Biomechanical Properties of Reperfused Transmural Myocardial Infarcts in Rabbits
During the First Week After Infarction
Implications for Left Ventricular Rupture

Carolyn M. Connelly, Soeun Ngoy, Frederick J. Schoen, and Carl S. Apstein

Left ventricular (LV) rupture potential was studied after transmural myocardial infarction (MI) in rabbits by measuring 1) the tensile strength of infarcted tissue strips, 2) the force required to initiate a tear (tear threshold) in the central infarcted region, and 3) the intracavitary pressure required to rupture the infarcted ventricle. During the first week after MI, infarcts resulting from a permanent coronary occlusion were compared with infarcts reperfused “late” (i.e., 3 hours) after coronary occlusion with a resultant hemorrhagic transmural infarct but no reduction in infarct size. The reperfused hemorrhagic infarcted strips had less tensile strength than strips from permanently occluded infarcts in the initial 24 hours after MI (16±1 versus 24±3 g/mm², p < 0.05), but the tear threshold and response to increased LV pressure were not influenced by infarct reperfusion at this time. By 3 days after MI, reperfused infarcts had equal tensile strength, had greater resistance to infarct tearing, and could withstand a greater LV distending pressure compared with permanently occluded infarcts. By 5 days after MI, reperfused infarcts maintained a greater tear threshold but had less tensile strength than permanently occluded infarcts, although all infarct values were equivalent or greater than normal LV values. By 7 days after MI, reperfused and permanently occluded infarcts were equally strong by all measurements. Thus, late reperfusion of transmural infarcts increased resistance to infarct tearing and LV rupture above that of nonreperfused permanently occluded infarcts by 3 days after MI and enhanced tissue strength after an initial 24-hour vulnerable period. These findings suggest that late reperfusion may accelerate myocardial healing after MI. (Circulation Research 1992;71:401–413)

Key Words • coronary occlusion • reperfusion • myocardial tearing • infarct healing • tear threshold • myocardial rupture

Left ventricular (LV) myocardial rupture is an important cause of mortality after acute myocardial infarction (MI) and occurs in an estimated 4–40% of post-MI deaths.1–8 It is thought that LV rupture occurs when transmural infarcts expand and stretch to the point of breachage within the first few days after MI,9,10 although rupture can also occur in the absence of wall thinning and at later times after MI.6,11 Suggested factors in LV rupture include hypertension and increased LV wall stress,2,4,8,12,13 decreased strength of infarcted tissue,10,14,15 increased inflammatory response,16,17 breakdown of the connective tissue network,16,18 and intramyocardial tearing.2,6,12,19

The effect of thrombolytic therapy on the incidence of cardiac rupture is currently being investigated,20,21 and a retrospective meta-analysis suggests that thrombolytic therapy potentiates cardiac rupture.22 Early thrombolytic therapy can reduce infarct size, and such infarct size reduction should reduce the risk of rupture.20,21 However, the effect of late reperfusion (i.e., too late to reduce infarct size) on post-MI healing and prognosis is controversial. Previous work from our laboratory has shown that a 1-hour coronary occlusion causes a transmural infarct in the rabbit, with no salvage of ischemic myocardium or reduction in infarct size if reperfusion occurs later than 1 hour after a coronary occlusion.23 Therefore, in the current study, we used a protocol of “late” reperfusion, where reperfusion occurred at 3 hours after coronary occlusion to ensure that such reperfusion would not reduce infarct size but could only affect post-MI healing. A previous study from our laboratory showed that such late reperfusion resulted in 3-week-old scar tissue with less collagen cross-linking and, subsequently, lower tensile strength than scar tissue from nonreperfused infarcts.14

The purpose of the current study was to measure the influence of late reperfusion on early infarct healing, independent of any effect of reperfusion to reduce infarct size. Therefore, we tested the LV rupture poten-
tial in late reperfused and nonreperfused rabbit infarcts during the first week of post-MI healing. Three different measurements were used to assess and compare the biomechanical properties of infarcted and normal LV tissue: 1) the tensile strength of tissue strips, 2) the force required to produce a tear through the infarct center ("tear threshold"), and 3) the distending pressure the infarcted ventricle could withstand without rupturing ("rupture threshold"). When rupture occurred, the site of rupture, through the infarcted region or the noninfarcted region, was also noted.

Materials and Methods

Coronary occlusion at the midpoint of the first marginal coronary artery in the rabbit of more than 1 hour in duration results in a transmural infarct encompassing approximately 25% of the LV in the anterolateral and apical regions at 24 hours after MI. Accordingly, New Zealand White male rabbits (1.5–2.0 kg) were anesthetized with sodium pentobarbital (50 mg/kg), intubated, and mechanically ventilated with a respirator (Harvard Apparatus, South Natick, Mass.) while a left thoracotomy was performed. The heart was suspended in a pericardial cradle, and the large marginal branch of the circumflex coronary artery was identified. The first marginal artery was either permanently occluded with 4-0 silk halfway between the apex and the atroioventricular groove (nonreperfused infarcts), or the artery was temporarily occluded with a snare ligation for 3 hours and then released to create a hemorrhagic reperfused transmural infarct without reducing infarct size (reperfused infarcts). Reflow was confirmed by the reedilation of the distal vessel and the appearance of reactive hyperemia. Penicillin (2,250 units/kg) was given after surgery to prevent infection.

Tensile Strength Study

Of the 94 animals undergoing surgery for the tensile strength study, 16 (17%) died before they could be killed for study (12 from the reperfused group and four from the nonreperfused group). Six animals had no infarct on inspection (6%). Thirty-six animals each were used for the reperfused groups and the nonreperfused groups and were killed at 1, 3, 5, and 7 days after infarction by an overdose of sodium pentobarbital. Eleven hearts from normal noninfarcted rabbits were used for control measurements.

The right and left atria and right ventricle were separated from the LV of each heart. The LV was opened by an incision along the anterior septum, and the infarct was separated from the myocardium and placed in oxygenated saline. Vertical strips of central infarcted tissue were excised by two parallel cuts along the long axis between the papillary muscles. The strips of infarcted tissue were clamped between two jaws of a mechanical testing device consisting of a constant-rate step motor, force transducer, and strip-chart recorder. After the resting length (Lo) of the infarcted strip was measured, the jaws were separated at a strain rate of 1 mm/sec until breakage of the tissue occurred. The force at the point of tissue breakage was determined from the chart recorder, and the length increase (∆L) was recorded by the strip-chart tracing, which was directly linked to the amount of jaw separation. Tensile strength was derived by calculating the force/cross-sectional area (CSA) at the point of tissue breakage. Strips were removed from the clamps and weighed to determine CSA by the following method:

\[
\text{Density} = \frac{\text{mass}}{\text{volume}} = \frac{\text{mass}}{(\text{length} \times \text{CSA})} = 1 \text{ g/cm}^3
\]

If

\[
\text{Length at rupture} = (\text{Lo} + \Delta L)
\]

then

\[
1 = \frac{\text{mass}}{((\text{Lo} + \Delta L) \times \text{CSA})} \quad \text{or} \quad \text{CSA} = \frac{\text{mass}}{(\text{Lo} + \Delta L)}
\]

Tear Threshold Study

Of the 109 rabbits entered in the tear threshold study, 19 died before the study began (17%, 14 from the reperfused group and five from the nonreperfused group), four were eliminated because of technical problems, and one was eliminated because of infection. The remaining study animals consisted of 46 nonreperfused infarcted and 39 reperfused infarcted rabbits.

At 1, 2, 3, 5, or 7 days after MI, the animals were killed, and their hearts were removed and placed in oxygenated saline. The right ventricle, atria, and septum were excised, leaving the LV free wall, which contained the anterior apical transmural infarct. A 4-0 silk suture was passed completely through the center of the infarct, from endocardium to epicardium, and the suture ends were tied to form a loop. The normal noninfarcted base of the LV free wall was clamped in a moveable jaw attached to a step motor. The suture loop was attached to a fixed second clamp connected to a strain gauge (force displacement transducer model FT03C, Grass Instrument Co., Quincy, Mass.). The step motor separated the jaws at a rate of 1 mm/sec until the increasing tension on the suture ultimately caused a tear in the infarcted or noninfarcted tissue as indicated by an abrupt decrease in suture tension (Figure 1).

Tear threshold was defined as the force required to initiate a tear through the infarct center. The suture either tore completely through the infarcted tissue with the force decreasing to zero (Figure 1B, complete tear), or the suture did not pull completely through the tissue but only created a rent in the infarct, and force did not decrease to zero after the partial tear occurred (Figure 1C, partial tear). Six normal noninfarcted rabbit hearts were tested with the suture placed through the anterolateral LV wall 5 mm from the apex, approximating the location of the infarct center.

Infarct and LV wall thicknesses were measured with a Vernier caliper. The infarcted region was dissected free and weighed separately from the remaining noninfarcted LV. An approximation of infarcted surface area was derived by dividing the weight of the infarcted tissue by the thickness. Tear threshold was "normalized" and expressed as force/tissue thickness. The time required to reach the tear threshold and the incidence of a complete or partial tear were noted.

LV Distending Pressure/Rupture Threshold Study

Of the 72 rabbits prepared for the LV distending pressure/rupture threshold study, 12 died before the study began (17%, five each in the reperfusion and permanently occluded groups after the occlusion and two before the occlusion). Five rabbits showed no infarct at the time of the study and were subsequently...
used as sham controls. Nine hearts were eliminated because of technical problems, resulting in a total of 23 rabbits with reperfused infarcts and 23 rabbits with nonreperfused infarcts available for the study.

The rabbits were killed at 1 and 3 days after MI, and the intact hearts were removed and placed in cold saline. A double-lumen balloon catheter was inserted into the LV through the left atrium across the mitral valve and secured with umbilical tape around the atrioventricular groove. One catheter was attached to a pressure transducer connected to a recorder, and the other catheter was attached to a stopcock and a 10-ml syringe filled with 0.9% saline connected to a mechanical pump. The pump injected pulses of saline from the syringe into the balloon within the LV chamber at a rate of 4 Hz. Additional saline was added through the stopcock to increase intracavitary pressure by 100-mm Hg increments every 2 minutes until the balloon ruptured through the LV.

The rupture location and the pressure (P) at which the LV ruptured were recorded. The LV or infarct wall thickness (Th) at the site of rupture was measured, and the balloon volume was used to derive the radius r (volume=4/3πr³) at rupture. LV wall stress at the rupture site was derived by the law of Laplace: wall stress=(0.01363)×P×r/2×Th.¹⁴

**Histological Analysis**

An additional 24 hearts were prepared for histology, including three from each reperfused and nonreperfused infarct group at 1, 3, 5, and 7 days after MI. Midventricular cross sections of the hearts were conventionally embedded in paraffin, cut with a thickness of 5–6 μm, and stained with hematoxylin and eosin (for overall morphology) and Movat's pentachrome stain (for connective tissue elements). They were graded semiquantitatively for assessment of the histological characteristics of the infarct without the graders' knowledge of the specific experimental group of a specimen. Histological features were graded 0 (absent) to 4 (most prominent) and included assessment of myocyte coagulation necrosis (with or without contraction bands), polymorphonuclear leukocyte and macrophage infiltration, collagen deposition, hemorrhage, and calcification.

**Statistics**

Measured values are expressed as mean±SEM. Differences among groups were tested by one-way analysis of variance. When the analysis of variance showed a significant difference among groups, an unpaired Student's t test was used to test the statistical significance of differences between specific mean values.²⁵ When only

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**Figure 1.** Schematic representation of apparatus for measuring the tear threshold of infarcts and normal myocardium in the heart. Panel A: Position of left ventricle (LV) in clamp preceding movement of clamp with step motor. Suture (4-0 silk) is placed 5 mm from LV apex in infarct center. Graph illustrates the force development. Panel B: Complete tear. As upper jaw separates from the bottom jaw attached to the strain gauge, the suture tears completely through the infarct as force and time are recorded. Complete tear is verified by force abruptly decreasing to zero. Panel C: Partial tear. As upper jaw separates from bottom jaw, an incomplete or partial tear is created in the tissue. The suture remains within the tissue, and force does not decrease to zero but is held at a higher force level.
two groups were analyzed, an unpaired Student’s t test was used to test statistical differences between specific mean values.

**Results**

**Tensile Strength Study**

Figure 2A illustrates the changes in tensile strength of reperfused and nonreperfused infarcted strips compared with the tensile strength of normal LV during the first week of healing after infarction. Tensile strength is the force at strip breakage divided by the cross-sectional area of the strip. The relative contributions of force (breakage threshold) and cross-sectional area at breakage to tensile strength of the tissue are shown in Figures 2B and 2C, respectively.

Reperfused infarcted tissue at 1 day after MI was the only tissue tested that had a tensile strength lower than that of normal LV. The decreased tensile strength of reperfused infarcts compared with that of normal LV resulted from a significantly smaller force at breakage (breakage threshold) \((p<0.05\) versus normal LV, Figure 2B) rather than any difference in strip dimension, because both reperfused infarcted tissue and normal LV had the same strip cross-sectional areas at breakage \((p=NS\) versus normal LV, Figure 2C). The tensile strengths of all other tissues at every time interval after MI were equal to or greater than that of normal LV, and all tensile strengths greatly exceeded a derived measure of normal physiological wall stress \((3 \text{ g/mm}^2=80\ \text{mm Hg})\).

The tensile strength of reperfused infarcted strips was less than that of nonreperfused infarcts at 1 and 5 days after MI \((p<0.05\), Figure 2A); however, reperfused and nonreperfused infarcted strips had the same force at breakage (Figure 2B) at all time intervals \((p=NS)\). Thus, at 1 day after MI, the lower tensile strength (force/cross-sectional area) in the reperfused infarcts was due to a larger cross-sectional area, because the force at breakage was the same in reperfused and nonreperfused infarcts. Since strips were cut at approximately the same width \((4 \text{ mm})\), the variation in strip cross-sectional area resulted from differences in infarct thickness, with reperfused strips having a thicker wall than nonreperfused strips. At 5 days after MI, both a lower breakage threshold and a larger cross-sectional area contributed to the significantly lower tensile strength of reperfused infarcts.

**Tear Threshold Study**

Table 1 reports the LV weights, infarct weights, and infarct surface areas from animals used in the tear threshold study during the first week after MI. LV weight and the calculated infarct surface area were similar in reperfused and nonreperfused infarcted hearts at all post-MI intervals, indicating equivalent infarct size in the two groups. Reperfused infarcts weighed significantly more than nonreperfused infarcts at 1, 2, 3, and 7 days after MI; however, hemorrhage and edema associated with reperfusion have been shown to contribute to increased infarct weight and wall thickness.\(^{26}\)

The tear tension was assessed by calculating the tension in the suture at the moment of tear initiation. Such suture tension was calculated as the maximum

![Figure 2. Graphs showing tensile strength of normal left ventricle (NL LV) and reperfused (Rep.) and nonreperfused (Non-Rep.) infarcts during the first week after myocardial infarction (MI). A, NL LV \((n=11\) strips); O, reperfused infarcted tissue \((n=8-12\) strips); ●, nonreperfused infarcted tissue \((n=8-12\) strips). Values are mean±SEM. Panel A: Tensile strength, the breakage threshold divided by the cross-sectional area at breakage. \(*p<0.025\) vs. NL LV, and **\(p<0.025\) vs. nonreperfused infarcts \((F=3.15-26.21; p<0.05\) at day 1 and \(p<0.01\) at days 5 and 7 after MI). Panel B: Breakage threshold, the peak force attained at breakage for the infarcted and NL LV strips. \(*p<0.01\) vs. NL LV \((F=3.51-20.9; p<0.05\) at days 1 and 5 after MI, and \(p<0.01\) at day 7 after MI). Panel C: Cross-sectional area at the point of breakage. Cross-sectional area of infarcted or NL LV strips at the point of breakage was measured as defined in text. \(*p<0.05\) vs. NL LV, and **\(p<0.025\) vs. reperfused infarct \((F=3.77-9.47; p<0.01\) at days 1 and 3 after MI, and \(p<0.05\) at day 7 after MI).force attained at tear threshold divided by the tissue thickness (force/thickness) and is detailed in Figure 3A. The tear tension or force/thickness in both infarct groups was equal to that of normal LV during the first 3
Table 1. Reperfused and Nonreperfused Infarct Weight, Surface Area, and Left Ventricular Weight

<table>
<thead>
<tr>
<th>Infarct weight (g)</th>
<th>Days after myocardial infarction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreperfused</td>
<td>0.41±0.04</td>
<td>0.39±0.04</td>
<td>0.40±0.02</td>
<td>0.46±0.06</td>
<td>0.39±0.03</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>0.63±0.06*</td>
<td>0.57±0.03*</td>
<td>0.55±0.03*</td>
<td>0.49±0.07</td>
<td>0.57±0.05*</td>
<td></td>
</tr>
<tr>
<td>Infarct area (mm²)</td>
<td>Nonreperfused</td>
<td>289±35</td>
<td>244±26</td>
<td>286±23</td>
<td>283±40</td>
<td>211±17</td>
</tr>
<tr>
<td>Reperfused</td>
<td>324±26</td>
<td>247±16</td>
<td>254±12</td>
<td>236±31</td>
<td>227±16</td>
<td></td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>Nonreperfused</td>
<td>2.37±0.11</td>
<td>2.62±0.08</td>
<td>2.51±0.12</td>
<td>2.62±0.11</td>
<td>2.68±0.11</td>
</tr>
<tr>
<td>Reperfused</td>
<td>2.51±0.13</td>
<td>2.54±0.13</td>
<td>2.52±0.14</td>
<td>2.57±0.17</td>
<td>2.50±0.11</td>
<td></td>
</tr>
</tbody>
</table>

Nonreperfused, nonreperfused infarcted tissue; reperfused, reperfused infarcted tissue (after a 3-hour occlusion); LV, left ventricular. Values are expressed as mean±SEM; n=7-11 hearts per group for given day. *p<0.05 vs. nonreperfused infarct weight.

days after MI but increased significantly above that of normal LV at 5 and 7 days after MI. The relative contributions of force (tear threshold) and thickness in reperfused and nonreperfused infarcts are shown in Figures 3B and 3C, respectively.

Reperfused infarcts resisted tearing earlier in the post-MI period than did nonreperfused infarcts (Figure 3B). At 1 day after MI, both reperfused and nonreperfused infarcts tore at a force 50% lower than did normal LV; however, by 3 days after MI, the tear threshold of reperfused infarcts was twice that of nonreperfused infarcts (p<0.05) and equaled that of normal LV. By 5 days after MI, the tear threshold of reperfused infarcts was significantly greater than the tear thresholds of both nonreperfused infarcts and normal LV (p<0.05), but by 7 days after MI, both reperfused and nonreperfused infarcts had equal tear thresholds that exceeded those of normal LV (p<0.05, Figure 3B).

Wall thickness. The wall thickness of both reperfused and nonreperfused infarcts was constant and less than that of normal LV wall during the entire post-MI week. Reperfused infarcts had a greater wall thickness than nonreperfused infarcts at all intervals after MI (Figure 3C). However, tear threshold progressively increased from day 2 to day 7 after MI without a significant increase in thickness (Figures 3B and 3C).

There was no consistent correlation between tear threshold and thickness values for individual infarcts in each group each day after MI, with the exception of permanent-occlusion nonreperfused infarcts at 3 days after MI (r=0.683, p=0.02). Hydroxyproline production begins at this time after MI in rabbits and may contribute to the correlation between wall thickness and tear threshold in the nonreperfused infarcts. Tear threshold in the reperfused infarcts did not correlate with wall thickness at 3 days after MI, because the wall thickness measurement in reperfused infarcts is complicated by residual hemorrhage (Figure 3C).

Incidence of a complete tear through the infarct. Table 2 shows the percentage of reperfused and nonreperfused infarcts exhibiting a complete tear after suture tension was increased (Figure 1B) at each day after MI. At 1 and 2 days after MI, both reperfused and nonreperfused infarcts tore completely, but by 3 days after MI, 50% of the reperfused infarcts resisted tearing and had only a partial tear (Figure 1C), whereas 91% of the nonreperfused infarcts tore completely. By 5 days after MI, the majority of reperfused infarcts resisted tearing (only 29% with a complete tear), whereas the majority of the nonreperfused infarcts tore completely once the tear threshold was attained (89% with a complete tear). By 7 days after MI, both infarct types resisted tearing, and the incidence of a complete tear decreased to 11% and 27% in the reperfused and nonreperfused infarcts, respectively (Table 2).

Attainment of tear threshold. Figure 4 summarizes and compares the time needed to attain the tear threshold and slope of the suture force/time curves in normal LV and in reperfused and nonreperfused infarcts at 1, 3, and 5 days after MI. A steeper slope indicates a combination of lesser elasticity and greater resistance to tissue tearing. At 1 day after MI, the reperfused infarcts achieved the same tear threshold but at a slightly steeper slope than did nonreperfused infarcts (Figure 4A). Normal LV attained a higher tear threshold with a steeper slope than did either type of infarct.

By 3 days after MI, the reperfused infarcts achieved the same tear threshold as normal LV but had a steeper slope and tear threshold than did nonreperfused infarcts (Figure 4B). At 5 days after MI, reperfused

Table 2. Incidence of Complete Tearing in Nonreperfused and Reperfused Infarcts

<table>
<thead>
<tr>
<th>Days after myocardial infarction</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>%</td>
<td>n/N</td>
</tr>
<tr>
<td>Nonreperfused infarcts</td>
<td>100</td>
<td>8/8</td>
<td>100</td>
<td>7/7</td>
<td>91</td>
<td>10/11</td>
<td>89</td>
</tr>
<tr>
<td>Reperfused infarcts</td>
<td>100</td>
<td>8/8</td>
<td>100</td>
<td>7/7</td>
<td>50</td>
<td>4/8</td>
<td>29</td>
</tr>
</tbody>
</table>

n/N. Number of infarcts with tearing entirely through the region/total number tested. For normal left ventricle, the incidence of complete tearing was 50% (3/6).
infarcts had a greater tear threshold but had the same slope as the nonreperfused tissue (Figure 4C).

**LV Distending Pressure/Rupture Threshold Study**

The average maximum rupture pressure in isolated reperfused and nonreperfused infarcted hearts is shown by rupture location in Figure 5A (1 day after MI) and
Figure 5B (3 days after MI), and the incidence of rupture by site is reported in Table 3. At 1 day after MI, both the reperfused and nonreperfused infarcted hearts required extremely high pressures to cause rupture (450–550±60–80 mm Hg) in nine of 13 hearts (69.2%) from each group (Figure 5A, Table 3), and rupture occurred at the infarct or infarct border in all cases. However, at 3 days after MI, none of the 10 reperfused infarcted hearts ruptured through the infarct or infarct border; these reperfused infarcted regions resisted the increasing pressure, and rupture consequently occurred through the noninfarcted septum (seven hearts) or noninfarcted LV free wall (three hearts) (Figure 5B, Table 3). In contrast, seven of 10 nonreperfused infarcted hearts ruptured through the infarct or infarct border at 3 days after MI (Figure 5B, Table 3).

Table 4 summarizes the calculated values for the LV and infarcted regions for wall stress, wall thickness, and chamber radius for each rupture location. Wall stresses were calculated by using Laplace’s law and assuming a

<table>
<thead>
<tr>
<th>Day 1 after MI</th>
<th>Inf</th>
<th>Inf Bdr</th>
<th>Inf or Bdr</th>
<th>Septum</th>
<th>LV wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Nonreperfused (N=13)</td>
<td>38.5</td>
<td>30.8</td>
<td>69.2</td>
<td>23.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Reperfused (N=13)</td>
<td>30.8</td>
<td>38.5</td>
<td>69.2</td>
<td>23.1</td>
<td>7.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 3 after MI</th>
<th>Inf</th>
<th>Inf Bdr</th>
<th>Inf or Bdr</th>
<th>Septum</th>
<th>LV wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Nonreperfused (N=10)</td>
<td>20.0</td>
<td>50.0</td>
<td>70.0</td>
<td>20.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Reperfused (N=10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

\[ n, \text{Number of ruptures; } N, \text{total number of hearts; Inf, infarcted region; Inf Bdr, rupture at the border of the left ventricular (LV) wall and infarct or at the border of the septum and infarct; Inf or Bdr, combined number of hearts that ruptured through either the middle of the infarct or the border of the infarct; MI, myocardial infarction; nonreperfused, nonreperfused infarcted tissue; reperfused, reperfused infarcted tissue (after a 3-hour occlusion).} \]
spheroid cavity as the balloon expanded in the arrested LV, and wall thickness was measured directly. In general, both reperfused and nonreperfused infarcted hearts were very resistant to rupture, because the pressures required to burst the hearts were superphysiological. Moreover, the calculated wall stresses were remarkably close to the superphysiological tensile strengths observed in the infarcted strip study.

A comparison of the tensile strengths of infarcted strips with the calculated wall stress at the moment of rupture in isolated hearts at 1 day after MI is reported in Table 5. Both the tensile strength and the wall stress at rupture in reperfused infarcts at 1 day after MI were less than those in normal LV (Table 5); however, the intracavitary pressure necessary to achieve the wall stress was beyond normal physiological limits (Table 4).

### Histological Findings

Histological grading revealed that reperfused infarcts had more hemorrhage than did nonreperfused infarcts throughout the post-MI week and had less polymorphonuclear leukocyte infiltration than did nonreperfused infarcts at 3 days after MI. By 7 days after MI, the grade for necrosis was less in reperfused compared with...
TABLE 5. Left Ventricular Wall Stress at Rupture Site Versus Tensile Strength 1 Day After Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>LV wall stress (g/mm²)</th>
<th>Tensile strength (g/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM  n</td>
<td>Mean±SEM  n</td>
</tr>
<tr>
<td>Reperfused</td>
<td>17±2*  4</td>
<td>16±1†  12</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>24±4  5</td>
<td>24±3  12</td>
</tr>
<tr>
<td>Normal LV</td>
<td>25±2  9</td>
<td>23±3  8</td>
</tr>
<tr>
<td>Septum</td>
<td>19±1  15</td>
<td>15±2*  8</td>
</tr>
</tbody>
</table>

LV, left ventricle (ventricular); n, number of myocardial strips; reperfused, reperfused transmural infarct; nonreperfused, non-reperfused transmural infarct.

Myocardial strips from noninfarcted hearts were used for the measurement of normal LV tensile strength. Measurement of wall stress in normal LV and septum includes all hearts (reperfused, nonreperfused, and shams) that ruptured through those regions. Two hearts that ruptured through the septum were excluded because of missing wall thickness values. Wall stress is calculated as 0.01363×pressure×radius²×wall thickness. Unpaired Student’s t test was used for comparisons (analysis of variance with F=3.5–3.8, p<0.03).

*p<0.05 vs. normal LV, and †p<0.02 vs. nonreperfused.

nonreperfused infarcts. There were no other apparent differences in histological appearance between the reperfused and nonreperfused infarcts during the post-MI week. These results are reported in Table 6.

**Discussion**

This study used three different measurements of tissue strength to assess post-MI LV rupture potential after a permanent coronary occlusion or in infarcts subjected to late reperfusion. There are no animal models of spontaneous cardiac rupture. Therefore, our study of the biomechanical properties of the infarct tissue, which assessed post-MI healing in terms of tissue strength, was used as a measure of post-MI rupture potential. In a previous study we found that late reperfusion decreased tensile strength and collagen cross-linking in 3-week-old scar tissue compared with scar tissue from age-matched nonreperfused infarcts. The current study focused on the early post-MI period of infarct healing, when LV rupture is more likely to occur in patients.

At 1 day after MI, we found that late reperfused infarcted tissue was weaker than both normal LV and nonreperfused infarcted tissue and thus potentially more vulnerable to rupture. This finding is in agreement with the results from the International Study of Infarct Survival (ISIS-2 trial), which showed an increased incidence of rupture in patients within 24 hours after thrombolytic therapy, although other clinical studies have not supported this finding. However, by 3 days after MI, late reperfused infarct tissue had attained strength equal to or greater than normal LV strength, whereas nonreperfused tissue was still weaker than normal LV tissue in tear threshold and propensity to rupture through the infarcted region when subjected to increased LV distending pressure.

**Tensile Strength Compared With Tear Threshold Measurements**

Tensile strength and tear threshold measure different biomechanical properties of healing infarcts. Tensile strength is a uniaxial measurement that varies with tissue strip orientation, because normal myocardium and healing infarcts are anisotropic tissues. The myocardial wall is composed of muscle fibers at different

TABLE 6. Histological Profile of Reperfused and Nonreperfused Infarcts at 1, 3, 5, and 7 Days After Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Days after myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>4.0±0.0</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>2.0±1.1</td>
</tr>
<tr>
<td>PMN</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>3.0±0.0</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Macrophage</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>Myocyte necrosis</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Calcification</td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>1.7±0.8</td>
</tr>
<tr>
<td>Nonreperfused</td>
<td>1.0±0.6</td>
</tr>
</tbody>
</table>

Reperfused, reperfused infarcts; nonreperfused, nonreperfused infarcts (permanent occlusion); PMN, polymorphonuclear leukocytes; myocyte necrosis, myocyte coagulation necrosis (with or without contraction bands). Values are mean±SEM histological score based on a 0–4 ranking, with 0 representing an absence of the feature and 4 representing the maximum. Three samples were evaluated in each group at each day after myocardial infarction.
depths and orientations as well as an extensive collagen network composed of collagen struts and microthreads.\textsuperscript{18,28} As the myocardium undergoes infarction, the necrotic muscle fibers exhibit myocyte slippage,\textsuperscript{29} whereas the collagen struts have been shown by Factor et al\textsuperscript{18} to disappear by 24 hours after MI.

The effect of tissue strip orientation and infarct composition on tensile strength was specifically examined in a previous study from our laboratory by Przyklenk et al.\textsuperscript{24} In 24-hour-old infarcted tissue from dogs, we found that midmyocardial infarcted strips that were elongated in a circumferential direction or parallel to fiber orientation had a higher tensile strength than did midmyocardial strips that were elongated in a longitudinal direction or perpendicular to fiber orientation.\textsuperscript{24,28} To minimize anisotropic differences in tensile strength in the current study,\textsuperscript{14} we cut the LV and infarcted tissue strips in the longitudinal direction.

Tissue composition can also influence scar tissue strength. In our previous study, tensile strength was highest in the epicardium and endocardium and was directly related to collagen content,\textsuperscript{24} suggesting that the connective tissue of the infarct is a key factor in maintenance of the structural integrity and geometry of the LV wall after myocyte necrosis occurs. Previously, we have shown that the cellular composition was similar in both late reperfused and nonreperfused 3-week-old rabbit scar tissue, each type containing approximately 75–80% fibrous tissue with some residual myocytes at the subepicardium and subendocardium. To minimize compositional differences in the current study, we compared only nonreperfused and late reperfused infarcts, since we previously had demonstrated their compositional similarity.

The tensile strength of infarcted strips was identical to the calculated wall stress at the rupture site in isolated hearts subjected to overdistension at 1 day after MI. Both measurements indicated that freshly infarcted tissue can withstand superphysiological stress levels without rupture (Table 5). These observations suggest that rupture should never occur and is obviously contrary to clinical observations.\textsuperscript{8,22} This contradiction suggests that progressive stress loading of infarcted tissue may not assess clinical rupture potential.

Since a number of studies have shown that myocardial rupture is preceded or caused by intramyocardial tearing,\textsuperscript{6,19,30} the tear threshold measurement may be a more useful parameter to assess susceptibility of the myocardial wall to tear and rupture. Both reperfused and nonreperfused tissue tore at subnormal force levels at 1 and 2 days after MI, a time when necrosis and phagocytosis are occurring.\textsuperscript{11,31,32} However, late reperfusion increased resistance to tearing relative to nonreperfused infarcts during the critical time, from 3 to 5 days after MI, when rupture frequently occurs in humans.\textsuperscript{5}

The path of the suture-initiated tearing through the tissue was always directly vertical for a complete tear (Figure 1B) and horizontal for a partial tear (Figure 1C). Complete tears occurred at 1 and 2 days after MI in virtually all infarcts (Table 2); that is, the suture cut straight through the tissue once the tear threshold was achieved. By 3 days after MI, the increased resistance to a complete tear in reperfused infarcts (50% with a complete tear versus 91% with a complete tear in the nonreperfused infarcts, Table 2) coincides with the increase in tear threshold observed in reperfused infarcts (Figure 2B).

Whittaker et al,\textsuperscript{33} using polarized light microscopy, have shown that collagen deposition in the healing infarct occurs in the same plane as the previous myocyte pattern. Moreover, Judgutt and Amy\textsuperscript{34} found that hydroxyproline content was highest in the endocardium and epicardium compared with the midmyocardium in both 7-day-old and 6-week-old infarcted tissue, suggesting that the endocardium and epicardium “heal first,” before the midmyocardium with circumferential fibers. Thus, a horizontal (partial) tear may represent an advanced state of healing, indicating a tear plane along the midmyocardial circumferential fibers but resistance to vertical tearing that is due to increased collagen at the subendocardial and subepicardial layers. Thus, the 50% horizontal tear rate in reperfused infarcts at 3 days after MI compared with only 9% in the nonreperfused infarcts (Table 2) also suggests accelerated healing in the reperfused infarcts.

**Infarct Wall Thickness and Biomechanical Measurements**

Hemorrhage and edema contribute to wall thickness in reperfused infarcts, whereas regional collapse of the myocardial vasculature secondary to coronary occlusion is responsible for the acutely decreased wall thickness in nonreperfused infarcts.\textsuperscript{14,30,35} Hence, greater wall thinning in nonreperfused infarcts contributed to a decreased cross-sectional area, because the width measurement during strip sectioning was constant. Thus, infarcted strips with greater wall thinning had a higher tensile strength for any given force at breakage (Figure 2).

Different tear thresholds were found in infarcted tissue despite similar wall thicknesses. The wall thickness in each individual infarct group was constant over the course of the week, with wall thickness in nonreperfused infarcts being less than that in reperfused infarcts ($p<0.05$) (Figure 3C). Therefore, the increasing force/thickness (tear tension) during the week in the tear study resulted from an increasing tear threshold (force, Figure 3B) rather than changes in wall thickness, implying that infarct composition was more important than wall thickness in reducing the tendency of infarcts to tear.

**Superphysiological Measurements**

Our biomechanical measurements demonstrated that late reperfusion reduced tissue strength and increased rupture potential below that of normal LV only during the first day after MI. However, tensile strength of infarcted strips, bursting pressures, and wall stress at rupture in isolated hearts were well beyond normal physiological limits, even at their nadir, 1 day after MI. The lowest measurement of tensile strength observed at 1 day after MI in reperfused tissue ($16 \pm 1$ g/mm\(^2\)) was five times a calculated normal systolic wall stress in the rabbit LV (3 g/mm\(^2\)) at a physiological systolic pressure of 80 mm Hg.\textsuperscript{14} Likewise, the wall stress at rupture in these infarcts was $17 \pm 2$ g/mm\(^2\) (Table 5) with a bursting pressure greater than 400 mm Hg (Table 4). These bursting pressures are consistent with an earlier study by Lerman et al\textsuperscript{27} that reported bursting pressures greater than 500 mm Hg in nonreperfused infarcted hearts.
A study by Lyon et al\textsuperscript{36} also found similar bursting pressures in noninfarcted lamb hearts; these authors discuss the sequential transfer of stresses to different structural elements with increasing pressure and diastolic tension in the noninfarcted heart. In the infarcted heart, similar transfer of stress may occur, as documented by Radhakrishnan et al\textsuperscript{37}, Bogen et al\textsuperscript{38}, and Weisman et al\textsuperscript{39} during infarct expansion and LV dilatation. However, there is a limit to the degree of cardiac compensation that occurs with LV chamber remodeling, and concurrent infarct expansion may lead to rupture or aneurysm formation.\textsuperscript{39}

In our current study, the incremental addition of pressure by pulsating fluid into the balloon in the LV chamber was designed to simulate the systolic paradoxical pulsation of the infarcted region. The extremely high pressures seen at rupture indicate that an infarcted rabbit LV in the early stages of healing is still very resistant to rupture and suggest that increased LV pressure alone is not sufficient to cause rupture. Nonetheless, myocardial rupture occurs in post-MI patients by some undefined mechanism.

The absence of myocardial rupture through the reperfused infarcted region in isolated hearts at 3 days after MI, although nonreperfused infarcts continue to rupture through the infarcted region, strongly suggests that late reperfusion accelerates healing and results in strengthening of the tissue by 3 days after MI. Both infarct types were equally strong by our measurements at 7 days after MI, suggesting that changes occurred earlier in the pattern of healing in reperfused tissue.

### Potential Mechanisms Contributing to Myocardial Rupture

Late reperfusion has been described as either accelerating healing\textsuperscript{35,40,41} or interfering with healing\textsuperscript{16,42–45} of necrotic infarcted tissue. Studies have shown an increased necrosis with contraction bands and advanced granulation tissue formation, indicating accelerated healing in reperfused infarcts.\textsuperscript{17,35,45} The structural elements in an infarcted region mainly include residual necrotic myocytes undergoing myocyte slippage and then degradation by phagocytosis. The collagen-producing fibroblasts do not appear until the second or third day after infarction, resulting in a 48-hour period during which the structural integrity of an infarct is maintained by the original collagen network and both necrotic and surviving myocytes.

Although some differences in the histological appearance were noted between the reperfused infarcted tissue and the nonreperfused tissue during the post-MI week, these differences were modest. At 1 day after MI, a greater degree of hemorrhage was observed in the reperfused infarcts compared with the nonreperfused infarcts and may have contributed to the observed lower tensile strength (Tables 5 and 6). At 3 days after MI, the increased tear threshold (Figure 3B) of reperfused infarcts and the resistance to rupture in the isolated reperfused infarcted hearts (Figure 5B) coincide with less leukocytic infiltration than is observed in the nonreperfused infarcts (Table 6). Similarly, at 3 days after MI in dogs, Roberts et al\textsuperscript{35} reported fewer polymorphonuclear leukocytes in reperfused infarcts compared with permanently ischemic infarcts.

A more rapid resolution of necrosis, as indicated by a lower score for myocyte necrosis (Table 6), was found within the reperfused infarcts compared with nonreperfused infarcts at 7 days after MI. This finding is in agreement with another study showing fewer residual myofibrils in reperfused rat infarcts at 7 days after MI and suggesting accelerated healing and more rapid removal of necrotic myofibrillar material with late reperfusion.\textsuperscript{46}

The connective tissue network has been the focus of recent interest regarding myocardial rupture. Weber et al\textsuperscript{47} have shown that there is a decrease in connective tissue at the site of rupture, and Factor et al\textsuperscript{18} have shown that the skeletal framework is altered in the central zone of infarction and suggest an association with myocardial rupture. Similarly, Atkinson et al\textsuperscript{48} have shown an increase in eosinophils associated with myocardial rupture and have suggested that proteolytic enzymes released from the leukocytes contribute to tissue weakness by destroying the connective tissue network. The reintroduction of blood flow to a necrotic region would bring leukocytes to the infarcted region and potentially begin the destruction of the collagen network. The degradation process may then stimulate the repair process, resulting in the tissue strengthening observed in the reperfused tissue. Reperfusion may also wash out chemotactic factors or increase oxidative metabolism and subsequent protein synthesis and thus accelerate healing.

A recent study in our laboratory examined the underlying mechanisms involved in myocardial rupture by quantitating the expression of collagen I, III, and IV as well as fibronectin in reperfused and nonreperfused infarcts.\textsuperscript{48} Fibronectin is a glycoprotein in the extracellular matrix that has been shown to be a key component of wound repair and may provide the structural framework on which new collagen is formed.\textsuperscript{49} We found that fibronectin mRNA expression increased dramatically in both infarct types at 1 day after MI, whereas collagen expression did not increase until 2 days after MI. Of particular relevance to the current study is an observed increase in fibronectin protein levels at 3 days after MI in reperfused infarcts, whereas fibronectin protein levels in nonreperfused infarcts did not increase until 5 days after MI.\textsuperscript{48} These results complement our current findings in the tear threshold study, in which a significant increase in tear threshold was observed in the reperfused infarcts at 3 days after MI, whereas nonreperfused infarcts did not show an increase in tear threshold until 5 days after MI (Figure 3B).

### Study Summary

This study expands our previous work on the effect of reperfusion on infarct healing\textsuperscript{44} by reporting a detailed examination of the biomechanical properties of reperfused and nonreperfused infarcted tissue during the critical first week of healing, when cardiac rupture is most likely to occur. We found that late reperfusion weakened the infarcted tissue, potentially making the tissue more vulnerable to rupture at 1 day after MI, but also increased resistance to rupture compared with the nonreperfused infarcts as early as 3 days after MI, suggesting an accelerated response to healing. The mechanisms underlying the initial decrease and later increased resistance to rupture remain to be elucidated.
Future studies correlating the increase in tissue stress with reperfusion to infarct composition and healing are clearly warranted. The precise definition of the weak link in the healing infarct needs to be determined if the desired goal of prevention of cardiac rupture is ever to be realized.

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