Role of Collagen in Acute Myocardial Infarct Expansion

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Background. We sought to determine if damage to the myocardial collagen network was the cause of infarct expansion.

Methods and Results. Using polarized light microscopy, we examined sections from rat hearts obtained at 1, 2, 3, and 4 days after permanent coronary artery occlusion. Three features of the collagen network likely to be important in resisting infarct expansion were assessed: collagen quality, collagen quantity, and collagen organization. We observed a decrease in the number of normally birefringent collagen fibers in the infarct as early as 1 day after infarction. This decrease correlated significantly with time ($r = -0.989, p < 0.001$). In addition, we found that the fewer normally birefringent collagen fibers present, the greater the degree of infarct expansion (assessed by measurement of total left ventricular cross-sectional area). At 4 days after infarction, we noted a loss of intermyocyte collagen struts and loss of interstitial space. These changes coincided with the onset of pronounced infarct expansion. The loss of collagen struts is consistent with the concept that expansion proceeds via slippage of myocytes previously tethered by the struts. The loss of interstitial space may represent the resolution of interstitial edema, which could further weaken the ventricular wall.

Conclusions. The correlation of infarct expansion with collagen damage and the loss of support provided by collagen struts suggest that collagen is important in maintaining structural integrity after acute myocardial infarction. (Circulation 1991;84:2123–2134)

Infarct expansion has been defined as the permanent, disproportionate regional thinning and dilatation of the heart after acute myocardial infarction. Expansion of the infarcted tissue is a frequent complication of transmural infarcts and is a complication that has been associated with congestive heart failure, cardiac rupture, and increased mortality. Although mechanisms to explain infarct expansion have been proposed, the exact cause of expansion is unknown.

Most previous studies have focused on changes that occur in the myocytes. However, the myocardium is more than just myocytes, and, if appropriate stains are used, an extensive collagen network can be seen. Collagen provides strength and stiffness to the myocardium. Furthermore, the organization of the collagen provides a structural framework for the myocytes and, as part of this support, collagen fibers provide myocyte-to-myocyte connections (referred to as collagen struts) that are thought to be important in tethering the cells. Thus, the myocardium contains a relatively extensive collagen network that appears mechanically and structurally well able to resist infarct expansion. Nevertheless, expansion does occur. To reconcile this apparent paradox, our hypothesis was that infarct expansion occurs because of damage to the collagen network of the heart. To test this hypothesis, we examined three features of the collagen network likely to be important in resisting infarct expansion: collagen quality, collagen quantity, and collagen organization.

Collagen analysis was performed using polarized light microscopy. The use of polarized light microscopy exploits the birefringent properties of the collagen, enabling easy detection of collagen fibers, indirect assessment of the molecular organization of the collagen, and measurement of collagen organization. We found that infarct expansion was associated with damage to the myocardial connective tissue matrix, including the apparent loss of collagen struts.

Methods

Female retired breeder Sprague-Dawley rats (250–370 g) were anesthetized with ketamine and xylazine.
The rats were intubated and ventilated with room air, a left thoracotomy was performed, and the pericardium removed. A stitch (using Ethicon 5-0 polypropylene suture and a C-1 taper needle) was then taken from the atioventricular groove to the pulmonary cone. At this point, the animals were randomly assigned to either 1 \((n=4)\), 2 \((n=6)\), 3 \((n=6)\), or 4 \((n=5)\) days of permanent coronary artery occlusion or to sham-operated controls \((n=5)\). In the occlusion groups a double knot was tied to occlude the left coronary artery, and in the controls the suture was removed. We monitored the electrocardiogram for ST segment elevation; if no elevation was seen, additional stitches were taken until ST elevation was observed. The chest was closed and the animal removed from the respirator. After regaining consciousness, the animals were returned to their cages. On the specified day, the rats were reanesthetized and their hearts were arrested in diastole by injection of potassium chloride. The hearts were then removed, the aortas cannulated and attached to a static pressure head so that the hearts were formalin fixed at a maintained distending pressure of 100 mm Hg. After fixation, the hearts were cut parallel to the atioventricular groove in four slices and photographed.

The animal experiments conducted as part of this study conformed to the position of the American Heart Association on research animal use.

**Gross Analysis**

The photographed slices were projected at a magnification of approximately \(\times 14\) and traced. We measured left ventricular (LV) wall thickness at three equally spaced locations in the infarcted ventricular free wall and in the center of the septum from the second slice up from the apex. In addition, we measured the area of the LV cavity and the total area of the LV.

**Index of Infarct Expansion**

The degree of infarct expansion was assessed using the LV cavity area and the total LV area. Expansion of the infarct will result in an increase in total LV area. If the expansion occurred without wall thinning, then there would be an equal increase in LV cavity area; however, if wall thinning did occur, then the increase in LV cavity area would be greater than the increase in total LV area. Wall thinning can occur either by a loss of tissue area or by redistribution of the existing tissue. We calculated tissue area by subtracting LV cavity area from total LV area. All of the area measurements were normalized for the mass of the heart (see “Appendix”). In addition, we also calculated the degree of expansion by using two previously published methods: 1) LV cavity area/LV area, and 2) (LV cavity area/LV area) × (uninfarcted septal wall thickness/infarcted LV free wall thickness) modified from Hochman and Choo.

**Infarct Size Assessment**

Hematoxylin and eosin–stained sections of the heart slices were projected at a magnification of approximately \(\times 10\) and traced, and the extent of the infarcted area was marked. If the infarct was not grossly visible (which was usually the case at 1 day after infarction), the section was examined microscopically and the edge of the infarct identified by the presence of contraction bands at the infarct border. The infarcted areas were then measured by planimetry and were expressed as a percentage of the total LV tissue area.

**Histological Analysis**

The heart slices were processed for paraffin embedding and sectioned at a thickness of 7 \(\mu m\). Sections from each slice were stained with hematoxylin and eosin or picrosirius red\(^{10}\) or by using James’s silver impregnation technique.\(^{11}\) All of the histological analysis was performed on sections from the second slice up from the apex of the heart, which always contained the central region of the infarct. All analysis was done with the investigator blinded to the identity of the samples.

**Collagen Analysis**

**Collagen quality.** If collagen in the infarct is damaged, then its mechanical properties may be affected and the support that the collagen provides could be compromised; therefore, we assessed the quality of the collagen. Silver-stained sections have been used to detect damage to the myocardial connective tissue matrix in conjunction with bright-field examination\(^{12,13}\) and with polarized light microscopy.\(^{14}\) We examined collagen fibers sampled at 25-\(\mu m\) intervals across the whole thickness of the LV wall by using a Nikon Optiphot polarizing microscope and a \(\times 40\) objective lens. When viewed with polarized light, normal silver-stained collagen fibers are uniformly birefringent and appear a gold-yellow color.\(^{15}\) Each sampled fiber was graded as 1) normal, 2) having reduced birefringence, or 3) nonbirefringent. The collagen fiber grading procedure was performed at four equally spaced locations around the ventricular free wall. We then calculated the average percentage of fibers in each category for each of the sections analyzed.

**Collagen quantity.** The amount of collagen in the wall will also influence strength and stiffness. We determined the collagen content in the LV free wall from histological sections by using a video analysis system. For this analysis, we used a \(\times 10\) objective lens. A monochrome, solid-state CCD (charge-coupled device) video camera (4810 series, Cohu Inc., San Diego, Calif.) was mounted on the vertical phototube of the microscope along with a \(\times 1\) relay lens. Images were digitized by a videoframe grabber (Truevision Inc., Indianapolis, Ind.) and displayed on a high-resolution monitor (PVM 1342Q Trinitron, Sony Corp. of America, Park Ridge, N.J.). We analyzed sections stained with picrosirius red. We first saved a bright-field image of the section illuminated through a red-absorbing,
blue glass filter (Melles Griot, Irvine, Calif.). In bright-field illumination of picrosirius red–stained sections, collagen fibers appear red and the myocytes yellow. Thus, with the filter, the monochrome camera produced an image composed of dark collagen fibers and bright myocytes. We then illuminated the same field of view with circularly polarized light. The brightness of the myocytes in this image was adjusted so that it was lower than in the filtered image. The filtered image was then subtracted from the polarized image, leaving only gray collagen fibers on a black background (corresponding to the area occupied by myocytes and interstitial space). Using the Java video analysis software package (Jandel Scientific, Corte Madera, Calif.), an intensity histogram of the image was plotted. Any pixel with a gray level greater than zero (i.e., not black) represented collagen. Thus, the amount of collagen in a field of view was expressed as the area fraction of bright pixels. Collagen content determined by similar video methods has been found to correlate with biochemical assessment of collagen content.5

We calculated the percentage of collagen content in three randomly chosen regions of the midmyocardium in each sham-operated control heart and in the central region of the infarct of each heart at 4 days postinfarction. We avoided the edges of the infarct, where newly produced collagen could be seen.

Collagen orientation. The third feature of the collagen network that may be important in resisting infarct expansion is collagen organization. We measured the two-dimensional orientation of collagen fibers in the midmyocardium in both the infarcted and noninfarcted tissue of each heart. Picrosirius red–stained sections were viewed with linearly polarized light. An eyepiece reticle was used to construct a 100-point grid over the tissue, which was examined using a ×40 objective lens. The orientation of each collagen fiber intersected by the grid points (or the nearest fiber, if the point fell on a myocyte or interstitial space) was then measured as described previously.7,16 The orientation distribution obtained from each area was plotted as a circular histogram and analyzed using circular statistics.17 We calculated the mean orientation angle and the angular deviation (the circular statistics equivalent of standard deviation) of each distribution.

Interstitial Space

Although we focused on collagen, we also noticed changes in the amount of interstitial space. These changes were quantified using point counting. We examined hematoxylin and eosin–stained sections by using a 100-point eyepiece reticle in conjunction with a ×10 objective. The percent volume fraction of interstitial space (Vvis), determined according to the equation

\[
V_{vis} = \left( \frac{P_i}{P_t} \right) \times 100\%
\]

(where P, equals the number of intersections with interstitial space and P, equals the number of inter-

<table>
<thead>
<tr>
<th>Days post-MI</th>
<th>Total LV tissue area (cm²)</th>
<th>Wall thickness (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV free wall</td>
<td>Septum</td>
</tr>
<tr>
<td>Sham (n=5)</td>
<td>0.56±0.02</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>1 (n=4)</td>
<td>0.62±0.03*</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>0.58±0.01</td>
<td>0.16±0.02*</td>
</tr>
<tr>
<td>3 (n=6)</td>
<td>0.56±0.01</td>
<td>0.15±0.01*</td>
</tr>
<tr>
<td>4 (n=5)</td>
<td>0.51±0.03*</td>
<td>0.14±0.01†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

MI, myocardial infarction; LV, left ventricle; Sham, sham-operated controls.

*p<0.05, †p<0.01 vs. sham-operated controls.

sections within the reference area), was measured in the center and at both edges of the infarcted and noninfarcted tissue. The average value was then calculated for both regions.

Statistics

Comparison of values was done using analysis of variance followed by pairwise comparison with the sham-operated controls by using Dunnett’s method,18 unless otherwise stated. All values are expressed as mean±SEM and were considered to differ significantly if p<0.05.

Results

Gross Analysis

The average values of LV tissue area, LV free wall thickness, and septal thickness are shown for each of the groups in Table 1. Statistically significant thinning of the LV free wall was seen at 2, 3, and 4 days after occlusion (p<0.01 versus control). There were no changes in septal thickness. We found an increase in LV tissue area at 1 day after occlusion (p<0.05 versus control) and a decrease in area at 4 days after occlusion (p<0.05 versus control).

![Figure 1. Graph showing relation between infarct expansion and time. Average values of left ventricular (LV) cavity area and total LV area (indicators of expansion) are shown at different times after occlusion (0 represents sham-operated controls). *p<0.01 vs. sham-operated controls for both LV cavity area and total LV area. **p<0.05 vs. sham-operated controls for LV cavity area.](http://circ.ahajournals.org/doi/10.1161/CIRCULATIONAHA.117.031520)
The average corrected values of LV cavity area and total LV area are shown in Figure 1 for all groups. There was a significant increase in both total LV area \((p<0.01\) versus control) and in LV cavity area \((p<0.01\) versus control) at 4 days after occlusion. In contrast, there was a significant decrease in LV cavity area \((p<0.05\) versus control) at 1 day after occlusion.

Table 2 shows the data for LV cavity area and total LV area (uncorrected for heart mass). The decrease in area at 1 day and the increase at 4 days after occlusion parallel the results obtained from the corrected values. Table 2 also shows the values obtained for the other indexes of expansion. Both of these indexes revealed significant expansion at 4 days after occlusion; however, the modified index of Hochman and Choo\(^9\) also indicated the presence of expansion at 2 and 3 days after occlusion.

**Infarct Size**

The percentage of the left ventricle occupied by the infarct was 44.9±6.2\%, 48.6±3.9\%, 51.1±1.9\%, and 44.7±5.5\% at 1, 2, 3, and 4 days after occlusion, respectively. There was no statistically significant difference in infarct size among the four groups.

**Collagen Analysis**

**Collagen quality.** Collagen fibers in the noninfarcted regions appeared mostly gold-yellow in color after silver staining when viewed with polarized light (Figure 2A); however, many collagen fibers in infarcted regions had lost their normal uniform birefringence and had a speckled appearance. Furthermore, some fibers appeared to be no longer birefringent (Figure 2B). The damage appeared to affect thin fibers to a greater extent than thick fibers (Figure 2B). These changes in collagen birefringence were seen only in the silver-stained sections and not in the sections stained with picrosirius red.

The percentage of normally birefringent collagen fibers across the ventricular wall is shown in Figure 3 as a function of time after occlusion. In addition to collagen birefringence being influenced by molecular organization, birefringence is also orientation dependent. Birefringence decreases as the angle between the fiber and the plane of the section increases and is zero when this angle is 90°. It is because of this orientation effect that the percentage of normally birefringent fibers in the sham-operated controls is less than 100%. We observed a reduction in the percentage of collagen fibers with normal birefringence at 1 day after occlusion \((p<0.05\) versus control). There was also a significant correlation between the reduction of normally birefringent collagen fibers and time after occlusion \((r=-0.989, p<0.001)\). Along with the decrease in normal collagen fibers, there was a corresponding increase in the number of fibers with reduced or absent birefringence.

The percentage of normally birefringent collagen fibers had a negative correlation with total LV area \((r=-0.424, p<0.05)\). Thus, the fewer normally birefringent collagen fibers present, the greater the degree of expansion (Figure 4).

**Collagen quantity.** The average percent area of the wall occupied by collagen was 7.6±0.7\% in the control group and 17.2±3.5\% at 4 days after occlusion. Although some new collagen was produced at 4 days (at the edges of the infarct), the reason for the apparent increase in collagen content is probably the decrease in wall thickness found at 4 days. If the wall thickness decreases, then the proportion of collagen will increase even if the absolute amount of collagen remains the same. In the infarcted tissue, the average decrease in ventricular wall thickness at 4 days was 26%. Although changes in wall thickness may not account for all of the increase in collagen content, it appears certain that there was no appreciable loss of collagen over the course of 4 days. In addition, we never observed any large regions that were totally devoid of collagen, even at the center of the 4-day infarcts.

**Collagen orientation.** The orientation of collagen was assessed from picrosirius red-stained sections examined using polarized light. The collagen fibers appeared red-orange or yellow-green, depending on their thickness.\(^{19}\) In noninfarcted tissue, most of the

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**Table 2. Expansion Parameters**

<table>
<thead>
<tr>
<th>Days after MI</th>
<th>Uncorrected LVC (cm(^2))</th>
<th>Uncorrected LV (cm(^2))</th>
<th>LVC/LV</th>
<th>((LVC/LV)\times(S/F))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.549±0.032</td>
<td>1.128±0.068</td>
<td>48.7±1.0</td>
<td>0.400±0.041</td>
</tr>
<tr>
<td>1</td>
<td>0.397±0.017*</td>
<td>0.932±0.017*</td>
<td>43.0±1.8*</td>
<td>0.446±0.078</td>
</tr>
<tr>
<td>2</td>
<td>0.577±0.040</td>
<td>1.170±0.085</td>
<td>49.4±0.5</td>
<td>0.597±0.041†</td>
</tr>
<tr>
<td>3</td>
<td>0.580±0.055</td>
<td>1.143±0.078</td>
<td>50.5±1.2</td>
<td>0.598±0.061†</td>
</tr>
<tr>
<td>4</td>
<td>0.765±0.060*</td>
<td>1.341±0.069†</td>
<td>57.0±2.8*</td>
<td>0.688±0.075*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
MI, myocardial infarction; Sham, sham-operated controls; LVC, left ventricular cavity area; LV, left ventricular area; S, uninfarcted septal wall thickness; F, infarcted ventricular free wall thickness.

\(*p<0.01, †p<0.05\) vs. Sham-operated controls.
fibers appeared wavy but they were generally aligned parallel to the long axis of the myocytes. We also observed collagen fibers aligned at oblique angles to the muscle (Figure 5A). Such fibers are the so-called collagen struts described by Robinson et al.4 In the infarcted tissue at 4 days after occlusion, the number of these struts appeared to be substantially reduced (Figure 5B).

We attempted to quantify these changes by measuring the orientation of the collagen fibers. Representative examples of the orientation distributions obtained from infarcted and noninfarcted tissue are shown in Figure 6. The collagen struts are represented by the outliers in the control distribution, which in this particular case are aligned at 45° or more from the mean of the distribution. The distribution from infarcted tissue at 4 days after occlusion contains no such outliers (i.e., no struts). The orientation distributions were compared statistically using Kuiper's test.17 Only at 4 days after occlusion did any of the collagen orientation distributions in the infarct differ from those obtained in noninfarcted tissue (three of five samples, p<0.05).

The presence or absence of collagen struts will be reflected in the angular deviation of the orientation distributions. The average angular deviation of the distributions for each group in both infarcted and noninfarcted tissue is shown in Figure 7. At 1 day after occlusion, there was a significant increase in the angular deviation of the collagen distributions in both infarcted and noninfarcted regions compared with control (p<0.05). This increase occurred because of an increase in waviness of the collagen. The angular deviations of the distributions obtained at 2 and 3 days after occlusion were not different from control values. In contrast, there was a significant decrease (p<0.05 versus control) in angular deviation in the 4-day infarcts. Two factors contributed to this decrease at 4 days after occlusion: 1) the collagen fibers became less wavy, and 2) there was an apparent loss of obliquely aligned collagen fibers (collagen struts). Such obliquely aligned collagen fibers were present in all of the distributions obtained from the other groups. To further analyze the distributions, we determined the number of collagen fibers that were aligned at more than 30° from the mean of the distribution (Table 3). The number of such fibers was significantly reduced in infarcted tissue (p<0.05, t test with Bonferroni correction for multiple comparisons) at 4 days after occlusion. Thus, visual inspection of the tissue in addition to analysis of the orientation distributions indicated that the number of collagen struts in the infarcted tissue was reduced at 4 days after occlusion.

**Interstitial Space**

The percent volume fraction of interstitial space in noninfarcted tissue was 11.1±0.9%. There was no significant change in interstitial space at either 1 (8.3±1.0%), 2 (10.0±1.6%), or 3 (10.1±0.9%) days after occlusion; however, interstitial space was significantly reduced at 4 (5.6±1.0%) days after occlusion (p<0.01 versus noninfarcted tissue).

**Discussion**

We found statistically significant damage to the myocardial collagen network as early as 1 day after
infarction. The extent of collagen damage correlated with total LV area (a marker for the degree of expansion), suggesting that collagen damage does play a role in infarct expansion. However, significant infarct expansion did not occur until 4 days after infarction. At that time, additional collagen damage was noted in the form of loss of collagen struts. Thus, it appears that the loss of struts may provide the extra damage necessary to allow significant expansion.

The apparent discrepancy between the results obtained using our index of expansion and that derived from Hochman and Choo\(^9\) arises from the definitions used. Previously published work has defined expansion to include both thinning and dilatation.\(^1\,\,^9\) The expansion index that we used (total LV cross-sectional area) is actually an index of dilatation. Both ratios in the modified index of Hochman and Choo are influenced by wall thickness. Therefore, the significant expansion seen at 2 and 3 days using the latter index (which was not indicated by our index) is probably caused by wall thinning without dilatation.

The finding of collagen damage after ischemia is consistent with previous studies. Using electron microscopy, Sato et al.\(^{20}\) observed collagen damage after only 40 minutes of ischemia in porcine myocardium. Silver-stained sections have also been used to detect apparent collagen degradation in both human and canine hearts several days after myocardial infarction.\(^{12}\) In the rat model of permanent coronary artery occlusion, biochemical assay for hydroxyproline has been used to detect collagen degradation 24 hours after occlusion.\(^{21}\)

What form does the collagen damage take? In the current study, we observed two kinds of collagen damage. The first was the loss of birefringence in silver-impregnated sections. Silver is thought to be bound by carbohydrates associated with the collagen fibers.\(^{15}\,\,^{22}\) When a birefringent material such as collagen is stained, the optical properties of the stained fiber will be determined by how the stain is bound. In the infarct, the collagen fibers were impregnated by silver and appeared black under bright-field observation; however, some of the fibers had reduced or absent birefringence. It therefore appears

<table>
<thead>
<tr>
<th>Days after occlusion</th>
<th>Sham</th>
<th>I</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.0±0.8</td>
<td>10.8±2.2</td>
<td>6.0±0.8</td>
</tr>
<tr>
<td>2</td>
<td>13.0±2.0</td>
<td>10.4±2.2</td>
<td>13.0±2.0</td>
</tr>
<tr>
<td>3</td>
<td>8.8±1.5</td>
<td>5.2±1.6</td>
<td>8.8±1.5</td>
</tr>
<tr>
<td>4</td>
<td>5.8±0.7</td>
<td>4.3±1.1</td>
<td>5.8±0.7</td>
</tr>
<tr>
<td>5</td>
<td>7.0±1.7</td>
<td>1.4±0.7*</td>
<td>7.0±1.7</td>
</tr>
</tbody>
</table>

Values represent percentage of collagen fibers aligned more than 30° from the mean of their distribution. The apparent increase at 1 day after occlusion reflects an increase in fiber waviness rather than an increase in the numbers of struts (see text).

Sham, sham-operated controls; N, noninfarcted tissue; I, infarcted tissue.

*p<0.05 vs. noninfarcted tissue value.

Figure 6. Circular histograms showing orientation of collagen fibers from a noninfarcted sham-operated control and from infarcted tissue at 4 days after occlusion. Width of each histogram bar represents 5°. Scale to the left of the histograms indicates percentage frequency of collagen fibers with a particular orientation. Zero on the inner scale denotes mean of the distribution; angular deviation (S) is shown in the center of each circle.

Figure 7. Bar graph shows average angular deviations of collagen fiber orientation distributions in noninfarcted tissue (panel A) (0 represents sham-operated controls). There was a significant increase in angular deviation at 1 day after infarction (p<0.05), indicating increased waviness of the collagen fibers. In infarcted tissue (panel B) there was an increase in angular deviation at 1 day after occlusion (p<0.05) and a decrease at 4 days after occlusion (p<0.02).
that the silver particles are no longer bound in the same way, perhaps because of degradation of the ground substance. We saw no similar reduction in birefringence of collagen fibers stained with picrosiris red, which is thought to bind directly to basic amino acid groups of the collagen molecule. Degradation of ground substance attached to collagen has been reported after myocardial ischemia; Sato et al. observed that glycoproteins bound to collagen fibrils were substantially reduced after 40 minutes of ischemia and virtually absent after 2 hours.

The second kind of collagen damage that we found was the loss of collagen struts that form lateral connections between neighboring myocytes. The apparent disappearance of the struts occurred at 4 days after infarction. Could the loss of struts be due to breakage of collagen fibers by overdistension during fixation? It has been reported that perimysial collagen fibers, which run parallel to the myocytes, are predominantly coiled at low (30 mm Hg) distending pressure but become straighter as pressure is increased. At distending pressures of 100 mm Hg and above, collagen fibers have been described as being virtually all straight with some torn fibers. This did not appear to be the case in our study. The measured orientation of the fibers shown in the orientation distributions indicates that the collagen fibers retain a degree of waviness. This waviness is also illustrated in Figure 5. In addition, the use of polarized light microscopy revealed waviness within fiber bundles in which the overall appearance was straight. Furthermore, loss of struts was only seen in the infarcted tissue at 4 days; uninfarcted tissue from the same hearts still contained struts. It might also be argued that the apparent loss of struts was an artifact produced by changes in the sectioning plane. However, as we measured and compared midmyocardial collagen fiber orientations in two regions of the same section, any deviation from longitudinal sectioning of the midmyocardium would affect both regions. Thus, it does not appear that the loss of collagen struts is an artifact produced either by our fixation or sectioning procedures.

We attempted to quantify the loss of struts in several ways. Statistical comparison of the orientation distributions in infarcted and noninfarcted tissue revealed that differences occurred only at 4 days (three of five samples) after occlusion, a difference caused in part by the loss of collagen struts. However, the method used (Kuiper’s test) does not adequately reflect the loss of struts because struts constitute a relatively small proportion of the total myocardial collagen content. The angular deviation of the distributions (Figure 7) was significantly lower at 4 days after occlusion, also indicating loss of struts. Similarly, the number of fibers aligned at more than 30° from the mean of the distribution was reduced in the infarct at 4 days (Table 3). Although collagen fibers with such orientations are not exclusively struts (the increase in the number of such fibers at 1 day after occlusion reflects increased waviness rather than an increased number of struts), their number certainly includes struts. Thus, quantitative analysis confirmed the qualitative observation that there was a reduction in the number of collagen struts present in the infarct at 4 days after occlusion.

Although we did find reduced numbers of collagen struts, we did not see the large loss of collagen fibers that had been reported previously. In the central regions of both human and canine infarcts, Factor et al. reported a virtual absence of normal silver-impregnated collagen. The discrepancy with our study may have resulted from differences in tissue preparation (frozen versus paraffin-embedded sections) between the two studies. However, our results do not preclude the loss of collagen fibrils smaller than the resolving power of our microscope.

What are the consequences of the observed collagen damage? We found that the decrease in the number of normally birefringent collagen fibrils correlated with infarct expansion. This finding suggests that the collagen is no longer as stiff as it was before occlusion; that is, for the same distending pressure, there was a greater degree of expansion in hearts with fewer normally birefringent collagen fibers. Collagen damage produced in the absence of myocyte necrosis by repeated brief occlusion of the left anterior descending artery has been found to result in an increase in systolic bulging, which may also suggest a decrease in collagen stiffness. Ground substance has been shown to be an important factor in the mechanical properties of collagen fibers. For example, tendons treated with α-amylase, which removes glycoproteins, exhibited decreased tensile strength and decreased stiffness. Thus, although the reason for the reduction in collagen birefringence that we observed is unknown, it is possible that the reduction in birefringence may be accompanied by a change in the mechanical properties of the collagen.

Collagen struts are thought to tether the myocytes, preventing overdistention of the sarcomeres and myocyte slippage. Therefore, if the struts are broken, the support provided by the collagen will be compromised, and myocytes will be free to slip past their neighbors. The concept of myocyte slippage has been invoked to explain infarct expansion. In the proposed model, thinning of the wall and dilatation of the ventricle occur by rearrangement of either individual myocytes or myocyte bundles. In rat myocardium examined 1–3 days after infarction, Weisman et al. found that the number of myocytes across the wall decreased as wall thickness decreased. The decrease in cell number was accompanied by a relatively small increase in cell density. The investigators therefore concluded that infarct expansion was mediated primarily by rearrangement of the cells, with a small contribution from stretching of myocytes. The authors suggested slippage of myocyte bundles rather than slippage of individual myocytes as the mechanism of expansion for two reasons: They noted changes in the relative orientation of subendocardial muscle bundles associated with expansion,
and they did not find any evidence for separation of individual myocytes. Using transmission electron microscopy, they observed that collagen struts linking myocytes within bundles were still present after infarction. However, their ultrastructural examination was limited to tissue obtained 1 day after infarction, a time when we also found no loss of collagen struts. We were unable to perform an analysis of the number of cells across the wall because the myocytes had frequently been replaced or obscured by inflammatory cells. However, our finding of collagen damage including loss of struts is consistent with the concept of slippage: Cells or bundles of cells will be unable to slip unless the collagen fibers that tether them are damaged. A similar lack of collagen struts has been reported in patients with dilated cardiomyopathy.27 Again, the resulting loss of intermyocyte tethering was suggested as the mechanism underlying the dilatation.

The ultimate test of the hypothesis that collagen is crucial in maintaining the structural integrity of the myocardium would be to prevent the collagen damage and thus prevent infarct expansion. However, before this hypothesis can be tested, it is important to know what causes the collagen damage. At present, the cause of the damage is unknown. Even though Cannon et al21 found collagen degradation at 24 hours after infarction, they found no degradation in rats made leukopenic by exposure to whole-body radiation. They therefore attributed collagen degradation to the action of inflammatory cell proteases. However, in our study, damaged collagen was seen in regions of the myocardium devoid of inflammatory cells. Collagen damage has also been found in canine myocardium after repeated brief coronary artery occlusions before polymorphonuclear leukocytes have infiltrated the area.13,14 Therefore, although leukocytes are certainly capable of degrading collagen and may in fact do so after infarction, there appear to be other mechanisms involved.

In addition to the collagen damage in infarcted tissue, we observed that the orientation distributions of collagen in both the infarcted and noninfarcted regions at 1 day after infarction had higher values of angular deviation than sham-operated controls; that is, the fibers were wavier. Why should these fibers be wavier than control when they were fixed at the same distending pressure? Collagen fibers become straighter as increasing strain is applied.28 Therefore, at 1 day after infarction, the fibers appear to be under less strain than control. We also found that the LV cavity was significantly smaller at 1 day after infarction. These findings suggest an increase in wall stiffness. Furthermore, we found a significant increase in tissue area and a trend toward a decrease in interstitial space. The presence of intracellular edema may explain these findings. Intracellular edema, which is an early indicator of cell damage, causes cell swelling, thus increasing tissue area and decreasing interstitial space. Later, as damage to the cell membrane increases, water would leak out of the cells and the edema would eventually be resolved. A significant increase in the water content of the infarct has been found in rats at 24 hours after infarction.29 The presence of swollen myocytes is also likely to increase the stiffness of the infarcted wall. Raya et al30 found an increase in the stiffness constant calculated from analysis of myocardial pressure–volume curves at 1 day after infarction compared with controls, which suggests an early increase in the passive stiffness of the infarcted ventricle. Mannisi et al29 found that rats treated with methylprednisolone, which completely suppressed the increase in edema at 24 hours, exhibited more pronounced early expansion than saline-treated controls. The investigators speculated that edema may be a factor in limiting early expansion.

We observed a significant loss of interstitial space at 4 days after infarction that coincided with the onset of pronounced infarct expansion. Loss of interstitial space could occur either because of an increase in absolute collagen content or a decrease in edema. Although some new collagen could be seen, it was located at the edges of the 4-day infarcts (regions that were avoided for both the collagen content and interstitial space measurements). In addition, new collagen fibers would be thinner than most of the existing fibers and so would appear green when stained with picrosirius red and viewed with polarized light.19 Large amounts of green collagen fibers were not seen in the 4-day infarcts except at the edges of the infarcts. Thus, the loss of interstitial space that we observed appears to be due to a decrease in edema. From the discussion in the preceding paragraph, it is tempting to speculate that resolution of edema (marked by the decrease in interstitial space) deprives the infarct of support, which, coupled with the collagen damage, allows pronounced expansion.

The concept that collagen is important in maintaining the structural integrity of the myocardium after infarction is supported by other studies. For example, Przyklenk et al31 found that the stiffness and tensile strength of myocardium correlated directly with collagen content. In a review of ventricular remodeling after myocardial infarction, Pfeffer and Braunwald32 stated that ventricular enlargement could be influenced by three interdependent factors: 1) infarct size, 2) infarct healing, and 3) ventricular wall stress. Each of these factors may be explained in terms of collagen damage. 1) Reduction of infarct size salvages muscle but may also salvage collagen. Presumably collagen in an area of salvaged muscle would no longer be exposed to agents responsible for collagen degradation and so collagen damage would be reduced. The location of the salvaged collagen may be the most important consideration. Early reperfusion of an occluded artery to salvage epicardial muscle significantly reduces the incidence of infarct expansion. If the salvaged epicardial tissue contained undamaged collagen, the ventricle would not expand because the undamaged collagen is suf-
sufficiently stiff to prevent expansion. 2) Infarct expansion appears to occur before appreciable amounts of collagen have been deposited in the infarct. Once collagen has been deposited in the infarct, the scar appears able to resist further expansion. These findings again suggest that normal collagen is able to withstand expansion but that damaged collagen is vulnerable. 3) Similar arguments apply when considering the third proposed mediator of ventricular enlargement, ventricular wall stress. Increased ventricular wall stress produced either by increased blood pressure or by increased afterload results in increased infarct expansion; ischemia-mediated damage would make the myocardial collagen less able to resist the increase in ventricular wall stress.

Summary

The myocardial collagen network is damaged after myocardial infarction. The extent of the collagen damage correlated with the degree of infarct expansion, and pronounced expansion appeared to occur after the loss of collagen struts. The finding of collagen damage appears to reconcile the paradox of how expansion can occur in a relatively stiff structure and also provides a unifying theme that is consistent with previous experimental and clinical data regarding factors that mediate expansion.

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Appendix

Many different methods have been used to quantify infarct expansion, ranging from semiquantitative scoring systems to dimensionless form factors to assess endocardial and epicardial surface contours. Infarct expansion will produce an increase in both LV cavity area and total LV cross-sectional area. These parameters appeared to be the simplest means of assessing expansion. However, such area measurements will be a function of the mass of the heart. We therefore determined the relation between total heart mass and area: Assuming that the heart is spherical, the volume of the heart, $V$, is given by the equation $V = \frac{4}{3} \pi r^3$, where $r$ is the radius of the sphere. The area, $A$, of a cross-section through the heart is given by $A = \pi r^2$; therefore, $V = A^{3/2}$. However, $V = m/r$, where $r$ is the density and $m$ the mass of the heart, thus $V = m/r$; therefore, substituting for $V$, $m = A^{3/2}$.

Therefore, to normalize the area measurements, the values should be divided by $m^{3/2}$. Figure 8A shows the non-normalized values of total LV area plotted as a function of heart mass; LV area increases as heart mass increases. There was a significant correlation between area and (mass) $m^{3/2}$ ($r = 0.948, p < 0.001$). When the values are normalized, the dependence of area on mass is removed (Figure 8B). The scatter in the values about the ideal horizontal line is partly caused by the presence of expansion in some of the hearts.

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