Myocardial Cell Hypertrophy After Myocardial Infarction With Reperfusion in Dogs

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Background. The potential role of myocardial cell hypertrophy in the ischemic zone in the mechanism of late recovery of regional contractile function after myocardial infarction followed by reperfusion has not been examined.

Methods and Results. Eight chronically instrumented, conscious dogs were subjected to 90–120 minutes of circumflex coronary artery occlusion followed by reperfusion. The thickness and function of the anterior (AT) and posterior (PT) walls was measured by ultrasonic gauges at control, during occlusion, and after reperfusion. After 3 weeks, cross-sectional areas of surviving cells were determined from subepicardial (epi), midwall (mid), and subendocardial (endo) regions in six dogs and compared with those from six animals without infarction, including three sham-operated control dogs. PT systolic wall thickening showed dyskinesia during occlusion but recovered after reperfusion to 48% of control at 1 week and 67% at 3 weeks. End-diastolic thickness of the PT wall increased markedly after reperfusion, but AT and PT walls were only slightly thicker (p = NS) than in control dogs at 3 weeks. Cross-sectional areas of reperfused dogs in the infarct region averaged 279 [PT_epi], 291 [PT_mid], and 317 μm² [PT_endo] and were significantly larger than in control animals (237 [PT_epi], 241 [PT_mid], and 233 μm² [PT_endo]). PT cell areas were significantly larger than AT cells, ENDO cell areas were larger than EPI cells (both p < 0.05), and ENDO cells of the AT wall were larger than those of noninfarcted dogs (p < 0.05).

Conclusions. In dogs with myocardial infarction followed by reperfusion, the cross-sectional areas of cells in the infarcted PT wall were larger than those in the noninfarcted AT wall, and within both the infarcted and noninfarcted zones, cell areas were larger in the endocardial than the epicardial region. In all regions of the infarcted wall and in the ENDO region of the noninfarcted wall, cell areas were generally larger than those of control dogs without infarction, and the control dogs showed no transmural differences in cell areas. The mechanisms responsible for this significant remodeling of the reperfused infarcted zone, which involves myocardial cellular hypertrophy, are unknown, but it is possible that hypertrophy of surviving regions of the infarcted wall played a role in the late recovery of regional function that accompanied this hypertrophic response. (Circulation 1992;86:1935–1944)

KEY WORDS • reperfusion • myocardial function • cells • myocardial infarction

Recovery of regional contractile function provides a marker for one of the important goals of coronary artery reperfusion, salvage of functioning left ventricular (LV) myocardium. A number of experimental studies in dogs have demonstrated that when coronary artery reperfusion is performed within 2–4 hours after coronary occlusion, there is some recovery of regional myocardial function. Little is known, however, about the mechanisms that underlie such recovery of regional and global ventricular function after reperfusion after prolonged periods of severe myocardial ischemia. Previous studies in this laboratory showed that coronary artery reperfusion after 3 hours of occlusion can result in very early return of regional function (assessed by cineradiography and opaque beads) at 30 minutes after reperfusion.1 This observation was later confirmed by experiments in chronically instrumented dogs, in which it was first shown that gradual recovery of regional function occurs over a period of 2–4 weeks after 2 hours of coronary occlusion followed by reperfusion, recovery being greatest at the margin of the ischemic zone but also observed in the central region despite subendocardial tissue loss.2 Reimer et al11 conducted studies on coronary artery occlusion with reperfusion in dogs using quantitative histology and reported epicardial sparing after 3 hours of coronary occlusion followed by reperfusion, as suggested in experiments by others showing sparing of outer wall myocardial creatine kinase at 1 week after 3
hours of coronary occlusion with reperfusion.\textsuperscript{4} Reimer et al\textsuperscript{11} further demonstrated a "wave front" of spreading necrosis from endocardium to epicardium over 6 hours. Sasayama et al\textsuperscript{12} reported that the thickness of infarcted wall after permanent coronary artery occlusion in dogs showed minimal thinning at 3 weeks, despite scar formation, suggesting the possibility of compensatory hypertrophy of undamaged cells in the infarct zone. Subsequent studies also documented recovery of regional function after reperfusion\textsuperscript{6-9} together with improved survival and reduced infarct size\textsuperscript{6,7} and biochemical recovery.\textsuperscript{8}

On the basis of these observations, we hypothesized that hypertrophy of surviving subepicardial cells and/or surviving islands of cells in the infarcted subendocardial or midwall areas might occur and contribute to regional functional recovery after coronary artery occlusion after reperfusion. Therefore, in this study in a canine model, we examined over 3 weeks the potential for regional cellular hypertrophy after reperfusion following 90–120 minutes of coronary occlusion together with assessment of regional wall dimensions and functions.

**Methods**

The animals in this study were handled according to the animal welfare regulations of the University of California San Diego, which are in accord with the animal use principles of the American Heart Association, and the protocol was approved by the Animal Use Committee of this institution.

**Animal Preparation**

Thirty mongrel dogs of both sexes weighing between 19.0 and 40.0 kg (mean weight, 28.3 kg; mean age, 1.8 years) were trained to lie on a table before surgical instrumentation. On the day of surgery, dogs were tranquilized with morphine (10.5 mg/kg i.m.) and anesthetized with sodium thiopental (20 mg/kg i.v.). After endotracheal intubation, anesthesia was maintained with isoflurane (1–2%). Arterial blood gases were measured repeatedly throughout the surgery, and ventilatory adjustment was made as necessary to keep PO\textsubscript{2} above 150 mm Hg and PCO\textsubscript{2} and pH within the physiological range. The heart was exposed through a left lateral thoracotomy in the fifth intercostal space, and the pericardium was opened. A high-fidelity micromanometer (Konigsberg P7) and a Tygon fluid-filled catheter (1.27-mm i.d.) were inserted through a stab wound in the apex to measure LV pressure. The micromanometer was calibrated by matching it to the ventricular pressure through the fluid-filled catheter (Statham P-23Db), and the zero pressure reference was taken at the estimated level of the right atrium. Silicon rubber catheters were inserted into the upper descending aorta and left atrium through the atrial appendage, and a pair of pacing electrodes was sutured on the left atrial appendage. The proximal left circumflex artery was dissected approximately 2 cm, and a Silastic occluder and a Doppler flow probe were placed around the artery. All visible coronary collateral vessels between the left anterior descending artery and the left circumflex artery were suture ligated.

For measuring LV wall thickness, pairs of ultrasonic crystals were implanted in the anterior (AT) and posterior (PT) walls by standard techniques.\textsuperscript{10} For PT wall thickness, two pairs of crystals were implanted, one for total wall thickness and the other to measure subepicardial wall thickness (Figure 1). Thickness of the PT transmural (PT\textsubscript{tm}) and PT epicardial (PT\textsubscript{ep}) sites was measured, and the PT endocardial (PT\textsubscript{endo}) thickness was calculated by subtracting the epicardial layer thickness from the total thickness. The pericardium was left open, and all wires and the catheters were passed subcutaneously to the back of the dog and brought through the skin between the scapulae. The thorax was evacuated through a chest tube in the sixth intercostal space. Cefazolin (500 mg/kg i.m.) was administered 1 hour before surgery and every 3 hours throughout the procedure for a total of three doses.

**Protocols**

Control studies were conducted 1–2 weeks after surgery, when the animals had recovered fully, and all data were obtained with the animal lying quietly on a table. Sedation and analgesia were used only at the time of coronary occlusion. After control hemodynamic recordings were collected at the intrinsic and several paced heart rates, the dog was given acepromazine (5–10 mg i.m.) and buprenorphine (0.3 mg i.m.) for analgesia and sedation. Radiolabeled microspheres (15.5 $\mu$m in diameter) were injected through the left atrial catheter, and a reference blood sample was withdrawn from the aortic catheter for 90 seconds, starting just before the microsphere injection. Before coronary occlusion, lidocaine (20–40 mg bolus) was injected through the LV catheter and 1–2 mg/min was then continuously infused to minimize arrhythmias. Coronary arterial occlusion was performed by inflating the Silastic occluder with air or water, and the occlusion was maintained for 90 minutes (in two dogs, the occlusion was 120 minutes). During occlusion, heart rate, ECG, LV and aortic pressures, wall thickness, and coronary flow (Doppler flowmeter) were continuously monitored.

\textbf{FIGURE 1. Schematic of the instrumentation.} Posterior dimension gauges were placed at the center of the region supplied by the left circumflex coronary artery (LCX). Brief coronary artery occlusion was used during instrumentation to ensure proper positioning of the dimension gauges. LA, left atrial; ISCH, ischemic; TM, transmural; EPI, epicardial; LV, left ventricular; LAD, left anterior descending coronary artery; AO, aortic.
After occlusion for 80 minutes, hemodynamic recordings were repeated, and another batch of microspheres was injected. Before reperfusion, a bolus of lidocaine (20–40 mg) was again administered, and when the heart rate was <130 beats per minute, the atrium was paced 20–30 beats per minute faster than the intrinsic rate for the first 15 minutes of reperfusion to further minimize dysrhythmias. Two hours after reperfusion, another set of recordings was made, and each dog was transferred to a recovery cage. Tocainide (600 mg/day p.o.) was given for the first 3 days. Hemodynamic recordings were repeated on days 1, 2, and 3 and at 1, 2, and 3 weeks. Radiolabeled microspheres were injected at 24 hours and 3 weeks after reperfusion. Atrial pacing was used to match heart rates to the control heart rate when necessary.

The animals were killed after 3 weeks of reperfusion. Under general anesthesia, a left thoracotomy was performed, the heart was exposed, and a fixative (2% glutaraldehyde in buffer and 4% paraformaldehyde) was infused via the coronary orifice of the left main trunk. The perfusion pressure was kept between 120 and 150 mm Hg. After the heart was properly fixed, transmural tissue blocks that contained a pair of crystals were cut and divided into the three layers (subepicardial, midwall, and subendocardial layers). The tissues were kept in buffer solution at 4°C. These tissues were used to calculate regional blood flow (RBF) and also were used to assess cell area and percent infarction.

**Data Acquisition and Analysis**

Data were recorded on an eight-channel chart recorder (Gould, Brush) and on 1.3-cm magnetic tape (Hewlett Packard, HP 3955D). Digitized data (sampling interval, 4 msec) were simultaneously recorded on a microcomputer for beat averaging. At least 10 consecutive beats were used for each observation. Measurements of myocardial wall thickness were made with a sonomicrometer (Triton Technologies, San Diego, Calif.). The measured parameters were heart rate, LV pressure (micromanometer), the first derivative of LV pressure (LV dP/dt), aortic pressure, and the regional wall thicknesses. End diastole was defined as the time coincident with the rapid onset of a positive LV dP/dt. End systole was defined as the point within 20 msec before peak negative dP/dt.

**Regional Myocardial Blood Flow**

Radiolabeled microspheres were used randomly and included $^{14}$Ce, $^{58}$Cr, $^{99}$Nb, $^{103}$Ru, $^{114}$In, $^{125}$I, $^{46}$Sc, and $^{113}$Sn. RBF was computed with a standard method as previously applied in this laboratory. RBFs in the ischemic area at control, during occlusion, and at 1 day of reperfusion were corrected for infarction as described by Reimer et al.

**Cell Area and Infarct Size Analysis**

Transmural blocks of tissue were cut across the ventricular wall, one from the infarcted region and one from the normal region, and these blocks were divided into endocardial, midwall, and epicardial thirds. In some animals, two blocks were available from the infarcted region, in which case the data from the two blocks were averaged.

Myocardial tissues were first used for RBF measurements, and then small blocks were cut from the endocardial, midwall, and epicardial tissue samples in a plane perpendicular to the epicardial surface. These were embedded in paraffin, sliced at a thickness of 5 μm, and stained with Milligan trichrome stain for analysis of scar formation. Percent infarction (percent scar formation) was measured by a computer-assisted point counting method (final magnification, ×25).

The remaining tissue in each of the endocardial, midwall, and epicardial samples was then divided into 15–20 pieces, excluding visible subendocardial scar. A binocular dissecting microscope was used to identify fiber cross- striations so that blocks could be cut as closely as possible perpendicular to the cross-striations. This yielded multiple blocks from each of the endocardial, midwall, and epicardial regions sampled by the larger transmural blocks from the infarcted wall and the normal zone. These small tissue blocks were then dehydrated and treated in OsO4. They were embedded in Araldite and sectioned at a thickness of 1 μm (LKB 8800 Ultramicromite), mounted on slides, and stained with toluidine blue. From these sections, a minimum of four and up to 12 sections (average, seven sections) were selected for cell area determinations, based on lack of an oblique cut (cell cross sections close to circular), presence of a visible nucleus, and unbroken cell membrane. When a section was selected, all cells in the section that met these criteria were counted.

Cell areas were measured on an imaging system connected to a computer (image processing and analysis program by Analytical Imaging Concepts, magnification ×400) from areas that appeared to contain no tissue edema. No tissue was used from the visibly scarred area, and cells directly adjacent to microscopic scar tissue were avoided, with at least one cell layer separating measured cells from scar. Measurements of cell areas were made by an observer blinded to the origin of samples.

**Animals Included in the Study**

Among 22 dogs undergoing surgical instrumentation in preparation for the complete protocol, five died during or after operation, and three dogs were later killed because of poor health or inadequate instrumentation. Coronary artery occlusion was performed in 18 dogs, of which four died after coronary artery occlusion or during reperfusion because of arrhythmias. We completed the protocol in 14 dogs, but six dogs were excluded from the study because of insufficient infarction. Criteria for insufficient infarction included no tissue necrosis on histological examination, percent wall thickening (%WT) >10% at 2 hours after reperfusion, or lowest subendocardial blood flow >0.2 ml·min⁻¹·g⁻¹, leaving eight dogs with significant subendocardial infarction for analysis. One of the two dogs that had 120 minutes of coronary occlusion was deleted from all analysis because of absence of a significant infarct; the other animal was included, but fixation was inadequate, and cell area determinations could not be made. In the eight dogs reported, hemodynamic parameters were obtained in seven; wall thicknesses, RBF, and cell areas were successfully measured in six dogs each.

Four additional dogs were used as shams for cell area (instrumented but no coronary artery occlusion); one of
the shams was dropped because of abnormal distribution of RBF at control. Another four dogs were not instrumented (control dogs), and tissue samples were taken for cell area determinations. We could not analyze cell area on one of the control dogs because of poor fixation. Because there were no statistical differences between controls and shams, the three control and three sham animals were pooled for statistical analysis.

**Statistical Analysis**

Cell area data were analyzed by a two-way repeated-measures ANOVA with a group factor.\textsuperscript{15} Hemodynamic parameters, wall thickness, systolic wall thickening, and RBF were analyzed with repeated-measures ANOVA. PT\textsubscript{endo}, wall thickness changes were not analyzed statistically because they were calculated from the PT\textsubscript{es} and PT\textsubscript{tm} data. Tukey’s test was used for post hoc tests, and \( p < 0.05 \) was considered significant. Data are shown as mean±SD unless otherwise stated.

**Results**

**Hemodynamic Parameters**

Representative original tracings are shown in Figure 2. Hemodynamic parameters under control conditions are shown in Table 1. The average heart rate was slightly increased during coronary occlusion but did not change significantly throughout the experimental period, and atrial pacing was not required. LV end-diastolic pressure during coronary occlusion and at 3 weeks was not significantly different from control. Peak LV pressure decreased slightly during occlusion and was significantly reduced at 2 hours after reperfusion. Peak positive dP/dt decreased slightly during early reperfusion, but there were no statistically significant changes.

**TABLE 1.** Hemodynamic Parameters at Control, Occlusion, and During Reperfusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Occlusion 80 minutes</th>
<th>2 hours</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>107.0±8.0</td>
<td>116.7±10.5</td>
<td>103.2±11.9</td>
<td>118.0±17.5</td>
<td>110.1±16.9</td>
<td>106.2±6.9</td>
<td>110.2±12.0</td>
<td>107.1±10.7</td>
<td>109.6±11.6</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>12.4±4.0</td>
<td>15.5±7.0</td>
<td>14.6±5.8</td>
<td>14.1±7.1</td>
<td>11.3±4.9</td>
<td>14.9±8.1</td>
<td>12.8±4.8</td>
<td>14.8±6.3</td>
<td>14.1±4.1</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>131.3±9.8</td>
<td>116.0±6.0</td>
<td>110.3±7.4*</td>
<td>115.8±12.7</td>
<td>120.2±11.5</td>
<td>126.3±14.7</td>
<td>126.9±16.5</td>
<td>131.4±12.1</td>
<td>131.1±13.8</td>
</tr>
<tr>
<td>dP/dt\textsubscript{max} (mm Hg/sec)</td>
<td>3,048±555</td>
<td>2,978±1,012</td>
<td>2,552±910</td>
<td>2,449±215</td>
<td>2,864±357</td>
<td>3,060±474</td>
<td>3,411±696</td>
<td>3,301±671</td>
<td>3,431±603</td>
</tr>
<tr>
<td>Mean AOP (mm Hg)</td>
<td>107.3±13.3</td>
<td>96.8±11.3</td>
<td>93.1±11.4</td>
<td>102.1±14.7</td>
<td>100.8±14.9</td>
<td>112.3±21.5</td>
<td>103.9±19.3</td>
<td>114.2±19.3</td>
<td>111.3±13.9</td>
</tr>
</tbody>
</table>

bpm, Beats per minute; LVEDP, left ventricular (LV) end-diastolic pressure; LVSP, LV peak systolic pressure; dP/dt\textsubscript{max}, peak positive dP/dt; dP/dt\textsubscript{min}, peak negative dP/dt; AOP, aortic pressure.

\( n = 7 \) (\( n = 5 \) for mean AOP).

*\( p < 0.05 \) vs. control.
Peak negative dP/dt was transiently decreased after reperfusion. At 3 weeks, none of the hemodynamic parameters were significantly changed from control values.

Systolic Wall Thickening and Wall Thickness

**Percent wall thickening.** Before coronary occlusion, percent systolic wall thickening (%WT) of the AT wall was 26.5%, and PT$_{im}$, PT$_{epi}$, and calculated PT$_{endo}$ were 24%, 19%, and 32%, respectively. The ischemic wall showed dyskinesia during occlusion, quickly recovered partially during the first 2 days after reperfusion, and then gradually improved further over the 3 weeks after reperfusion (shown as PT$_{im}$ and PT$_{epi}$ in Figure 3). PT$_{im}$ recovered to 67% of control by 3 weeks. Recovery of systolic wall thickening appeared somewhat greater for PT$_{epi}$ than for PT$_{endo}$ (Table 2); however, the scatter in the calculated values of PT$_{endo}$ was wide. The %WT of PT$_{im}$ remained significantly depressed after reperfusion (Table 2).

The %WT of the AT wall tended to increase during coronary occlusion, but the changes were not significant ($p=NS$). At 3 weeks of reperfusion, %WT of AT tended to be higher than control, but the difference was not statistically significant (Table 2).

**End-diastolic wall thickness.** Under control conditions, end-diastolic wall thickness of the AT wall, PT$_{im}$, PT$_{epi}$, and calculated PT$_{endo}$ were 11.6, 12.4, 7.2, and 5.2 mm, respectively. PT$_{epi}$ was 58% and PT$_{endo}$ 42% of PT$_{im}$ (Table 2). After reperfusion, PT$_{im}$ thickness increased markedly above control (Figure 3), then gradually decreased, and at 3 weeks after reperfusion it was not significantly different from the control level (Table 2). AT (control) wall thickness increased slightly during the last two weeks ($p=NS$). At 3 weeks, all wall thicknesses were not significantly different from control (Table 2).

**Regional Blood Flow**

The RBF data in the dogs with infarction are shown in Table 3. Values are corrected for infarction at control, occlusion, and 1 day after reperfusion. Values

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**Table 2. Wall Thickness and Systolic Thickening at Control, Occlusion, and During Reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Occlusion 80 minutes</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 hours</td>
</tr>
<tr>
<td><strong>EDWT (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT (n=5)</td>
<td>11.6±2.8</td>
<td>11.6±2.8</td>
<td>11.7±2.5</td>
</tr>
<tr>
<td>PT$_{im}$ (n=6)</td>
<td>12.4±2.0</td>
<td>11.9±2.3</td>
<td>15.18±4.5*</td>
</tr>
<tr>
<td>PT$_{epi}$ (n=6)</td>
<td>7.2±0.8</td>
<td>6.8±1.0</td>
<td>8.6±2.2</td>
</tr>
<tr>
<td>PT$_{endo}$ (n=5)</td>
<td>5.2±1.5</td>
<td>5.1±1.6</td>
<td>6.4±2.7</td>
</tr>
<tr>
<td><strong>%WT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT (n=5)</td>
<td>26.5±6.5</td>
<td>32.2±11.9</td>
<td>32.4±11.2</td>
</tr>
<tr>
<td>PT$_{im}$ (n=6)</td>
<td>23.9±4.5</td>
<td>-1.8±5.2*</td>
<td>-0.4±3.4*</td>
</tr>
<tr>
<td>PT$_{epi}$ (n=6)</td>
<td>19.1±9.7</td>
<td>-0.9±4.0*</td>
<td>-0.1±3.6*</td>
</tr>
<tr>
<td>PT$_{endo}$ (n=5)</td>
<td>32.1±25.5</td>
<td>-2.1±17.6</td>
<td>-0.4±13.7</td>
</tr>
</tbody>
</table>

EDWT, end-diastolic wall thickness; AT, anterior wall; PT$_{im}$, transmural posterior wall; PT$_{epi}$, epicardial half of posterior wall; PT$_{endo}$, endocardial half of posterior wall (obtained by subtracting PT$_{epi}$ from PT$_{endo}$); %WT, percent systolic wall thickening. Numbers in parentheses designate the number of animals.

* $p<0.05$ vs. control (PT$_{endo}$ was not used in the statistical analysis because it is dependent on both PT$_{im}$ and PT$_{epi}$).
at 3 weeks after infarction are not corrected, because no change in tissue volume is expected in the brief time between injection and the termination of the experiment. The time course of transmural RBF distribution in dogs with infarction is shown in Figure 4. During coronary occlusion, there was subendocardial ischemia. One day after reperfusion and at 3 weeks, the RBF had recovered, and at 3 weeks there were no significant differences from 1 day and control across the wall in the ischemic zone (Table 3). The AT wall RBF remained normal throughout the experiment (Figure 4).

**Scar Formation**

Scar formation was found in all three layers in the samples from the PT walls of dogs with infarction. Percent infarction (average percent scar formation) was greatest in the subendocardium and least in the subepicardium: 4.8% in the subepicardium, 10.0% in the midmyocardium, and 34.0% in the subendocardium. Scar formation >1% was not found in the AT walls in any of the dogs without infarction or in either wall of the sham-operated dogs.

**Myocardial Cell Areas**

Mean cell cross-sectional areas were larger in dogs with infarction than in dogs without infarction, and the ranges of distribution were wider in infarcted dogs. Cell areas in all dogs are shown in Table 4, in which the data represent the average of cells measured. The average number of cells counted from each subendocardial, midwall, or subepicardial region was 159±73.

The averaged cell areas of dogs with infarction were significantly larger than those of dogs without infarction in all regions of the PT (infarcted) wall and in the subendocardial region of the AT (normal) wall: 279 versus 237 μm² (PTepi, 18% increase), 291 versus 241 μm² (PTmid, 21% increase), 317 versus 233 μm² (PTendo, 37% increase), and 275 versus 218 μm² (ATendo, 26% increase) (Table 4, Figure 5). In dogs with infarction, the endocardial cell areas were significantly larger than the epicardial cell areas in both the AT and PT walls (Figure 6), and the endocardial–to–epicardial cell area ratios were abnormal (Figure 7). In dogs with infarction, cell areas were significantly larger in the PT wall than in the AT wall (Figure 6).

**TABLE 3. Regional Blood Flow of Dogs With Infarction**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control (corr)</th>
<th>Occlusion 80 minutes (corr)</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepicardium (ml · min⁻¹ · g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>1.07±0.24</td>
<td>1.07±0.24</td>
<td>1.09±0.42</td>
<td>0.95±0.25</td>
</tr>
<tr>
<td>PT</td>
<td>1.22±0.29</td>
<td>1.07±0.24</td>
<td>0.36±0.19*</td>
<td>0.91±0.33</td>
</tr>
<tr>
<td>Midwall (ml · min⁻¹ · g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>1.34±0.27</td>
<td>1.34±0.27</td>
<td>1.38±0.53</td>
<td>1.33±0.28</td>
</tr>
<tr>
<td>PT</td>
<td>1.56±0.29</td>
<td>1.34±0.27</td>
<td>0.19±0.10*</td>
<td>1.39±0.38</td>
</tr>
<tr>
<td>Subendocardium (ml · min⁻¹ · g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>1.45±0.37</td>
<td>1.45±0.37</td>
<td>1.57±0.50</td>
<td>1.41±0.37</td>
</tr>
<tr>
<td>PT</td>
<td>1.76±0.29</td>
<td>1.45±0.37</td>
<td>0.16±0.04*</td>
<td>1.26±0.30</td>
</tr>
</tbody>
</table>

n=6. corr, Values of PT are corrected for infarction; AT, anterior wall; PT, posterior wall (average from two blocks).

*p<0.05 vs. control.

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**FIGURE 4.** Graphs showing transmural regional blood flows in the subepicardium (EPI), midwall (MID), and subendocardium (ENDO) in the ischemic (PT, posterior) and nonischemic (AT, anterior) regions. Data are plotted on the ordinate at four time points. CONT, control; OCLL, during coronary occlusion at 80 minutes and at 1 day and 3 weeks (WK) after reperfusion. Data are shown as mean±SD.
In dogs without infarction, differences in cell areas between the AT and PT walls or across the walls were not significant (Figure 5).

**Discussion**

It is generally agreed that compensatory myocardial hypertrophy of the volume overload type often occurs in normal regions after myocardial infarction, but little is known about the response of surviving cells in the infarcted area. Hypertrophic responses in the ischemic zone could have an important role in the functional recovery of that region and the whole heart after myocardial infarction, particularly after reperfusion. Considering that myocardial necrosis after infarction is often nontransmural in human subjects, especially after successful thrombolysis, coronary angioplasty, or spontaneous recanalization in the acute phase, the present study in dogs was designed to evaluate the potential for surviving cells in a zone associated with nontransmural myocardial infarction to undergo hypertrophy during the time that recovery of regional function is under way after reperfusion.

**Responses of Regional Wall Motion and Thickness**

The relatively small hemodynamic changes observed suggest that the extent of subendocardial infarction in these dogs at 3 weeks was not large, a supposition supported by the observed degree of subendocardial necrosis (34%); however, shrinkage of the scar in the infarct region occurs^{16} and would not be manifested in our estimates of percent necrosis, leading to underestimation of the original degree of necrosis. The early

**Table 4. Averaged Cell Sizes of Dogs With and Without Infarction**

<table>
<thead>
<tr>
<th>Infarction (+)</th>
<th>PT (μm²)</th>
<th>AT (μm²)</th>
<th>Endo/epi</th>
<th>Infarction (−)</th>
<th>PT (μm²)</th>
<th>AT (μm²)</th>
<th>Endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Mid</td>
<td>Endo</td>
<td>Epi</td>
<td>Mid</td>
<td>Endo</td>
<td>Epi</td>
</tr>
<tr>
<td>Dog 1</td>
<td>381.2</td>
<td>390.7</td>
<td>401.1</td>
<td>297.1</td>
<td>295.5</td>
<td>345.6</td>
<td>1.05</td>
</tr>
<tr>
<td>Dog 2</td>
<td>264.0</td>
<td>285.6</td>
<td>342.7</td>
<td>259.3</td>
<td>245.2</td>
<td>248.4</td>
<td>1.30</td>
</tr>
<tr>
<td>Dog 3</td>
<td>371.7</td>
<td>370.1</td>
<td>402.6</td>
<td>303.8</td>
<td>280.4</td>
<td>319.7</td>
<td>1.08</td>
</tr>
<tr>
<td>Dog 4</td>
<td>233.0</td>
<td>240.0</td>
<td>257.3</td>
<td>199.8</td>
<td>194.1</td>
<td>261.9</td>
<td>1.10</td>
</tr>
<tr>
<td>Dog 5</td>
<td>176.9</td>
<td>222.6</td>
<td>216.8</td>
<td>162.4</td>
<td>186.9</td>
<td>194.6</td>
<td>1.23</td>
</tr>
<tr>
<td>Dog 6</td>
<td>247.0</td>
<td>238.1</td>
<td>279.7</td>
<td>200.3</td>
<td>252.3</td>
<td>277.0</td>
<td>1.13</td>
</tr>
<tr>
<td>Average</td>
<td>279.0*</td>
<td>291.2*</td>
<td>316.7*</td>
<td>237.1</td>
<td>242.4</td>
<td>274.5*</td>
<td>1.15*</td>
</tr>
<tr>
<td>SD</td>
<td>81.0</td>
<td>72.5</td>
<td>77.5</td>
<td>58.1</td>
<td>44.2</td>
<td>53.5</td>
<td>0.10</td>
</tr>
</tbody>
</table>

PT, posterior (wall); AT, anterior; Endo, subendocardium; Epi, subepicardium; Endo/epi, cell size ratio of Endo to Epi; Mid, midwall; infarction (+), dogs with infarction; infarction (−), dogs without infarction; S, sham dogs; C, control dogs.

*p<0.05 vs. dogs without infarction.

**Figure 5.** Bar graph showing comparison of cell areas between dogs with infarction and without infarction. Data are shown as mean±SD. EPI, subepicardium; MID, midwall; ENDO, subendocardium.
rapid increases of wall thickness in the ischemic zone at end diastole likely represent edema after reperfusion (Figure 3). At the end of the study period (3 weeks), the end-diastolic thicknesses of the AT wall, PT\textsubscript{m}, and PT\textsubscript{epi} walls were slightly increased, but changes were not statistically significant, and in the absence of measures of global ventricular geometry, these responses cannot be interpreted in terms of myocardial hypertrophy. However, any increase in chamber diameter or scar formation in the infarcted wall should reduce the transmural wall thickness, suggesting that the lack of significant change in the transmural thickness of the infarct zone (Figure 3) was a result of regional hypertrophy. We hypothesize that the recovery of regional function represents a combination of resolution of stunning during the first week and later changes mediated, at least in part, by hypertrophy primarily of the outer wall, which was ischemic during the coronary occlusion (Figure 4). In the present study, however, these relative contributions of hypertrophy to functional recovery cannot be determined with certainty. Others have reported that the epicardial half of the wall shows better early recovery from stunning than the transmural wall\textsuperscript{17}

**Myocardial Cellular Hypertrophy**

The fact that gross cardiac hypertrophy is often associated with myocardial infarction in human subjects is known from postmortem studies\textsuperscript{18–20} and recent postmortem findings have reported myocardial cellular hypertrophy in the outer third of the infarcted wall in some human subjects\textsuperscript{20} as well as in the noninfarcted wall\textsuperscript{21} 1 month or more after infarction. Studies in rats with permanent coronary artery occlusion have demonstrated that myocardial cellular hypertrophy occurs late after infarction,\textsuperscript{22–28} but hypertrophy can be an early event occurring within 1 week, as also demonstrated by studies in other models such as pressure overload.\textsuperscript{29} It has been shown that myocardial cellular hypertrophy in rats was present in the noninfarcted wall with large infarctions,\textsuperscript{27,28} and Olivetti et al\textsuperscript{22} demonstrated hypertrophic responses of surviving myocardium to be greatest close to the site of transmural infarction. In rats, the infarction caused by permanent coronary artery occlusion is usually transmural, with little spared myocardium in the outer wall. Also, in rats with transmural infarction and heart failure that were studied late (up to 6 months), Zimmer et al\textsuperscript{28} reported that hypertrophy was evident only by examining cell length (cross-sectional areas unchanged).

Our observations concern responses with smaller infarcts after coronary artery reperfusion at an earlier time and in a different species. To the best of our knowledge, they represent the first in which myocardial cellular responses to coronary occlusion with reperfusion have been examined transmurally in both noninfarcted and infarcted regions. In these dogs, at 3 weeks after reperfusion, the infarcted wall showed recovery to 67% of control systolic %WT, and myocardial cellular hypertrophy was observed both in the AT (noninfarcted) wall and in surviving cells throughout the PT (infarcted) wall, with cells in the infarcted region being larger than in normal regions and with the largest cells being in the subendocardium. The reason for the observed abnormal endocardial/epicardial ratio of cell area in the infarcted region is uncertain, but variations in regional wall stress may be one factor. An abrupt decline of LV performance after coronary occlusion

**Figure 6.** Bar graph showing comparison of cell areas within dogs with infarction in anterior (AT) and posterior (PT) walls. Data are shown as mean±SD. EPI, subepicardial; MID, midwall; ENDO, subendocardium.

**Figure 7.** Graph showing subendocardial-to-subepicardial (endo/epi) ratios of cell areas of all dogs in the anterior (AT) and posterior (PT) walls plotted as individual points and mean±SD.
causes LV dilation, at least for a period, and therefore regional wall stress will probably increase in both infarcted and normal walls. The subsequent abnormal chamber geometry could produce abnormal stress distributions. It has been reported that stretch of cultured myocardial cells produces a hypertrophic response, and such an effect around the infarcted site, as well as temporary regional ischemia, might evoke regional hypertrophy through activation of growth or other factors affecting upregulation of contractile protein gene expression. Thus, the pattern of hypertrophy in the reperfused zone noted in the present study suggests that a significant role is played by local factors, which remain to be investigated.

There was variability in the degree of cell area enlargement, but the reason for this finding is not entirely clear. At 3 weeks, there was a good inverse correlation between the percent scar formation in the subendocardial sample and the systolic %WT normalized to the control %WT (r = 0.99, p < 0.002), increased percent scar being correlated with decreased wall function as previously reported. One animal with infarction in which myocardial cell areas were unusually small (dog 5) had the least percent scar in the subendocardial region at 3 weeks (8%), at which time function had returned to normal. In other animals, the degree of subendocardial scar ranged between 18% and 70%, but there were no significant correlations between scar in the subendocardium (or the subendocardium plus the midwall) and cell areas (subendocardial or mean), although subendocardial cells were clearly larger in the infarcted than the control wall in four animals (infarcted wall) and five animals (AT wall). Thus, although there was cell area enlargement in the infarcted and normal regions, which could have contributed to the return of function observed in each animal, a mechanism for the considerable diversity of the response in cell areas cannot be identified in this relatively small series of animals.

Limitations of the Study

There are several limitations to the present study. We evaluated regional rather than global ventricular performance or geometry. Thus, the effect of the severity of the infarction and subsequent recovery on overall LV performance was not evaluated. Substantial changes in global LV performance appear unlikely, because hemodynamic parameters such as mean aortic pressure, LV end-diastolic pressure, and peak systolic LV pressure were unchanged at 3 weeks after reperfusion. We did not attempt to measure myocardial infarct size in these animals because of the heavy instrumentation, the need to remove blocks at the site at which instrumentation was implanted, and previous knowledge that the infarcted area would be rather small in this reperfusion model. However, we examined the infarct histologically at a central site to ensure the presence of tissue necrosis and to exclude dogs without significant infarct. We examined cellular hypertrophy as the cell cross-sectional area, without assessment of cell length or volume. The cell area measurements alone showed significant hypertrophy, however, consistent with the observed preservation of ventricular end-diastolic wall thickness in the ischemic region (despite subendocardial infarction). Cell length changes have been documented with more chronic transmural infarction in rats associated with LV dilation. Stretching of cells with changes in cell length could have further increased cell volume, and even in the presence of hypertrophy, elongation could have caused no change or a reduction in cell cross-sectional area, as observed in rats with transmural infarction; if such changes had occurred in our experiments, they would have resulted in underestimation of the degree of hypertrophy, based on cross-sectional cell areas. However, with the relatively small nontransmural infarcts produced in the present study, significant ventricular dilation would not be expected at 3 weeks, as supported by the lack of LV end-diastolic wall thinning of the noninfarcted AT wall at any time point (Table 2). Cell areas were relatively consistent within groups, except for one dog in the infarct group that showed unusually small cells but with a markedly abnormal endocardial/epicardial cell ratio. Because the same tissue preparation methods were used in all animals, and cell areas are consistent with previous reports, comparisons between the infarcted and noninfarcted groups should be valid.

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