No Reflow and Extent of Infarction During Maximal Vasodilation in the Porcine Heart

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To explore the relation between myocardial and vascular injury in the generation of the no-reflow phenomenon, the pressure-flow relation during maximal vasodilation after coronary artery reperfusion was studied in the open-chest porcine model. During both endogenous and maximal vasodilation with intracoronary adenosine, pressure-flow (P/Q) plots were constructed before and after 20-minute (n = 9) or 40-minute (n = 17) circumflex artery occlusions. Decreases in circumflex vascular bed conductance were represented by downward shifts in P/Q plot regression lines. No significant change occurred in P/Q line slope or pressure at zero flow 30 minutes after release of the 20-minute occlusion, and no infarction was found. After release of the 40-minute occlusion, a small but insignificant decrease in P/Q line slope occurred during endogenous vasodilation. However, during maximal vasodilation, a significant (p<0.01) decrease in P/Q line slope was present during reperfusion compared with preocclusion corresponding to a decrease in vasodilatory reserve (P/Q line slope = 1.52 ± 0.14 ml/min/mm Hg preocclusion vs. 1.03 ± 0.13 at 15 minutes reperfusion). Pretreatment with aspirin did not prevent this decrease in vascular conductance during maximal vasodilation. Total circumflex, as well as subendocardial, midmyocardial, and subepicardial blood flows, was measured with radioactive microspheres. There was a good correlation between the extent of infarction measured by triphenyltetrazolium chloride staining and the decrease in vascular conductance during maximal vasodilation for all three myocardial layers as well as for the total circumflex vascular bed. Hence, the degree of no-reflow correlates closely with the extent of infarction during maximal vasodilation (but not during endogenous vasodilation) and is not altered by aspirin therapy. (Circulation 1988;78:462–472)

Interest in preserving acutely ischemic myocardium has led to interventional techniques aimed at reestablishing blood flow to jeopardized myocardium.1–5 However, on establishing reperfusion after prolonged coronary occlusion, flow to the previously ischemic region is heterogeneous and lower than to the nonischemic region. This observation has been labeled the "no-reflow" phenomenon.6,7 The beneficial effects of restoring blood flow after ischemia may be limited because of this.

The pathogenesis of the no-reflow phenomenon is not well understood. Proposed mechanisms include capillary compression by myocardial tissue edema8–10 or myocardial ischemic contracture,11–12 direct ischemic microvascular injury with endothelial cell swelling,13–16 and increases in vasomotor tone.17–18 Rheologic factors have also been implicated including microvascular obstruction by red blood cell sludging,16 leukocyte plugging,19 or platelet aggregates either formed locally or embolized from larger coronary thrombi.20–22 However, the contributions of myocardial tissue damage, microvascular effects, and rheologic factors to the postischemic flow reduction with reperfusion are unknown. For example, it is not known whether a reduction in postischemia maximum blood flow occurs without infarction or whether the maximum blood flow during reperfusion is directly affected by the extent of myocardial cell damage.

The goal of the present study was to determine whether the no-reflow phenomenon was primarily related to myocardial damage, to vascular damage or dysfunction, or to both. First, we sought to establish whether reperfusion after severe ischemia without myocardial infarction produced an increase in vascular resistance and reduced maximum coronary flow. Then, we investigated whether flow reductions

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seen during reperfusion after severe ischemia resulting in myocardial damage correlated with the extent of infarction. The conducting properties of the reperfused vascular bed were evaluated not only during autoregulation but also during maximal vasodilation with intracoronary adenosine, which eliminates the effects of vasoactive substances and the metabolic requirements of the myocardium on blood flow. Pretreatment with aspirin was performed in a group of animals to determine if platelet aggregates contribute to the no-reflow phenomenon.

Materials and Methods

The methods described are similar to those we have previously used.23,24

Surgical Preparation and Instrumentation

Experiments were performed in pigs of either sex weighing 26–45 kg. They were premedicated with xylazine (2 mg/kg i.m.) and ketamine (10 mg/kg i.m.) and then anesthetized throughout the experiment with morphine sulfate (1 mg/kg/hr i.v.) and ketamine (10 mg/kg/hr i.v.). The animals were intubated and ventilated with oxygen-enriched air by a piston respirator. Tidal volume, respiratory rate, and percent inspired oxygen were adjusted to maintain arterial pH between 7.35 and 7.45, PCO2 between 35 and 45 mm Hg, and PO2 greater than 100 mm Hg.

Aorta and inferior vena cava catheters were placed through a groin cutdown. The heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. Catheters were inserted into the left atrium and the left ventricle for injection of radioactive microspheres and measurement of left ventricular pressure, respectively. The proximal-to-midcircumflex coronary artery was dissected free and instrumented from proximal to distal with a small silicone rubber catheter (i.d., 0.3 mm; o.d., 0.6 mm) inserted by the Herd-Barger25 technique for infusion of adenosine, an electromagnetic flow probe (2.0–3.0 mm), an inflatable hydraulic occluder, and a second silicone rubber catheter to measure distal coronary artery pressure.

Pressures were measured with Statham P23Gb (Cleveland, Ohio) strain gauges calibrated with a mercury manometer at the start of each experiment. The coronary catheter system has an undamped natural frequency of 44 Hz and a damping ratio of 0.45. Circumflex coronary flow was measured with a Statham 2202-2 flowmeter system, and zero flow was set and frequently checked during the experiment by brief complete cessations of flow produced by the occluder. Circumflex coronary artery phasic and mean pressures and flows, left ventricular pressure, and electrocardiogram were recorded with an eight-channel Brush recorder at a paper speed of 50 mm/sec.

Experimental Protocol

Pressure-flow (P/Q) relations were obtained by inflating the hydraulic occluder to achieve eight to 12 randomly chosen distal coronary artery pressures and flows, from control pressure and flow to complete occlusion of the circumflex artery. At each level of constant occluder inflation, the phasic and mean coronary flows and pressures were recorded at the end of 10 seconds, which allowed the distal coronary pressure to stabilize. It has been previously documented that steady-state pressure and flow are reached within 10 seconds.23 The obstruction was released by 20 seconds, and the animal was allowed to stabilize before the next P/Q point was measured.

P/Q plots were constructed for each pig from the eight to 12 distal circumflex pressures and corresponding flows and were analyzed with linear regression analysis to obtain P/Q line slopes for ease of comparison between groups. The coronary P/Q relation is a useful descriptor of the function of the coronary circulation. The slope of the relation represents the conductance, which is the inverse of the resistance (conductance = 1/resistance). Thus, an increase in the slope of the relation represents a decrease in vascular resistance, whereas a decrease in slope represents an increase in resistance. The P/Q relation has been shown previously to curve leftward at low perfusion pressures; thus, the pressure axis intercept at zero flow extrapolated from linear regression analysis is typically higher than the measured pressure at zero flow (Pzf).23 In this study, the Pzf's reported are the measured values.

To remove the effects of vasomotor tone and varying myocardial oxygen demand on coronary artery flow, measurements were also performed during maximal vasodilation with adenosine infused into the proximal circumflex coronary catheter at a rate of 40 μg/kg/min in an infused volume less than 3% of the total circumflex flow. We have previously shown that maximal vasodilation is achieved at a dose of 20 μg/kg/min, and twice this infusion rate was arbitrarily chosen and shown not to affect systemic blood pressure.24 Maximal vasodilation was confirmed by a 10-second period of circumflex occlusion showing an absence of reactive hyperemia.

Heart rate, left ventricular pressure, and P/Q relations were initially measured during autoregulation and then during adenosine infusion. Coronary occlusion was produced for 20 minutes in one group of animals and for 40 minutes in another group with the hydraulic occluder. Lidocaine (3 mg/kg i.v.) was given as a bolus before occlusion followed by a constant intravenous infusion of 2 mg/min over 2 hours. Heart rate, left ventricular pressure, and electrocardiogram were monitored during the occlusion. Deflation of the occluder was performed gradually over 5 minutes to limit ventricular ectopy. If required, defibrillation was performed promptly across the chest wall. In both groups, heart rate, left ventricular pressure, and P/Q relations were remeasured at 15 minutes of reperfusion during adenosine infusion, at 30 minutes during autoregulation, and finally at 45 minutes, again during adenosine infusion. Sufficient time was allowed for the vasodila-
tory effect of adenosine to end and flow to return to baseline before producing circumflex occlusion or performing autoregulation measurements at 30 minutes of reperfusion.

In addition, in the 40-minute occlusion group, regional myocardial flows were measured by the radioactive labeled microsphere technique at three different times: during adenosine infusion before occlusion, just before release of the 40-minute occlusion (no adenosine given), and during adenosine infusion at 60 minutes after release of the occlusion. Radioactive microspheres (diameter, 11 μm; labeled with Ce141, Cr51, or Ru103. New England Nuclear, Boston, Massachusetts) were injected into the left atrium over 30 seconds while a reference sample was withdrawn from the ascending aorta at a constant rate for 2 minutes. The microspheres were suspended in 0.9% saline with 0.01%/Tween 80 and were agitated for 15 minutes before injection. The number of microspheres was calculated so that the smallest sample of myocardium for which flow was calculated contained approximately 800 microspheres at control coronary flows. Five pigs of the 40-minute occlusion group were pretreated with aspirin, 35 mg/kg i.v., after instrumentation but before coronary occlusion.

Analysis of Infarction and Regional Blood Flow

To identify the individual coronary artery vascular beds, the heart was excised 3 hours after release of the coronary occlusion, and 30 ml each of Cardiogreen dye, Evan’s blue dye, and saline solution were injected simultaneously into the right, left anterior descending, and circumflex (distal to the occluder) coronary arteries, respectively. The great vessels, aorta, right ventricle, and large epicardial vessels were removed, and the left ventricle was cut transversely into four to six slices of 1-cm thickness parallel to the atrioventricular sulcus. Each slice was then incubated in a 1% solution of triphenyltetrazolium chloride (TTC) buffered in 0.2 M Tris buffer to pH 7.8 at approximately 37°C for 5–7 minutes. TTC forms red precipitates in the presence of intact dehydrogenase enzyme systems, giving a clear demarcation of the normal TTC stained tissue from unstained infarcted tissue. A close correlation has been found between infarct size measured by TTC staining and that measured by microscopy. The slices were then removed and rinsed briefly, and the basal surface of each slice was photographed. The slices were reassembled to maintain appropriate spatial geometry and placed in 10% buffered formalin for 3–7 days.

Infarct size was measured from the gross photographs of the TTC-stained slices by projecting 35-mm transparencies of the basal surface on the screen and tracing the total left ventricular section, the circumflex bed, and the infarcted zones within the circumflex bed. Later, the traced circumflex bed was divided equally into subendocardial, midmyocardial, and subepicardial layers. The traced copies were digitized with an electronic digitizing pad linked to a Cubicomp 3-D solid modeling system (Hayward, California) and an IBM-AT computer. Spatial resolution was within 10⁻³ in. Values of circumflex bed as a percentage of the total left ventricle, the percent infarction of the circumflex bed, and the percent infarction of the subendocardial, midmyocardial, and subepicardial layers within the circumflex bed were computed.

Tissue perfused distal to the coronary occluder, as well as tissue from the left anterior descending and right coronary artery, was identified by the dye injections in each slice. Each vascular bed was divided into three layers—a subendocardial, a midmyocardial, and a subepicardial layer. Tissue samples of approximately 1 g were counted for 5 minutes with a programmable Nuclear Data ND 600/660 multichannel analyzer (Schaumburg, Illinois) connected to a Packard instrument changer. Coronary flow was calculated for the three layers in the three vascular beds for the three sample periods and expressed in milliliters per minute per gram wet tissue.24,27

Statistical Analysis

Hemodynamic data, percent myocardial infarcted, and microsphere coronary blood flows before and after ischemia are presented with respective mean±SEM. Multiple comparisons were made by one-way ANOVA with repeated measures. Range testing was done with Tukey’s procedure. Correlations between circumflex pressure versus flow and regional blood flows and vascular conductances versus extent of infarction were obtained with linear regression analysis. The regression lines were compared for different slopes and elevations with one-way analysis of covariance. p<0.05 was considered to be statistically significant.

Results

Data were collected from a total of 26 pigs. Defibrillation for ventricular fibrillation at the time of reperfusion was performed in five pigs. In each, sinus rhythm was promptly restored, and these animals were included in the data analysis. Twenty-minute and 40-minute circumflex occlusions were performed successfully in nine and 17 pigs, respectively. Hemodynamic measurements including coronary P/Q relations were obtained in five of nine of the 20-minute occlusion group and 16 of 17 of the 40-minute occlusion group. The other four pigs of the 20-minute occlusion group underwent TTC staining only for analysis for infarction. Ten pigs in the 40-minute occlusion group received radioactive microsphere injections for measurement of regional blood flows and TTC staining for infarct sizing. Five of these pigs were pretreated with aspirin (35 mg/kg i.v.). Both mean and end-diastolic instantaneous coronary P/Q relations were analyzed. Because the trends and conclusions were the same for both, only the mean data will be presented.
TABLE 1. Hemodynamic Measurements and Pressure-Flow Regression Line Slopes Before, During, and After 20-Minute Circumflex Occlusion (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>Adenosine (15 min reperfusion)</th>
<th>Reperfusion</th>
<th>Adenosine (45 min reperfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autoregulation</td>
<td>Adenosine</td>
<td>Occlusion</td>
<td>Autoregulation</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>99.4 ± 4.2</td>
<td>102.8 ± 4.8</td>
<td>104.4 ± 6.2</td>
<td>110.4 ± 7.5</td>
</tr>
<tr>
<td>LVESP (mm Hg)</td>
<td>116.0 ± 6.4</td>
<td>119.8 ± 5.9</td>
<td>108.2 ± 7.7</td>
<td>108.8 ± 7.1</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>11.0 ± 0.8</td>
<td>12.2 ± 1.2</td>
<td>15.8 ± 6.7</td>
<td>12.6 ± 1.1</td>
</tr>
<tr>
<td>Mean Pzf (mm Hg)</td>
<td>17.4 ± 2.4</td>
<td>14.5 ± 1.4</td>
<td>. .</td>
<td>16.5 ± 1.1</td>
</tr>
<tr>
<td>Mean P/Q slope</td>
<td>0.30 ± 0.10</td>
<td>1.22 ± 0.07</td>
<td>. .</td>
<td>1.15 ± 0.07</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
HR, heart rate; LVESP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Pzf, distal coronary pressure when the coronary artery is occluded; P/Q, pressure-flow.

* p<0.05 vs. preocclusion value; † p<0.001 vs. preocclusion value.

Twenty-Minute Occlusion Group

Hemodynamics. Heart rate, left ventricular peak systolic pressure, and left ventricular end-diastolic pressure for pigs undergoing 20-minute circumflex occlusion are shown in Table 1. During endogenous vasodilation, no significant difference was present between the values for reperfusion or occlusion versus preocclusion for any of these variables except for heart rate, which increased significantly from 99.4 to 108.4 beats/min during reperfusion (p<0.01). During maximal vasodilation, heart rate was higher during reperfusion, but this increase was significant only at 45 minutes (p<0.05). Left ventricular peak systolic pressure was significantly lower at 15 minutes and 45 minutes of reperfusion during maximal vasodilation compared with preocclusion (p<0.05). The slight increase in left ventricular end-diastolic pressure during reperfusion was not statistically significant.

Pressure-flow relations and extent of infarction. P/Q plots were obtained during endogenous vasodilation in five pigs. Mean P/Q plots during endogenous vasodilation displayed the typical plateau of autoregulation with a break point between 40 and 50 mm Hg. At distal coronary artery pressures less than the break point, flows became pressure dependent. The slopes of the P/Q plots and the Pzfs 30 minutes after reperfusion were unchanged from the preocclusion values (Table 1).

Similarly, P/Q plots during adenosine infusion were obtained before occlusion and 15 and 45 minutes after reperfusion. As expected, the slopes of these P/Q relations are much higher than those during autoregulation, indicating a lower vascular resistance. The slopes of the P/Q lines after reperfusion do tend to decrease slightly compared with the preocclusion lines; however, this small shift is not statistically significant. The slopes actually increased after reperfusion in several of the animals. Hearts from four pigs that received 20 minutes of circumflex occlusion were stained with TTC, and all failed to demonstrate necrosis. Because vasodilatory reserve after release of the 20-minute coronary occlusion was not altered, regional blood flows with microspheres were not evaluated.

Forty-Minute Occlusion Group

Hemodynamics. The hemodynamic measurements for 11 pigs undergoing 40-minute occlusion and not pretreated with aspirin are shown in Table 2. During endogenous vasodilation, heart rate was significantly higher during reperfusion compared

TABLE 2. Hemodynamic Measurements and Pressure-Flow Regression Line Slopes Before, During, and After 40-Minute Circumflex Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>Adenosine (15 min reperfusion)</th>
<th>Reperfusion</th>
<th>Adenosine (45 min reperfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autoregulation</td>
<td>Adenosine</td>
<td>Occlusion</td>
<td>Autoregulation</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>101.3 ± 5.2</td>
<td>105.4 ± 6.5</td>
<td>109.5 ± 5.9</td>
<td>125.9 ± 6.9*</td>
</tr>
<tr>
<td>LVESP (mm Hg)</td>
<td>135.9 ± 10.1</td>
<td>131.6 ± 9.5</td>
<td>114.8 ± 9.1†</td>
<td>106.1 ± 8.4†</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>12.4 ± 1.2</td>
<td>12.5 ± 1.3</td>
<td>15.6 ± 1.5*</td>
<td>13.8 ± 1.4</td>
</tr>
<tr>
<td>Mean Pzf (mm Hg)</td>
<td>18.5 ± 1.1</td>
<td>16.3 ± 0.8</td>
<td>. .</td>
<td>19.1 ± 1.5</td>
</tr>
<tr>
<td>Mean P/Q slope</td>
<td>0.40 ± 0.05</td>
<td>1.52 ± 0.14</td>
<td>. .</td>
<td>1.03 ± 0.13†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 11).
HR, heart rate; LVESP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Pzf, distal coronary pressure when the coronary artery is occluded; P/Q, pressure-flow.

* p<0.05 vs. preocclusion value; † p<0.001 vs. preocclusion value; ‡ p<0.001 vs. preocclusion value.
Mean preocclusion (p<0.001). Similarly, during maximal vasodilation, left ventricular systolic pressure was significantly lower during reperfusion compared with preocclusion (p<0.001). Left ventricular end-diastolic pressure was significantly increased only during circumflex occlusion (p<0.05).

**Pressure-flow relations.** P/Q relations were obtained in 11 pigs undergoing 40-minute circumflex occlusions. P/Q plots during reperfusion were uniformly shifted rightward and downward. Representative plots during autoregulation and during maximal vasodilation are shown for one pig in Figure 1.

The mean P/Q regression line slopes and Pzfs for the 11 pigs before and after 40-minute circumflex occlusion are tabulated in Table 2. For autoregulation, slopes were computed with only the points with distal coronary pressures less than 50 mm Hg. During autoregulation, the decrease in slope during reperfusion compared with preocclusion was small and not statistically significant, whereas the Pzf increased significantly. However, during maximal vasodilation, the slopes were 33% lower than the preocclusion values (p<0.01). Thus, vasodilatory reserve was decreased from 280% to 155%. Slopes at 15 minutes and 45 minutes of reperfusion were not significantly different. Pzfs were significantly increased by 45 minutes of reperfusion (p<0.05).

**Aspirin-Pretreated 40-Minute Occlusion Group**

**Hemodynamics.** Hemodynamic measurements and P/Q relations were obtained in five additional pigs undergoing 40-minute circumflex occlusion and pretreated with aspirin (Table 3). During both autoregulation and maximal vasodilation, heart rate was significantly increased. Peak left ventricular systolic pressure was significantly decreased during reperfusion compared with preocclusion (p<0.05 or p<0.01). These hemodynamic changes were the same as for pigs not pretreated with aspirin (Table 2).

**Pressure-flow relations.** Slopes for the P/Q relations during autoregulation were not significantly different during reperfusion compared with during

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**Figure 1.** Circumflex pressure-flow (P/Q) plots during autoregulation and maximal vasodilation for one experiment before coronary occlusion and during reperfusion. Note rightward shift of P/Q plots after 40 minutes of coronary occlusion.

**Table 3.** Hemodynamic Measurements and Pressure-Flow Regression Line Slopes Before, During, and After 40-Minute Circumflex Occlusion in Pigs Pretreated With Aspirin

<table>
<thead>
<tr>
<th>Preocclusion</th>
<th></th>
<th></th>
<th>Reperfusion</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Autoregulation</td>
<td>Adenosine</td>
<td>Occlusion</td>
<td>Adenosine (15 min reperfusion)</td>
<td>Autoregulation (30 min reperfusion)</td>
<td>Adenosine (45 min reperfusion)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>92.0±10.4</td>
<td>94.4±10.9</td>
<td>112.4±13.0</td>
<td>135.2±5.5*</td>
<td>141.8±5.6*</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>120.2±8.7</td>
<td>127.6±12.0</td>
<td>108.4±2.9</td>
<td>92.0±3.4*</td>
<td>90.2±4.4*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>11.6±2.1</td>
<td>10.4±1.0</td>
<td>15.0±1.9</td>
<td>8.6±0.4</td>
<td>6.8±1.8</td>
</tr>
<tr>
<td>Mean Pzf (mm Hg)</td>
<td>14.8±1.4</td>
<td>14.9±1.7</td>
<td>...</td>
<td>13.8±1.0</td>
<td>17.4±1.5</td>
</tr>
<tr>
<td>Mean P/Q slope (ml/min/mm Hg)</td>
<td>0.56±0.14</td>
<td>2.28±0.63</td>
<td>...</td>
<td>1.51±0.31</td>
<td>0.48±0.16</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5).

HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Pzf, distal coronary pressure when the coronary artery is occluded; P/Q, pressure-flow.

*p<0.01 vs. preocclusion value; †p<0.05 vs. preocclusion value.
Regional Blood Flow

In 10 pigs of the 40-minute occlusion group, radioactive microspheres were injected before occlusion and at 60 minutes of reperfusion during adenosine infusion. Radioactive microspheres were also injected during occlusion without adenosine infusion. The decreases in microsphere flows with occlusion and with reperfusion are shown in Figure 2. The low microsphere flows during occlusion confirm that these pigs had minimal collateral flow to the ischemic region. As expected, the greatest decreases in flows during reperfusion occurred in the subendocardial layer (71%) with the least fall noted in the subepicardial layer (38%). The percent reduction in flow in the midmyocardial layer (61%) was intermediate between the subendocardial and subepicardial layers. For all three layers, the reperfusion microsphere flows were significantly lower than the preocclusion flows ($p<0.001$). The mean percent reduction in flow in each layer was different from the other layers ($p<0.05$).

Infarct Size

Hearts from the same 10 pigs given radioactive microspheres were stained with TTC to determine the extent of infarction as a percentage of the area at risk (Table 4). The circumflex bed accounted for nearly 30% (range, 24–40%) of the left ventricular mass as measured by the planimetric method. Both subendocardial and midmyocardial layers were infarcted to a significantly ($p<0.01$) greater extent than the subepicardial layer. On the average, two thirds of the subendocardial and midmyocardial layers and one third of the subepicardial layers sustained infarction. Although a slightly greater percentage of the midmyocardial layer was infarcted as compared with the subendocardium, the difference was not statistically significant.

The percent transmural infarction for the circumflex vascular bed varied greatly, ranging from 14% to 80%. The greatest variation in the extent of infarction occurred in the subepicardial layer (4–77%). The correlations between the percent transmural infarction and the left ventricular systolic pressure and double product (heart rate × systolic pressure) at 40 minutes of coronary occlusion were good ($r=0.75$ and 0.68, respectively). The correlations between extent of infarction and heart rate and left ventricular end-diastolic pressure were poor ($r=0.24$ and 0.07, respectively). Thus, the variability in infarct size was related to the myocardial oxygen demand in a given pig.

Relation Between Infarct Size and Regional Blood Flow

Regional blood flows during reperfusion with maximal vasodilation were expressed as a percent of their preocclusion flows and plotted against their respective percent vascular bed infarcted (Figure 3). There is a close correlation between the extent of infarction as a percentage of the area at risk and the blood flow during adenosine vasodilation as a percentage of its preocclusion adenosine vasodilation flow across the wall ($r=-0.79$), as well as for all three layers (subendocardial $r=-0.84$, midwall $r=-0.79$, and subepicardial $r=-0.76$). The slopes of the regression lines for the subendocardial, midmyocardial, and subepicardial layers are not significantly different from each other ($p>0.25$). The regression line for the subendocardial layer is not significantly lower than the regression lines for the other two layers ($p=0.10$ for the $y$-axis intercepts).
TABLE 4. Extent of Infarction Expressed as Percentage of Area at Risk Measured in Pigs Undergoing 40-Minute Circumflex Occlusion

<table>
<thead>
<tr>
<th>Percent circumflex of total left ventricle</th>
<th>Percent infarction of circumflex</th>
<th>Percent infarction by layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.9 ± 1.7</td>
<td>47.1 ± 7.5</td>
<td>Endo 63.0 ± 7.5* Mid 67.1 ± 9.4* Epi 36.0 ± 8.6</td>
</tr>
</tbody>
</table>

Endo, subendocardial layer; Mid, midmyocardial layer; Epi, subepicardial layer.
*p < 0.01 vs. subepicardial extent of infarction. Values are mean ± SEM.

A decline in reperfusion blood flow may not be the most accurate marker of the no-reflow phenomenon because both perfusion pressure and vascular resistance are determinants of flow. Vascular conductance (represented by the slope of the P/Q relation) is the increment of coronary blood flow expected for a given change in perfusion pressure and more accurately represents the blood conducting properties of the vascular bed. In the present study, regional vascular conductances were calculated by dividing the microsphere flows by their respective differences between mean distal coronary pressures and Pqfs.

The reperfusion transmural vascular conductances as a percentage of their preocclusion values are plotted versus the percentage of the circumflex area at risk that infarcted in Figure 4. A good ($r = -0.74$) linear correlation exists between the decline in transmural vascular conductance and the extent of infarction. With the exception of one animal with a small infarction (14% of the area at risk), reperfusion vascular conductances were lower than preocclusion values. The slope of the regression line ($y = 109 - 1.0x$) indicates that with enlarging infarcts, the decrease in vascular conductance as a fraction of its preocclusion value is closely correlated to the extent of infarction.

Similar plots were obtained for the vascular conductances of the subendocardial, midmyocardial, and epicardial layers of the circumflex vascular bed versus area at risk that infarcted (Figure 4). A good linear correlation between vascular conductance and extent of infarction exists for all three layers (subendocardial $r = -0.87$, midwall $r = -0.77$, and subepicardial $r = -0.73$). As with the transmural case, the regression equations for the subendocardial and midmyocardial layers have a slope close to $-1.0$ indicating that the decline in vascular conductance is close in magnitude to the extent of infarction. The slopes of the regression equations for the three layers are not significantly different ($p > 0.25$). The regression line for the subendocardial layer is not significantly lower than the regression lines for the other two layers ($p = 0.07$ for the y-axis intercepts).

**Figure 3.** Reperfusion blood flows by layer during maximal vasodilation (expressed as percentages of preocclusion values) vs. respective percent areas at risk infarcted. See text for discussion.
**Discussion**

The present study investigated changes in coronary vascular function and their relation to myocardial damage during the 1st hour of reperfusion in open-chest pig models. After 20 minutes of coronary occlusion followed by reperfusion, there was no evidence of infarction by TTC staining and no loss of vascular function as judged by P/Q relations during either autoregulation or maximal vasodilation. After 40 minutes of coronary occlusion, a small but insignificant decrease in vascular conductance was found during autoregulation. However, during maximal vasodilation, transmural vascular conductance was significantly reduced, and the degree of reduction correlated well with the extent of infarction. Because 15-minute and 45-minute reperfusion vascular conductances were not significantly different, the changes in vascular resistance were nearly completed by 15 minutes of reperfusion. Pretreatment with aspirin did not modify the process, suggesting that platelet aggregation, prostaglandins, and the release of vasoactive substances by platelets were not important factors.

Of interest, a good correlation was found during maximal vasodilation between the extent of infarction (expressed as a fraction of the area at risk) and the decline in blood flow or vascular conductance for all three myocardial layers as well as for the entire circumflex bed. This makes intuitive sense if almost all the blood flow after reperfusion occurs through the remaining noninfarcted tissue in the area at risk. This supports the view that myocardium that can be reperfused is probably viable. It also implies that after acute occlusion, the change in vascular conductance during maximal vasodilation might be a way to quantify infarct size.

The relation between reperfusion and myocardial damage has been examined in several dog studies. Using thioflavin S dye, Kloner et al. reported reperfusion defects (no-reflow) in areas of microvascular damage after release of 90-minute, but not 40-minute, coronary occlusions in dogs. Ultrastructural evidence of microvascular damage occurred only in tissue samples with myocardial cell injury. Later, using more sensitive radioactive microspheres, Kloner et al. reported severe reductions in flow to the subendocardial and midmyocardial layers (partially infarcted) with a moderate reduction in flow to the subepicardial layer (largely noninfarcted) at 1 hour after release of a 3-hour coronary occlusion. In dogs subjected to 2 hours of coronary occlusion, Cobb et al. demonstrated hyperemic microsphere flows in all layers during the first 15 minutes of reperfusion, with lower flows in myocardial samples with greater amounts of infarction. By 4 hours of reperfusion, coronary flow was decreased in regions with greater than 50% infarction compared with nonischemic regions.

The results of the present study complement and extend those of Kloner et al. and Cobb et al.
After a prolonged coronary occlusion of insufficient duration to produce infarction, vascular conductance was not altered, in agreement with thioflavin S tracer histological studies, and vasodilatory reserve was not reduced. After coronary occlusion of sufficient duration to produce infarction, a reduction in flow and vascular conductance occurred that was more severe in regions with greater necrosis. Furthermore, the magnitude of this reduction in vascular conductance measured during maximal vasodilation and the extent of infarction were closely related for all three layers.

Another important finding of the present study was that the reduction in vascular conductance measured during maximal vasodilation was nearly completed by 15 minutes of reperfusion. In contrast, during the first 15 minutes of reperfusion, Cobb et al? reported hyperemic blood flows to the previously ischemic region despite infarction. Thus, reduction in vascular conductance with P/Q relations, especially during maximal vasodilation as in the present study, is a more sensitive measure of vascular impairment. Finally, it is unlikely that platelet aggregates, prostaglandins, and vasoactive substances released by platelets contribute to the decline in maximum vascular conductance during the 1st hour of reperfusion because pretreatment with aspirin did not prevent the decline in vascular conductance.

In the present study, the extent of infarction averaged 14% of the left ventricular mass for the 40-minute occlusion group. This extent of infarction is in agreement with findings of other investigators for swine subjected to prolonged circumflex occlusion and postmortem human hearts examined 3–16 days after infarction. The large variability in extent of transmural infarction of the circumflex vascular bed can be explained in part by differences in myocardial oxygen demand during coronary occlusion. Recently, adenosine has been shown to inhibit neutrophil-mediated endothelial cell damage and reduce infarct size if administered immediately at the time of reperfusion. It is possible that the infusion of adenosine before occlusion and at 15 minutes and 45 minutes of reperfusion may have prolonged the duration of coronary occlusion necessary to produce infarction and mildly reduced the extent of transmural infarction in the 40-minute occlusion group.

The extent of infarction measured 3 hours after reperfusion averaged two thirds of the area at risk for the subendocardial and midmyocardial layers and one third for the subepicardium. The subendocardium, by virtue of its location away from epicardial vessels and proximity to the relatively high left ventricular intracavitary pressures, is known to be most vulnerable to ischemic injury during coronary occlusion. Because a rim of subendocardium was not infarcted, probably through local oxygen transport from blood in the left ventricular cavity, the extent of infarction was nearly the same for the subendocardial and midmyocardial layers.

Controversy exists over whether the transmural location of the myocardium influences the relation between the regional blood flow and the degree of infarction after acute coronary occlusion. In dogs subjected to acute coronary occlusion without reperfusion, Rivas et al found that for a given regional blood flow, the extent of infarction in the endocardial samples exceeded the percentage of infarction in the epicardial samples. He surmised that the relation between blood flow and extent of infarction varied in different layers of the myocardium. In contrast, Hess and Bache found no difference in the degree of reduction of blood flow for a given percentage of infarction across transmural layers. In the present study, the regression lines for reperfusion blood flow and vascular conductance for the three layers were not significantly different, although there was a trend toward lower blood flow and vascular conductance in the subendocardial layer for any given extent of infarction (Figure 4). The reason for this is unknown, but a reasonable hypothesis is that the interstitial pressure is higher in the inner layer after infarction because of local tissue edema.

Limitations

A limitation of the present study is that no attempt was made to dissociate regional blood flow to infarcted versus noninfarcted tissue within each layer. Thus, it cannot be determined if the decrease in flow corresponding to a decrease in vascular conductance resulted from complete occlusion of the small vessels in the infarcted zone or a more uniform reduction in the perfusion (and conductance) of the entire area at risk. However, because no infarction or change in vascular conductance during maximal vasodilation with adenosine occurred in the 20-minute occlusion group, it is far more likely that the decrease in vascular conductance results from no reflow or markedly reduced flow in the infarcted zone with little if any decline in the surrounding noninfarcted tissue.

A second limitation is that the sensitivity and specificity of our method for measuring vascular function are not clearly established. Adenosine was chosen because it produces maximal vasodilation of small arterioles and precapillary resistance vessels, which are expected to be influenced by intramyocardial pressure and edema. However, because adenosine is a potent endothelial cell–independent vasodilator, a normal vasodilatory response to adenosine does not preclude more subtle abnormalities of vascular response, including endothelial cell swelling or leakage across endothelial cell barriers. Also, the vascular response to endothelial cell dependent vasodilators may differ from that to adenosine.

Finally, the close correlation between the extent of infarction and the degree of vascular dysfunction suggests but does not prove a cause-and-effect relation. However, the lack of change in vascular
Implications

It has been demonstrated experimentally in animals and clinically in humans that myocardium is available for salvage by reperfusion after less than 6 hours of acute coronary occlusion. It has been suggested by other investigators that the presence of residual flow after reperfusion may be valuable in assessing the efficiency of interventions for reperfusion. The present study supports this belief. A high vascular conductance with maximal vasodilation shortly after reperfusion indicates a greater proportion of the myocardium may have been salvaged. Conversely, a very low vascular conductance after reperfusion indicates a larger infarction relative to the area at risk. Thus, measurement of the vascular conductance during maximal vasodilation 1 hour after reperfusion may allow prediction of the quantity of myocardium at risk for necrosis if coronary reocclusion should occur.

References


Key Words: • adenosine • coronary artery conductance • pressure-flow relation • reperfusion
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