Evaluation of transmural distribution of viable muscle by myocardial strain profile and dobutamine stress echocardiography

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Maruo T, Nakatani S, Jin Y, Uemura K, Sugimachi M, Ueda-Ishibashi H, Kitakaze M, Ohe T, Sunagawa K, Miyatake K. Evaluation of transmural distribution of viable muscle by myocardial strain profile and dobutamine stress echocardiography. Am J Physiol Heart Circ Physiol 292: H921–H927, 2007. First published September 29, 2006; doi:10.1152/ajpheart.00019.2006.—Transmural distribution of viable myocardium in the ischemic myocardium has not been quantified and fully elucidated. To address this issue, we evaluated transmural myocardial strain profile (TMSP) in dogs with myocardial infarction using a newly developed tissue strain imaging. TMSP was obtained from the posterior wall at the epicardial left ventricular short-axis view in 13 anesthetized open-chest dogs. After control measurements, the left circumflex coronary artery was occluded for 90 min to induce subendocardial infarction (SMI). Subsequently, latex microbeads (90 μm) were injected in the same artery to create transmural infarction (TMI). In each stage, measurements were done before and after dobutamine challenge (10 μg·kg⁻¹·min⁻¹ for 10 min) to estimate transmural myocardial viability. Strain in the subendocardium in the control stage increased by dobutamine (from 53.6 ± 17.1 to 73.3 ± 21.8%, P < 0.001), whereas that in SMI and TMI stages was almost zero at baseline and did not increase significantly by dobutamine [from 0.8 ± 8.8 to 1.3 ± 7.0%, P = not significant (NS) for SMI, from −3.9 ± 5.6 to −1.9 ± 6.0%, P = NS for TMI]. Strain in the subepicardium increased by dobutamine in the control stage (from 23.9 ± 6.1 to 26.3 ± 6.4%, P < 0.05) and in the SMI stage (from 12.4 ± 7.3 to 27.1 ± 8.8%, P < 0.005), whereas that in the TMI stage did not change (from −1.0 ± 7.8 to −0.7 ± 8.3%, P = NS). In SMI, the subendocardial contraction was lost, but the subepicardial showed a significant increase in contraction with dobutamine. However, in TMI, even the subepicardial increase was not seen. Assessment of transmural strain profile using tissue strain imaging was a new and useful method to estimate transmural distribution of the viable myocardium in myocardial infarction.

Myocardial strain reflects regional myocardial function. With the recent advancement of tissue Doppler echocardiography, myocardial strain can be obtained noninvasively (3, 33) and has been reported to be useful to quantify regional myocardial systolic function in ischemic heart disease (9, 11, 24, 29, 36). The recently developed myocardial strain imaging system provides us myocardial strain in each wall layer and shows its distribution in a form of transmural myocardial strain profile (TMSP; see Ref. 1). Thus combination of TMSP and dobutamine stress echocardiography (DSE), which has been used for the assessment of myocardial viability (18), is expected to demonstrate transmural distribution of viability. There have been no methods to visualize distribution of myocardial viability over the ventricular wall in myocardial infarction, and such method would provide important information in the clinical situation.

In the present study, to assess the transmural extent of myocardial infarction, we investigated TMSP in subendocardial and transmural myocardial infarction dog models and quantified the transmural heterogeneity of myocardial viability using myocardial strain imaging with DSE.

MATERIALS AND METHODS

Experimental subjects and settings. We used 13 mongrel dogs (weighing 27.3 ± 2.2 kg). After induction with intravenous pentobarbital sodium (25 mg/kg body wt), they were anesthetized with 2% isoflurane with oxygen. A median sternotomy was performed, the pericardium was split from apex to base, and, after the instrumentation, the edges of the pericardial incision were loosely resutured. A 5-Fr. micromanometer-tipped catheter (model MPC-500; Millar Instruments, Houston, TX) was positioned in the left ventricle through the apex to obtain peak systolic left ventricular pressure and peak positive and negative dP/dt. Electrocardiogram (ECG) was monitored from limb leads. Left ventricular pressure signals and ECG were digitized online. The care and use of animals was in strict accordance with the guiding principles of the American Physiological Society, and the experimental protocol was approved by the National Cardiovascular Center Committees on Animal Experiments.

Experimental protocol. A 6-Fr. sheath was placed in the right femoral artery, and an angioplasty balloon catheter was positioned in the proximal segment of the left circumflex coronary artery by the standard catheterization technique. DSE (dobutamine infusion at 10 μg·kg⁻¹·min⁻¹ for 10 min) was used to assess myocardial viability. At the control stage, echocardiographic and hemodynamic measurements were done before and after DSE. A subendocardial myocardial infarction was created by inflating the balloon for 90 min.


- Control

<table>
<thead>
<tr>
<th>Measurement</th>
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<tr>
<td>DSE 10 min</td>
<td>DSE 10 min</td>
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- SMI by coronary artery occlusion (CAO)

<table>
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<tr>
<th>CAO 90 min</th>
<th>Measurement</th>
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<tr>
<td>Inflation</td>
<td>DSE 10 min</td>
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<tr>
<td>Deflation</td>
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- TMI by microbeads embolization (MBE)

<table>
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<tr>
<th>MBE 60 min</th>
<th>Measurement</th>
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<tr>
<td>60 min</td>
<td>DSE 10 min</td>
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Fig. 1. Experimental protocol. DSE, dobutamine stress echocardiography; SMI, subendocardial myocardial infarction; TMI, transmural myocardial infarction.

(SMI stage; see Refs. 8 and 10), and DSE was performed during balloon inflation. After balloon deflation, 200–300 mg of latex microbeads (diameter 90 μm) were slowly injected in the same artery in 60 min to create a transmural myocardial infarction (TMI stage; see Refs. 7, 12, 15). At the TMI stage, DSE was performed 60 min after microbead embolization to complete myocardial infarction and to avoid ventricular instability to dobutamine challenge, and measurements were done before and after DSE (Fig. 1).

Ultrasound data acquisition. A commercially available ultrasound scanner (PowerVision 8000 3.5-MHz transducer; Toshiba, Tokyo, Japan) was used to obtain the epicardial left ventricular short-axis images at the level of basal and midventricle by tissue Doppler imaging. Recordings were stored in the form of digital loops of two cardiac cycles with 96–102 frames/s for subsequent analysis (33).

Tissue strain imaging. Strain is defined by the equation below and expresses the deformation of an object,

$$Strain = \frac{(L - L_0)}{L_0}$$

where $L_0$ is the length of an object before deformation and $L$ is that after or during deformation. In echocardiography, $L_0$ is usually a muscle length at end diastole, and myocardial strain is used to express the deformation of local myocardial segments (4, 33).

In the present study, myocardial radial strain image was obtained from off-line analysis by using a research software TDI-Q (Toshiba; see Ref. 3). To obtain a strain image, TDI-Q software first calculates the myocardial displacement of all pixels of tissue by integrating myocardial velocity over a certain period. Because the frame rate was 96–102 frames/s, the step size for integration was 9.8–10.4 ms. Next, strain is obtained by evaluating the change of distance between pairs of two points defined on all pixels on the image by utilizing the displacement values. The distance of all two-pixel pairs at the initial time frame is equivalent to “$L_0$” on the above equation and set at 3 mm in this study (17). The initial time frame is set at end diastole to evaluate contraction; in other words “deformation” of the myocardium occurring in systole.

To measure local strain accurately, it is indispensable to obtain local velocity accurately. Therefore, the present myocardial strain imaging system has adopted tissue Doppler tracking and angle-correction techniques. Tissue Doppler tracking is an automatic motion tracking technique based on tissue Doppler information (30). By integrating a velocity of an indexed point on the ventricular wall known from tissue Doppler imaging, we can obtain myocardial displacement and predict the point where that point moves next. By repeating this procedure, the system can automatically track the motion of the point. With this technique, the influence of myocardial translation can be neglected. The angle-correction technique enables us to partly overcome the Doppler incident angle dependency that is inherent in Doppler echocardiography, as previous reports described (3, 26, 32). To correct the Doppler incident angle, a contraction center is set at the center of the left ventricular cavity at end systole in the left ventricular short-axis view. Next, the software automatically calculates the tissue velocity toward the contraction center ($V_{motion}$) by dividing the velocity toward a transducer ($V_{beam}$) by the cosine of the angle (θ) between the Doppler beam and the direction to the contraction center as follows:

$$V_{motion} = \frac{V_{beam}}{\cos\theta}$$

With the use of these two techniques, the research software TDI-Q automatically cancelled the effect of myocardial translation and angle dependency, accurately providing myocardial velocity, displacement, and strain (3). In the previously described experiments, the displacement data obtained by this method correlated with true displacement ($r = 0.99, P < 0.0001$; see Ref. 26).

Myocardial radial strain distribution over the myocardium is obtained as M-mode color-coded images, and the profile of distribution (TMSP) at end systole is shown as in Fig. 2. Bright color indicates high strain, and dark color indicates low strain. We obtained TMSP at basal and midinferolateral walls at end systole. We divided the myocardium into subendocardial and subepicardial half-layers by the midpoint of the myocardium at end systole. Mean strain values in the subendocardial half-layer and in the subepicardial half-layer were calculated by averaging strain values over each layer.

![Fig. 2. Color-coded strain imaging, M-mode strain imaging, and transmural myocardial strain profile imaging in the control stage. Left: myocardial strain imaging of the left ventricular short axis at end systole. Red color means myocardial thickening. A white bar indicates an M-mode cursor. Middle: color-coded M-mode myocardial strain imaging obtained at the left ventricular posterior wall. The subendocardium is brighter than the subepicardium, indicating that the subendocardium contracts more vigorously. Right: transmural strain profile at end systole. The strain was highest at the subendocardium and lowest at the subepicardium, and the transmural strain showed a linear profile. LV, left ventricular wall.](H922 TRANSMURAL MYOCARDIAL VIABILITY DISTRIBUTION)
Histopathological studies. Establishment of subendocardial and transmural infarction by these techniques has been confirmed in our preliminary study and other previous studies (8, 10). We assessed the degree of extension of myocardial infarct also in the present study. At the end of the SMI stage in four dogs and the TMI stage in seven dogs, the heart was excised and cut into five to seven equally distant short-axis slices. Each slice was stained with hematoxylin-eosin and Masson's trichrome (Fig. 3). A pathologist who was blind to the experimental data examined the hearts histologically and measured the degree of infarct extension at the basal and midinferolateral walls, as previously reported (2). On each enlarged photomicrograph of the hearts, three to five transmural radii in the infarcted area were traced perpendicular to the endocardial and epicardial borders. The distance from the endocardial border to the external limit of the infarcted zone was measured and was expressed as a percentage of the distance between the endocardial and epicardial borders as an index of infarct extension, 100% being fully transmural and 0% being no infarction.

Table 1. Hemodynamic parameters in control, SMI, and TMI stages

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<th>Baseline</th>
<th>DSE</th>
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<td>Control (n = 13)</td>
<td>SMI (n = 11)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>133±17</td>
<td>128±27</td>
</tr>
<tr>
<td>LVP, mmHg</td>
<td>123±10†</td>
<td>108±24</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2,169±484**‡‖</td>
<td>1,577±347†</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−2,531±408*† ‖</td>
<td>−1,824±606*†</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD; n, no. of dogs. DSE, dobutamine stress echocardiography; SMI, subendocardial myocardial infarction; TMI, transmural myocardial infarction; HR, heart rate; LVP, peak systolic left ventricular pressure; +dP/dt, peak positive dP/dt; −dP/dt, peak negative dP/dt. P < 0.05 vs. DSE values (*), vs. SMI values (‡), and vs. TMI values (†).
Reproducibility. Myocardial strain was measured by two independent observers and by one observer two times a week apart in 10 randomly selected segments to determine interobserver and intraobserver variability. The variability was assessed as the absolute difference between two measurements expressed as a percentage of their mean values. The interobserver variability was $6.5 \pm 5.5$ and $9.5 \pm 7.5\%$ for the subendocardial and subepicardial strains, respectively. The intraobserver variability was $7.2 \pm 4.9$ and $9.3 \pm 3.7\%$ for the subendocardial and subepicardial strains, respectively.

Statistical analysis. Hemodynamic data were obtained as an average of three to five consecutive beats. Statistical analyses were done with commercially available software (StatView 5.0; SAS Institute). Data are expressed as mean values $\pm$ SD. Comparisons of parameters among the stages were made by one-way ANOVA for repeated measures, followed by Scheffe’s test. The Wilcoxon signed-ranks test was used to compare parameters before and after DSE. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Hemodynamic and histopathological data. Measurements were done in 13 dogs in the control stage, in 11 dogs in the SMI stage, and in 7 dogs in the TMI stage. Because of a large infarct created by the procedure, two dogs did not survive in the SMI stage and four dogs in the TMI stage. The absolute value of peak systolic left ventricular pressure and peak positive and negative $dP/dt$ decreased gradually with the advancement of the stage. However, heart rate showed no significant changes. Both positive and negative $dP/dt$ significantly increased in response to dobutamine administration (Table 1 and Fig. 4).

The degree of infarct extension was assessed at 14 sites from 4 dogs after the SMI stage and at 20 sites from 7 dogs after the TMI stage. The infarct extension index was $24.9 \pm 7.8\%$ for the SMI stage and $76.1 \pm 9.9\%$ for the TMI stage. Typical examples of the histopathological findings for both subendocardial and transmural infarcts are shown in Fig. 3.

Strain value in each stage. Myocardial strain was obtained at 25 segments in the control stage, at 20 segments in the SMI stage, and 11 segments in the TMI stage. Figure 5 shows representative TMSPs in each stage. In the control stage, myocardial strain was highest in the subendocardium and declined linearly toward the subepicardium. After DSE, TMSP...
was uniformly uplifted, indicating the enhancement of contractility. In the SMI stage, subendocardial strain was almost zero before and after dobutamine challenge. In contrast, subepicardial strain increased after dobutamine, suggesting the presence of myocardial viability in the subepicardium. In the TMI stage, TMSP was almost flat before and after DSE, showing loss of myocardial viability through whole layers (Table 2).

Figure 6 shows changes in the subendocardial and subepicardial mean strain. Strain in the subendocardial half-layer was lower in the SMI and TMI stages than in the control stage (53.6 ± 17.1 vs. 0.8 ± 8.8 and −3.9 ± 5.6%, both *P < 0.001*).

There were no significant differences in the subendocardial strain between the SMI and TMI stages (*P = not significant (NS)*). Strain in the subepicardial half-layer was lower in the TMI stage (−1.0 ± 7.8%) than that in the SMI stage (12.4 ± 7.3%, *P < 0.001*) and that in the control stage (23.9 ± 6.1%, *P < 0.001*).

Subendocardial strain in the control stage increased with DSE (53.6 ± 17.1 vs. 73.3 ± 21.8%, *P < 0.001*), whereas that in the SMI (0.8 ± 8.8 vs. 1.3 ± 7.0%, *P = NS*) and in the TMI stage (−3.9 ± 5.6 vs. −1.9 ± 6.0%, *P = NS*) showed no significant increase. Subepicardial strain in the control stage (23.9 ± 6.1 vs. 26.3 ± 6.4%, *P < 0.05*) and in the SMI stage (12.4 ± 7.3 vs. 27.1 ± 8.8%, *P < 0.005*) increased with DSE. It did not increase after DSE in the TMI stage (−1.0 ± 7.8 vs. −0.7 ± 8.3%, *P = NS*). Subepicardial strain after DSE showed no significant differences between the control and SMI stages (*P = NS*). These results showed that myocardial viability in the subepicardium was preserved in the SMI stage, whereas that in the TMI stage was lost.

**DISCUSSION**

In the present study, we analyzed the transmural distribution of viable muscle in myocardial infarction using echocardiography. Contraction in the subendocardium was lost and did not increase with dobutamine in either subendocardial infarction or transmural infarction models. On the other hand, subepicardial contraction was increased in subendocardial infarction but not in transmural infarction. These results showed that, with TMSP, we could quantify the transmural distribution of myocardial strain and identify the transmural differences in a local inotropic reserve in the viable and infarcted myocardium. The TMSP with DSE was useful to estimate the heterogeneity of transmural myocardial viability in SMI and TMI.

**Transmural heterogeneity of myocardial viability.** The left ventricular myocardium demonstrates transmural heterogeneity of strain distribution. It has been reported that, under normal circumstances, the subendocardial myocardium receives more blood flow and consumes more oxygen than the subepicardial one (20, 28, 35). Moreover, there is a transmural gradient of contractile function in the left ventricular wall, with greatest amount of thickening occurring in the subendocardial myocardium (6, 25). Clinically, these results were noninvasively confirmed in healthy subjects with tissue Doppler tracking technique (31). In the present study, strain value in the subendocardial layer was greater than that in the subepicardial layer in the control stage, being consistent with those of previous experimental and clinical studies. The linear decline pattern in TMSP was not observed in myocardial infarction. TMSP would potentially be useful for more detailed and innovative

**Table 2. Subendocardial and subepicardial strain in control, SMI, and TMI stages**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>SMI (n = 20)</th>
<th>TMI (n = 11)</th>
<th>Control</th>
<th>SMI</th>
<th>TMI</th>
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<tbody>
<tr>
<td>Endo strain</td>
<td>53.6 ± 17.1*+‡‡</td>
<td>0.8 ± 8.8</td>
<td>−3.9 ± 5.6</td>
<td>73.3 ± 21.8‡‡</td>
<td>1.3 ± 7.0</td>
<td>−1.9 ± 6.0</td>
</tr>
<tr>
<td>Epi strain</td>
<td>23.9 ± 6.1*+‡‡</td>
<td>12.4 ± 7.3*†</td>
<td>−1.0 ± 7.8</td>
<td>26.3 ± 6.4†‡</td>
<td>27.1 ± 8.8†</td>
<td>−0.7 ± 8.3</td>
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Data are presented as means ± SD; *n*, no. of dogs. Endo strain, subendocardial strain; Epi strain, subepicardial strain. *P < 0.05 vs. DSE values (*), vs. SMI values (‡), and vs. TMI values (†).
evaluations of transmural myocardial function experimentally and clinically.

**Effect of ischemia and dobutamine on transmural heterogeneity.** After coronary artery occlusion, myocardial necrosis begins first in the endocardium and then progresses toward the epicardium with an increase in the occlusion time (8, 13). In our present study, we confirmed histologically that the SMI stage induced subendocardial infarction and the TMI stage induced transmural myocardial infarction. We observed continuous progression of myocardial dysfunction from the subendocardium to the subepicardium in myocardial infarction using echocardiography.

In acute animal models of reversible postischemic dysfunction and myocardial infarction, improved wall thickening during inotropic stimulation accurately differentiated reversible from fixed dysfunction and provided a better early assessment of viability than assessment of resting function alone (18). In clinical studies, contractile reserve by low-dose dobutamine was an independent predictor of functional recovery for myocardial infarction, which was superior to the other clinical criteria (23). In this experimental subendocardial infarction model, subendocardial strain showed no significant increase in response to inotropic stimulation, whereas subepicardial strain increased, indicating that the subepicardial myocardium was still viable. In the transmurally infarcted myocardium, myocardial strain of both subendocardium and subepicardium did not show significant increase. Therefore, the present method using TMSP and DSE is useful to visualize and quantify the contractile reserve and viability of both the subendocardium and the subepicardium.

**Clinical implications.** Because the prognosis of patients with subendocardial infarction is better than that with transmural infarction, assessment of the transmurality of myocardial necrosis and ischemia is an important clinical issue for patients with acute myocardial infarction or with chronic myocardial ischemia (22, 27). However, it has been difficult to make a diagnosis of subendocardial infarction by two-dimensional echocardiography. Some previous studies have shown that strain rate or strain echocardiography was useful to differentiate subendocardial infarction from transmural infarction (2, 34). We also obtained similar results using a new method of visualizing transmural myocardial strain distribution. Because the transmurality of necrosis is an important determinant of ultimate infarct size, its knowledge would be helpful in making therapeutic decisions for myocardial infarction (16, 34). Thus it is clinically helpful that we can quantify the transmural myocardial viability and necrosis extent. Furthermore, we could estimate the myocardial viability of each layer with DSE, enabling us to diagnose the stunned myocardium and predict myocardial functional recovery after myocardial infarction.

The present imaging system can be applied for the clinical evaluation of the various heart diseases characterized by subendocardial myocardial dysfunction such as anthracycline cardiotoxicity, syndrome X, hypertrophic cardiomyopathy, and dilated cardiomyopathy (14, 19, 21).

**Study limitations.** There was a possibility that some dogs in the subendocardial infarction models might develop transmural infarction. However, the 90-min ischemic period chosen for the subendocardial infarction model was similar to the previous studies, and it did result in subendocardial infarction (8, 10). In the present study, we showed histological evidence of subendocardial infarction after the SMI stage in parts of dogs. Furthermore, the difference in strain between the subendocardial and transmural infarction models was very prominent and consistent in each dog in the present study. These suggested that dogs after the SMI stage developed myocardial infarct almost only in the subendocardial layer.

We did not validate myocardial strain values using other methods, such as sonomicrometry. However, sonomicrometry is not always suitable to assess transmural distribution of myocardial strain, as shown in Fig. 5. We believe our measurement should be accurate because the displacement data obtained by our method were shown to be accurate (3, 21).

In conclusion, the quantitative analysis of transmural myocardial strain distribution could assess transmural differences in local inotropic reserve within the viable and infarcted myocardium. In subendocardial infarction, the subepicardial myocardial strain showed an increase in contraction with dobutamine. However, in transmural infarction, this increase was lost. Assessment of transmural strain profile using tissue strain imaging was useful to quantify transmural distribution of the viable myocardium in SMI and TMI.

**ACKNOWLEDGMENTS**

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**GRANTS**

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