Experimental Evaluation of the Extent of Myocardial Dyssynergy and Infarct Size by Two-dimensional Echocardiography

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SUMMARY The extent of left ventricular (LV) dyssynergy was assessed noninvasively in 19 dogs with two-dimensional echocardiographic short-axis sections during myocardial ischemia and infarction. After coronary occlusion, two-dimensional echocardiography uniformly indicated an increase in LV end-diastolic volume and a decrease in LV ejection fraction. Two-dimensional echocardiographic measurements of dyssynergy were evaluated and compared in three subgroups against (1) the extent of LV dyssynergy determined by force-gauge mapping during 10 coronary occlusions of 30-60 minutes' duration in eight open-chest dogs, (2) infarct size delineated by nitroblue tetrazolium (NBT) staining of left ventricular slabs after 48 hours of left anterior descending coronary artery (LAD) occlusion in five closed-chest dogs, and (3) NBT infarct size after 3-hour LAD occlusion followed by 45 hours of reperfusion in six closed-chest dogs. Linear regression analysis of results from these three comparisons gave good correlations (r = 0.89) for groups 1 and 2; in group 2, the extent of dyssynergy by two-dimensional echocardiography was consistently greater than infarct size by NBT. In group 3, the correlation was poor (r = 0.39). These results suggest that an adequate estimate for the extent of LV dyssynergy or infarct size may be obtained with two-dimensional echocardiography during myocardial ischemia or infarction, but not in the presence of coronary reflow, which causes an acute discrepancy between myocardial viability and function.

WE recently reported techniques for visualizing the left ventricle in dogs with two-dimensional echocardiography (2-D echo) and for quantifying left ventricular mass and volume.1-4 Preliminary studies also showed that quantitative analysis of left ventricular (LV) regional wall motion during myocardial ischemia and infarction by 2-D echo was feasible.5-6 A noninvasive method for estimating the severity and extent of myocardial dyssynergy would be extremely useful for evaluating spontaneous changes during myocardial ischemia and effects of preventive or therapeutic interventions. Meltzer et al.9 reported that estimations of myocardial dyssynergy by 2-D echo correlated well with infarct size measured by another independent technique, even during pharmacologic interventions.

In the present study, we analyzed the left ventricle using 2-D echo in both short and long-axis cross-sectional views to evaluate the extent of myocardial dyssynergy during coronary artery occlusion and reperfusion in dogs. The ultrasound measurements of dyssynergy were compared with the extent of myocardial dyssynergy estimated by force-gauge mapping and the size of myocardial infarction delineated by histochemical staining with nitroblue tetrazolium (NBT).

Methods

Experimental Procedure

Nineteen dogs that weighed 20-40 kg were anesthetized with morphine (2 mg/kg i.m.) and sodium pentobarbital (30 mg/kg i.v.) and were placed on a Harvard respirator while in the right lateral decubitus position. The dogs were separated into three groups. The eight dogs in group 1 underwent a thoracotomy through the fourth left intercostal space to expose the myocardium for the purpose of force-gauge recordings. Recordings with 2-D echo from the right chest wall were obtained before (control) and 10-20 minutes after occlusion of the proximal left anterior descending coronary artery (LAD) or circumflex coronary artery. A force-gauge recording was obtained during the control period, and force-gauge mapping of the myocardial wall in the vicinity of the occlusion was performed 20-30 minutes after occlusion of a single coronary artery. In two of the eight dogs, a second coronary artery was occluded for further comparison of 2-D echo and force-gauge mapping.

In the five closed-chest dogs in group 2, 2-D echo recordings were obtained before and 48 hours after proximal LAD occlusion. Using aseptic techniques, coronary occlusion was performed by passing an occlusive plug through a #8F, thin-wall, polyethylene catheter into a preselected location of the LAD. The dogs were allowed to recover in a special care unit and 48 hours after occlusion were reanesthetized with sodium pentobarbital. Recordings of 2-D echo from
the right chest wall were performed before occlusion of the LAD and 48 hours after occlusion. The dogs were given an overdose of sodium pentobarbital, and the hearts were excised, rapidly frozen and cut transversely into 0.7-cm-thick slabs. The site and extent of myocardial infarction were assessed in these slabs by NBT staining.

In the six closed chest-dogs in group 3, the coronary artery was occluded by inflating a #4F intracoronary balloon in the proximal LAD, as described previously. The LAD occlusion was maintained for 3 hours and followed by intracoronary balloon deflation, resulting in reperfusion, which continued for 45 hours. The dog was then reanesthetized and 2-D echo recordings were obtained; after sacrifice, the excised hearts were studied with NBT for site and extent of myocardial infarction as in group 2.

2-D Echo Examination

A phased-array, 84°, ultrasonic sector scanner (Varian Model 3000) was used to perform the 2-D echo studies. Echocardiographic examination of the heart was performed according to methods developed and extensively used in our laboratory. Briefly, with the dog on its right side, the transducer was directed upward from below against the right side of the chest at the fourth or fifth intercostal space. The transducer was tilted and moved along the right chest wall from the direction of the left ventricular base toward the apex. The location of each short-axis image was easily determined using visualized intracardiac anatomic landmarks and knowledge of cardiac section anatomy. Short-axis cross-sectional images were obtained for several levels of the left ventricle: mitral valve leaflets; high, middle and low levels of the papillary muscles; and proximity of the apex. Short-axis sections were recorded at four to eight levels as the transducer was directed in a scan from base to apex; an effort was made to record with approximately equal spacing between sections. By turning the transducer 90°, an LV long-axis cross-section was obtained. Based on anatomic considerations, specific long-axis sections could be relocated repeatedly. All short- and long-axis LV views were recorded on videotape and played back for analysis. Endocardial and epicardial outlines were traced onto a transparent paper from video stop frames in end-diastole and end-systole (fig. 1A), timed to correspond to the onset of the QRS and the end of the T-wave, respectively. Delineation of LV outlines and akinetic or dysskinetic segments was further facilitated by videotape viewing of the motion of the heart against the background of the traced stop frames. The superimposed end-diastolic and end-systolic outlines of short-axis cross-sections were closely examined to define regions of dyskinesis, akinesis or extreme hypokinesis. Although two small apical segments showed some inward motion, we included these in the region of poor function. In such small segments, it is difficult to determine the true origin of a deviation from the surrounding predominant wall motion. However, this kind of judgment was left to the discretion of the observer; thus, another observer might choose to separate the smaller regions from the large region. The potential differences in results due to observer judgments are represented by the results of the reproducibility analysis. The following sectional areas were determined by planimetry: (1) short-axis area of segments of the myocardial wall exhibiting poor function, (2) short-axis area of the epicardial outline at end-diastole, and (3) short-axis area of the endocardial outline at end-diastole and end-systole. Length of the left ventricle was defined as the distance from the mitral-aortic junction to the apex in a long-axis view. All the raw 2-D echo measurements were corrected by a calibration factor measured from a grid on the screen of the Varian 3000 system that had been checked with objects of known dimensions (phantom box).

LV volume (V) was calculated at end-diastole and end-systole from short-axis areas at four to eight levels from base to apex, and LV length (L) was calculated using Simpson's reconstruction. LV length was divided by the number of short-axis cross-sections to form an arbitrary and identical height (h) for each section. The intraluminal volume of each of the short-axis cross-sectional slabs was then calculated by multiplying the planimetered endocardial area by its height (h), except in the apical region, for which the volume was calculated using an ellipsoid-segment formula. The total intraventricular volume was then obtained by summation of volumes of individual sections:

\[
V = (A_1 + A_2 + A_3 + A_4)h + \frac{A_2h}{2} + \frac{\pi}{6}h^3
\]

For each 2-D echo short-axis section, the portion of the myocardial wall judged to be dyskinetic, akinetic or extremely hypokinetic was also planimetered to establish the extent of dyssynergy (fig. 1B). In the first group of eight open-chest dogs, separate dyssynergic areas were measured in each short-axis section for both the LV free wall and septal regions. Each of these areas was multiplied by its appropriate slab height, and corresponding volumes were summed for short-axis sections extending from base to the apex of the left ventricle. The total dyssynergic muscle volume was then multiplied by the density of myocardium (1.055) to obtain the mass of dyssynergic myocardium. In each dog, this mass was compared directly, and also as a percent of the 2-D echo reconstructed total LV mass, to the corresponding dyssynergic mass delineated by force-gauge mapping.

In the dogs in groups 2 and 3 with myocardial infarction (five and six closed-chest dogs, respectively), echocardiographic short-axis sections were first matched with the corresponding transverse LV slabs at autopsy by comparison of anatomic landmarks (e.g., mitral valve, papillary muscle) (fig. 2), and relative sectional diameters. The 2-D echo cross-sections were then examined without knowledge of NBT results for site and extent of myocardial dyssynergy. The videotape was played back at normal and slow speeds to assist in judging the site of
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Figure 1. (A) Echocardiographic short-axis section of the left ventricle at the papillary muscle level at end-diastole and end-systole. ANT = anterior; POST = posterior; LAT = lateral; SEP = septal. The continuity of endocardial and epicardial outlines at end-systole is diminished at end-diastole. (B) Echocardiographic tracing of epicardial and endocardial short-axis cross-sections at several levels of the left ventricle at both end-diastole (solid line) and end-systole (dotted line) after occlusion of the middle left anterior descending coronary artery. The akinetic region is outlined in each section (speckled area); this region was planimetered in each section and mass was obtained as described in the text. In the middle and low papillary sections, the akinetic region is located in the anterior and septal walls of the left ventricle. Note the good contraction around the entire perimeter of the mitral valve and high papillary sections. Compare with designated left ventricular levels of figure 3 from the same dog.

Data Analysis

In the study of eight open-chest dogs, the size of the dyssynergic zone was estimated by 2-D echo and force-gauge mapping and a correlation was obtained using linear regression analysis. In the studies of 11 closed-chest dogs, the size of the dyssynergic zone in LV short-axis sections by 2-D echo was correlated with NBT analysis of equivalent LV slabs.

Figure 2. Comparison of nitroblue tetrazolium stain for infarct size in one anatomic left ventricular section (right) and the corresponding two-dimensional echocardiographic analysis for dyssynergy (left). Both infarct and dyssynergy are located in the anterior and septal portions of this low left ventricular section.
with infarct size in equivalent anatomic slabs by NBT staining, using linear regression analysis.

Reproducibility

Two-dimensional echocardiographic recordings obtained by one observer from nine dogs were used to study the reproducibility of wall motion analysis. The reproducibility of 2-D echo recordings was not studied here. Assessment of the extent of wall motion abnormality was performed on 28 LV short-axis sections by two observers. The percentage of dyssnergy in each section was estimated by each observer; linear regression analysis was performed and a mean error was calculated.

Myocardial Force-gauge Mapping

In eight open-chest dogs, a miniature piezoresistive force gauge with four 1-cm-long pins was inserted into the myocardial wall at 20–25 locations in and surrounding the occluded zone (fig. 3). The very thin pins of the gauge caused minor bleeding at the epicardium, and repeated insertion and removal in two normal hearts revealed no measurable depression of developed force in that region of myocardium. For each experiment, a map of predetermined gauge locations was drawn before the coronary occlusion. After echocardiographic examination 20–30 minutes after occlusion of a coronary artery, the force gauge was inserted for about 20 seconds into each of the predetermined locations. Force recordings thus obtained are shown in figure 3. Normal function was defined as a positive systolic force development and ischemic dysfunction was defined as either zero or negative force development. At the end of the experiment, the dysfunctioning region of the myocardium delineated in this way was excised from the remaining myocardium and weighed separately for comparison with the echocardiographic determinations.

NBT Infarct Size

Hearts to be stained by NBT were frozen after excision to preserve the myocardial enzymes. The ventricular cavities were packed with cotton to prevent distortion of the cardiac contour during freezing. After 50 minutes of refrigeration, the heart was sectioned transversely from apex to base, 7 mm apart. Each slab was weighed after removal of the right ventricular free wall and incubated in NBT for 30 minutes at 37°C. The margins of the epicardium, endocardium, and unstained (infarcted) areas in the top and bottom of each section were traced onto transparent paper and planimetrized for the area and percentage of infarction for each section. Each slab was fixed in formalin for 2 days and processed for histologic slides. The margins of the infarcted areas in each section were confirmed by histologic examination. Figure 2 shows a comparison of NBT-delineated infarct size and extent of dyssnergy delineated by 2-D echo for corresponding anatomic and echocardiographic sections of the LV.

Results

In the first study, 10 occlusions were performed in eight open-chest dogs. The effects of coronary artery occlusion on LV end-diastolic volume (LVEDV) and ejection fraction derived by 2-D echo are shown in figure 4. As a result of the occlusion, LVEDV increased in eight instances and remained the same in one instance, and ejection fraction decreased in all nine instances. Mean LVEDV increased from 54.5 ± 23.1 to 70.2 ± 20.4 ml and ejection fraction declined from 0.53 ± 0.12 to 0.31 ± 0.13. These changes were statistically significant. If only the first occlusion is considered in each dog, the change in mean values remains statistically significant (p < 0.05) for both LVEDV and ejection fraction. For 10 occlusions in eight dogs, comparison of the extent of dyssnergic development and ischemic dysfunction was defined as either zero or negative force development. The speckled area of the left ventricle represents the area of ischemic dysfunctioning determined by force mapping; the myocardium was sliced along the dotted line and the excised myocardium was weighed for comparison with echocardiographic determination. The approximate location of echocardiographic short-axis sections on the left ventricle is noted here for comparison with figure 1B (same dog).
myocardium in the LV free wall estimated by 2-D echo and by force-gauge mapping (fig. 5) gave a correlation coefficient of 0.891 when results were expressed in grams of myocardium and a correlation coefficient of 0.888 when results were expressed as a percentage of the total LV mass. Standard errors of estimate were 6.8 g and 3.8%, respectively. The extent of LV free wall dyssynergy estimated by force-gauge mapping ranged from 12.2–53.1 g and from 10.7–30.0% of the LV mass.

In the second study of five closed-chest dogs that underwent a 48-hour occlusion, 16 echocardiographic short-axis sections were analyzed individually for extent of dyssynergy and compared with infarct size estimated by NBT staining of corresponding anatomic

**Figure 4.** Individual and mean changes of left ventricular end-diastolic volume (A) and ejection fraction (B) for nine coronary occlusions in eight dogs. Left ventricular end-diastolic volume (mean ± SD) increased significantly, from 54.4 ± 23.1 ml to 70.2 ± 30.4 ml, and left ventricular ejection fraction (mean ± SD) decreased significantly, from 0.53 ± 0.12% to 0.31 ± 0.13%.

**Figure 5.** (A) Comparison of two-dimensional echocardiography (2-D echo) and force-gauge mapping for quantification of dysfunctioning myocardium expressed as grams of left ventricular free wall. Ten coronary occlusions were performed in eight open-chest dogs. Linear regression analysis showed a good correlation. (B) Comparison of 2-D echo and force-gauge mapping for quantification of dysfunctioning myocardium expressed as a percentage of the left ventricular free wall mass. Ten coronary occlusions were performed in eight open-chest dogs. Linear regression analysis showed a good correlation.
short-axis sections at levels from base to LV apex. Normal echocardiographic short-axis sections with no dyssynergy were not included in this comparison. Infarct size by NBT staining ranged from 3–79% of the LV short-axis section (mean 31.8%). The resulting correlation coefficient for linear regression analysis was 0.886 (fig. 6) and the standard error of estimate was 10.6%. The data in figure 6 show that the echocardiographic extent of dyssynergy is generally greater than histochemical infarct size, but the two measurements approach an equivalence as infarct size increases.

In the third study of six closed-chest dogs that underwent a 3-hour occlusion and 45 hours of reperfusion, 16 echocardiographic short-axis sections were analyzed individually for extent of dyssynergy and compared with NBT analysis of corresponding anatomic sections. Normal echocardiographic short-axis sections with no dyssynergy were not included in this comparison. NBT staining ranged from 0–45% of the LV short-axis section; the mean value, 13.3%, was significantly smaller than the 31.8% for dogs with 48-hour occlusions. In addition, infarcts in the reperfused dogs were characteristically more patchy. In contrast to the 48-hour occlusion study, the correlation coefficient for this study was poor ($r = 0.39$) and the SEE was 8.3% (fig. 7).

The interobserver reproducibility in 2-D echo measurements of the extent of myocardial dyssynergy was evaluated in 28 short-axis sections. The percentage of each section that exhibited dyssynergy was estimated by the two observers and compared by linear regression analysis and the mean percentage difference. The correlation coefficient was 0.895 and the mean difference was 7.8 ± 1.6%.

**Discussion**

Two-dimensional echocardiography has been used to quantify LV mass and volume in dogs. The innovative feature with this technique, currently unobtainable with other methods, is the ability to delineate endocardial wall motion in vivo with a series of short-axis cross-sections from base to apex of the left ventricle. Because of the potential to characterize LV geometry and wall motion more comprehensively than previously possible, we investigated the possibility of quantifying the extent of myocardial dyssynergy during ischemia and infarction. Radial wall motion was analyzed by superimposition of end-diastolic and endsystolic images, and dyssynergy was defined as dyskinesis, akinesis or extreme hypokinesis. The reproducibility of measuring extent of dyssynergy was acceptable in that a difference of 7.8% was found between two observers. Thus, errors involved in the echocardiographic measurement of myocardial dyssynergy are probably not an important limitation to the use of the technique, at least when endocardial outlines are well defined.

In the present study, comparison of the extent of LV dyssynergy measured by 2-D echo with that estimated by myocardial force-gauge mapping showed good agreement ($r = 0.89$). Although both techniques provide estimates of the extent of LV dyssynergy, they measure different components of segmental function.
Two-dimensional echocardiography measures radial wall motion at the endocardial surface, whereas the force gauges record myocardial force development tangential to the surface of the left ventricle. Both the techniques, however, showed dramatic changes in the central ischemic region after coronary artery occlusion; radial wall motion measured echocardiographically declined from normal inward motion to either zero or outward motion, and myocardial force recorded by the gauges declined from positive to zero or negative values. Both measurements of segmental function unequivocally reflect the loss of contractile ability in the ischemic region. Thus, it is not surprising that estimates of the extent of LV dyssnergy by these two techniques correlate well. Further comparison of the echocardiographic extent of LV dyssnergy with infarct size delineated by NBT staining for individual short-axis cross-sections also correlated well \( (r = 0.89) \) for dogs with 48-hour occlusion. The implication of this finding is that the quantity of necrotic or infarcted myocardium after long periods of myocardial ischemia is reflected in the extent of myocardial dyssnergy measured echocardiographically. Inspection of data points in the linear regression analysis (fig. 6) reveals that the extent of myocardial dyssnergy measured by 2-D echo was consistently greater than the histochemical infarct size. This is not an unexpected result, as experimental evidence has indicated the possibility of a border zone of ischemic myocardium surrounding infarcted myocardium.\(^{12, 13}\)

Other reports have suggested that myocardial dysfunction appears to spread beyond the region of ischemia to involve adjacent normal myocardium during experimental coronary occlusion.\(^{14, 16}\)

In the study of Meltzer et al.,\(^{9}\) the extent of abnormal LV wall motion measured by 2-D echo correlated well with infarct size estimated by technetium pyrophosphate \( (r = 0.82) \), but the dyssynergic zone was generally smaller than the infarct size. The reason for the discrepancy between our findings and those of Meltzer and co-workers is unclear, although there are differences between the two studies. In their study, 2-D echo analysis of LV wall motion was performed primarily using the long-axis section, which provides a less comprehensive delineation of overall LV wall motion than serial short-axis sections. Use of a single 2-D echo long-axis image to estimate a zone of dyssnergy requires an assumption that wall motion abnormalities are symmetric about the plane of the long-axis section. This might be expected to yield errors in the presence of regional coronary occlusions in which the ischemic myocardium may lie predominantly on one side of the plane. Meltzer et al.\(^{9}\) suggested that more effective use of short-axis sections would possibly give better results, especially when all motion abnormalities are not symmetric about the plane of long-axis imaging. The other important difference is that histochemical staining was used to delineate infarct size in our study whereas radionuclide imaging was used in the study of Meltzer et al. It is unclear whether the two techniques measure the same indexes of myocardial ischemic injury.

In the present study, myocardial infarcts were smaller and more patchy in the dogs that underwent reperfusion than in the dogs that underwent occlusion only. Thus, as described previously,\(^{18}\) restoration of coronary arterial blood flow even after 3 hours of occlusion of the proximal LAD appears to result in the salvage of some ischemic myocardium, distributed in a patchy manner. However, the extent of echocardiographic LV dyssnergy had no consistent relationship with histochemical infarct size after coronary artery reperfusion (fig. 7). This poor correlation suggests that reperfusion of a coronary artery occlusion may cause dissociation of the normal expected relationship between tissue viability and contractile function. More specifically, reperfusion causes a prolonged depression of function in primarily ischemic but viable myocardium\(^{17}\) after only a short period of coronary occlusion (15 minutes). Even after 3 hours of LAD occlusion (as in group 3 of the present study), function in the ischemic region may return almost completely to normal after 7 days of reperfusion, with only minimal infarct size.\(^{14}\) However, the early phase of reperfusion is associated with myocardial cell swelling, edema and hemorrhages that result in inadequate coronary reflow, arrhythmias and functional derangements.\(^{15}\) These effects, in addition to the patchy nature of the infarcts after reperfusion in the present study, may have been responsible for the poor correlation between the extent of LV dyssnergy and infarct size.

Increased myocardial wall thickness in both end-diastole and end-systole also indicates the development of generalized myocardial edema with reperfusion;\(^{18}\) thus, in the present study, edema and cell swelling may have interfered with contractile function of viable muscle in the reperfused region. These observations may have important clinical implications. Some patients with acute myocardial ischemia or infarction may actually have incurred an episode of coronary artery occlusion (or insufficiency) followed by reflow. Patients that have recently undergone coronary artery bypass surgery would present a similar problem. The results of the present study suggest that if the presence of reperfusion can be ruled out in the clinical setting, the extent of LV dyssnergy by 2-D echo should indicate the extent of infarction or ischemia. Because the clinical situation most often involves multiple coronary lesions of varying degree and duration, conditions are not as simple as in the present experimental situation. Because the distinction between ischemia and infarction cannot be performed well with 2-D echo alone, clinical estimation of infarct size with 2-D echo may not be feasible.

Changes in myocardial wall motion are actually the result of numerous influences, including alterations in contractility, preload and afterload. Accordingly, a pharmacologic intervention, such as propranolol, which depresses contractility but improves perfusion and thus viability of ischemic myocardium,\(^{18}\) might increase the extent of LV dyssnergy but at the same time reduce the eventual infarct size. Thus, the use of 2-D echo short-axis images may be reliable for es-
imating the extent of dyssynergy during myocardial ischemia or infarct size during myocardial infarction, but may not be reliable for estimating infarct size in the presence of an intervention that causes an acute dissociation between myocardial viability and contractile function.

In conclusion, the present study shows that 2-D echo characterization of LV wall motion using serial short-axis sections is reproducible and may be useful in quantifying the extent of LV dyssynergy during myocardial ischemia. The echocardiographic estimation correlated well with force-gauge mapping and histochemical infarct size during coronary artery occlusion. The correlation, however, was poor after coronary artery reperfusion, which implies that echocardiographic measurement of the extent of LV dyssynergy may not reflect true infarct size after the use of interventions that cause temporary depression of contractile function in viable myocardium.

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