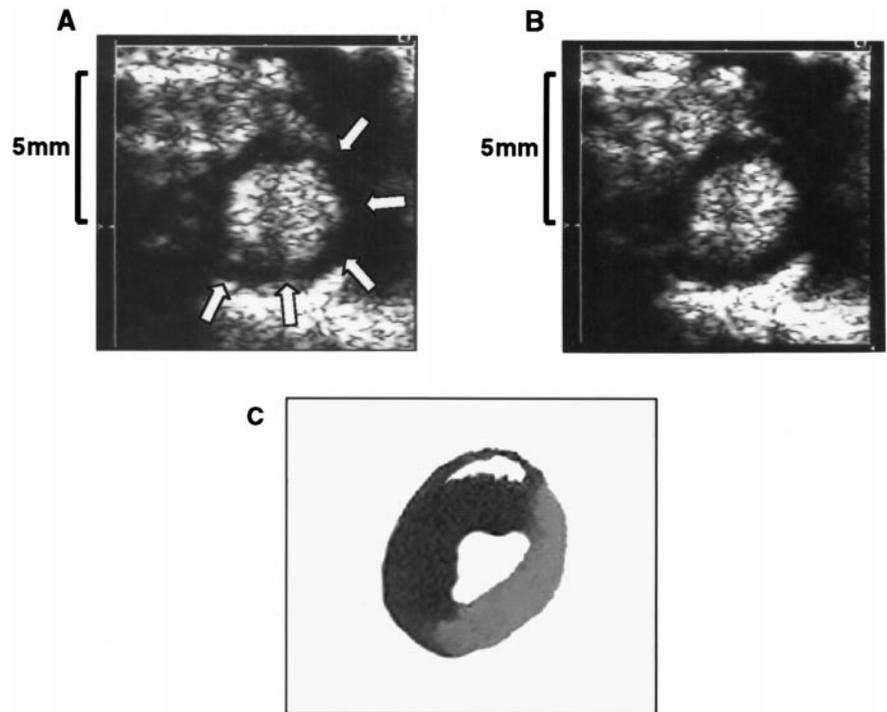


Fig. 2. Representative echocardiographic images and a pathological sample obtained from an infarcted heart. *A*: end diastole, *B*: end systole, *C*: sectioned heart stained with Evans blue dye. The area that exhibited wall motion abnormality is indicated by arrows (*A*). This area is well-matched with the area of hypoperfusion seen in the pathological sample.

pathologically normal finding. Agreement between echocardiographically normal wall motion and a pathologically normal finding in basal, midpapillary, and apical levels were 99%, 88%, and 71%, respectively, after contrast injection.

Figure 3 shows correlation between total segment number of wall motion abnormalities by echocardiography and hypoperfusion by pathology. There was a good correlation in global LV function between echocardiography and pathology ($r = 0.89$).

DISCUSSION

We demonstrated that detection by echocardiography of segmental wall motion abnormalities in awake mice is feasible. The definition of the endocardial border for the purpose of assessing segmental wall motion

is significantly improved by the use of a contrast agent. To the best of our knowledge, this is the first report to demonstrate feasibility of echocardiographic assessment of regional wall motion abnormalities with intravenous injection of a contrast agent from the tail vein in awake mice. The present technique has two critical advantages. First, serial assessment of segmental wall motion is possible in murine hearts with myocardial infarction because it can be repeatedly performed without invasive procedures, e.g., cannulation into the femoral or jugular vein. Second, the entire procedure for the present method can be carried out with the subject in an awake state without hemodynamic perturbation from anesthetic agents.

Assessment of wall motion abnormality. Because the novel treatments to improve regional function, such as

Table 2. Agreement between echocardiographically assessed regional wall motion abnormalities and pathologically determined hypofusion with and without contrast injection

	AS	A	AL	PL	I	IS	Total
Basal level							
C+	3/3 (100)	13/15 (87)	14/15 (93)	5/5 (100)	3/3 (100)	0/1 (0)	38/42 (90)
C-	3/3 (100)	11/15 (69)	13/15 (87)	4/5 (80)	2/3 (67)	0/1 (0)	33/42 (77)
Midpapillary level							
C+	4/4 (100)	36/36 (100)	51/52 (98)	36/45 (80)	21/23 (91)	1/1 (100)	149/161 (93)
C-	3/4 (75)	23/33 (70)	38/45 (84)	27/35 (77)	18/21 (86)	1/1 (100)	110/139 (79)
	S	A	L	I	Total		
Apical level							
C+	4/5 (80)	38/39 (97)	43/44 (98)	4/8 (50)	89/96 (93)		
C-	1/1 (100)	8/13 (62)	13/17 (76)	3/5 (60)	25/36 (69)		

Data are expressed as the number of segments in which echocardiographic and pathological diagnosis were in agreement per the number of visualized segments. Numbers in parentheses represent agreement percentage. AS, anteroseptal wall; A, anterior wall; AL, anterolateral wall; PL, posterolateral wall; I, inferior wall; IS, inferoseptal wall; S, septal wall; L, lateral wall.

Table 3. Agreement between echocardiographically assessed normal wall motion and a pathologically normal finding with and without contrast injection

	AS	A	AL	PL	I	IS	Total
Basal level							
C+	72/72 (100)	59/59 (100)	58/60 (97)	70/71 (99)	71/71 (100)	71/71 (100)	401/404 (99)
C-	59/60 (98)	53/55 (96)	50/55 (91)	60/65 (92)	59/61 (97)	53/55 (96)	334/351 (95)
Midpapillary level							
C+	56/60 (93)	17/32 (53)	14/19 (74)	26/27 (96)	42/43 (98)	59/63 (94)	214/248 (88)
C-	24/27 (89)	4/18 (22)	17/21 (81)	8/9 (89)	17/20 (85)	20/24 (83)	90/119 (76)
	S	A	L	I	Total		
Apical level							
C+	41/48 (85)	5/21 (24)	11/18 (61)	34/42 (81)	91/131 (71)		
C-	13/24 (54)	2/7 (29)	3/6 (50)	8/19 (42)	26/56 (46)		

Data and abbreviations are given in Table 2.

angiogenesis (16), are evolving, application of these methods to mice may be useful for research. Accordingly, for investigation of these effects, the technique to assess regional wall motion in murine hearts should be developed.

Although echocardiographic assessment of murine hearts has practical limitations due to their small size and rapid HR, it has become more accessible because of recent improvement of echocardiographic systems. Our use of high-frequency (13 MHz), high-frame rate (162 Hz) echocardiography provided images of excellent quality. In the present study, HR of awake mice was 360–600 beats/min. Because the frame rate was set at 162 Hz, 16–27 frames/beat was assigned for evaluation, and this was adequate to diagnose segmental wall motion abnormalities in awake mice.

Scherrer-Crosbie et al. (11) also evaluated LV wall motion abnormalities in anesthetized mice using a three-dimensional echocardiographic approach. In their report, HR of the mice was 465 ± 85 beats/min preoperatively and 485 ± 83 beats/min postoperatively. They successfully evaluated wall motion abnormalities of these murine hearts using a 13-MHz linear array ultrasound probe with a frame rate of 130 Hz.

Impact of contrast enhancement in murine echocardiography. In our model, myocardial infarction due to left coronary artery ligation is mainly observed in the

apical portion of the LV. To improve opacification the chamber for better identification of the endocardium of these areas, contrast enhancement was especially useful.

Optison, octafluoropropane-filled, human, albumin-coated microspheres of 3.6–5.4 μm in diameter (1, 9), were employed in the present study. It has been reported that Optison has a longer half-life than other agents (1), and this becomes especially useful in studies of awake mice, because the optimal position of the probe is not always aligned for scanning immediately after the agent is infused. Use of this contrast agent significantly improved LV opacification and improved the ability to visualize the endocardial border, a critical feature for assessment of wall motion abnormality based upon two-dimensional images. In noncontrast echocardiography in mice, identification of the right ventricle, and determination of septal insertion sites are often difficult. However, with the addition of contrast, the RV cavity is markedly enhanced and the septal wall is clearly identified. Similarly, structures, such as the papillary muscles, are clearly observed as well. Accordingly, proper probe orientation and selection of the proper myocardial slice are enhanced by the use of a contrast agent.

Echocardiographic assessment of awake mice. Because cardiovascular disease is a dynamic process, analytic methods that can be repeated over time are required (6). One of the most common tools is the echocardiogram, a noninvasive procedure that provides the investigator with a wealth of information about the structural and functional characteristics of hearts under study. Also, echocardiography is a powerful tool to assess wall motion abnormality.

Echocardiography has been performed previously in anesthetized mice because of difficulty in animal handling. However, because anesthetic agents have positive or negative inotropic effects (7, 14) and because adjustment of depth of anesthesia is especially difficult using peritoneal injection, which is most commonly used in murine studies, echocardiography should be performed in an awake state.

Similarly, it is equally advantageous to make assessment of regional wall motion abnormalities when the

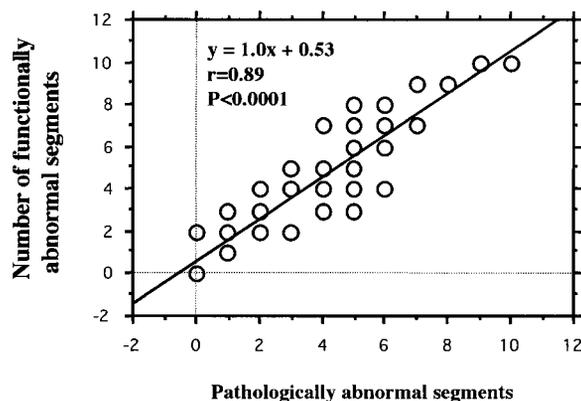


Fig. 3. Correlation between total segment number of wall motion abnormalities in echocardiography and that of hypoperfusion by pathological findings.

mice are in an awake state. Because of the anatomic considerations (see below), ligation of the left coronary artery may result in infarction and/or ischemia of the entire LV free wall. Because some anesthetic agents, e.g., xylazine, can exert negative inotropic effects, it can be difficult to identify regional wall motion abnormalities in studies performed under anesthesia.

Anatomic features of the murine heart after left coronary ligation. Mice have a single left coronary artery running on the LV anterior or lateral wall, not along the interventricular groove as in humans. A branching vessel emanating from the left anterior descending (LAD) coronary artery, which is analogous to the left circumflex artery, is often found (8). Therefore, as shown in this study, infarction was most frequently found in the lateral walls at the midpapillary level. The anterior wall was also affected, but less frequently than the lateral walls. The inferior wall, which is rarely affected by ligation of LAD in larger mammals, exhibited infarction in a significant number of animals. Because a large part of the septal wall is fed by the right coronary artery in mice, the septal wall was not significantly affected.

The apex exhibited a similar distribution of infarction. Specifically, the lateral walls were most frequently affected and the anterior wall was also significantly affected. However, the apical inferior wall was less frequently affected, possibly because of collaterals from the right coronary artery.

Limitations. Because imaging of basal segments was generally excellent and basal segments were not typically infarcted after LAD ligation, there was a high agreement between echocardiography and pathology for diagnosis of hypoperfusion. However, in the mid-papillary and apical levels, agreement between each method for a normal segment was decreased in the anterior wall. As shown in the present pathological results, these areas might frequently include the border zone between infarcted and normal area. There were technical reasons for discordant pathological and echocardiographic findings beyond those associated with analyzing echocardiographic images of small mouse hearts. During echocardiographic analysis, the orientation of the segment was determined by the end-diastolic image. In preparing the pathological specimens, we used potassium hydrochloride to attempt diastolic cardiac arrest. However, the pathological samples did not always have the same configuration as those segments in end diastole identified by echocardiography. Because of this difference of configurations between the images obtained from echocardiography and pathological samples, these areas might potentially exhibit disagreement. For the same reason, the apical level, which might have blood supply from the right coronary artery, showed decreased agreement.

In the present study, we induced regional wall motion abnormalities by ligation of only the left coronary artery, not the right coronary artery, because of technical difficulty. However, distribution of myocardial infarction is variable in each mouse because mice have

a single left coronary artery system and variable branches. Thus the present model includes various areas of myocardial infarction.

In conclusion, contrast two-dimensional echocardiography can be performed on awake, nonsedated mice, which allows for accurate assessment of function without confounding factors related to anesthesia. This technique was used for reliable diagnosis of segmental wall motion abnormalities that correlated with regions of hypoperfusion.

REFERENCES

1. Cohen JL, Cheirif J, Segar DS, Gillam LD, Gottdiener JS, Hausnerova E, and Bruns DE. Improved left ventricular endocardial border delineation and opacification with OPTISON (FS069), a new echocardiographic contrast agent. Results of a phase III multicenter trial. *J Am Coll Cardiol* 32: 746–752, 1998.
2. Fentzke RC, Korcarz CE, Shroff SG, Lin H, Sandelski J, Leiden JM, and Lang RM. Evaluation of ventricular and arterial hemodynamics in anesthetized closed-chest mice. *J Am Soc Echocardiogr* 10: 915–925, 1997.
3. Gardin JM, Siri FM, Kitsis RN, Edwards JG, and Leinwand LA. Echocardiographic assessment of left ventricular mass and systolic function in mice. *Circ Res* 76: 907–914, 1995.
4. Hoit BD, Khan ZU, Pawloski-Dahm CM, and Walsh RA. In vivo determination of left ventricular wall stress-shortening relationship in normal mice. *Am J Physiol Heart Circ Physiol* 272: H1047–H1052, 1997.
5. Hoit BD, Khoury SF, Kranias EG, Ball N, and Walsh RA. In vivo echocardiographic detection of enhanced left ventricular function in gene-targeted mice with phospholamban deficiency. *Circ Res* 77: 632–637, 1995.
6. James JF, Hewett TE, and Robbins J. Cardiac physiology in transgenic mice. *Circ Res* 82: 407–415, 1998.
7. Kass DA, Hare JM, and Georgakopoulos D. Murine cardiac function: a cautionary tail (Editorial). *Circ Res* 82: 519–522, 1998 [Erratum. *Circ Res* 83: 115, 1998].
8. Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, Hawkins HK, Berens K, and Ballantyne CM. Myocardial ischemia and reperfusion: a murine model. *Am J Physiol Heart Circ Physiol* 269: H2147–H2154, 1995.
9. Mor-Avi V, Korcarz C, Fentzke RC, Lin H, Leiden JM, and Lang RM. Quantitative evaluation of left ventricular function in a transgenic mouse model of dilated cardiomyopathy with 2-dimensional contrast echocardiography. *J Am Soc Echocardiogr* 12: 209–214, 1999.
10. Patten RD, Aronovitz MJ, Deras-Mejia L, Pandian NG, Hanak GG, Smith JJ, Mendelsohn ME, and Konstam MA. Ventricular remodeling in a mouse model of myocardial infarction. *Am J Physiol Heart Circ Physiol* 274: H1812–H1820, 1998.
11. Scherrer-Crosbie M, Steudel W, Hunziker PR, Liel-Cohen N, Ullrich R, Zapol WM, and Picard MH. Three-dimensional echocardiographic assessment of left ventricular wall motion abnormalities in mouse myocardial infarction. *J Am Soc Echocardiogr* 12: 834–840, 1999.
12. Scherrer-Crosbie M, Steudel W, Ullrich R, Hunziker PR, Liel-Cohen N, Newell J, Zaroff J, Zapol WM, and Picard MH. Echocardiographic determination of risk area size in a murine model of myocardial ischemia. *Am J Physiol Heart Circ Physiol* 277: H986–H992, 1999.
13. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, Silverman NH, and Tajik AJ. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography American Society of Echocardiography Committee on

- Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 2: 358–367, 1989.
14. **Takuma S, Suehiro K, Sciacca R, Shimizu J, Yamamoto N, Mendoza L, Wang J, Burkhoff D, Di Tullio MR, and Homma S.** Assessment of anesthetic superiority of gas anesthesia in mice echocardiography (Abstract). *J Am Soc Echocardiogr* 12: 1-380, 1999.
 15. **Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, and Ross J Jr.** Trans-thoracic echocardiography in models of cardiac disease in the mouse. *Circulation* 94: 1109–1117, 1996.
 16. **Ware JA and Simons M.** Angiogenesis in ischemic heart disease. *Nat Med* 3: 158–164, 1997.
 17. **Youn HJ, Rokosh G, Lester SJ, Simpson P, Schiller NB, and Foster E.** Two-dimensional echocardiography with a 15-MHz transducer is a promising alternative for in vivo measurement of left ventricular mass in mice. *J Am Soc Echocardiogr* 12: 70–75, 1999.

