Partial Coronary Stenosis Is Sufficient and Complete Reperfusion Is Mandatory for Preconditioning the Canine Heart

Michel Ovize, Karin Przyklenk, and Robert A. Kloner

Repeated brief episodes of total coronary artery occlusion (i.e., severe ischemia), each separated by brief periods of reperfusion, reduce infarct size after a subsequent sustained ischemia. The intensity of the ischemic injury during these coronary artery occlusions and the role of the following transient reflow have been poorly understood. Therefore, our objective was to determine whether moderate preconditioning ischemia induced by partial coronary artery stenosis (reducing coronary flow to approximately 50% of its baseline values), with or without a brief period of total reperfusion, could precondition the canine myocardium. Dogs were randomized to receive one of three preconditioning “treatments”: the R(−) group underwent 15 minutes of partial coronary stenosis without subsequent brief reperfusion (n=8); the R(+) group underwent 15 minutes of partial coronary stenosis followed by 10 minutes of full reflow (n=8); and the control group underwent no intervention (n=8). All dogs then underwent 1 hour of total coronary artery occlusion and 4.5 hours of reperfusion. Both treated groups were equally and moderately ischemic during partial stenosis: myocardial blood flow in the inner two thirds of the left ventricular wall averaged 0.25±0.05 and 0.31±0.07 ml/min per gram in the R(−) and R(+) groups, respectively (p=NS). Furthermore, all three groups were equally and severely ischemic during sustained total occlusion: myocardial blood flow in the inner two thirds of the left ventricular wall averaged 0.06±0.05, 0.05±0.03, and 0.07±0.03 ml/min/g in control, R(−), and R(+) groups, respectively (p=NS). Infarct size (expressed as percentage of the area at risk) in the R(+) group was 9.0±2.5%, which was significantly smaller (p<0.01) than the value of 22.8±5.5% observed in control animals. Stenosis followed by full reflow in the R(+) group did not, however, attenuate contractile dysfunction during the sustained total coronary artery occlusion and did not preserve function in peri-infarct tissue after reperfusion. In contrast, stenosis without reperfusion in the R(−) group did not limit infarct size (28.4±5.4%, p=NS versus control) and did not preserve wall motion during total occlusion/reperfusion. Therefore, we conclude that 1) partial coronary artery stenosis can precondition the heart, 2) complete reflow after the preconditioning ischemia is mandatory to induce myocardial protection, and 3) preconditioning with moderate ischemia does not preserve contractile function. (Circulation Research 1992;71:1165-1173)

Key Words • preconditioning • coronary stenosis • moderate ischemia • reperfusion • contractile function

Repeated brief episodes of reversible ischemia limit infarct size after a subsequent sustained ischemic insult.1 This phenomenon has been termed preconditioning and has now been demonstrated in various animal species, including dogs, pigs, rabbits, and rats.1–4 There is also recent evidence that preconditioning may occur in the human heart.5

In experimental preparations, the preconditioning regimen has, in most cases, consisted of one or more brief episodes of total coronary artery occlusion (i.e., severe ischemia), each separated by brief episodes of reperfusion. Several studies have addressed the number of brief ischemic episodes that are required to achieve a reduction in infarct size.6,7 However, the importance of the intensity of the preconditioning ischemia has not been investigated: specifically, it is not known whether moderate ischemia, induced by a partial coronary artery stenosis, can protect the heart from a subsequent episode of total coronary artery occlusion. In addition, it is not known whether complete restoration of blood flow after brief ischemia is necessary to trigger the preconditioning response.

Therefore, we addressed these issues in a canine preparation of partial coronary artery occlusion, in which coronary blood flow was reduced to approximately 50% of its baseline value during the preconditioning regimen. Our specific objectives were to determine whether 1) partial coronary artery stenosis is sufficient to precondition the ischemic myocardium; 2) moderate preconditioning ischemia, induced by partial coronary artery stenosis, can improve the recovery of postischemic contractile function after the subsequent sustained ischemic insult; and 3) full restoration of blood flow between preconditioning ischemia and the sustained ischemic insult is necessary to achieve a reduction in infarct size.
Materials and Methods

This study conformed to the position of the American Heart Association on research animal use, followed the University of Southern California Code of Ethics for the Humane Treatment of Animals, and was approved by the Institutional Animal Care and Use Committee of the Hospital of the Good Samaritan.

Surgical Preparation

Thirty-six mongrel dogs of either sex, weighing 15–25 kg, were sedated with morphine sulfate (15 mg s.c.), anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and ventilated with room air. Cannulas were inserted into the left jugular vein (for administration of drugs and fluids) and the left carotid artery (for measurement of heart rate and blood pressure and for withdrawal of reference blood samples for measurement of myocardial blood flow). A throracotomy was performed in the fourth left intercostal space, and the heart was suspended in a pericardial cradle. A 5F microtipped pressure transducer (Millar Instruments, Inc., Houston, Tex.) was positioned within the left ventricular (LV) cavity via the left atrial appendage for measurement of the LV pressure and its first derivative (dP/dt). Through the same incision, a cannula was inserted in the left atrium for later injection of radiolabeled microspheres for measurement of regional myocardial blood flow (RMBF).

One pair of ultrasonic crystals, used to assess regional contractile function, was positioned in the center of the soon-to-be-ischemic left anterior descending coronary artery (LAD) bed, as previously described. Crystals were inserted via small scalpel incisions into the mid-myocardium at a separation of 6–12 mm and oriented parallel to the minor axis of the heart. Regional contractile function, LV dP/dt, and arterial and LV pressures were monitored continuously throughout the experiment on a recorder (Gould Inc., Cleveland, Ohio).

After the crystals were positioned, a segment of the LAD was isolated, usually just distal to its first major diagonal branch. The animals were allowed 15 minutes after these surgical procedures to reach steady state.

Experimental Design

After the surgical procedure, dogs were randomly assigned to one of three groups, the control group or one of two “treatment” groups (Figure 1). In the treatment groups, an ultrasonic flow probe was placed around the LAD for continuous measurement of mean coronary blood flow during the treatment period. Just proximal to the flow probe, a plastic micrometric constrictor was then placed around the coronary artery. To avoid manipulation of the coronary artery during the treatment period, the control dogs received neither of these two devices. Another 10 minutes was allowed to reach steady state. Then, all dogs underwent a 25-minute treatment period.

Control group. The control group underwent no intervention during the 25-minute period.

R(−) group. The R(−) group underwent no intervention for the first 10 minutes. Then, the constrictor was tightened such that coronary blood flow was reduced to approximately 50% of its baseline value for 15 minutes. This particular degree of stenosis was chosen because, in this canine model, it usually results in a mean subendocardial blood flow of 0.20–0.40 ml/min per gram (i.e., moderate ischemia). Once the degree of stenosis had been adjusted (within approximately 1 minute), no attempt was made to further improve the precision of the coronary flow reduction. This stenosis was then maintained for 15 minutes. Without releasing the stenosis, the LAD was then abruptly occluded with vascular clamps at the site of the stenosis; that is, full reflow was not restored in these animals after the episode of moderate ischemia. Immediately after the LAD had been occluded, the flow probe and the constrictor were removed.

R(+) group. For the R(+) group, at the onset of the 25-minute treatment period, the constrictor was tightened such that coronary blood flow was reduced to approximately 50% of its baseline value. This stenosis was maintained for 15 minutes. The stenosis was then abruptly released and removed, allowing full restoration of blood flow. This brief period of reperfusion after the 15-minute stenosis phase lasted for 10 minutes. The LAD was then abruptly occluded with vascular clamps, and the flow probe was removed.

After completion of this treatment period, all dogs underwent a 60-minute period of total coronary artery occlusion followed by 4.5 hours of reperfusion.

At the end of each experiment, the LAD was reclosed, and 0.5 mg/kg Unisperse Blue Pigment (CIBA-GEIGY, Hawthorne, N.Y.) was injected into the coronary vasculature via the left atrial appendage to delineate the in vivo area at risk. With this technique, the previously nonischemic myocardium appears blue, whereas the previously ischemic myocardium (area at risk) remains unstained. Under deep anesthesia, the hearts were stopped by intracardiac injection of potassium chloride (40 meq) and excised for further analysis.

Hemodynamics and Contractile Function

Measurements of heart rate, arterial pressures, and segment shortening were made at baseline (i.e., before treatment) and immediately before the sustained occlu-
sion in all groups. In the $R(-)$ and $R(+)$ groups, hemodynamics, coronary blood flow, and segment shortening were measured 10 minutes after stenosis. In the $R(+)\text{ group, coronary blood flow was also measured immediately after release of the stenosis. In all groups, hemodynamics and segment shortening were then monitored throughout the sustained total coronary artery occlusion. In both treated groups, myocardial blood flow was also measured 10 minutes after tightening the stenosis.

**Measurement of Regional Myocardial Blood Flow**

RMBF was assessed by injection of microspheres labeled with either $^{141}\text{Ce}$, $^{86}\text{Nb}$, or $^{103}\text{Ru}$ (New England Nuclear, Boston). In all dogs, myocardial blood flow was measured 30 minutes into the sustained total coronary artery occlusion. In both treated groups, myocardial blood flow was also measured 10 minutes after tightening the stenosis.

**Analysis**

**Area at risk and area of necrosis.** All hearts were cut into five to seven transverse slices parallel to the atrioventricular groove. After right ventricular tissue had been removed, the heart slices were weighed. The basal surface of each slice was photographed for later measurement of the area at risk. Then, each slice was incubated for 10 minutes in a 1% solution of triphenyltetrazolium chloride at 37°C. This method has been shown to reliably identify necrotic myocardium, which appears pale, from viable myocardium, which stains brick red.12 The position of the ultrasonic crystals with respect to the necrotic area was checked at this time. The slices were then rephotographed. Enlarged projections of these slides were traced for determination of the boundaries of the area at risk and area of necrosis. Extent of the area at risk and the area of necrosis was quantified by computerized planimetry and corrected for the weight of the tissue slices. Total weight of the area at risk and the area of necrosis was then calculated and expressed as a percentage of the total LV weight.

**Regional myocardial blood flow.** Tissue blocks were cut from the center of the LAD bed and from the circumflex bed and subdivided into endocardial, midmyocardial, and epicardial segments. RMBF in these three segments was then determined by the technique of Domenech et al.13 In addition, myocardial blood flow in the inner two thirds of the LV wall was calculated from the weighted means of the endocardial and midmyocardial blood flows.

**Hemodynamics and segment shortening.** Heart rate and arterial blood pressure were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. LV $\Delta p/\Delta t$ was used to define the timing of the cardiac cycle for segment length measurements with the ultrasonic crystals: end-diastolic lengths were measured at the onset of the rapid increase in LV $\Delta p/\Delta t$, whereas end-systolic lengths were measured at peak negative LV $\Delta p/\Delta t$. End-diastolic and end-systolic lengths were obtained from three well-separated cardiac cycles in each sample period, averaged, and used to compute segment shortening, an index of regional systolic contractile function defined as follows: $SS=\{[\text{mean EDL}−\text{mean ESL}]/\text{mean EDL}]\times100\%$, where SS is segment shortening. EDL is end-diastolic length, and ESL is end-systolic length.9 Segment shortening during each sample period was normalized and expressed as a percentage of the respective baseline values. Because one purpose of this study was to assess contractile function in the viable peri-infarct tissue, segment shortening data were not included in the final analysis if the crystals were positioned within a necrotic area.

**Exclusion criteria.** Dogs with high collateral blood flow during coronary occlusion and/or a small area at risk were excluded from the final analysis. Specifically, our standard exclusion criteria, established before the onset of the protocol, were values of RMBF in the "ischemic" subendocardium $>0.20\text{ ml/min per gram during sustained total LAD occlusion and/or an area at risk occupying <10% of the LV. In addition, no attempt was made to resuscitate dogs that developed ventricular fibrillation at any time during the experiment.

**Statistics**

All measurements are expressed as group mean±SEM. Comparisons of RMBF and infarct size among groups were performed by analysis of variance. If significant $F$ ratios were obtained, comparisons between control and treatment groups were then made by means of two-tailed Dunnett's test.14 Differences in the relation between infarct size and RMBF were evaluated by analysis of covariance for linear regressions, with infarct size (as percentage of the area at risk) as the dependent variable and myocardial blood flow in the inner two thirds of the LV wall as the covariate. For comparisons of hemodynamics, coronary blood flow, and segment shortening measurements, we used analysis of variance with Bonferroni's correction for multiple comparisons. A value of $p<0.05$ was considered indicative of a statistically significant difference.

**Results**

**Mortality and Exclusions**

Table 1 summarizes exclusions from the study due to lack of ischemia or death from ventricular fibrillation. Of the thirty-six dogs entered into the study, two were excluded because they had subendocardial blood flow $>0.20\text{ ml/min per gram (no ischemia) during the sustained period of total coronary artery occlusion: one dog in the control group and one dog in the R(−) group. Ten dogs in the study succumbed to ventricular fibrillation and were not resuscitated: five died during the initial 25 minutes of sustained occlusion, and five died at the onset of reperfusion.

Data are thus presented for the 24 dogs (eight in each group) that successfully completed the study.

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<th>Table 1. Mortality and Exclusions</th>
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<td>Control (n=10)</td>
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<td>$R(-)$ (n=12)</td>
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<td>$R(+)$ (n=14)</td>
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VF, ventricular fibrillation; control, dogs that underwent no intervention; $R(-)$, dogs that underwent 15 minutes of moderate ischemia without a subsequent reperfusion; $R(+)$, dogs that underwent 15 minutes of moderate ischemia with a subsequent 10-minute reperfusion.

No ischemia indicates subendocardial blood flow $>0.20\text{ ml/min per gram during coronary occlusion.}
Coronary Blood Flow During the Treatment Period

Baseline coronary flow was similar in both treated groups, averaging 21 ± 3 and 19 ± 1 ml/min in R(−) and R(+) groups, respectively. The stenosis reduced the coronary blood flow to an average of 38 ± 9% and 42 ± 4% of baseline values in R(−) and R(+) groups, respectively (p < 0.01 versus baseline for both groups). This reduction in coronary flow did not vary significantly during the 15-minute stenosis period in both groups (Table 2).

After the release of the stenosis, all R(+) dogs experienced the expected transient hyperemic response, with an approximately fivefold increase in coronary flow over baseline values. Immediately before the sustained coronary artery occlusion, coronary blood flow in the R(+) group had returned to baseline values but was still significantly greater than that observed in the R(−) group (p < 0.01).

Table 2. Coronary Blood Flow

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<tr>
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<th>Coronary blood flow (ml/min)</th>
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<tr>
<td></td>
<td>Stenosis</td>
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<td>Baseline</td>
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<tr>
<td>R(−) (n=8)</td>
<td>21±3</td>
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<td>R(+) (n=8)</td>
<td>19±1</td>
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R(−), dogs that underwent 15 minutes of moderate ischemia without a subsequent reperfusion; R(+), dogs that underwent 15 minutes of moderate ischemia with a subsequent 10-minute reperfusion.

* p<0.01 vs. baseline.
† p<0.01 vs. R(−).

Hemodynamics

Data are reported in Table 3 for 23 dogs: seven control (1 excluded due to a technical problem), eight R(−), and eight R(+) dogs. Heart rate and mean arterial pressure did not differ among groups at any time during the experiment.

Regional Myocardial Blood Flow

As expected, both treated groups were moderately ischemic during the stenosis phase, with RMBF (Table 4) in the inner two thirds of the LV wall averaging 0.25 ± 0.05 versus 0.31 ± 0.07 ml/min per gram in R(−) and R(+) groups, respectively (p = NS between groups). During the sustained total coronary occlusion, all three groups were severely ischemic: RMBF in the inner two thirds of the LV wall was 0.06±0.05, 0.05±0.03, and 0.07±0.03 ml/min per gram in control, R(−), and R(+) groups, respectively (p = NS among groups). The reduction in myocardial flow between moderate ischemia during the treatment period and sustained total coronary occlusion was statistically significant in all three layers for both treated groups (p < 0.05).

Infarct Size

The area at risk (as percentage of the LV weight) was not different among groups: 22.9±1.4% in control, 23.6±1.7% in R(−), and 22.6±1.0% in R(+) dogs (Figure 2).

Infarct size in control dogs averaged 22.8±5.5% of the area at risk (Figure 2). In the R(−) group, infarct size was not significantly different from that in the control group, averaging 28.4±5.4% of the area at risk (p = NS). In contrast, R(+) dogs experienced a signifi-

Table 3. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Stenosis</th>
<th>Preocclusion</th>
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<th>Time elapsed after reperfusion (min)</th>
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<td>HR (bpm)</td>
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<tr>
<td>Control</td>
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<td>149±8</td>
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<tr>
<td>R(−)</td>
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<td>R(+)</td>
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<td>145±7</td>
<td>137±7</td>
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<tr>
<td>SBP (mm Hg)</td>
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<tr>
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<tr>
<td>R(−)</td>
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<td>129±8</td>
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<tr>
<td>R(+)</td>
<td>135±8</td>
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<td>137±7</td>
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<td>DBP (mm Hg)</td>
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<tr>
<td>Control</td>
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<td>R(+)</td>
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<td>MBP (mm Hg)</td>
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<tr>
<td>Control</td>
<td>132±4</td>
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<td>135±4</td>
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<td>114±6</td>
<td>115±6</td>
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<td>R(+)</td>
<td>121±7</td>
<td>121±7</td>
<td>122±6</td>
<td>122±7</td>
<td>117±6</td>
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</table>

HR, heart rate; bpm, beats per minute; control, dogs that underwent no intervention; R(−), dogs that underwent 15 minutes of moderate ischemia without a subsequent reperfusion; R(+), dogs that underwent 15 minutes of moderate ischemia with a subsequent 10-minute reperfusion; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure. Values are mean±SEM.

There was no significant difference among groups.
cant reduction in infarct size, to a mean of 9.0±2.5% of the area at risk (p<0.01 versus control).

These observations were further confirmed when infarct size (expressed as percentage of the area at risk) was plotted as a function of myocardial blood flow to the inner two thirds of the LV wall during total coronary artery occlusion (Figure 3). Data from the control animals exhibited the expected inverse relation between collateral blood flow and infarct size. The regression relationship for the R(-) group was similar to that observed for the control group, demonstrating no reduction in infarct size in this group (Figure 3, left panel). In contrast, all R(+) data points lie below the regression line (Figure 3, right panel). In fact, the regression line of the R(+) group differed significantly with respect to the control line (F=8.83; p<0.05 by analysis of covariance), indicating that partial coronary artery stenosis, provided it was followed by full reperfusion, limited infarct size irrespective of the amount of collateral blood flow during total coronary artery occlusion.

Regional Contractile Function

Segment shortening data were not available for one control dog and one R(+) dog because of technical problems. Data from three R(-) dogs were excluded because ultrasonic crystals were positioned within a necrotic area. Thus, data are reported for 19 dogs: seven control, five R(-), and seven R(+) dogs.

All R(-) and R(+) dogs exhibited hypokinetic or dyskinetic in the ischemic LAD bed during stenosis: segment shortening averaged -29.7±21.7% of baseline in the R(-) group and 5.2±10.3% of baseline in the R(+) group (p<0.05 versus control for both groups), but there was no significant difference for R(-) versus R(+) groups (Figure 4). Immediately before the sustained coronary artery occlusion (i.e., 10 minutes after release of the stenosis), R(+) dogs were hypokinetic (segment shortening averaged 66.2±8.6% of baseline), but this did not differ significantly from the value of 94.7±3.8% observed in the control group (Figure 4).

All groups were equally dyskinetic throughout the sustained coronary occlusion (Figure 4), with segment shortening averaging -28% to -53% of baseline values among the three groups (p=NS). In addition, all groups were equally stunned throughout reperfusion (Figure 4): at 4.5 hours after reflow, segment shortening averaged only 2.6±16.6%, 0.3±10.7%, and -3.6±14.1% of baseline values in the control, R(-), and R(+) groups, respectively (p=NS).

Discussion

In the present study, we observed that moderate reduction in myocardial perfusion induced by a partial coronary artery stenosis, provided it is followed by total/complete reperfusion, can limit infarct size caused by a subsequent sustained total coronary artery occlusion. This reduction in infarct size could not be explained by differences in the size of the area at risk, in

<table>
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<th>Table 4. Regional Myocardial Blood Flow</th>
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<td>Regional myocardial blood flow (ml/min per gram)</td>
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<tr>
<td>Ischemic LAD bed</td>
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<td>Control</td>
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<td>R(-)</td>
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<tr>
<td>Nonischemic CX bed</td>
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<tr>
<td>Control</td>
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<tr>
<td>R(-)</td>
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Endo, subendocardial blood flow; Mid, midmyocardial blood flow; Epi, subepicardial blood flow; LAD, left anterior descending coronary artery; control, dogs that underwent no intervention; R(-), dogs that underwent moderate ischemia without a subsequent reperfusion; R(+), dogs that underwent moderate ischemia with a subsequent reperfusion; CX, circumflex. Values are mean±SEM.

There was no difference among groups at any time point of the experiment.

FIGURE 2. Bar graph showing mean values of infarct size. Control, dogs that underwent no intervention; R(-), dogs that underwent 15 minutes of moderate ischemia without a subsequent reperfusion; R(+), dogs that underwent 15 minutes of moderate ischemia with a subsequent 10-minute reperfusion. The area at risk (AR) is expressed as a percentage of the left ventricular weight (LV), and the area of necrosis (AN) is expressed as a percentage of AR in all three groups. There was no significant difference in the size of AR among groups. Infarct size was significantly reduced in the R(+) group (*p<0.01 vs. control) but not in the R(-) group.
hemodynamics, or in myocardial blood flow during the sustained ischemic insult.

Infarct Size Limitation

Partial coronary artery stenosis can trigger ischemic preconditioning. The first important observation of this study is that moderate ischemia, induced by a partial coronary artery stenosis, is sufficient to “trigger” preconditioning.

In most previous studies, brief episodes of total coronary artery occlusion, resulting in severe myocardial ischemia, were used to precondition the heart. Consequently, the only way to vary the preconditioning ischemic insult was to modify the duration of coronary artery occlusion. For example, Li et al showed in the canine model that one episode of 5-minute coronary occlusion was as efficient as six or 12 episodes of the same duration. Van Winkle et al demonstrated that two episodes of 2 minutes of coronary artery occlusion was insufficient to precondition the rabbit heart, yet one 5-minute episode reduced infarct size. We recently observed in the canine model that one episode of 2.5 minutes of total coronary artery occlusion induced preconditioning, although the reduction in infarct size was less dramatic than that observed with longer durations of preconditioning ischemia. Overall, it appears from these previous investigations that a minimal duration of ischemia is necessary to trigger preconditioning.

The present study addressed the issue of the intensity of preconditioning ischemia in terms of the magnitude of the reduction of myocardial blood flow. We found that moderate preconditioning ischemia can trigger further ischemic tolerance. As a result of a partial stenosis acutely reducing coronary blood flow to \( \approx 50\% \) of its baseline value, the mean blood flow in the inner two thirds of the LV wall averaged 0.31±0.07 ml/min per gram during the preconditioning regimen in the R(+) group. This finding is in agreement with previous studies that have shown that graded reductions in coronary flow result in graded reductions in myocardial blood flow in all myocardial layers.

Mandatory role of brief total reperfusion. The second major finding of this study is that complete reperfusion after the preconditioning ischemia is mandatory to protect the heart. R(−) dogs underwent a reversible ischemic insult during the treatment period similar to that in the R(+) dogs. However, they did not exhibit a reduction in infarct size.

It might be questioned whether the 10-minute “no-intervention” period preceding stenosis in the R(−) group could have been responsible for the absence of reduction in infarct size in this group. To address this issue, we submitted five additional dogs to a preconditioning regimen consisting of 25 minutes of partial stenosis not followed by complete reperfusion. In this group, coronary flow was reduced for 25 minutes to 54±9% of baseline, resulting in moderate ischemia similar to that in the R(−) and R(+) groups. As illustrated in Figure 5, infarct size in these dogs did not differ from that obtained in the control or R(−) groups. This indicates that the 10-minute no-intervention period preceding the partial stenosis episode did not interfere with preconditioning. Our study suggests that the absence of total reperfusion after the reversible ischemic insult is responsible for the lack of preconditioning effect in the R(−) group.

The importance of the duration of the brief episode of reperfusion has been addressed in previous studies. Murry et al have reported that the protective effect of preconditioning in the canine model is partially lost when the duration of reperfusion between brief and sustained ischemia is extended to 2 hours. Similar
However, there are observations have been made in the rat and rabbit models of preconditioning.18,19

However, the importance of complete reperfusion for myocardial protection has not been investigated. Given the inherent complication in eliminating reperfusion from the preconditioning regimen, why did we assess the effects of R(−) and R(+). First, it must be stressed that, despite the lack of total reperfusion in this group, the ischemic bed did receive approximately 50% of normal blood flow during the stenosis. This limited perfusion presumably facilitated the washout of at least some of the noxious metabolites accumulated in the moderately ischemic tissue. Second, recent studies have demonstrated that partial coronary artery occlusion can induce a metabolic adaptation to ischemia.20-22 For example, indexes such as myocardial lactate production, pH, and phosphocreatine recovery to baseline values despite persistent moderate flow deprivation. We did not assess whether 15 minutes of coronary stenosis induced metabolic adaptation in our model, but even if this were the case, it was clearly not sufficient to limit infarct size after subsequent severe ischemia in the R(−) group. Rather, it appears that total reperfusion after moderate ischemia is needed to achieve the preconditioning effect.

How can total reperfusion render ischemic preconditioning effective? One obvious difference between R(−) and R(+) groups is the presence of the hyperemic response after release of the stenosis in the R(+) group. However, according to a recent study in our laboratory, it is unlikely that this transient fivefold increase in coronary flow per se can explain preconditioning.23 In this previous study, dogs were preconditioned by coronary cyclic flow variations caused by repeated formation/dislodgment of platelet thrombi upon a severe coronary stenosis with local endothelial damage. Although we used a similar partial coronary stenosis in this previous study, the endothelial damage triggering the formation of platelet thrombi resulted in severe preconditioning ischemia during the nadir of the flow variations. More importantly, in this previous protocol, the stenosis was not released during the treatment period; thus, although coronary flow returned to baseline values at the peak of the flow variations, hyperemia did not occur. Cyclic flow variations did, however, limit infarct size caused by subsequent ischemia, suggesting that hyperemia per se is not the sole stimulus for myocardial protection. In the present study, coronary flow was persistently higher in the R(+) group after reflow/hyperemia than in the R(−) group during stenosis. Thus, any deleterious metabolite produced during the preceding phase of moderate ischemia could have been better washed out in the R(+) group, limiting further accumulation during the subsequent sustained ischemia.

Reperfusion after an ischemic insult results in a burst of oxygen-derived free radical production, which has been suggested to play a role in preconditioning.24 However, Iwamoto et al25 recently reported that the protective effect of preconditioning was not prevented by oxygen free radical scavengers. Murry et al26 proposed that preconditioning may be caused by myocardial stunning that results from the initial reversible
ischemia/reperfusion injury, but the incompatibility in the time courses of myocardial stunning and preconditioning makes this hypothesis unlikely. Even further doubt concerning the protective effect of stunning was raised by a recent study in which we observed a significant reduction in infarct size with a preconditioning regimen that did not stun the ischemic bed before the sustained coronary artery occlusion.15

Thus, although reperfusion after the initial preconditioning ischemic insult appears mandatory to induce preconditioning, the reason for this remains unclear.

Preconditioning With Partial Coronary Artery Stenosis and Contractile Function

We have recently demonstrated in the canine model that preconditioning with four episodes of 3-minute total coronary artery occlusion significantly reduces infarct size but does not improve the recovery of contractile function in the peri-infarct tissue.20 In the present study, despite a significant reduction in infarct size and only a mild deterioration of contractile function before the sustained ischemia, R(+) dogs experienced similar dyskinesia during the total coronary artery occlusion and a similar degree of stunning throughout the 4.5 hours of reperfusion. Although we cannot rule out the possibility that contractile function in R(+) dogs may recover faster during the following hours or days, the present results confirm that preconditioning does not preserve contractile function in the canine model, at least during the initial hours of reflow. This also confirms previous studies suggesting that the mechanisms of preconditioning and stunning are likely not related.15,26

Potential Limitations

A potential concern in our protocol is that triphenyltetrazolium chloride staining might not provide an accurate assessment of infarct size. However, there is convincing evidence that triphenyltetrazolium chloride staining reliably distinguishes irreversibly injured from viable myocardium at 6 hours after the onset of ischemia,12 i.e., within the time frame of this study. Second, our acute protocol cannot definitively determine whether the reduction in infarct size in the R(+) group represents true salvage (as opposed to delayed death) of myocytes. In fact, some pharmacological agents (such as the iron chelator deferoxamine) have been reported to acutely reduce infarct size assessed at 4 hours after reperfusion, but these agents failed to provide sustained protection when infarct size was measured 1 day after reflow.27 However, Murry et al.11 have provided histological evidence that the reduction in infarct size observed with preconditioning persists at 4 days of reperfusion, demonstrating that preconditioning does, in fact, salvage myocardium rather than merely provide acute protection with later development of necrosis. In any case, our data indicate that preconditioning with moderate ischemia can acutely limit infarct size caused by subsequent coronary artery occlusion.

Conclusion

The present study demonstrates that moderate ischemia, induced by a partial coronary artery stenosis, is sufficient to acutely precondition the canine heart, provided it is followed by complete reperfusion. Preconditioning with moderate ischemia does not, however, preserve contractile function during the initial hours after reflow. Although any extrapolation must be made with caution, this study raises the possibility that recurrent anginal attacks not associated with total coronary artery occlusion may precondition the human heart.

References

Partial coronary stenosis is sufficient and complete reperfusion is mandatory for preconditioning the canine heart.

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