The Relationship of Vascular Injury and Myocardial Hemorrhage to Necrosis After Reperfusion

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SUMMARY Early reperfusion may salvage ischemic myocardium; late reperfusion often intensifies morphologic changes of necrosis and causes hemorrhage. To determine whether hemorrhage after reperfusion increases the extent of myocardial infarction, six closed-chest, anesthetized dogs underwent balloon occlusion of the left anterior descending coronary artery for 5.5 hours, followed by 30 minutes of reflow. Colloidal carbon was injected distal to the balloon before reperfusion to label injured vessels. After sacrifice, the area of myocardial necrosis was measured by planimetry of 1-cm-thick serial slices of left ventricle stained with triphenyl tetrazolium chloride. Areas of hemorrhage and vascular injury were also measured. In all hearts, the extent of hemorrhage and vascular injury was less than the extent of necrosis (10.2 ± 4.6% vs 19.8 ± 8.6% [mean ± SD], p < 0.01). Further, hemorrhage was always within the area of necrosis, primarily in the subendocardial portion. Hemorrhage after reperfusion occurred only in necrotic tissue where carbon labeling indicated severe vascular injury before reperfusion, suggesting that the hemorrhage was the consequence of preexisting microvascular injury, not its cause.

RESTORATION of blood flow to ischemic myocardium would seem to be a rational way of limiting the extent of ischemic injury. Studies in dogs have shown that if reperfusion is instituted less than 20 minutes after coronary occlusion, normal myocardial blood flow, function and morphology eventually return. However, if 40 minutes or more elapse before flow is restored, normal perfusion may not be restored, function may not recover fully or may even worsen temporarily and the morphologic signs of myocardial injury may be more pronounced than would be expected after occlusion of the same duration not followed by reperfusion. Morphologically, with reperfusion one may observe marked cell swelling and disruption, extensive deposition of calcium, diffuse contraction band change and marked vascular damage with marked hemorrhage into the myocardium; when coronary occlusion is not followed by reperfusion, these changes are unusual or not extensive. Whether reperfusion merely makes already irreversibly injured myocardium look worse or whether it actually extends myocardial necrosis is controversial. Because surgical bypass and transluminal angioplasty techniques are applied now for early reperfusion of myocardium in patients with evolving myocardial infarction, this matter is clinically relevant.

The goal of this study was to determine whether reperfusion hemorrhage actually extends infarction or occurs in myocardium that was already irreversibly injured before reperfusion.

Methods

Six mongrel dogs that weighed 22–25 kg were anesthetized with morphine (1.5 mg/kg), followed 45–60 minutes later by i.v. pentobarbital (30 mg/kg) supplemented by doses of 2 mg/kg every 30 minutes. The dogs were intubated and artificially ventilated 10 times/min at tidal volumes of 20 ml/kg. Heparin (100 IU/kg) was given hourly for anticoagulation. Aortic
pressure and the ECG were monitored continuously. An angiographic catheter was inserted through the left carotid artery and advanced under fluoroscopic guidance into the ostium of the left main coronary artery. A double-lumen 2½F catheter with a distal lumen and a balloon (also 2½F deflated) 2 mm from the tip was then inserted 2–3 cm into the left anterior descending coronary artery through the lumen of the angiographic catheter. The balloon was inflated with diluted Renografin until cylindrical distortion was visualized fluoroscopically; then, the angiographic catheter withdrawn into the aorta. This degree of inflation causes pressure distal to the balloon to decrease to less than 20 mm Hg, indicating complete coronary occlusion (unpublished observations).

The occlusion was maintained for 5½ hours. Then, to label myocardial vessels with ischemic injury, 0.1 ml/kg of filtered colloidal carbon suspension (Pelikan Co., biologic black ink) was injected over 5 minutes distal to the inflated balloon through the distal lumen of the catheter (fig. 1).

The carbon was allowed to circulate for 15 minutes so it could be cleared from the bloodstream and noninjured vessels by the reticuloendothelial system. The clearing of carbon from the circulation and noninjured vessels before reperfusion was confirmed initially by the absence of carbon in centrifuged blood and later by its absence in areas of nonischemic myocardium. Thus, any vessel labeled with carbon must have been labeled before reperfusion. The balloon was then deflated to allow reperfusion of the myocardium for 30 minutes. Reperfusion arrhythmias (premature ventricular complexes) were not frequent or severe and were abolished by the administration of a 20-mg bolus of lidocaine. The dogs were sacrificed and their hearts excised and cut into 1-cm-thick transverse slices. Each slice was incubated in triphenyl tetrazolium chloride (TTC), a histochemical stain for dehydrogenase activity that identifies areas of necrosis grossly.10 A planimeter was used to measure areas of necrosis, areas with carbon-labeled, injured vasculature and areas of hemorrhage in all gross heart slices and giant whole-mount histologic sections of each slice. The use of whole-mount sections of entire slices of ventricle prepared and stained with hematoxylin-eosin facilitated the localization and quantitation of histopathologic changes. For each heart, the extent of vascular injury, hemorrhage and necrosis was measured in each slice and expressed as a percentage of the entire left ventricle. The paired t test was used to compare the percentages of necrosis and vascular injury in each heart.

The initial 5½-hour period of ischemia was chosen to allow enough time for gross and microscopic recognition of the infarction11–18 and for development of sufficient injury to result in hemorrhage after reperfusion.14–16 The 5-minute duration of carbon injection was chosen so the injection could be performed in a short time, yet at low pressure. The 15-minute period of carbon circulation was chosen to allow time for the carbon to be cleared from the blood and noninjured vessels before beginning deflation of the balloon so that no labeling would occur after reperfusion. The 30-minute reperfusion period was to allow enough time for reperfusion injury to occur.1,2

Thus, hemorrhage outside the area of necrosis and away from areas of carbon-labeled vessels would suggest an increase in the volume of injured tissue; if all the hemorrhage were from previously injured labeled vessels within the confines of the original area of necrosis, additional tissue destruction would not be expected.

Results

Grossly, areas of nonnecrotic myocardium that stained with TTC appeared red and areas of vascular injury and hemorrhage that trapped carbon appeared black (figs. 2 and 3). Areas of necrosis of myocardium without vascular injury or hemorrhage remained brown (figs. 2 and 3). The areas of blackening were predominantly subendocardial, with sparing of the peripheral portions of the infarct (figs. 2 and 3).

Microscopic examination of giant whole-mount histologic sections confirmed gross findings. Microscopically, infarcts delineated by TTC showed increased eosinophilia and waviness of fibers, spaces presumed to represent interstitial edema and early infiltration of polymorphonuclear leukocytes at their margins. Gross and microscopic determinations of infarct size correlated well (unpublished observations). Areas of hemorrhage were predominantly subendocardial and completely within the areas of necrosis (fig. 3), as defined both grossly and microscopically. In each slice from each heart, the area of hemorrhage seen microscopically was less extensive than the area of blackening seen grossly (fig. 3). In addition, areas of hemorrhage were always smaller than the areas of vascular labeling and always occurred within regions containing labeled, injured vessels. Although hemorrhages were severe and confluent in the subendo-
cardium, they were milder and patchy toward the periphery of the zone of vascular injury. Light microscopy showed the appearance of injured vessels within the necrotic areas (fig. 4). Carbon could be found lining vessels, in carbon thrombi and in interstitial areas (fig. 4). No carbon was found in myocardium outside the areas of necrosis as delineated by TTC. Autopsy study of liver, spleen and lymph nodes confirmed the trapping of circulating carbon by the reticuloendothelial system (fig. 5).

Planimetry of areas of necrosis and vascular injury in the six dogs showed that the area of necrosis was greater than the area of vascular injury (table 1). For all hearts, 52% of the region of necrosis contained labeled, injured vasculature (10.2 ± 4.6% vs 19.8 ± 8.6%, p < 0.01) (table 1). Hemorrhagic areas were more difficult to planimeter accurately from histologic sections because they were patchy peripherally; they constituted 50–60% of the regions with injured vessels.

**Discussion**

Several investigators, using a variety of experimental models, have shown that early reperfusion can salvage jeopardized but viable ischemic myocardium,
occlusion and measured serial serum CK levels to estimate infarct size 24 hours after occlusion. While 56% of animals had smaller infarcts, 44% were thought to have larger infarcts on the basis of the CK measurements. Extensive hemorrhage of the myocardium was noted grossly in some animals and was thought to be causally related to greater elevations of serum CK. Vatner et al.20 showed that reperfusion enhances the release of CK from the myocardium (washout phenomenon), resulting in greater recovery of CK in blood per gram of infarcted myocardium. Thus, in reperfusion experiments, higher blood CK levels do not necessarily reflect larger infarcts.

Mathur and associates20 found that reperfusion after 2 hours of occlusion improved function in dyskinetic and akinetic left ventricular wall in 42% of dogs and reduced infarct size in 50%, but caused hemorrhagic infarcts in some of the dogs, suggesting to them that early reperfusion of ischemic myocardium may be harmful. Bulkley and Hutchins44 studied 58 patients who died after coronary artery bypass surgery and found areas of necrosis with hemorrhage in the distribution of patent grafts in 15 patients. These investigators also suggested that with interruption of coronary perfusion followed by reflow, the reperfusion itself may be causing the necrosis. In the studies cited above, however, one cannot be certain that the hemorrhagic, necrotic areas were not already irreversibly injured before reperfusion.

Unlike previous studies, the present study was designed to determine whether hemorrhage associated with reperfusion causes extension of infarction. Areas of severe vascular injury were labeled with carbon before reflow to determine whether hemorrhage occurred outside as well as inside these areas of injured vessels. The area of ischemic necrosis was identified by use of the TTC technique, a reliable gross indicator of myocardial necrosis,7,8 with excellent correlation between gross and microscopic measurement of infarct size. In all dogs, hemorrhage occurred without exception in the area of vessels injured before reperfusion and was confined to the central and subendocardial portions, which are known to be the most severely

resulting in infarcts smaller than would be expected with permanent occlusion.14 22 If reperfusion is instituted after more than 6 hours of occlusion, little or no myocardial salvage results in the dog.20 21 Bresnahan et al.14 instituted reflow after 5 hours of coronary

<table>
<thead>
<tr>
<th>Dog</th>
<th>MI size (% LV)</th>
<th>Area of vascular injury (% LV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.6</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>15.6</td>
<td>7.3</td>
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<tr>
<td>3</td>
<td>10.7</td>
<td>3.7</td>
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<tr>
<td>4</td>
<td>33.8</td>
<td>16.2</td>
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<tr>
<td>5</td>
<td>23.3</td>
<td>9.8</td>
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<tr>
<td>6</td>
<td>22.6</td>
<td>14.3</td>
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<tr>
<td>Mean</td>
<td>19.8 ± 8.6</td>
<td>10.2 ± 4.6*</td>
</tr>
</tbody>
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*p < 0.01.
Abbreviations: MI = myocardial infarction; LV = left ventricle.
ischemic and to undergo irreversible damage earliest.25

Higginson et al.,26 using labeled erythrocytes and microspheres, found that hemorrhages only occurred where myocardial blood flow was less than 21% of control before reperfusion, supporting our data showing that hemorrhages occur only within the infarcts in the most severely ischemic regions. That hemorrhage depends on the severity of preexisting ischemia is also indicated by the fact that the extent of hemorrhage is directly proportional to the duration of the preceding ischemia.27-31

Whether or not reperfusion could have significantly increased the amount of myocardial necrosis by destroying "islands" or "peninsulas" of viable myocardium within the borders of the ischemic area cannot be answered directly on the basis of this study. However, this seems unlikely because (1) hemorrhages occurred in the most severely ischemic subendocardium, where viable myocardium would not be likely to be present, and (2) studies of global and regional function in this model showed no further impairment of function after reperfusion, which one might expect if additional myocardial necrosis were occurring (unpublished observations). Whether reperfusion hemorrhage might interfere with infarct healing and predispose to rupture, aneurysm formation or infarct expansion29 must also be considered, but cannot be answered by this short-term study. There is, however, some experimental evidence that reperfusion may actually benefit healing by accelerating the proliferation of granulation tissue.29

The lack of hemorrhage in subepicardial and lateral portions of the infarct observed in this study is of interest. Recent studies by Reimer et al.30 have shown that there is a wave front phenomenon of cell death in myocardial infarction with irreversible injury involving the most severely ischemic subendocardium first and then subepicardium. Klomer and associates31 showed ultrastructurally that within any region of ischemic myocardium, irreversible myocardial cell injury occurs before vascular endothelial injury, and that damaged vessels only occur in regions where there has already been irreversible ischemic injury to the myocardium. Thus, it is not surprising that toward the subepicardial portion of an infarct, where myocardial cell death evolves later, there is even more of a delay in the evolution of vascular endothelial injury, with less labeling by carbon and less hemorrhage.

In conclusion, in this experimental model, hemorrhage with reperfusion occurred only within necrotic tissue where there had been severe vascular injury and irreversible myocardial injury before reperfusion. There is no evidence to suggest that reperfusion per se resulted in an increase in the amount of myocardial necrosis, despite being associated with striking morphologic changes within the infarcts. These findings may have an important clinical implication, because one potential problem of early reperfusion often discussed is extension of infarction due to reperfusion hemorrhage. The results of this study suggest that hemorrhage after reperfusion is the consequence of preexisting vascular injury and not likely to cause infarct extension.

Acknowledgment
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