Reperfusion Alters the Relation Between Blood Flow and the Remaining Myocardial Infarction

Alan Chu, MD, and Frederick R. Cobb, MD

This study evaluated whether or not reperfusion of ischemic myocardium 2 hours after occlusion alters the basic relation between myocardial blood flow and infarction occurring during permanent occlusion. Awake mongrel dogs chronically instrumented with proximal circumflex coronary occluders were subjected to permanent occlusion (group A, n=10) or occlusion followed by reperfusion 2 hours later (group B, n=11). Myocardial blood flow was quantified with radioactive microsphere injections before, 6 hours after occlusion (group A), immediately before release, and 4 hours after reperfusion (group B). Three days later, the dogs were killed, and the heart was sectioned systematically into approximately 80 1–2-g circumferential and transmural samples for radioactive counting and histologic infarct quantification. Epimyocardial and endomyocardial samples from the permanent occlusion group (A) and the reperfused group (B) were separated by infarct range and related to regional myocardial blood flow measurements. In groups A and B, regional myocardial blood flow in endomyocardial and epimyocardial layers were inversely related to the extent of infarction. For given degrees of infarction, myocardial blood flow was significantly higher (>twofold) in the reperfused group. Myocardial samples with extensive infarction (51–75%) showed only mild (20–30%) reductions in blood flow when compared with nonischemic regions in the reperfused group. Thus, although early reperfusion may salvage ischemic myocardium, these studies showed that reperfusion causes a new relation between blood flow to the ischemic region and eventual histologic infarct size. When myocardial blood flow is used as an index of myocardial salvage after reperfusion, the basic relation obtained from permanent occlusion studies substantially overestimates the extent of myocardial salvage and underestimates the degree of remaining infarction. (Circulation 1989;79:884–889)

In previous studies, we described the relation between regional myocardial blood flow during permanent coronary artery occlusion and subsequent histologic myocardial infarction. Although infarction was directly related to the severity of early ischemia, comparable degrees of ischemia resulted in greater infarction in the endocardial compared with epicardial regions. Studies from our laboratory and others have shown that although reperfusion of myocardium at 2 hours after coronary occlusion salvages ischemic myocardium, a significant fraction of the ischemic zone develops infarctions. Studies have also shown that after reperfusion at this early interval, blood flow initially increases throughout the ischemic region but then subsequently decreases in the nonsalvaged infarcted region. Thus, areas subjected to prolonged ischemia followed by reperfusion are characterized by variable infarction and variably reduced blood flow. Although myocardial blood flow measurements after coronary thrombolysis have been used as an index of myocardial salvage in patients experiencing acute myocardial infarction, studies have not determined whether or not the basic relation between regional myocardial blood flow measured after permanent coronary occlusion and subsequent infarction is altered after reperfusion of ischemic myocardium.

The present study was designed to determine whether or not reperfusion alters the basic relation between regional blood flow and histologic infarc-
tion that is observed in the setting of permanent occlusion. The studies were performed in awake chronically instrumented dogs to avoid the influences of anesthesia and acute surgery.

Methods

Twenty-eight mongrel dogs anesthetized with thi-amylyl sodium (60–80 mg/kg i.v.) were subjected to left thoracotomy. Heparin-filled polyvinyl catheters were inserted into the left atrium through the left atrial appendage and to the aortic root through the internal thoracic artery. In 14 dogs (group A), a snare-type occluder was placed around the left circumflex coronary artery proximal to the first marginal branch, whereas in the remaining 14 dogs (group B), a pneumatic-type occluder was implanted in the same location. The catheters and tubings were tunneled to a subcutaneous pouch at the base of the neck. Studies were performed 7–14 days after surgery. The catheters and occluders were exteriorized from the subcutaneous pouch after local lidocaine infiltration anesthesia. The dogs were loosely restrained and allowed to adapt to the laboratory environment for 30–45 minutes. The animals were studied lying on their right side in a quiet and dimly illuminated room. Aortic pressure, left atrial pressure, and electrocardiogram were recorded throughout the study. In group A, the coronary vessel was permanently occluded. In group B, the pneumatic cuff was inflated to occlude the coronary artery for 2 hours. The occluder was then deflated to restore coronary flow. Morphine sulfate was given intravenously in 2-mg boluses (total dose 10 mg) during occlusion to minimize discomfort. Lidocaine (2 mg/kg i.v.) was also given just before occlusion. Four dogs in group A and three in group B fibrillated during the study and were excluded.

Myocardial blood flow was quantified with radioactive microspheres (9 ± 1 μm) labeled with $^{125}$I, $^{141}$Ce, $^{51}$Cr, $^{113}$Sn, $^{85}$Sr, $^{95}$Nb, or $^{46}$Sc as previously described. Microsphere suspension (1 ml) was injected into the left atrium during 5–10-second intervals and flushed with 5–10 ml saline. Beginning 5 seconds before the microsphere injection and continuing for 90 seconds, a reference blood sample was collected from the aortic catheter with a Harvard withdrawal pump (South Natick, Massachusetts). Blood flow was measured before, 6 hours after permanent occlusion in group A ($n = 10$), immediately before release of the 2-hour occlusion, and 4 hours after reperfusion in group B ($n = 11$). Previous studies from our laboratory showed that immediately after a 2-hour coronary occlusion followed by reperfusion, blood flow increased throughout the ischemic region. Blood flow in the previously ischemic region then gradually decreased and was maximally decreased 4 hours after reperfusion.

Three days later, the dogs were killed with an overdose of thiamylal sodium and potassium chloride. The hearts were removed and fixed in 10% buffered formalin. After removal of the atria, right ventricle, and epicardial fat, the left ventricle was sliced into four transverse rings. The rings were further subdivided into circumferential regions that were subsequently cut into transmural layers. This sampling technique yielded approximately 80 samples of 1–2 g each. The samples were weighed and counted in a Packard gamma scintillation spectrometer (Downers Grove, Illinois) with appropriate window settings for each isotope. A dedicated digital computer corrected background spillover activity. Blood flow was calculated with the following formula: $Q_m = Q_r \cdot C_m/Cr$, where $Q_m$ is myocardial blood flow (ml/min), $Q_r$ is reference blood flow (ml/min), $C_m$ is counts per minute of myocardial sample, and $Cr$ is counts per minute of reference blood. Myocardial blood flow was divided by the sample weight and expressed as milliliters per minute per gram.

The myocardial samples were then embedded in paraffin, and two-step histologic sections were made for each tissue sample. The histologic sections were stained with hematoxylin and eosin. Myocardial infarction was defined as the presence of complete or partial cellular dissolution, inflammatory cell infiltrate or loss of normal cellular structure. Infarct size of each sample was determined by planimetry of the image of each individual histologic section from a projection microscope with a graph pen attached to a dedicated computer. Isolated islets of infarcted tissue were individually planimetered and summed. The sum total weight of the infarcted tissue in each sample was calculated, and infarct size was expressed as a percentage of each tissue sample. For each group of dogs, samples were separated into endocardial and epicardial regions and classified into five groups having infarction ranges of 0–25%, 26–50%, 51–75%, 76–90%, and 91–100%. The myocardial flow of each sample was expressed as percent normal region flow and averaged for each infarct range in each dog. The mean absolute flow and percent normal flow values in each infarct range of the reperfused and permanent occlusion groups were calculated and compared by Student’s $t$ test for unpaired data. The reproducibility of this method of infarct quantification has been previously established.

Results

The hemodynamic measurements before and 6 hours after occlusion (i.e., 4 hours after reperfusion) of both the permanent occlusion group (A) and reperfused group (B) are shown in Table 1. At each interval, no significant difference occurred in hemodynamic measurements between the two groups of dogs.

The endocardial and epicardial myocardial blood flow of the permanent occlusion group and the reperfused group were separated according to their infarct range and illustrated in Figures 1 and 2 and Tables 2 and 3. Blood flow and percent normal flow measurements of samples from the same infarct
TABLE 1. Hemodynamic Measurements Before and 6 Hours After Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean left atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>6 Hr</td>
<td>Before</td>
</tr>
<tr>
<td>Group A</td>
<td>85±7</td>
<td>115±7</td>
<td>96±5</td>
</tr>
<tr>
<td>Group B</td>
<td>87±6</td>
<td>101±9</td>
<td>100±2</td>
</tr>
</tbody>
</table>

Data are mean±SD.

range in each dog were averaged. These averaged values were subsequently used to calculate the mean absolute flow and mean percent normal flow for each infarct range in each group. Regional myocardial blood flow in endocardial and epicardial layers of the normal regions or the ischemic regions 2 hours after occlusion was comparable between group A and B dogs. Six hours after occlusion, flow to the reperfused region in group B dogs was significantly increased compared with the flow in group A dogs (Table 2). Regional myocardial blood flow in both endocardial and epicardial layers was inversely related to the extent of infarction in the permanently occluded and reperfused groups. Reperfusion, however, resulted in a new relation between infarction and blood flow. Mean endocardial flow to the ischemic region 6 hours after occlusion in the permanently occluded group was 0.44±0.06 ml/min/g or 50±9% normal flow in the 0–25% infarct range, and it decreased to 0.06 ml/min/g or 7±3% normal flow in the 91–100% infarct range. The flow in the reperfused group was 1.10±0.09 ml/min/g or 106±14% normal flow in the 0–25% infarct range, and it decreased to 0.39±0.05 ml/min/g or 38±6% normal flow in the 91–100% infarct range (p<0.05). Mean epicardial flows to the ischemic zone were higher overall than the endocardial values: ranging from 0.59±0.04 ml/min/g or 64±6% normal flow to 0.10±0.00 ml/min/g or 9±1% normal flow in the permanently occluded group compared with 1.20±0.11 ml/min/g or 126±13% normal flow to 0.43±0.11 ml/min/g or 38±8% normal flow in the reperfused group 6 hours after occlusion. Relative to the permanent occlusion situation, reperfusion resulted in hyperperfusion in the regions containing residual infarction. For a given degree of infarction, myocardial blood flow was significantly increased (≥twofold) in reperfused dogs so that even minimally decreased (relative to normal myocardium) myocardial flow, measured 4 hours after reperfu-

FIGURE 1. Bar graph of relation between myocardial blood flow (expressed as percent normal region flow±SEE) in the subepicardium and percent histologic infarction in individual myocardial samples. The number on top of each bar indicates the number of dogs in each group. The 76–90% and 91–100% infarction groups were excluded from statistical analyses because of small number of dogs.

FIGURE 2. Bar graph of relation between myocardial blood flow (expressed as percent normal region flow±SEE) in the subendocardium and percent histologic infarction in individual myocardial samples. The number on top of each bar indicates the number of dogs in each group.
TABLE 3. Relation Between Myocardial Blood Flow Quantified at 6 Hours After Occlusion and Infarct Size

<table>
<thead>
<tr>
<th>Infarct (%)</th>
<th>Endocardial MBF (%)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>50±9</td>
<td>64±6</td>
<td>126±13*</td>
</tr>
<tr>
<td>26–50</td>
<td>42±5</td>
<td>39±5</td>
<td>93±12*</td>
</tr>
<tr>
<td>51–75</td>
<td>24±3</td>
<td>22±3</td>
<td>78±16*</td>
</tr>
<tr>
<td>76–90</td>
<td>15±3</td>
<td>13±5</td>
<td>62±24†</td>
</tr>
<tr>
<td>91–100</td>
<td>7±3</td>
<td>9±1</td>
<td>38±8†</td>
</tr>
</tbody>
</table>

Data are mean±SD based on percent normal region blood flow.

Group A, no reperfusion; group B, reperfusion after 2 hr of occlusion.

*p<0.05, compared with corresponding group A values; †statistical analyses not performed because of small number of dogs.
interval, blood flow was increased above control flow values throughout the ischemic region, indicating a marked stimulus to vasodilation. Approximately 15 minutes after reperfusion, the hyperemic response had decreased with blood flow in the reperfused regions approximating preocclusion values. Blood flow subsequently decreased in regions containing extensive infarction. We observed that blood flow was maximally reduced at 4 hours, and no further reduction occurred in flow between 4 hours and 3 days after reperfusion. Previous studies showed that the reperfused region is characterized by a highly variable eventual infarction and variably reduced blood flow. Studies have not established whether or not the basic relation between regional myocardial blood flow and infarction as observed in the setting of permanent occlusion is altered after reperfusion. The present study addresses this question by relating regional myocardial blood flow to the final extent of histologic infarction in two models of ischemic injury. One group of animals was subjected to permanent occlusion for the duration of the study, and the other group of animals was subjected to 2 hours of ischemia followed by the reperfusion. Blood flow measurements 6 hours after onset of coronary occlusion or 4 hours after reperfusion were compared with histologic infarction quantified at 3 days. Blood flow was measured at these intervals because previous studies showed that maximum reductions in flow were present after reperfusion at this interval.4

Our study clearly shows that for comparable degrees of infarction, blood flow was substantially higher in the animals subjected to reperfusion compared with those subjected to permanent occlusion. The data showed that in the reperfusion setting, myocardial samples may contain extensive (51–57%) infarction, with only mild (about 20–30%) reductions in blood flow compared with nonischemic regions. Reperfusion thus alters the basic relation between regional myocardial blood flow and the final extent of histologic necrosis. Although blood flow was reduced in areas with extensive infarction, reperfusion resulted in a relative hyperperfusion compared with the permanent occlusion situation. Studies by Bloor and White10 showed that phasic flow was increased after reperfusion in areas subjected to 2, 6, 24, and 72 hours of ischemia despite highly variable infarction.

Even though reperfusion altered the basic relation between blood flow and infarction, alterations in myocardial blood flow after reperfusion were related to the extent of infarction. Thus, this new relation between blood flow and infarction after reperfusion may be used to identify areas of extensive infarction. This study does not clarify whether or not these relations are also influenced by varying the period of ischemia before reperfusion. This study thus provides quantitative information about a new relation between the final extent of histologic infarction and regional myocardial blood flow measured after reperfusion, and consequently, it has important implications that should be considered in interpreting blood flow measurements in early reperfusion intervals. Clinical studies in patients with acute myocardial infarction have used imaging techniques such as the distribution of $^{201}$TI to assess the effects of coronary thrombolysis.5,6 Increases in thallium uptake after thrombolysis have been interpreted to indicate salvage of ischemic myocardium. Previous studies by Buja et al11 showed that the status of myocardial perfusion is an important determinant for the different morphologic patterns of myocardial necrosis in different regions of infarction and for detection of infarctions with $^{201}$TI. Studies from our laboratory have further shown that the distribution of $^{201}$TI activity is directly related to myocardial blood flow as measured by microsphere techniques in normal, ischemic, and infarcted myocardium.12 Although $^{201}$TI activity may reflect the distribution of blood flow in the setting of reperfusion, blood flow as measured by any technique is likely to seriously overestimate the degree of salvage. The degree of overestimation would be expected to be influenced by the timing of the blood flow measurements, for example, the earlier the measurement, the greater the overestimation. Okada and Pohost13 and Granato et al14 in open-chest anesthetized dogs subjected to 2 hours of occlusion of the left anterior descending coronary artery, also showed normal or increased $^{201}$TI activity measured up to 1 hour after reperfusion in areas that contained myocardial infarction. The relation between blood flow and extent of histologic infarction was not systematically quantified.

The present study does not address whether the reduced perfusion has a role in mediating the final extent of infarction or whether the reduced perfusion resulted from a reduced metabolic stimulation from irreversibly injured myocytes, ischemic injury to the vasculature, or other factors. Blood flow in normal myocardium is closely coupled to the myocardial metabolic activity.15,16 Based on previous studies that have examined the effects of prolonged ischemia on regional wall motion, it is likely that contractile function, which is a major stimulus to metabolic activity, was markedly reduced in the reperfused area17 and may have contributed to the reductions in perfusion. However, blood flow measurements after reperfusion were directly related to the extent of infarction, indicating that the ischemic injury was the major factor in reducing perfusion.

This study shows that reperfusion alters the basic relation between regional myocardial blood flow and the final extent of infarction. For a given degree of infarction, reperfusion compared with permanent occlusion, resulted in a relative hyperperfusion of the remaining histologic infarction. Reperfusion thus resulted in a new quantitative relation between blood flow and infarction. Although the degree of reduction in blood flow after reperfusion was directly related to the extent of infarction, substantial in-
fraction occurred in regions with only mild reductions in blood flow.

Acknowledgments
We acknowledge the technical assistance of Robert H. Murdock Jr. and Marjorie Grubb; the Durham VA Medical Media Section for illustrations, and Cathie Collins for her expert secretarial assistance.

References

KEY WORDS • reperfusion • myocardial blood flow • myocardial infarct size
Reperfusion alters the relation between blood flow and the remaining myocardial infarction.
A Chu and F R Cobb

Circulation. 1989;79:884-889
doi: 10.1161/01.CIR.79.4.884
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 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:
http://circ.ahajournals.org/content/79/4/884

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/