Heparin Accelerates Coronary Collateral Development in a Porcine Model of Coronary Artery Occlusion

Susan M. Carroll, PhD; Francis C. White, MS; David M. Roth, PhD; and Colin M. Bloor, MD

Background. Coronary collaterals develop in response to an ischemic stimulus. However, collateral growth is not sufficient to result in the complete recovery of coronary reserves. Using a porcine model of gradual coronary artery occlusion, we investigated the effect of continuous heparin infusion on coronary collateral development.

Methods and Results. We placed ameroid constrictors on the left circumflex coronary artery of 16 minipigs; the ameroid constrictors completely occluded the left circumflex coronary artery at 10±1 days. Half of the animals also were instrumented with subcutaneously placed osmotic pumps and catheters that delivered heparin (300 units/h) into the external jugular vein. At 2, 3, and 4 weeks, we assessed blood flow at rest and during vasodilation using radioactive microspheres. Our results indicate that the animals receiving heparin restored resting myocardial blood flow to normal levels at or before 2 weeks; in contrast, we did not see normal resting myocardial blood flow levels in the untreated-ameroind animals until 3 weeks. Under vasodilated conditions, untreated-ameroind animals experienced a severe loss of coronary reserves at 2 weeks. Although this improved with time, these animals still were significantly underperfused at 4 weeks. In contrast, in the heparin-treated animals, coronary reserves returned to near-normal levels between 3 and 4 weeks. In addition, infarct size was significantly smaller in the heparin-treated animals.

Conclusions. These experiments suggest that the administration of heparin in the early phases of gradual coronary occlusion accelerates the rate of return of normal blood flow under resting conditions, substantially increases the recovery of coronary reserve, and reduces the risk of infarction. (Circulation 1993;88:198-207)

Key Words • ischemia • angiogenesis • myocardial infarction • heparin • collaterals

A ngiogenesis normally does not occur in the adult heart. However, in response to an ischemic stimulus such as the occlusion of a coronary artery, new collateral vessels develop.1-4 In addition, vessel remodeling and new vessel synthesis in the bed at risk occur.5-7 Studies in dogs have shown that this species possesses an extensive endogenous collateral circulation. The growth of new collateral vessels in the dog after slow coronary artery occlusion is a rapid process8,9 that results in normal blood flow to the region at risk, maintenance of coronary reserves, and reduction of infarct size in the region at risk.10 In contrast, humans and pigs have limited endogenous collateral circulation.11-13 Using a porcine model, we previously have shown that pigs respond to an ischemic stimulus with a rapid burst of collateral development, and although the newly synthesized collateral vessels allow the restoration of normal resting blood flow levels after coronary artery occlusion, coronary reserve levels remain severely compromised.12,14,15

In the past decade, several potent angiogenic agents, including a variety of growth factors, have been described. The usefulness of these factors in facilitating blood vessel growth in the heart is an important field of investigation. Several studies have demonstrated that heparin is a potent angiogenic agent.16,17 In addition, there is evidence suggesting that administration of heparin in vivo has positive effects on collateral development in the heart. For example, heparin, in conjunction with exercise in humans, increased the level of blood vessel development in patients with stable effort angina.18-20 Heparin also accelerates collateral growth in dogs subjected to brief repeated coronary occlusion.21 In addition, Unger and coworkers22 found that after an ischemic stimulus, localized delivery of heparin to a specific vessel increased the proportion of collateral flow originating from that vessel. However, in this canine model, the total amount of collateral flow did not increase.22

We have developed a porcine model to study the rate of blood vessel growth in response to ischemia. We and others have shown by a variety of methods that angiogenesis occurs in animals in which an ameroid constrictor occludes the left circumflex coronary artery.12,15,23 In

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Supported in part by National Institutes of Health NHLBI grant HL-32670.

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All editorial decisions for this article, including selection of reviewers and the final decision, were made by David G. Harrison, MD, as guest editor. This procedure applies to all manuscripts with authors from the University of California San Diego or UCSD Medical Center.

Received July 30, 1992; revision accepted February 27, 1993.
the present study, we examined the effect of constant heparin infusion on collateral development in pigs. Our results suggest that heparin greatly increases the rate of collateral development and facilitates the rapid recovery of coronary reserves.

Methods

Animal Preparation

The animals in this experimental protocol were handled according to the guidelines for animal care of the American Physiology Society. The Animal Subjects Committee of the University of California, San Diego approved this protocol. We sedated the pigs with ketamine (25 mg/kg i.m.), atropine (0.05 mg/kg i.m.), and sodium thialmylal (20 mg/kg i.v.), and then intubated and maintained them on 1% to 2% halothane anesthesia during an aseptic surgical procedure. A left thoracotomy was performed at the fourth intercostal space, and Silastic catheters were placed in the left atrium and proximal descending aorta. The proximal left circumflex coronary artery was dissected free of surrounding tissue for a length of 1 to 1.5 cm. A metal-encased amerosid constrictor with a 1.8- to 2.0-mm lumen (manufactured in this laboratory; material obtained from K.-G. Ulrich, Montreal, Quebec, Canada) was placed around the dissected artery. We selected the amerosid constrictor with the proper luminal dimensions to provide a tight fit to the coronary artery. The lumen was oval rather than circular; this shape allows complete closure of the amerosid in a narrow time window. Occlusion of the vessel occurs gradually as the walls of the artery are pinched together by the swelling amerosid. In contrast with circular constrictors, closure may occur by thrombus formation, resulting in acute occlusion of the vessel. We have used the oval amerosid occluder in 30 pigs and have never observed a thrombus. We pretested four oval amerosid constrictors in body temperature saline with excised arteries and found that the amerosids closed completely in 10±1 days.

Myocardial Blood Flow Measurements

Regional myocardial blood flows were measured with radiolabeled microspheres (15 μm diameter, E.I. Du Pont de Nemours & Co., Inc., Boston, Mass) with standard techniques. Briefly, microspheres (approximately 6×10⁶) were injected into the left atrium during arterial blood withdrawal at a constant rate. Using the ratio of the radioactivity of the arterial blood sample to the withdrawal flow rate, we calculated regional blood flow (mL·min⁻¹·g⁻¹). We used a matrix inversion technique to correct for overlapping spectral peaks from the radionuclides used in a given study. Formalin-fixed myocardial tissue was cut into transmural thirds, weighed, and placed in glass counting vials to determine the radioactivity with a gamma spectrometer (model 5901, Packard Instruments, Downers Grove, III).

At the completion of the study (28 to 29 days after the initial surgery), all animals were euthanized with an overdose of pentobarbital (1.25 mg/kg i.v.). The hearts were carefully dissected free of adherent tissue and excised from the thorax. We placed polyethylene catheters in the proximal portions of the left anterior descending, the left circumflex coronary artery beyond the amerosid, and the right coronary arteries. Simultaneously, 60 mL of colored dyes (Trypan blue, acridine orange, and Monastral green) were quickly injected into the three catheters to delineate the perfusion beds of the main coronary arteries and to define the bed at risk. We have previously shown that there is minimal washover of the dyes through the collateral vessels into the different beds. Thus, the intersection of the dyes defines the collateral margin and the boundaries of the left circumflex coronary artery and left anterior descending beds. We fixed the hearts in buffered 10% formalin and excised the portion of the left circumflex coronary artery at the site of the amerosid constrictor. The amerosid occluder was removed to determine the status of the vessel lumen. Complete closure of the lumen was present in all animals.

The fat and major vessels were trimmed from the heart and cut into five transverse rings 1 to 1.5 cm thick from base to apex. We separated the region of the left circumflex coronary artery perfused during the acute canulation experiment and delineated by the Trypan blue dye from the various rings and weighed it. The perfused left circumflex coronary artery region was calculated and expressed as a percentage of the total left ventricular weight. One transmural section of this region from each of the two most basal rings weighing 3 to 4 g and lying at least 15 mm inside the dye border of the region was removed and divided into subendocardial, midmyocardial, and subepicardial layers. Similarly, two transmural sections of myocardium perfused by the left anterior descending coronary artery were removed from the second and third most basal rings of the heart and divided into three layers. We have previously shown that blood flow in these regions is representative of blood flow in the left circumflex coronary artery and left anterior descending beds, respectively. In addition, the left circumflex coronary artery region sampled will not be contaminated by tissue from the left anterior descending bed. Blood flows per gram of myocardium were normalized and were corrected in the collateral region for the presence of myocardial infarction. Pieces of myocardium with infaracts had corrected flow increases proportional to the size of the infarct. In this study, this meant that coronary collateral blood flows increased from 0% to 13% above measured flows, thus reflecting the amount of infarction in an individual piece of myocardium. On six thin transmural slices of tissue, each about 1 cm², we used histologic analysis and a quantitative morphometric point-counting technique to determine infarct size in the collateral region. Four slices were selected from different quadrants to reflect the total bed, and two slices were from the piece of tissue used for blood flow analysis. The slides were stained with Masson’s trichrome, which clearly stains the infarcted area in green.

Left atrial pressures were measured. We found no differences between groups under the same conditions; therefore, these data were not used in the calculation of coronary blood flow resistances.Resistance was calculated as mean blood pressure divided by regional blood flow and expressed as mm Hg per mL·min⁻¹·g⁻¹. Data for normal control blood flows and resistances at rest and during adenosine infusion were obtained from 10 normal conscious pigs from a previous study. We also calculated the ratios of the endocardial left circumflex coronary artery to endocardial left anterior descending.
and of epicardial left circumflex coronary artery to epicardial left anterior descending resistances. These data provided a way of statistically comparing the ratios obtained in this study to ratios obtained from normal pigs.

**Experimental Groups**

Animals were Yucatan minipigs obtained from a local breeder (S&S Farms, Ranchita, Calif). The data for the normal controls (10 pigs) are from a previous study. The 16 pigs used in this study were males (average weight, 34±2 kg) and were divided at random into two groups. The first group (eight pigs) had ameroid placement but no pharmaceutical intervention (designated "untreated-ameroid"). The second group (eight pigs) had ameroid placement and continuous heparin infusion (designated "heparin-ameroid"). Alzet osmotic pumps (model 2ml-2, Alza, Inc., Palo Alto, Calif) were loaded with 2 mL of porcine intestinal heparin (60,000 units/mL; Sigma Chemical, St. Louis, Mo) dissolved in phosphate-buffered saline. We installed two pumps in each animal subcutaneously at the time of the initial surgery so that the attached catheter would pump into the external jugular vein. The vein remained patent by using a small purse string attached to a 1-mm vinyl catheter. The pumps were exchanged for new ones at 14 days (after the 14-day study) using thialylmal anesthesia (25 mg/kg i.v.). Heparin perfusion rate was approximately 150 units/h per pump for a total of 300 units/h.

We measured indirectly the amount of heparin infused using the activated partial prothrombin time (APTT) with reagents from Dade (Miami, Fla). In three untreated-ameroid pigs and three heparin-ameroid pigs, APTT was measured hourly for 24 hours at 12 days after the initial surgery. We obtained blood from carefully flushed arterial catheters that had had no heparin cap for at least 24 hours before the test. In addition, all animals received at least two tests per week administered at random, and three human volunteers had the same tests performed on venous blood samples for comparison.

One animal in the untreated-ameroid group died 9 days after ameroid implantation of the occluder, apparently from ventricular fibrillation, and was excluded from the study. However, examination of the constrictor in the pig that fibrillated revealed that, as expected, the device had occluded the left circumflex coronary artery. These data indicate that the constrictor closed 9 to 11 days after instrumentation (also see below).

**Experimental Protocols**

After 14, 21, and 28 days, the variables recorded on an eight-channel ink recorder (model 7848-A, Hewlett-Packard, Andover, Mass) while the animals stood quietly in a darkened room in the laboratory were aortic pressure, left atrial pressure, and heart rate (using bipolar surface ECG).

We gave six doses of radiolabeled microspheres to pigs at rest or during an infusion of adenosine (1.25 mg · min⁻¹ · kg⁻¹) into the left atrial catheter. Previous results indicated that there is complete vasodilation with this dose (also see Fig 1).

It was important to know the status of the ameroid occluder, especially at the early time points. Four pigs were euthanized at the finish of the 14-day study to see if all the pigs had closed ocluders (two in the untreated-ameroid group and two in the heparin-ameroid group). All the pigs had completely occluded left circumflex coronary arteries. Because we used specially designed tightly fitting ocluders and because all the pigs euthanized at 14 days plus the one pig that died at 9 days had completely occluded arteries, it is presumed that all the pigs had occluded arteries at the time of the first study. In addition, it was verified that all the pigs had completely occluded left circumflex coronary arteries at the time of euthanasia.

**Statistical Analysis**

Comparison of statistical data was carried out on CRUNCH 4 from Crunch Software Corp. (San Francisco, Calif). Intergroup comparisons were made using ANOVA repeated measures with Newman-Keuls posthoc comparison. Between-group comparisons were made using one-way ANOVA. Using the Student's mean t test, we compared (1) left circumflex coronary artery with left anterior descending, (2) untreated-ameroid animals at a given time to heparin-ameroid animals at the same time, and (3) untreated-ameroid animals or heparin-ameroid animals at 14, 21, and 28 days with the normal controls. All values are presented as mean±SEM values. P<.05 values were considered significant. Data from the untreated-ameroid group represent seven pigs at 14 days and five pigs at 21 and 28 days. Data from the heparin-ameroid group represent eight pigs at 14 days and six pigs at 21 and 28 days. Data for the normal controls represent ten pigs.

**Results**

**Swine Model of Coronary Occlusion**

We have developed a porcine model of coronary artery occlusion that allows the study of collateral development. An ameroid constrictor is placed on the left circumflex coronary artery of pigs. The constrictor occludes the artery gradually resulting in complete closure between 10 and 14 days. Because the shape of
TABLE 1. Effect of Heparin Infusion on Blood Pressure and Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>14 Days</th>
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<th>28 Days</th>
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<td>Heparin-ameroid</td>
<td>Untreated-ameroid</td>
<td>Heparin-ameroid</td>
<td>Untreated-ameroid</td>
<td>Heparin-ameroid</td>
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<td>Blood pressure (mm Hg)</td>
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<td>99±4</td>
<td>99±2</td>
<td>110±7</td>
<td>104±5</td>
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<td>Adenosine</td>
<td>59±2*</td>
<td>50±2*</td>
<td>56±3*</td>
<td>56±4*</td>
<td>54±2*</td>
<td>51±2*</td>
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<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rest</td>
<td>123±13</td>
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<td>114±9</td>
<td>136±8</td>
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<td>126±2</td>
</tr>
<tr>
<td>Adenosine</td>
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<td>114±6</td>
<td>110±4</td>
<td>126±7</td>
<td>98±4</td>
<td>106±7</td>
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</tbody>
</table>

Values are mean±SEM. Blood pressure for the normal controls was 102±3 at rest and 60±1 with adenosine. Heart rate for the normal control was 109±3 at rest and 104±3 with adenosine. *Significant (P<.05) comparing blood pressure with adenosine to blood pressure without adenosine within a group of animals tested at the same time.

The constrictor is ellipsoid, closure results by the gradual pinching together of the artery walls rather than by the acute formation of a thrombus within the vessel (see “Methods”). This model results in small (generally 15% or less) uniform infarcts in the left circumflex coronary artery bed at risk but minimal contractile dysfunction. Blood flow through the remaining coronary arteries in the left ventricle is minimally affected by this procedure.

Previous studies have shown that collateral vessels developed during the period of occlusion, with the bulk of development occurring in the first 3 weeks after surgery. In this model, we have observed many of the pathophysiological responses of the chronically ischemic myocardium. This suggests that collateral development decreases dramatically after the initial early phase, thus resulting in a deficit in coronary reserves. In an effort to develop therapies that accelerate the rate or prolong the period of collateral formation, we have assessed the effect of heparin infusion on collateral growth. Small pumps capable of delivering a continuous dose of heparin were placed in the external jugular vein of animals containing the atheroconstrictor. Heparin was administered continuously from the time of surgery to the time at which the animals were euthanized. These animals are referred to as the heparin-ameroid group. Animals containing the atheroconstrictor but without heparin infusion are referred to as the untreated-ameroid group. Normal controls refer to animals that did not undergo surgery and had no interventions.

Hemodynamics

Mean aortic blood pressures, heart rates, and left atrial pressures at the time of microsphere blood flow measurements were recorded. In both the untreated-ameroid and atheroconstrictor-herpian groups, left atrial pressures at 14 days were 17±3 mm Hg; by day 28, the atrial pressures had decreased to a mean of 12±2 mm Hg. Mean aortic blood pressures under resting conditions were somewhat low in the 14- and 21-day groups compared with normal controls but increased to normal level by 28 days (Table 1). During adenosine infusion, mean aortic blood pressures decreased significantly in both groups as expected.

Heart rates were similar in both groups during resting conditions at all time periods and were slightly higher than those of normal controls, perhaps reflecting increased metabolic needs (Table 1). During adenosine infusion, heart rates tended to decrease slightly, but the changes did not reach significant levels.

Heparin Blood Levels

In swine, heparin has a dose-dependent half-life of about 90 minutes. To assess the level of heparin activity in the experimental animals in this study, we performed the APTT test on the atheroconstrictor-ameroid pigs. This test indirectly measures the amount of heparin in the serum by assessing the time required to form blood clots in an in vitro system.

Figure 2 presents APTT values from three pigs in each group. These values were obtained from hourly samples taken over a 24-hour period. The heparin-ameroid group increased APTT by more than threefold compared with the untreated-ameroid group. Human volunteers showed APTT of about 36±3 seconds using the same kits. Pigs sampled at random had values that were not significantly different from those shown in this figure. These data show that the continuous heparin infusion system was adequate to maintain a constant increase in APTT in pigs.

**Fig 2.** Plot of activated partial prothrombin times (APTTs) for untreated-ameroid (●) and heparin-ameroid (○) animals. Tests were performed as described in “Methods” to indirectly measure the anticoagulant activity of heparin in the blood. Each point is the mean of values from three animals. Errors indicate 1 SEM. The x axis indicates the times during a 24-hour period that the tests were performed.
TABLE 2. Effect of Heparin Infusion on Endocardial and Epicardial Blood Flows and Resistances in the Left Anterior Descending Coronary Artery and Occluded Left Circumflex Coronary Artery Beds

<table>
<thead>
<tr>
<th></th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
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<tbody>
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<td></td>
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<td>Heparin-ameroid</td>
<td>Untreated-ameroid</td>
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<td>A. Endocardium</td>
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<tr>
<td>Rest</td>
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<tr>
<td>Blood flow</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LCCA</td>
<td>0.74±0.12*††</td>
<td>1.35±0.14</td>
<td>1.25±0.15</td>
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<tr>
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<td>Resistance</td>
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<tr>
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<td>143±27*††</td>
<td>68±10</td>
<td>85±13</td>
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<tr>
<td>LAD</td>
<td>66±7†</td>
<td>67±11</td>
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<tr>
<td>Adenosine</td>
<td></td>
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<tr>
<td>Blood flow</td>
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<tr>
<td>LCCA</td>
<td>0.76±0.10*††</td>
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<tr>
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<tr>
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<tr>
<td>LCCA</td>
<td>86±10*††</td>
<td>42±3*††</td>
<td>44±3*††</td>
</tr>
<tr>
<td>LAD</td>
<td>27±1*††</td>
<td>22±1</td>
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<tr>
<td>B. Epicardium</td>
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<td>Rest</td>
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<td>Blood flow</td>
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<tr>
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<tr>
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<tr>
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<td>22±2</td>
<td>19±1†‡</td>
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See text and "Methods" for description of treatments and measurements.

Blood flow is reported as mL · min⁻¹ · g⁻¹. Resistance is mean blood pressure divided by blood flow and is reported as mm Hg per mL · min⁻¹ · g⁻¹.

*Significant (P<.05) comparing the left anterior descending coronary artery (LAD) value under the same conditions.

††Significant (P<.05) comparing the untreated-ameroid with the heparin-ameroid under the same conditions.

‡‡Significant (P<.05) comparing the untreated-ameroid or the heparin-ameroid with normal control.

Effect of Heparin on Collateral Blood Flow and Resistance

Resting and vasodilated endocardial blood flows and resistance values in the nonoccluded left anterior descending and the occluded left circumflex coronary artery regions are shown in Table 2A. In the left circumflex coronary artery region in both the untreated-ameroid and heparin-ameroid groups, the pathway for normal blood flow has been occluded by the ameroid constrictor so that flow through this region represents the collateral circulation. The values shown for the left anterior descending represent blood flow and resistance in the nonischemic region of the heart. Blood flow in the left anterior descending in pigs with constriction of the left circumflex coronary artery is somewhat increased compared with normal controls, although the level of increase generally is not statistically significant (Table 2A and 2B). One component that may contribute to the small increase in blood flow in the left anterior descending bed is that blood flow may increase due to the greater metabolic needs of the left anterior descending bed, as its function increases to compensate for decreased function in the left circumflex coronary artery region.

In untreated-ameroid animals at 14 days, resting blood flow (0.74±0.12; all blood flow values are expressed as mL · min⁻¹ · g⁻¹) was severely depressed in the left circumflex coronary artery region compared with the homologous left anterior descending region (1.50±0.15) or with the left circumflex coronary artery region.
of the normal control (1.24±0.05). In contrast, resting blood flow in the 14-day heparin-ameroid animals (1.35±0.14) is approximately twofold greater than that observed in the 14-day untreated-ameroid group and is not significantly different from either the corresponding left anterior descending region (1.41±0.16) or the left circumflex coronary artery of the normal control (1.24±0.05). These data demonstrate that heparin infusion accelerates the rate at which resting blood flow is restored after coronary artery occlusion and suggests that heparin infusion potentiates the development of collateral vessels. In the heparin-ameroid animals, normal blood levels are regained at or before 14 days after surgery, whereas the untreated-ameroid animals regain normal blood flow levels between 14 and 21 days. The resistance values calculated under resting conditions also support this conclusion.

To evaluate coronary reserve levels, endocardial blood flows and resistances were determined under vasodilated conditions. The animals were given adenosine (1.25 mg·kg⁻¹·min⁻¹), which allows for maximal vasodilation27 (also see Fig 1). In the presence of adenosine, blood pressures were not significantly different among the untreated-ameroid and heparin-ameroid groups compared with the normal controls (Table 1). As expected, blood flows increased and resistance values decreased in the left anterior descending region of all the groups during vasodilation compared with resting conditions. These values are comparable to the normal control.

In the left circumflex coronary artery region of the ameroid-control animals, there is no significant increase in blood flow with adenosine compared with resting conditions at 14, 21, or 28 days. This suggests that the left circumflex coronary artery of the untreated-ameroid group is underperfused at all time points during vasodilated conditions. Resistance values indicate that these animals are severely underperfused at 14 days (86±10; all resistance values are expressed as mm Hg per mL·min⁻¹·g⁻¹) compared with the corresponding left anterior descending region (27±1) or normal controls (22±1). The level of underperfusion is reduced gradually and coronary reserves are restored slowly from 14 to 28 days in the untreated-ameroid animals, but reserve levels are still diminished at 28 days (38±2) compared with normal controls (22±1). We have determined previously that reserve levels remain diminished at 4 months after surgery in the ameroid-control group.12

In contrast, resistance values for the left circumflex coronary artery are significantly lower at 14 days in the heparin-ameroid animals (42±3) compared with the untreated-ameroid group (86±10). This is due to the higher blood flow in the heparin-ameroid group at 14 days. It is noteworthy that blood flow in the left circumflex coronary artery is not increased with adenosine at this early time point, indicating that there is little collateral reserve in either group of animals. However, blood flow and resistance values improve rapidly with heparin infusion so that by 28 days the resistance level in the heparin-ameroid group (21±2) is comparable to the normal controls (22±1). These results demonstrate that heparin treatment facilitated the restoration of coronary reserve levels within the 28-day period, again suggesting that collateral development is modulated positively by heparin.

In the epicardium (Table 2B), blood flows values slightly increased compared with normal controls were observed in both the heparin-ameroid and untreated-ameroid groups in the left anterior descending region at rest, reflecting differences in metabolic demands. In the left circumflex coronary artery bed at 14 days, the blood flows (1.58±0.11) and resistance values (39±3) for the untreated-ameroid group under vasodilated conditions indicate that the epicardium of these animals was underperfused compared with normal controls. In contrast, in the heparin-ameroid animals at 14 days, blood flows (2.15±0.11) and resistance values (24±2) were not significantly different than normal controls, suggesting that heparin accelerated the growth of collateral vessels in the epicardial region. Midmyocardial blood flows and resistances also were calculated but are not presented; these values were intermediate between the endocardial and the epicardial results.

Collateral-to-Coronary Resistance Ratios

To normalize the effects of the different perfusion pressures during adenosine infusion on blood flow measurements, we calculated the collateral (left circumflex coronary artery)-to-coronary (left anterior descending) resistance ratios from the data in Table 2 (see “Methods” for calculations). Statistical comparisons were made with the values obtained from normal controls.27 For normal controls, the calculated ratios of left circumflex coronary artery to left anterior descending resistance were 1.02±0.02 (at rest) and 0.98±0.01 (with adenosine) for the endocardium and 1.05±0.04 (at rest) and 1.04±0.03 (with adenosine) for the epicardium.

Fig 3 shows resistance ratios for the endocardium and epicardium of the untreated-ameroid and heparin-ameroid groups under resting and vasodilated conditions. Fig 3A shows that at 14 days, there is a significant difference between the resistance ratios under resting conditions of the untreated-ameroid and the heparin-ameroid animals. The heparin-treated animals show normal resistance ratios (1.04±0.04) at this early time, indicating that heparin treatment resulted in a rapid return to normal blood flow. In contrast, the left circumflex coronary artery bed in the untreated-ameroid animals is underperfused as indicated by the resistance ratio of 2.13±0.23. At 21 and 28 days, the ratios for both groups are close to unity.

During adenosine infusion, the ratio of collateral to coronary resistances represents a measure of coronary collateral reserve.27 As shown in Fig 3B, the high resistance ratios in the untreated-ameroid group indicate diminished coronary collateral reserves. Reserve levels in the untreated-ameroid animals improve over time; however, at 28 days the ratio is still significantly higher (1.62±0.07) than seen in normal controls. Additional measurements made at longer times demonstrate that reserve levels remain below normal even at 4 months after ameroid placement.12,23 Continuous heparin treatment significantly increases the level of coronary reserves at 14 days compared with the untreated-ameroid animals, but the heparin group is still underperfused at this early time. Remarkably, by 28 days, the ratio had decreased further to 0.93±0.04, which is not significantly different from normal controls.
Infarct Size

Fig 4 shows the effect of heparin infusion on infarct sizes. We determined infarct sizes from histology slides by point counting and expressed them as a percentage of the bed at risk. The size of the bed at risk was obtained from weighing the excised, dyed left circumflex coronary artery bed. These bed sizes were 22±1% and 23±1% of the total left ventricular weight in the untreated-ameroid and heparin-ameroid groups, respectively, and there was no difference in bed sizes between the groups. The untreated-ameroid group had infarcts representing 5.8% of the endocardial left circumflex coronary artery bed and 0.8% of the midmyocardial left circumflex coronary artery bed, whereas the heparin-ameroid group had infarcts representing 2.8% of the endocardial left circumflex coronary artery bed and no infarction of the midmyocardial left circumflex coronary artery bed. This indicates that heparin infusion reduced infarct size by approximately 50%.

Discussion

The development of collateral vessels is the single most important parameter in minimizing myocardial infarction due to coronary artery occlusion. In humans, collateral development does not fully restore normal blood flow to the bed at risk (for reviews of coronary collaterals, see Cohen31). Studies have shown that the number of new collateral vessels in humans usually is insufficient to meet the demands of the stressed myocardium (ie, exercise) and that these vessels contain less smooth muscle than found in other arteries.11 Inadequate collateral development may be partly responsible for the angina associated with coronary artery disease. Due to its obvious clinical importance, several groups have sought to develop treatments that would positively affect the rate or extent of collateral growth.

A good animal model for the development of coronary collaterals is necessary to test the efficacy of various interventions on collateral growth. Two models of collateral development in response to cardiac ischemia have been studied extensively. In dogs, which contain an abundant native collateral circulation, collateral development is rapid and extensive and occurs mainly epicardially.32,33 In pigs, collateral development also is rapid, but because of the paucity of native collateral vessels, the final outcome of collateral angiogenesis is limited, resulting in predominantly endocardial and midmyocardial vessels that are comparable in number to those found in human hearts with ischemic disease.32,33 The pig model used in this study, that is, constriction of the left circumflex coronary artery by an
amoerd occluder, has been characterized extensive-ly. Using autoradiography to detect the incorpo-
ration of $^3$H-thymidine into the dividing nucleus, we
have shown previously that there is a dramatic increase
in the number of replicating endothelial and smooth
muscle cells after vessel closure. The maximum level
of cell division occurs early, at or before 2 weeks after
surgery, and then declines to near-normal levels by 8
weeks. This is consistent with the data presented here
and in previous studies showing that normal resting
blood flow is restored within the first 3 weeks after
amoerd placement. However, these animals still expe-
rience chronic episodic ischemia, for example, during
exercise or while feeding, because coronary reserves are
not fully restored even after 4 months of recovery.

It is unclear why angiogenesis declines by 8 weeks in
the presence of this continued ischemic stimulus, but this
observation does suggest that therapeutic interventions
designed to increase blood vessel development need to
be initiated soon after the onset of ischemia.

The results presented here indicate that application of
an angiogenic agent can profoundly affect the devel-
opment of blood vessels in the ischemic heart. In the
heparin-treated animals, restoration of normal blood
flow to the region at risk occurred by (or before) 2
weeks after placement of the amoerd constrictor,
whereas normal flow did not return in the untreated
animals until 3 weeks. Because 2 weeks was our first
time point, it is possible that restoration of blood flow
occurred even earlier. Significantly, we also observed
rapid and nearly complete recovery of coronary re-
serves in the heparin-treated animals. In the untreated-
amoerd group at 28 days, collateral reserve levels were
significantly decreased compared with normal controls.
In contrast, in the heparin-amooerd group, reserve
levels were essentially identical in the normally perfused
and collateral-dependent regions during maximum va-
sodilation (see Fig 3B). Schaper and coworkers have
shown that dogs, which generally are considered to be
better collateral formers than pigs, do not fully restore
reserve levels in the collateral-dependent region. This
suggests that heparin administration promoted a rem-
arkable degree of collateral development. In a recent
study, we have shown that collateral vessels in the
untreated-amooerd group have a significant lack of
smooth muscle development, which may influence their
capacity to respond to vasodilators. In future studies,
it will be of interest to determine if the number or
composition of the collateral vessels is affected by the
heparin treatment.

In this model of gradual coronary artery occlusion,
infarct size generally is small, resulting in less than 15%
infarction of the left circumflex coronary artery bed.
However, we were able to detect a significant decrease
in infarct sizes in the heparin-amooerd group. We
believe that this is due to the increase in collateral blood
flow in the heparin-amooerd group around the time of
closure of the left circumflex coronary artery. Alter-
nately, it is possible that heparin inhibited the formation
of a thrombus in the constrictor and thereby allowed for
more gradual occlusion of the artery. This seems un-
likely because thrombotic occlusion generally occurs
with circular amoerd occluders and not with oval
occluders such as the kind used in our studies.

We had reported previously that heparin did not
increase collateral development in pigs with amoerd
constrictors. However, in that study, the animals were
given heparin twice daily as bolus injections. APTT time
measurements, taken at hourly intervals, showed that
the serum from heparin-injected animals had 10- to
20-fold increases in clotting times at shortly after hepa-
rin administration, but by 3 hours after injection, clotting
times had returned to normal (F.C. White and C.M.
Bloor, unpublished data). This suggests that the
rapid clearance of heparin from the circulation may
account for the negative results obtained in our initial
study.

Although our studies do not address the mechanisms
of heparin effect, we hypothesize that heparin increases
both the rate of restoration of blood flow and the rate of
the recovery of coronary reserves by increasing the
absolute extent to which angiogenesis occurs. There is
considerable evidence that heparin is angiogenic in vivo,
and several reports have shown that heparin positively
affects blood vessel formation in the heart. Fujita et al
studied the effect of a single daily dose of heparin on the
collateral formation in dogs subjected to repeated brief
coronary artery occlusions. Their results demonstrated
that heparin decreased the number of occlusions re-
quired to promote collateralization, indirectly measured
as the disappearance of systolic dysfunction. From this
evidence, the authors suggested that heparin acceler-
ated the rate of growth of collateral vessels. In sub-
sequent clinical studies, they examined the effect of
heparin with and without exercise on patients with
stable effort angina. From these studies, the authors
concluded that heparin increases collateralization by
potentiating the action of an ischemia-derived angi-
genetic factor. Although only a small group of patients
was studied, this investigation is encouraging regarding
the use of heparin as a therapeutic agent.

Recently, Unger et al developed a novel dog model
to study collateralization. These investigators implanted
the internal mammary artery into the left anterior
descending bed and subsequently occluded the left
anterior descending, rendering the bed collateral-
dependent. Infusion of heparin directly into the implanted
artery increased the proportion of blood flow through
this vessel approximately 2.5-fold. This localized gain in
flow was not due to an increase in the absolute level of
collateral flow but rather was achieved by a decrease in
flow from other sources, presumably because the endog-
enous collateral flow was sufficient to meet the require-
ments of the bed at risk. Therefore, although this study
dramatically demonstrates the angiogenic effect of hepa-
rin, it also indicates that the enormous potential for
collateral growth in the dog makes this species a limited
model for human collateral development.

Heparin appears to increase angiogenesis by several
mechanisms, one of which involves its interaction with
angiogenic growth factors, such as acidic and basic
fibroblast growth factors. Heparin protects both these
factors from inactivation and potentiates the activity of
acidic fibroblast growth factor. Heparin also releases
basic fibroblast growth factor from the extracellular
matrix, thereby making this factor available for interac-
tion with endothelial cells. Recently, it has been shown
that cell surface heparinlike molecules are required for
the binding of basic fibroblast growth factor and

vascular endothelial growth factor\(^{52}\) to high-affinity receptors on the cell surface. These authors suggest that heparin, present either as a component of cell surface proteoglycans or free in solution, induces a conformational change in the growth factor that allows it to interact with its receptor. Importantly, angiogenic growth factors have been identified in the ischemic heart as well as in the normal heart.\(^{39-46}\)

Another mechanism by which heparin may be angiogenic is its ability to increase protease activity. Proteolytic destruction of the extracellular matrix is an important step in the development of new blood vessels. Proteases such as metalloproteases and plasminogen are secreted from sprouting capillary endothelial cells (for a review, see Pepper and Montesano\(^{50}\)). Heparin also stimulates the secretion and activation of plasminogen activator.\(^{46,48}\) In addition, it recently has been shown that plasminogen activator inhibitor–1 binds to heparin.\(^{50}\) This interaction potentiates the neutralization of plasminogen activator inhibitor–1 by thrombin, thereby favoring proteolysis.

In summary, we have demonstrated that heparin has a dramatic positive effect on the restoration of blood flow within the first 2 weeks after closure. Remarkably, heparin treatment potentiated the complete recovery of reserve levels in the collateral-dependent bed. In addition, the treatment afforded the animal significant protection from infarction. This model will be useful in testing the therapeutic value of other angiogenic treatments in the compromised heart and in defining the period most susceptible to such treatments.

Acknowledgments

We thank Kim Ciero for help in preparing the manuscript and Dr. M. Dan McKirnan for help with the statistical analysis.

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Circulation. 1993;88:198-207
doi: 10.1161/01.CIR.88.1.198
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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