Progression of myocardial injury during coronary occlusion in the collateral-deficient heart: a non-wavefront phenomenon

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Leshnower BG, Sakamoto H, Hamamoto H, Zeeshan A, Gorman JH 3rd, Gorman RC. Progression of myocardial injury during coronary occlusion in the collateral-deficient heart: a non-wavefront phenomenon. Am J Physiol Heart Circ Physiol 293: H1799–H1804, 2007. First published July 20, 2007; doi:10.1152/ajpheart.00590.2007.—It is widely accepted that, during acute coronary occlusion, ischemic cell death progresses from the subendocardium to the subepicardium in a wavefront fashion. This concept, which implies that the subendocardium is the most susceptible myocardial region to ischemic injury, was established using a canine model with an extensive system of subepicardial coronary collaterals. In humans, particularly in those with coronary artery disease, there is a wide range in the distribution and functional capacity of the collateral circulation, which may affect the pattern of infarct evolution. Using an ovine model with a limited system of preformed subendocardial coronary collaterals, we characterized the effect of increasing lengths of ischemia on regional blood flow and infarct size in three regions of the ventricular wall: subendocardium, midmyocardium, and subepicardium. Our results demonstrate that the myocardium and microvasculature in these three regions are equally susceptible to injury after 45 min of ischemia. When ischemic time is increased to 1 h, infarct size in the midmyocardium (90 ± 2%) is greater than in the subendocardium (76 ± 4%, \( P = 0.004 \)) and subepicardium (84 ± 5%, \( P = 0.13 \)). Microvascular dysfunction assessed as a percentage of baseline flow is also greater in the midmyocardium (14 ± 5%) compared with the subendocardium (20 ± 3%, \( P = 0.23 \)) and subepicardium (51 ± 9%, \( P = 0.007 \)). These findings suggest that, in subjects with a limited system of coronary collateral circulation, the midmyocardium is the most susceptible myocardial region to ischemia and the subendocardium is the most resistant. Myocardial viability during coronary occlusion appears to be primarily determined by the distribution and functional capacity of the collateral circulation.

myocardial infarction; reperfusion; microcirculation

In mammalian species, the subendocardium is considered to be the most susceptible region of the myocardium to ischemic injury. During acute coronary occlusion, it is widely accepted that myocardial cell death progresses as a transmural wavefront from the subendocardium toward the subepicardium with increasing lengths of ischemia. This concept is the result of a series of ischemia-reperfusion experiments in the canine, which have been cited over 1,100 times over the past three decades (18, 19). The wide extrapolation of the wavefront concept to human infarct pathophysiology tacitly assumes that there are minimal differences between the coronary anatomy of humans and dogs. The canine coronary circulation contains a well-developed native system of functional, preformed epicardial collaterals, which results in the persistence of 15–30% of baseline blood flow during coronary occlusion (2, 9, 14). In the normal human heart, a native system of preformed collaterals is also present, but is much less developed, located primarily in the subendocardium, and its physiological relevance remains controversial (4, 7, 23, 26).

In patients with coronary artery disease (CAD), it has been shown that a robust system of collaterals can develop over time, which is able, in some cases, to significantly mitigate the effects of an acute coronary occlusion (15). Approximately one-third of patients with CAD develop a system of coronary collaterals that is capable of preventing myocardial ischemia during coronary artery occlusion (15). The development of a functionally significant coronary collateral system has been linked to the presence of high-grade, flow-limiting atherosclerotic lesions, which reduce the luminal diameter by ≥70% (1, 13). The majority of myocardial infarctions have, however, been shown to occur in the presence of mild- to moderate-, rather than high-grade atherosclerotic lesions, which do not provide a strong stimulus for collateral circulation development (1, 5, 8, 20).

Thus there is a wide range in the distribution and functional capacity of the human coronary collateral circulation. The wavefront pattern is most likely representative of infarct evolution in patients with well-developed collaterals. Limited data exist regarding the transmural progression of myocyte death in collateral-deficient models, which may more accurately represent the anatomy of the coronary circulation found in a substantial subset of humans with CAD. We, therefore, conducted a series of experiments using an ovine model of ischemia-reperfusion. The sheep was selected because of its paucity of preformed collaterals and the consistent nature of these animals’ coronary anatomy (3, 10–13, 24).

Materials and Methods

Surgical protocol. Thirty-five Dorset male hybrid sheep weighing 35–40 kg were used in this study. Animals were treated under experimental protocols approved by the University of Pennsylvania’s Institutional Animal Care and Use Committee and in compliance with National Institutes of Health Publication No. 85–23, revised 1996. Anesthesia was induced with thiopental sodium (10–15 mg/kg iv), and sheep were intubated, anesthetized with isoflurane (1.5–2%), and ventilated with oxygen (Drager anesthesia monitor, North American Drager, Telford, PA). Fluid-filled catheters were placed in a femoral artery and internal jugular vein for the continuous measurement of blood pressure and the administration of intravenous medications. A Swan-Ganz catheter (131h-7F, Baxter Healthcare, Irvine, CA) was placed in the right ventricle via the right jugular and pulmonary arteries. The catheter was connected to a fluid-filled manometer and an oximeter, which were connected to an automated computer system for continuous blood pressure and oxygen saturation monitoring. Swan-Ganz catheter data were recorded at regular intervals throughout the experiment to assess the stability of the animals' hemodynamic status. A Swan-Ganz catheter (131h-7F, Baxter Healthcare, Irvine, CA) was placed in the right ventricle via the right jugular and pulmonary arteries. The catheter was connected to a fluid-filled manometer and an oximeter, which were connected to an automated computer system for continuous blood pressure and oxygen saturation monitoring. Swan-Ganz catheter data were recorded at regular intervals throughout the experiment to assess the stability of the animals' hemodynamic status.
introduced into the pulmonary artery through the internal jugular vein. Animals underwent a left thoracotomy, and silicone vascular loops (Quest Medical, Allen, TX) were placed around the left anterior descending artery and its second diagonal branch, which is 40% of the distance from the apex to the base of the heart. Occlusion of these arteries at these locations has produced a well-characterized model of anteroapical myocardial infarction in our laboratory (3, 11, 12).

Arterial blood pressure, heart rate, surface electrocardiograms (ECG), and rectal temperature were continuously monitored (Hewlett Packard 78534C; Palo Alto, CA) throughout the protocol in all animals. A hyper/hypothermia unit (Medi-Therm III, Gaymar Industries, Orchard Park, NY) was used to maintain core temperature of 39 – 40°C in sheep. Arterial blood gases were measured in all animals, and the mean pH was maintained at 7.40 ± 0.04 throughout the protocol.

Experimental protocol. After instrumentation and collection of microsphere data before coronary ischemia, sheep were assigned into groups and underwent coronary artery occlusion for 45 min (n = 6), 1 h (n = 9), 2 h (n = 8), 3 h (n = 6), or 6 h (n = 6). Before the induction of ischemia, a prophylactic antiarrhythmic regimen of 150 mg amiodarone, 1 mg/kg of lidocaine, and 1 g magnesium sulfate was administered intravenously. These agents have proven to greatly reduce malignant arrhythmias without having a significant impact on infarct size in this sheep model of ischemia-reperfusion (unpublished data from our laboratory). Next, coronary snares were tightened to produce an ischemic region of the left ventricle (LV). Ischemia was confirmed by a visible color change in the ischemic myocardial region and ST segment elevations on the ECG. Microsphere data were collected again after 30 min of ischemia. At the end of the ischemic period, coronary snares were loosened, and the previously ischemic myocardium was reperfused for all animals. The reperfused myocardium typically exhibited a visible hyperemic response. Microsphere data were collected upon release of the coronary snares and after 3 h of reperfusion.

Analysis of area at risk and infarct size. At the completion of the protocol, the coronary snares were retightened; vascular clamps were used to occlude the aorta, pulmonary artery, and inferior vena cava; and the right atrium was incised. One milliliter per kilogram of Evans blue dye (Sigma, St. Louis, MO) was injected via the left atrium to delineate the ischemic myocardial risk area (AR). All animals were euthanized via an injection of potassium chloride into the left atrium.

Table 1. Effects of increasing lengths of ischemia on the transmural distribution of the infarct

<table>
<thead>
<tr>
<th>Ischemic Time</th>
<th>n</th>
<th>Endo</th>
<th>Mid</th>
<th>Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 min</td>
<td>6</td>
<td>47±10</td>
<td>47±13</td>
<td>35±8</td>
</tr>
<tr>
<td>1 h</td>
<td>9</td>
<td>76±4</td>
<td>90±2*</td>
<td>84±3</td>
</tr>
<tr>
<td>2 h</td>
<td>8</td>
<td>84±1</td>
<td>96±1*</td>
<td>91±3</td>
</tr>
<tr>
<td>3 h</td>
<td>6</td>
<td>84±2</td>
<td>97±2*</td>
<td>96±1*</td>
</tr>
<tr>
<td>6 h</td>
<td>6</td>
<td>87±2</td>
<td>99±0.4*</td>
<td>98±1*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a percentage of the infarct of the area at risk; n, no. of animals. Endo, subendocardium; Mid, midmyocardium; Epi, subepicardium. *P < 0.05 compared with Endo.
Multiple points around the circumference, and individual arcs were divided into three equal regions (subepicardium, midmyocardium, subendocardium), and the I/AR was measured in each region using planimetry. \*P < 0.01 from subendocardium.

Next, the heart was excised, and the LV was sectioned perpendicular to its long axis into six slices. The thickness of each slice was measured with a digital micrometer, and all slices were photographed. All slices were then incubated in 2% triphenyltetrazolium chloride (TTC) at 37°C for 20 min and rephotographed. All photographs were imported into an image analysis program (Image Pro Plus, Media Cybernetics, Silver Spring, MD).

Myocardium unstained by Evans blue dye was determined to be the AR (Fig. 1A). Infarct area was determined by incubating the myocardium in TTC. TTC is a colorless dye, which is reduced to a brick-red colored precipitate in the presence of the coenzyme NADH (6). During reperfusion of previously ischemic myocardium, NADH is washed out of all necrotic myocytes (6). This results in a clear delineation of viable myocardium, which stains brick-red, and nonviable myocardium, which is visualized as an unstained, pale color (Fig. 1B).

Computerized planimetry (Image Pro Plus, Media Cybernetics) was used to measure AR and infarct areas. AR is expressed as a percentage of the LV (AR/LV), and infarct size is expressed as a percentage of the LV (AR/LV), and infarct area is measured for the AR and I/AR for the entire LV. Transmurality analysis. Using advanced planimetry techniques (Image Pro Plus, Media Cybernetics), a transmural analysis was performed on the AR in the second slice from the apex to evaluate the spread of ischemic cell death within different regions of the myocardium. The second slice was selected because of its consistent appearance following ischemia and reperfusion from prior experiments using this model (3, 11). After basic planimetry was completed, the radius of the left ventricular wall was divided into three equivalent lengths at multiple points around the circumference, and individual arcs were created, which connected these radial points. Next, these arcs were connected circumferentially to form concentric ellipses, which divided the AR into three statistically equivalent areas (subendocardium, midmyocardium, and subepicardium; P < 0.05). AR and I/AR were measured and calculated in each of the three areas of myocardium (Fig. 1).

Regional blood flow measurements. In all animals, fifteen million color-coded, 15.5-μm-diameter NuFlow Fluorescent microspheres (IMT Laboratories, Irvine, CA) were injected to measure the degree of ischemia during coronary occlusion and to study the effects of increasing ischemic time on microvascular integrity after reperfusion. Injections were made at baseline, after 30 min of ischemia, at the onset of reperfusion, and after 180 min of reperfusion. Reference blood samples were taken at all time points. At the end of the experiment, in a similar fashion to the transmural analysis described above, the AR from the second slice from the apex in each animal was isolated and circumferentially sectioned into three equivalent areas: subendocardium, midmyocardium, and subepicardium. The three different areas of myocardium and reference blood samples were analyzed using flow cytometry for microsphere content by IMT Laboratories. Regional perfusion was calculated using the following formula: \[ Q_m = (C_m \times Q)/C \], where \( Q_m \) is myocardial blood flow per gram (ml/min-g^-1), \( C_m \) is microsphere count per gram of tissue in sample; \( Q \) is withdrawal rate of the reference blood sample (ml/min); and \( C \) is microsphere count in the reference blood sample. Regional blood flow (RBF) values were normalized and expressed as a percentage of baseline flow.

Statistics. Measurements are reported as means ± SE. ANOVA was used for all comparisons between groups, and repeated-measures ANOVA was used for all comparisons within groups. All analyses were completed using SPSS version 11.0 (SPSS, Chicago, IL). Statistically significant differences were established at P < 0.05.

RESULTS

The mean AR for the entire LV for all 35 sheep was 23 ± 1%, which is consistent with our past experience with this model. I/AR measured 39 ± 5% after 45 min of ischemia. I/AR significantly increased vs. the 45-min group after 1 h (72 ± 4%), 2 h (84 ± 2%), 3 h (93 ± 1%), and 6 h (96 ± 1%) of ischemia (Fig. 2).

I/AR was measured in the subendocardium, midmyocardium, and subepicardium of the second slice from the apex (Table 1, Fig. 1C). Forty-five minutes of ischemia created a homogenous injury in the three regions of the ventricular wall. Infarct size in the subendocardium (47 ± 10%), midmyocardium (47 ± 13%), and subepicardium (35 ± 8%) were similar (P > 0.05 for all comparisons). When ischemic time was increased to ≥1 h, the morphology of the infarct changed. The ischemic injury became significantly larger in size and more heterogeneous in its distribution. After 1 h of ischemia, the midmyocardial infarct size increased to 90 ± 2%, which was

<p>| Table 2. Effects of increasing lengths of ischemia on the transmurality analysis of regional blood flow |
|------------------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Ischemic Time</th>
<th>RBF During Ischemia, %Baseline</th>
<th>RBF at Reperfusion, %Baseline</th>
<th>RBF After 3 h of Reperfusion, %Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endo</td>
<td>Mid</td>
<td>Epi</td>
</tr>
<tr>
<td>45 min</td>
<td>3 ± 1†</td>
<td>5 ± 2†</td>
<td>6 ± 3†</td>
</tr>
<tr>
<td>1 h</td>
<td>3 ± 0.2*†</td>
<td>1 ± 0.3†‡</td>
<td>7 ± 5†</td>
</tr>
<tr>
<td>2 h</td>
<td>4 ± 2†</td>
<td>4 ± 2†</td>
<td>35 ± 12†</td>
</tr>
<tr>
<td>3 h</td>
<td>0.5 ± 0.3†</td>
<td>0.2 ± 0.1†</td>
<td>0.4 ± 0.3†</td>
</tr>
<tr>
<td>6 h</td>
<td>0.1 ± 0.0†</td>
<td>0.1 ± 0.0†</td>
<td>0.4 ± 0.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a percentage of baseline flow. RBF, regional blood flow. All comparisons are made within groups. *P < 0.05 from Mid; †P < 0.05 from baseline; §P < 0.05 from Endo; $P < 0.05 from onset of reperfusion.
greater than the subendocardial infarct size (76 ± 4%, \( P = 0.004 \)) and subepicardial infarct size (84 ± 3%, \( P = 0.13 \)). After 2 h of ischemia, the midmyocardial infarct size increased to 96 ± 1%, which was greater than the subendocardial infarct size (84 ± 2%, \( P = 0.001 \)) and subepicardial infarct size (91 ± 3%, \( P = 0.14 \)). As ischemic time progressed to 6 h, cell death in the midmyocardium and subepicardium approached 100%, while 13% of the subendocardium remained viable (Table 1, Fig. 3).

The results of the transmural analysis of RBF are presented in Tables 2 and 3 and Fig. 4. For all animals, the mean RBF values in the subendocardium, midmyocardium, and subepicardium measured 2.4 ± 0.6, 2.4 ± 0.7, and 3.6 ± 1% of baseline flow, respectively, which confirmed the lack of significant preformed collaterals in sheep.

After 45 min of ischemia, there was a transmural hyperemic response at the onset of reperfusion, but only a mild degree of microvascular dysfunction after 3 h of reperfusion. As ischemic time was increased to 1 h, microvascular blood flow immediately upon reperfusion was mildly increased in the subendocardium (159 ± 49%) and midmyocardium (190 ± 24%) and mildly decreased in the midmyocardium (84 ± 42%) relative to baseline. After 3 h of reperfusion, a significant no-reflow effect was observed in all three regions of the ventricular wall (subendocardium, 20 ± 3%, \( P = 0.001 \); midmyocardium, 14 ± 5%, \( P = 0.03 \); subepicardium, 51 ± 9%, \( P = 0.06 \)).

When ischemic time was increased to 2 h, a significant transmural injury to the microvasculature was evident at the immediate onset of reperfusion (subendocardium, 35 ± 12%, \( P = 0.01 \); midmyocardium, 21 ± 8%, \( P = 0.001 \); subepicardium, 64 ± 9%, \( P = 0.04 \)). Again, the reduction in microvascular blood flow was most severe in the midmyocardium. Blood flow in the midmyocardium at the onset of reperfusion diminished to 7 ± 2 and 5 ± 2% in the 3- and 6-h groups, respectively. Injury to the subendocardial and subepicardial microvasculature also became more severe with increased ischemic time; however, perfusion in these regions remained significantly better than in the midmyocardium, even after 6 h of ischemia (Tables 2 and 3, Fig. 4).

**DISCUSSION**

The concept that ischemic myocardial cell death begins in the subendocardium and progresses in a wavefront fashion toward the subepicardium has been uniformly accepted for nearly three decades (18, 19, 25). Little attention has been directed toward understanding how the coronary collateral circulation impacts this phenomenon. The wavefront concept, which was developed in a canine model with a native system of well-developed epicardial coronary collaterals, implies that the subendocardium is the most vulnerable region of the myocardium to ischemic injury (18, 19). Due to variability in the development of the coronary collateral circulation in humans and other mammalian species, we hypothesized that this pattern of infarct progression is not an inherent property of mammalian myocardium, but rather dependent on the distribution and functional capacity of the coronary collateral circulation. To investigate this question, we characterized the evolution and distribution of ischemic cell death with increasing lengths of ischemia using an ovine model.
Sheep were selected because their coronary circulation contains a very limited system of collaterals (3, 10–13, 24).

We demonstrated that, after 45 min of ischemia, cell death was not localized to the subendocardial region, but rather distributed equally throughout the ventricular wall. When ischemic time was increased to 1 h, the size and distribution of the infarct were significantly altered. Infarct size within the entire AR increased from 39 to 72%, and a transmural gradient in myocardial necrosis was established in which cell death was consistently greatest in the midmyocardium and least in the subendocardium. When ischemic duration progressed to 6 h, 98% of midmyocardial and subepicardial myocardium was dead, yet 13% of the subendocardium (usually in the layer closest to the LV cavity) remained viable (Fig. 5). This pattern of myocardial salvage after such prolonged (6 h) ischemia most likely resulted from a subendocardial collateral system concentrated on the endocardial edge of the LV wall. Such subendocardial collateral systems have been described in the pig and humans, but have not been definitively documented in sheep (7, 21, 22). If a narrow subendocardial collateral network or system of sinusoidal vessels communicating directly with the LV cavity exist in sheep, our assessment of blood flow using microspheres was not sensitive enough to demonstrate it. An alternative explanation would be the direct diffusion of oxygen from the LV chamber; however, this seems unlikely given the distance of diffusion required (>1 mm).

The reperfusion blood flow data demonstrated a heightened susceptibility of the midmyocardium to ischemia-reperfusion induced microvascular injury. After 45 min of ischemia, a mild microvascular injury was discernable, which affected blood flow to the subendocardium, midmyocardium, and subepicardium equally. When ischemic time was increased to 1 h, the injury became heterogeneous, with blood flow greatest in the subepicardium and least in the midmyocardium. After 2 h of ischemia, blood flow to the midmyocardium had decreased to 21 ± 8% of baseline and was almost absent (5 ± 2%) after 6 h of ischemia.

The transmural gradient in myocardial perfusion that developed after ischemia and reperfusion in sheep was different from that described in the dog in the initial wavefront experiments (18, 19). In those studies, canine coronary blood flow was consistently greatest in the subepicardium and least in the subendocardium (18). We found a gradient in postreperfusion RBF that was also consistently greatest in the subepicardium, but least in the midmyocardium. It is unclear what is responsible for this variation in the regional distribution of microvascular injury; however, the profound difference in the anatomy of the collateral circulation that exists between the two species is a likely contributing factor.

These data are consistent with the work using rabbits of Reffelmann and colleagues (16, 17) and clearly demonstrate that the immediate postreperfusion severity and progression of the no-reflow phenomenon in the myocardium at risk is strongly dependent on the duration of ischemia. With shorter ischemic times, the microvascular injury immediately upon reperfusion is relatively mild, but progresses during the reperfusion period. With longer ischemic intervals, a profound microvascular injury is apparent immediately upon reperfusion.

The data generated in this study strongly suggest that the wavefront progression of myocardial death described nearly three decades ago is not an inherent response of the mammalian myocardium to ischemia, but rather a result of the collateral coronary circulation that commonly occurs in the dog. The implication of these experimental findings for patients is difficult to assess, since the functional significance of the human.

Fig. 4. Regional analysis of differences in regional blood flow (RBF) relative to preischemic (baseline) values after increasing lengths of ischemia and 3 h of reperfusion. The AR was divided into three equal regions (subepicardium, midmyocardium, subendocardium). *P < 0.01 from midmyocardium.

Fig. 5. Photographs of the ovine left ventricle after 6 h of ischemia and 3 h of reperfusion. A: Evans blue dye to delineate the ischemic AR. AR is the unstained myocardium. B: TTC staining to delineate viable (brick red) from nonviable (pale) myocardium in the AR. The arrows indicate areas of viable subendocardial myocardium.
coronary collateral system varies widely in normal subjects and is even more variable in patients with CAD (15, 23, 26). The infarct morphology of patients with a functionally adequate, “protective” system of collaterals is likely better represented by the wavefront pattern of infarct progression. Patients with limited collateral development are likely to be best modeled by the sheep pattern of ischemic cell death, in which the midmyocardium is the most sensitive myocardial region and the subendocardium is the most resistant. The dramatic difference in infarct progression during coronary occlusion that occurs between the dog and the sheep supports the conclusion that the anatomy of the coronary collateral circulation is the primary determinant of infarct distribution following myocardial ischaemia and reperfusion.

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GRANTS

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