EXPERIMENTAL STUDIES

Echocardiography-Derived Left Ventricular End-Systolic Regional Wall Stress and Matrix Remodeling After Experimental Myocardial Infarction

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OBJECTIVES
We tested the hypothesis that regional end-systolic left ventricular (ESLV) wall stress is associated with extracellular matrix remodeling activity after myocardial infarction (MI).

BACKGROUND
Increased left ventricular (LV) wall stress is a stimulus for LV enlargement, and echocardiography can be used to estimate regional wall stress. A powerful validation of a noninvasive method of estimating wall stress would be predicting cellular responses after a MI.

METHODS
Echocardiographic images were obtained in rats 1, 7, 14 or 21 days after coronary ligation (n = 11) or sham surgery (n = 5). End-systolic left ventricular wall stress was calculated by finite element analysis in three regions (infarcted, noninfarcted and border) from short-axis images. Matrix metalloproteinase-9 (MMP-9) and macrophage density were determined by immunohistochemistry, and positive cells were counted in high power fields (hpf).

RESULTS
Average ESLV wall stress was higher in rats with MI when compared to shams irrespective of time point (p < 0.01), and ESLV wall stress in the infarcted regions increased with time (25.1 ± 5.9 vs. 69.9 ± 4.4 kdyn/cm², day 1 vs. 21; p < 0.01). Matrix metalloproteinase-9 expression was higher in infarcted and border regions when compared to noninfarcted regions (22.1 ± 25.7 vs. 0.10 cells/hpf, respectively; p < 0.01). Over all regions, ESLV wall stress was associated with MMP-9 (r = 0.76; p < 0.001), macrophage density (r = 0.72; p < 0.001) and collagen content (r = 0.67; p < 0.001). End-systolic left ventricular wall stress was significantly higher when MMP-9 positive cell density was greater than 10 cells/hpf (45 ± 20 vs. 14 ± 10 kdyn/cm²; p < 0.001).

CONCLUSIONS
Regional increases in ESLV wall stress determined by echocardiography-based structural analysis are associated with extracellular matrix degradation activity. (J Am Coll Cardiol 1999;33:835–42) © 1999 by the American College of Cardiology

Experimental and human studies have identified left ventricular (LV) remodeling as an important prognostic factor after myocardial infarction (MI) (1–4). The extent of ventricular dilation is directly related to the magnitude of the initial damage (5–7), but subsequent changes in ventricular geometry may also be dependent on the effect of distending forces and the tissue healing process (8). It has been postulated that LV wall stress may have important consequences in infarct expansion and further structural changes in LV geometry (5), although the cellular mechanisms of these effects are not fully understood.

To date, most noninvasive attempts to measure LV wall stress have been limited to two- and three-dimensional models that were represented by simplified geometry analyses (9,10). The accuracy of these simplified models is compromised in the setting of the complex geometrical deformations that occur in the infarcted ventricle (11). Finite element analysis, an engineering technique utilized to study complex structures, can overcome some of these limitations (12–15) and can be applied to echocardiographic images. Although computational biomechanical modeling such as finite element analysis probably adds accuracy to LV wall stress measurements, any modeling methodology should be supported by biological evidence.

Dynamic pathologic processes involving extracellular matrix rearrangement mediate many of the morphological...
changes that occur after MI in both infarcted and peri-infarcted regions. Matrix metalloproteinases (MMPs) are a family of enzymes that play a crucial role in digestion of specific extracellular matrix components (16), and enhanced activity of MMPs have been demonstrated after experimental infarction (17).

In the absence of a reference standard for directly measuring stress, a powerful validation of a noninvasive method of estimating LV regional wall stress would be predicting regional pathophysiologic changes that follow an infarction. In addition, understanding the relationship between wall stress and MMP expression could provide insight into the role of mechanical forces in the processes involved in ventricular remodeling. The present study tested the hypothesis that focal MMP expression is associated with increased echocardiographically determined regional end-systolic left ventricular (ESLV) wall stress in an experimental model of MI.

**METHODS**

**Animals and surgery.** Thirty female Wistar rats, ranging in age from 8 to 12 weeks and in weight from 150 to 250 g, underwent coronary artery ligation for the production of MI. Overall survival rate was 53%. Only infarct sizes above 30% of the LV were used, resulting in 11 infarcted and 5 sham-operated animals that were included in the present study. Surgical procedures have been described in detail elsewhere (1). Briefly, animals were anesthetized with ether, intubated with a polyethylene endotracheal tube, and ventilated by a positive pressure rodent respirator (Columbus Inc., Columbus, Ohio). Following a left-sided thoracotomy, the heart was gently exteriorized and the left atrial appendage retracted to facilitate the ligation of the left coronary artery. In the sham procedure, a silk suture was inserted into the myocardium of the left ventricle and removed without ligation. All rats were housed under identical conditions and given food and water ad libitum.

**Image acquisition and hemodynamic study.** At each serial time point (1, 7, 14 and 21 days), groups of three to four rats were reanesthetized with ether to perform a hemodynamic study and to acquire echocardiographic images for structural analysis. The right carotid artery was cannulated with a 2-F Millar micromanometer that was advanced to the left ventricle. Echocardiographic and hemodynamic measurements were made under light ether anesthesia and spontaneous respiration. Short-axis two-dimensional echocardiographic images then were obtained with an ultrasound system (Sonos 1500; Hewlett-Packard Medical Products, Andover, Massachusetts) with a phased-array 5.0- to 7.5-MHz transducer. Three to five loops (each of them containing at least 10 heart beats) of LV short-axis images at the midpapillary level were recorded on S-VHS tapes. Simultaneously measurements of the systolic and end-diastolic LV pressures were made with image recordings.

**Tissue collection.** The rats were sacrificed immediately after the echocardiographic and hemodynamic studies. Their hearts were excised and the right and left ventricles were separated. A transverse section approximately 3 to 5 mm in length was obtained at the midventricular level, to assure the inclusion of papillary muscle sections. Tissue sections were embedded in O.C.T. compound (Miles, Elkhart, Indiana) and frozen in 2-methylbutane chilled with liquid nitrogen. Tissue blocks were stored at −80°C until sectioning.

**Structural analysis.** For each animal, the best transverse view of the left ventricle at the midpapillary level was chosen and, from the same frame at end-systole, the endocardial and epicardial contours were digitized. Digitized curves were imported into a computer simulations software package (IDEAS, SDRC, Inc.), and a solid surface and a finite element mesh were generated. The range of elements between models was 450 to 900, with a four-node brick element mesh. Loading conditions were applied to each individual element face on the endocardium. One node was restrained to move only in the radial direction, and one node next to the septum was restrained in all degrees of freedom. End-systolic left ventricular pressures measured simultaneously with image acquisition were used as loading pressures. The myocardial material properties were assumed to be homogeneous, isotropic and incompressible. A plane strain and minimal strain linear elastic finite element solution was employed. A Young’s modulus of $2.1 \times 10^8$ kdyn/cm$^2$ was assumed to ensure a minimal strain model, and a Poisson’s ratio of 0.49 represented near incompressibility. Von Mises stress, which represents a nondirectional parameter combining the effects of shear and normal forces, was calculated for each element and for the whole transverse section. In addition, changes in material properties were assigned to the infarcted segments according to previously reported estimates (18). Briefly, the models were solved with five- or 20-fold increases in the elastic modulus in the infarcted segment. The infarcted segment was spatially defined according to abrupt changes in wall thickness and wall motion analysis from the echocardiographic images. For simplicity, stresses derived from models that used a 20-fold increase in the elasticity modulus are reported here, as fivefold increases did not substantially change the solution from homogeneous models. Although the myocardium can behave as a nonlinear material, particularly during diastole, we did not incorporate nonlinearity.

**Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>ESLV</td>
<td>End-systolic left ventricular stress</td>
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<tr>
<td>hpf</td>
<td>High power fields</td>
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<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>TIMP</td>
<td>Tissue inhibitor of metalloproteinases</td>
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because the left ventricle deformations were considered to be very small.

The main spatial marker used to guide the correlation between echocardiographic images and histologic sections was the location of the papillary muscles. The correlations were calculated based on the assumptions that 1) the dyskinetic areas by echocardiography correspond to areas with reduced thickness in histologic sections, and 2) the pattern of infarction in rats is generally predictable. Based on these assumptions, the models were evaluated in three different standard regions: infarcted, noninfarcted and border regions (Figure 1A and C). Regional Von Mises ESLV wall stress then was calculated in these subdivisions. To standardize this assessment, these three regions were measured beginning at 0°, 90° and 180°, respectively, in all models (Fig. 1C). Regional wall stress analysis was done in duplicate by the same observer; reproducibility exceeded 99%. In addition, wall thickness was also evaluated in the predefined regions, and a mean of three measurements in each segment was used for statistical analysis.

**Immunohistochemistry.** Staining was performed on frozen sections after fixation in cold acetone (−20°C). The sections were incubated with protein block serum-free (X0909; Dako Corp., California) for 5 min and then incubated with primary antibodies at room temperature for 1 h. After washing in phosphate-buffered saline, species-appropriate biotinylated secondary antibodies (E0464; Dako A/S, Denmark) were applied, followed by avidin–peroxidase complexes (Vector Laboratories). The following primary antibodies were used: monoclonal antihuman MMP-9 (2C10; British Biotech Co., United Kingdom), monoclonal antihuman tissue inhibitor of metalloproteinases-1 (TIMP-1) (IM32L; Oncogene Science) and monoclonal antirat macrophage (ED1, Serotec Ltd., United Kingdom).

From each section, cells positive for MMP-9 and macrophages were counted in three distinct high power field (hpf) pictures in each of the three predefined regions (infarcted, noninfarcted and border) in the infarcted rats and in three random regions in the sham-operated rats. Cell counts expressed as a mean of these three high power fields.
were used to calculate the associations. Cell counting was done blindly, and intraobserver reproducibility was greater than 95%.

**Collagen content.** Myocardium collagen area fraction was determined by quantitative morphometry of sirius red-stained sections. Fresh-frozen sections (6 μm) were rinsed with distilled water and incubated with 0.1% sirius red F3BA (Polyscience Inc., Warrington, Pennsylvania) in saturated picric acid. Sections were then rinsed with 0.01 N HCl for 1 min twice and then immersed in distilled water. After dehydration with 70% ethanol for 30 s, sections were visualized under polarized light and photographed with the same exposure time for each section. The predefined regions were scanned and analyzed by morphometry using a personal computer–based quantitative 24-bit (16.2 million unique combinations) color image analysis system. Collagen area fraction was calculated as the sum of all connective tissue divided by the sum of muscle areas and connective tissue in the visual field of the section. This approach predicts the proportion of myocardium occupied by fibrillar collagen and closely correlates with the hydroxyproline concentration of the tissue (19).

**Statistical analysis.** Data are presented as mean ± standard deviation. The two-tailed Student t test and analysis of variance were used to compare normally distributed continuous variables between groups. The Tukey test was used to correct for multiple comparisons. The association between regional LV wall stress and positive cell counts was evaluated by Spearman rank coefficients. P < 0.05 was considered statistically significant.

**RESULTS**

**End-systolic left ventricular wall stress.** Infarcted rats had significantly higher average ESLV wall stress when compared to sham-operated rats (29.0 ± 1.2 vs. 4.9 ± 11.6 kdyn/cm²; p = 0.001, Fig. 1B and D), despite a consistent decrease in ESLV pressure (103 ± 11 vs. 112 ± 8 mm Hg in the shams; p < 0.05). Irrespective of the time point (n = 33 regions), infarcted regions showed a higher ESLV wall stress than border regions (42.2 ± 20 vs. 28.2 ± 12.5 kdyn/cm²; p < 0.01), and border regions had higher levels than noninfarcted segments (28.2 ± 12.5 vs. 16.5 ± 6.5 kdyn/cm²; p < 0.01). In general, LV wall stresses in both infarcted and border regions tended to increase with time. From day 1 to day 21, mean ESLV wall stress increased (p < 0.01) from 25.1 ± 5.9 to 69.9 ± 4.4 kdyn/cm² in the infarcted region, and tended to increase from 18.7 ± 5.5 to 37.9 ± 8.9 kdyn/cm² in the border region (Fig. 2).

**Immunohistochemistry.** Immunoreactive MMP-9 was detected in all infarcted rats in a reproducible pattern throughout the infarcted segments and border regions. Matrix metalloproteinase-9 expression was negligible in the sham-operated rats and in the noninfarcted regions of the infarcted rats (0.1 cell/hpf) and was higher in infarcted and border regions when compared with noninfarcted regions (22.1 vs. 25.7 vs. 0.10 cells/hpf, respectively; p < 0.01). Expression on the 1st day after the infarction was modest and restricted to the border regions, but increased sharply after 7 days. From the 1st to the 7th day after the infarction, MMP-9 expression increased (p < 0.05) from 0.4 ± 0.5 to 49.3 ± 18.4 cells/hpf in the infarcted region and from 4.4 ± 1.3 to 37.8 ± 11.5 cells/hpf in the border region. Positive cells were still present 21 days after the infarction in both infarcted (33.8 ± 7.4/hpf; Fig. 3A) and noninfarcted regions (28.8 ± 1.5/hpf). Tissue inhibitor of metalloproteinases-1 immunoreactivity was found in smooth muscle cells surrounding intramyocardial vessels and in the same areas where collagen tended to accumulate (see below) in the infarcted rats 7, 14 and 21 days after the MI (Fig. 4).

Immunoreactive macrophage distribution resembled MMP-9 distribution. Positive cells were present mainly in the border regions on the 1st day after the MI, and peaked on the 7th day after the infarction (20.3 ± 18 vs. 71.2 ± 25.2 cells/hpf from day 1 to day 7; p < 0.05). Irrespective of the time point, positive cells were concentrated in the infarcted (39.7 ± 31.3 cells/hpf) and border regions (37.8 ± 22 cells/hpf; Fig. 3B); no positive cells were observed in the noninfarcted regions. Double immunostaining revealed that some macrophages expressed MMP-9 (Fig. 3C), although there were positive cells for MMP-9 that did not stain as macrophages and some macrophages that did not express MMP-9.

**Collagen content.** At the 1st day after the MI, there was a significant increase in collagen positive area in the infarcted segments when compared with sham-operated rats (4.8 ± 1.3 vs. 11.2 ± 2.9; p < 0.01) and, as expected, collagen content in the infarcted regions increased with time (11.2 ±
2.9 vs. 41.8; from day 1 to day 21, p < 0.05). Collagen tended to accumulate next to the endocardial and epicardial surface and, interestingly, away from the regions where MMP-9 was expressed (Fig. 4).

Matrix metalloproteinase-9 and macrophages versus regional LV wall stress. As we identified an increased focal expression of immunoreactive MMP-9 and macrophages after the infarctions, we hypothesized that echocardiography-based left ventricular wall stress distribution would correlate with these two biological variables. The associations between MMP-9 and macrophage cell density with ESLV wall stress were analyzed among all predefined regions of infarcted and sham-operated rats at all time points after infarction. Regional LV wall stress among these regions correlated significantly with the number of cells stained for MMP-9 (Spearman coefficient = 0.76; p < 0.001). A significant correlation was also shown between macrophage expression and regional wall stress (r = 0.72; p < 0.001). These associations were also significant when the regions were dichotomized according to the density of positive cells. Regions with more than 10 positive cells/hpf for MMP-9 and macrophages had significantly higher regional ESLV wall stress than those with less than 10 cells/hpf (45.1 ± 20.1 vs. 14.3 ± 10.4 kdyn/cm² for MMP-9, p < 0.001; and 41.1 ± 19.7 vs. 12.6 ± 9.6 kdyn/cm² for macrophages, p < 0.001; Fig. 5). Regional collagen content was also positively associated with regional wall stress (r = 0.67; p < 0.001). Finally, wall thickness was weakly and inversely associated with regional ESLV wall stress (r = −0.33; p = 0.02), and ESLV pressure was not significantly associated with average stress (r = 0.14; p = 0.6).

Varying material properties. When the models were solved with a 20-fold increase in the elastic modulus of the infarcted tissue, there was a consistent increase in wall stress of the infarcted regions with minor changes in the noninfarcted segments. With this approach, the association between regional wall stress with MMP-9 expression (r = 0.79; p < 0.001), macrophage positivity (r = 0.76; p < 0.001) and collagen content (r = 0.68; p < 0.001) remained highly statistically significant.

DISCUSSION

In this study of experimental MI, we identified a progressive increase in regional ESLV wall stress associated with the expression of immunoreactive MMP-9 and macrophage infiltration. Compelling evidence implicates both extracellular matrix degradation and mechanical forces in the geometrical changes that occur in the infarcted ventricle (5,17). Noninvasive methods of studying LV regional wall stress may provide important information that global measures of LV function and wall stress lose. Our data suggest that regional ESLV wall stress can identify areas where biological processes are active with a higher predictive power than the simpler parameters systolic wall thickness and LV pressure.

Wall stress measurements. Myocardial stress has been traditionally viewed as an important stimulus for the remodeling of the heart, but even in the open chest animal, direct stress measurements are technically difficult (20). Noninvasive estimates of average stress in the left ventricle have proven useful in numerous clinical settings, such as evaluating contractile function in the presence of valvular disease (21–23). These estimates of LV wall stress are based on idealized LV geometries and are particularly useful when studying LV contractility under different loading conditions. However, these simplified analytic methods cannot estimate regional stress in distorted ventricles. The association of increased regional wall stress with MMP expression, macrophage density and collagen content described by
the current study provides experimental evidence supporting the concept that focal increases in wall stress represent areas of remodeling activity.

In previous studies, finite element analysis of regional LV stress has correlated with stress calculations based on models of the left ventricle represented as a noncircular cylinder with nonuniform thickness and increasing geometrical complexity (24). Lessick et al. (25) used magnetic resonance imaging and finite element analysis to demonstrate that wall stress indices increased after acute experimental ischemia, predominantly in the ischemic segments. Our data support the concept that wall stress in the infarcted area increases substantially after an acute ischemic insult and that this increase is sustained and progressive up to three weeks after the MI. Recently, finite element analysis has also been used to study LV mechanics in three-dimensional reconstruction of the canine left ventricle. These studies demonstrated that prestretching of the myocardial laminae may be a primary mechanism of residual shear stress, and that there is significant nonhomogeneity of fiber stress through different regions of the ventricle (14,15).

Extracellular matrix remodeling. The morphological rearrangement that occurs after a MI is partially mediated by pathologic processes involving reorganization of extracellular matrix components (26–28). Cleutjens et al. demonstrated a transient increase in collagenase activity in the infarcted left ventricle which began two days after the infarction, peaked at day seven and declined thereafter, together with a concomitant contribution in collagenolytic activity from gelatinases (MMP-9 and MMP-2) (17). Studies of collagen content after experimental MI also emphasize the importance of the balance between collagen synthesis and degradation in LV remodeling (28–30). In our study, MMP-9, TIMP-1 and collagen content were evaluated as mediators of the degradation pathways involved in this balance. Interestingly, regions where collagen was densely accumulated and organized were adjacent to but distinct from the areas where MMP-9 and macrophages were present. The source of MMP-9 and other MMPs in intact and damaged myocardial tissue is incompletely defined. It has been suggested that the cells responsible for MMP-13 transcription in a rat model of infarction are fibroblast-like cells and not leukocytes or endothelial cells.
(17). However, many other cells, such as neutrophils, fibroblasts and other stromal cells, can produce MMPs (30,31) in the infarcted and peri-infarcted tissue. Matrix metalloproteinase synthesis is under intricate control, involving a variety of physiologic and pharmacologic stimuli, and some evidence suggests that mechanical events could contribute to this regulation. James et al. (32) demonstrated induction of collagenase gene expression by mechanical injury in vascular smooth muscle cells. In human atherosclerotic coronary lesions, overexpression of MMP-1 occurs in regions of increased circumferential stress in the fibrous cap (33).

Limitations. In this study, ESLV wall stress measurements were based on two-dimensional short-axis reconstructions of infarcted left ventricles. Although the association between stress and matrix remodeling activity was very good, three-dimensional reconstructions could theoretically add additional accuracy to the stress calculations. It is also important to note that we chose Von Mises stress as the stress parameter, as this scalar stress parameter does not depend on the coordinate system. The authors acknowledge that most biological materials are frequently nonlinear, and the diastolic myocardium in particular is highly nonlinear and has both viscoelastic and poroelastic passive properties. Nonlinearity was not incorporated in this study because the constitutive equations for peak or end-systolic myocardial behavior are incompletely described. In addition, the left ventricle was modeled at end-systole as quasi-steady, that is, it is expected that the stiffness component dominates any viscous or inertial contributions. The maximum strains would be so small that even a highly nonlinear material law would yield identical results. The models also assumed that the stress-free state is the same as the end-systolic state. The presence of residual stress could potentially have interfered with our results (14,34).

Conclusions. We have demonstrated that regional overexpression of MMP-9 and accumulation of macrophages and collagen correlate with increased mechanical stress (end-systolic) in the infarcted left ventricle. The associations observed in this study, however, do not establish a cause and effect relationship. Further studies are needed to understand the pathways by which mechanical forces can result in expression of MMPs and activate the remodeling process.

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