Quantitative Effect of Early Coronary Artery Reperfusion in Baboons

Extent of Salvage of the Perfusion Bed of an Occluded Artery

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SUMMARY We examined the extent to which ischemic myocardium was salvaged by reperfusion using a method that allowed expression of the volume of infarction as a percentage of the volume of the perfusion bed of the occluded artery (region at risk of infarction). In eight baboons, the left anterior descending coronary artery (LAD) was occluded for 2 hours, after which perfusion was restored. A control group of eight baboons underwent an identical protocol, but perfusion was not restored. Twenty-four hours after occlusion, microvascular dyes were injected into the LAD and adjacent arteries to delineate the perfusion bed of the occluded artery. The volume of infarction and volume of the perfusion bed were determined planimetrically. The mean percentage of the perfusion bed infarcted in the control baboons was 94.2 ± 3.5% and 50.1 ± 5.8% in the reperfused baboons. Hence, the mean percentage of the perfusion bed infarcted was reduced by 44.1% in the reperfused group compared with the control group (p < 0.001). In reperfused baboons, hemorrhage occurred in the region of infarction but did not result in infarct extension. We conclude that reperfusion after 2 hours of coronary occlusion results in substantial salvage of ischemic myocardium in the baboon.

CORONARY ARTERY REPERFUSION initiated early after acute coronary artery occlusion may result in a reduction in ultimate infarct size, but the duration of coronary occlusion after which reperfusion results in significant salvage of ischemic myocardium remains unclear. Some experimental animal studies have shown a significant reduction in infarct size when reperfusion was initiated within the first few hours after occlusion.1-7 Other studies have shown that reperfusion initiated within the same time period results in either no significant change2, 8-10 or an increase in infarct size.11 These diverse experimental findings may be attributed to methodologic differences. When comparing control and reperfused groups, most investigators have compared absolute infarct volumes or infarct volumes expressed as a percentage of left ventricular volume. However, the size of an infarct resulting from coronary artery occlusion varies considerably at any given anatomic site,12, 13 owing to inherent differences in the distribution of the anatomic perfusion bed of an occluded coronary artery. Thus, considerable error is introduced when absolute infarct volumes or infarct volumes expressed as a percentage of the left ventricle are used to quantitatively assess the degree of myocardial salvage achieved by reperfusion. We circumvented this problem by expressing infarct volume as a percentage of the volume of myocardium at risk of infarction and using each animal as its own control.14-16

We used a microvascular dye injection technique that accurately delineates the anatomic perfusion bed of an occluded artery (region at risk of infarction).17 We compared the percentage of the perfusion bed of an occluded coronary artery that undergoes ultimate infarction in control and reperfused baboons to quantitatively assess the effects of reperfusion on infarct size. We studied baboons because their coronary anatomy closely resembles that in man.18, 19 The results may help clarify the time interval during which coronary revascularization in man may result in significant salvage of acutely ischemic myocardium in the absence of a well-established collateral circulation.

Methods

Surgical Preparation

Sixteen baboons that weighed 13–18 kg were sedated with intramuscular ketamine hydrochloride, 10 mg/kg. Anesthesia was induced and maintained by i.v. sodium thiopental, 2 mg/kg. Standard lead II of the ECG was monitored throughout the procedure. Baboons were intubated with a cuffed endotracheal tube and ventilated with a volume respirator to maintain an arterial pH of 7.40 ± 0.05. Supplemental oxygen was administered to maintain an arterial oxygen saturation of at least 95%. Arterial pressure (AP) was monitored by a #8F catheter advanced through the femoral artery. Under sterile conditions, a left thoracotomy was performed and the heart suspended in a pericardial cradle. A 3-0 Mersilene snare was placed around the left anterior descending coronary artery (LAD) distal to the first diagonal branch. An electromagnetic flow probe was placed around the pulmonary artery to monitor cardiac output with a Biotronex flowmeter (BL 610). Left atrial pressure was monitored by a catheter inserted through the left atrial appendage and left ventricular pressure by an 18-gauge cannula inserted through the apex of the left ventricle. The rate of rise of the left ventricular pressure (dP/dt) was derived with a resistance-capacitance differentiating circuit. The hemodynamic variables were continuously monitored during the procedure.
Epicardial Electrode Mapping

Two rows of nine unipolar atraumatic epicardial electrodes (spaced at 5 mm intervals) were sutured to the left ventricle parallel to its minor axis such that they overlay the anticipated region of ischemia. The electrograms were monitored with a Gould Brush Mark 260 recorder with multiple ECG couplers at a paper speed of 25 mm/sec and a sensitivity of 1 mV/mm. The frequency response of the ECG coupler with preamplifier is linear from 0.14 Hz to 1 KHz and the frequency response of the recorder is linear from DC to 40 Hz. The electrodes were used to measure ST-segment deflections during the periods of occlusion and reperfusion.

Experimental Protocol

In eight baboons, the LAD was occluded for 2 hours, after which perfusion was restored. After control hemodynamic measurements and epicardial ECGs were recorded, the LAD was occluded by tightening the snare. Hemodynamic measurements and epicardial ECGs were recorded at 15-minute intervals thereafter. Two hours after occlusion, perfusion of the LAD was restored by releasing the occluding snare. An i.v. lidocaine bolus, 1 mg/kg, was administered to control premature ventricular complexes. The left atrial and left ventricular pressure lines were removed 4 hours after reperfusion and the chest was closed. Lead II ECG, arterial pressure and cardiac output were monitored for 24 hours after coronary occlusion. An i.v. bolus of heparin, 5000 U, was given to prevent blood clotting within the coronary arteries and allow for subsequent microvascular dye injections. The baboons were sacrificed by rapid infusion of a saturated solution of potassium chloride and their hearts immediately excised.

A control group of eight baboons underwent an identical protocol, except that perfusion of the LAD was not restored.

Delineation and Histologic Assessment of the Perfusion Bed

Cannulas (i.d. 0.58 mm) were placed into the LAD at the site of previous occlusion, the proximal LAD, the left circumflex coronary artery, and the right coronary artery. These arteries were then infused simultaneously and at equal flow rates with colored microvascular dyes (Microfil, Canton Biomedical Products), which readily pass through the capillary beds. This technique allows accurate delineation of the perfusion bed subserved by an occluded coronary artery. After fixation of the hearts in 10% formalin for 3 days, serial cross sections of the left ventricle were then made at 3-mm intervals parallel to its minor axis. A microscopic slide was made from each cross section and stained with hematoxylin-eosin. The perfusion bed of the occluded artery was clearly visualized on the paraffin tissue block of the left ventricular cross section and was marked on the corresponding histologic slide by superimposing the slide on the tissue block. Microscopic examination of each histologic slide allowed the area of infarction to be also marked on the slides. The pattern of infarction with early coronary reperfusion is different from that with permanent coronary occlusion. With early reperfusion, a confluent central zone of infarction and a surrounding peripheral zone of patchy infarction are present. These areas were marked on each histologic slide. An enlarged tracing was made from each slide with a slide projector. The areas of the perfusion bed, central infarction and patchy infarction were determined by planimetry of the tracing. These areas were multiplied by the thickness of each cross section to give the volumes for each section. Summation of the volumes of each tissue section yielded the total volume of the perfusion bed (Vp), total volume of central infarction (Vc) and total volume of patchy infarction (Vp) for each baboon.

Statistical Analysis

The significance of hemodynamic and histologic changes in control and reperfused baboons was examined by t test. The relationship between Vp and Vc was examined for both groups of baboons by regression analysis. The correlation coefficient and the standard error of the estimate are given for all regression equations. The significance of differences in the variances, slopes, and elevations between regression lines was determined by F test. All values are given as mean ± SEM.

Results

Delineation of the Perfusion Bed of the Occluded Coronary Artery

Cross sections of the left ventricle in both control and reperfused baboons showed that the perfusion bed of the occluded coronary artery was readily delineated by the microvascular dye. The distinct demarcation of perfusion bed boundaries as well as the extensive midwall hemorrhage characteristically observed in the reperfused baboons are shown in figure 1. Because of the increased amount of necrosis in control baboons, the

Figure 1. Cross section of the heart from a reperfused baboon. The perfusion bed of the previously occluded left anterior descending coronary artery (yellow) is readily delineated from the adjacent perfusion beds (red). Extensive midwall hemorrhage is evident within the perfusion bed of the occluded artery and was characteristically observed in the reperfused baboons.
perfusion bed of the occluded artery was not as densely filled with dye as it was in the reperfused baboons. Nonetheless, the perfusion bed of the occluded artery was still readily visualized. The perfusion bed of the occluded artery in the control group showed no evidence of hemorrhage.

**Histologic Changes in Control and Reperfused Baboons**

Microscopic examination of left ventricular cross sections stained with hematoxylin-eosin in control baboons showed extensive coagulation necrosis in the perfusion bed of the occluded artery. Necrotic myocardial cells showed the characteristic changes of pyknosis and karyolysis and retained their distinct cellular outlines with cross striations remaining in register. There was no hemorrhage or patchy infarction in the perfusion bed. In the reperfused baboons, the areas of infarction were completely contained in the perfusion bed. Infarcts characterizedly consisted of a central confluent zone of infarction surrounded by a peripheral zone of small foci of patchy infarction. The central zone of infarction showed coagulation necrosis with pyknosis, karyolysis and loss of cross striations. The absence of contraction bands within this central zone is probably due to the no-reflow phenomenon. In the zone of patchy infarction some contraction band necrosis was also evident. The infarcts involved the subendocardial and midwall regions of the perfusion bed while myocardial preservation was observed in the subepicardial and lateral regions. Hemorrhage occurred extensively in the central area of infarction and to a lesser extent in the patchy areas of infarction, but no hemorrhage was observed outside of the infarcted regions.

**Percentage of the Perfusion Bed Infarcted in Control and Reperfused Baboons**

Figure 2 shows the mean percentage of the perfusion bed of the occluded coronary artery infarcted in control and reperfused baboons. In the control group, the mean percentage of the perfusion bed infarcted (\( V_i \)/\( V_{pb} \times 100 \)) was 94.2 \( \pm \) 3.4%. Patchy infarction was not observed in the control baboons. In the reperfused group, the mean percentage of the perfusion bed infarcted was 50.1 \( \pm \) 5.8% and the mean percentage of the perfusion bed showing patchy infarction (\( V_p \)/\( V_{pb} \times 100 \)) was 6.2 \( \pm \) 3.5%. With perfusion restored after 2 hours of coronary occlusion, the mean percentage of the perfusion bed undergoing ultimate infarction was reduced by 44.1\% when comparing the control and reperfused groups (\( p < 0.001 \)).

The regression lines in figure 3 show the relationship between \( V_i \) and \( V_{pb} \) for control and reperfused baboons. The variances and the slopes between the two regression lines are not significantly different, but the differences in elevation between regression lines was highly significant (\( p < 0.005 \)).

In reperfused baboons, when \( V_{pb} \) was smaller than 8 cm\(^3\) (or 15% of the volume of the left ventricle) the percentage of \( V_{pb} \) infarcted was substantially less than when \( V_{pb} \) was greater than 8 cm\(^3\). The mean percentage of the \( V_{pb} \) infarcted was 35.0 \( \pm \) 4.0% for the smaller perfusion beds and 59.1 \( \pm \) 6.0% for the larger perfusion beds (\( p < 0.05 \)). In the control baboons, the mean
percentage of $V_{\text{pl}}$ infarcted was independent of the size of the perfusion bed.

**Epicardial ST-segment Changes**

Epicardial ST-segment elevation occurred immediately after coronary occlusion in both the control and reperfused groups and remained constant during the 2-hour postocclusion monitoring period, indicating ischemia of the underlying myocardium. In the reperfused group, successful restoration of antegrade perfusion after release of the occluding snare was indicated by a rapid decrease in epicardial ST-segment elevation, the appearance of arterialized blood in the previously occluded artery, and the resolution of cyanosis in the ischemic region.

**Hemodynamics**

Table 1 summarizes the hemodynamic results for the control and reperfused baboons for the preocclusion and 2-hour postocclusion recording periods. There was no significant difference between groups in any of the hemodynamic measurements at either recording period.

Figure 4 shows the hemodynamic effects of occlusion and reperfusion for the reperfused groups of baboons. Occlusion resulted in a significant increase in LAP ($p < 0.001$) and a significant decrease in peak $dP/dt$ ($p < 0.05$) within the first few minutes of occlusion. No significant changes occurred in heart rate, arterial pressure or cardiac output. Similarly, reperfusion resulted in a significant increase in left atrial pressure ($p < 0.01$) as well as a significant decrease in peak $dP/dt$ ($p < 0.05$). The hemodynamic changes with reperfusion returned to prerereperfusion values within 30 minutes. No significant changes occurred in heart rate, arterial pressure or cardiac output within the first few minutes after reperfusion.

**Discussion**

We previously showed that after acute coronary occlusion in the baboon, injection of microvascular dye into the occluded artery and adjacent arteries (direct method) or injection of dye only into the adjacent arteries (defect method) accurately delineates the anatomic perfusion bed of the occluded artery. In both the control and reperfused baboons, the perfusion bed of the occluded artery was readily delineated using the direct method. In the control group, however, the microvascular dye directly injected into the previously occluded LAD only partially filled its perfusion bed because of increased vascular necrosis. Although the perfusion bed of the occluded artery could have been successfully delineated by the defect method alone (due to the minimal collateralization between perfusion beds in the baboon heart), the color contrast provided by the addition of the direct injection facilitated delineation of the perfusion bed. Comparison of the percentage of the perfusion bed infarcted in control and reperfused baboons allowed more accurate quantitative assessment of the effects of reperfusion on infarct size.

Coronary reperfusion after coronary occlusion lasting 15 minutes or less results in total salvage of ischemic myocardium. However, the results of studies of the effect of early coronary artery reperfusion at intervals later than 15 minutes after occlusion have been widely divergent. Smith et al. showed in monkeys that after 2 hours of coronary occlusion, reperfusion resulted in a 47% mean reduction in the percentage of the left ventricle infarcted, compared with monkeys with chronic occlusion. Bolooki et al. showed in the dog heart that after 2 hours of occlusion, reperfusion resulted in a 56% mean reduction in the percentage of the left ventricle infarcted. In contrast, O'Brien et al. and Mathur et al. found no significant change in the percentage of the left ventricle infarcted when coronary reperfusion was initiated 2 hours after occlusion. Although Mathur et al. noted an increased number of small infarcts in the reperfused dogs, infarct size varied widely, probably because of differences in the size of the perfusion bed of the occluded artery. In the present study, when the error introduced by variability in perfusion bed size was controlled, reperfusion resulted in substantial salvage of ischemic myocardium in all baboons.

Inherent variations in infarct size can occur not only as a result of differences in the size of the perfusion bed of an occluded artery but also as a result of differences in the degree of collateralization to this perfusion bed. The importance of variations in available collateral blood flow to variations in infarct size in the dog has been emphasized by the studies of Reimer and Jennings. Variations in infarct size due to differences in collateral blood flow do not occur in baboons, as collateralization between perfusion beds is minimal. Thus, because the error introduced by variability in collateralization is additionally controlled for, the selective effects of reperfusion on the percentage of the perfusion bed infarcted could be determined more accurately in the present study.

Studies in the monkey, pig and dog have also shown discrepant findings with regard to the degree of myocardial salvage when reperfusion is initiated 3–5

### Table 1. Hemodynamic Values for Control and Reperfused Baboons Before and 2 Hours After Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>2 hours postocclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125 ± 8</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>Reperfused</td>
<td>117 ± 9</td>
<td>122 ± 8</td>
</tr>
<tr>
<td><strong>AP (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>114 ± 8</td>
<td>112 ± 16</td>
</tr>
<tr>
<td>Reperfused</td>
<td>114 ± 5</td>
<td>108 ± 4</td>
</tr>
<tr>
<td><strong>LAP (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.4 ± 0.9</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Reperfused</td>
<td>2.2 ± 0.3</td>
<td>3.7 ± 0.8*</td>
</tr>
<tr>
<td><strong>CO (ml/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1376 ± 98</td>
<td>1593 ± 223</td>
</tr>
<tr>
<td>Reperfused</td>
<td>1403 ± 147</td>
<td>1284 ± 160</td>
</tr>
<tr>
<td><strong>Peak dP/dt (mm Hg/sec)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2503 ± 274</td>
<td>2263 ± 356</td>
</tr>
<tr>
<td>Reperfused</td>
<td>2563 ± 201</td>
<td>2163 ± 209</td>
</tr>
</tbody>
</table>

*p < 0.05 vs value before occlusion.

**Abbreviations:** HR = heart rate; AP = mean arterial pressure; LAP = mean left atrial pressure; CO = cardiac output; peak $dP/dt$ = peak value of rate of rise of left ventricular pressure.
hours after acute coronary occlusion. These differences are probably related to variability in the size of the perfusion bed and, in the dog, to the variability in collateral flow. The results of these studies reinforce the need for experimental models that control for variations in perfusion bed size and collateralization and provide for more accurate quantitative assessment of the effects of coronary reperfusion.

**Reperfusion Hemorrhage and Its Effect on Infarct Size**

Hemorrhage associated with reperfusion has been reported by several investigators. 9, 11, 22-25 Mathur et al. 9 and Bresnahan et al. 11 suggested that hemorrhage associated with reperfusion might have extended the region of infarction. In our study, extensive hemorrhage was evident in the reperfused baboons, but was clearly confined to the region of infarction. This hemorrhage may have enlarged the volume of infarction and reduced the size of the reported differences in the percent of the perfusion bed infarcted between control and reperfused baboons. Overestimation of anatomic infarct size has been reported by Reimer and Jennings; 26 however, it seems unlikely that reperfusion hemorrhage resulted in infarct extension and more likely that hemorrhage reflects associated vascular damage. This is in agreement with the findings of Kloner et al., 23 Reimer et al. 24 and Fishbein et al., 25 who showed that after reperfusion, areas of hemorrhage were always confined within the area of myocardial cell necrosis. Kloner et al. showed that ultrastructural evidence of microvascular damage lagged behind ultrastructural evidence of myocardial cell necrosis, which suggests that microvascular damage is not a primary cause of myocardial cell damage. 23

**Effect of Reperfusion on Left Ventricular Function**

Reperfusion resulted in a decrease in the indexes of global left ventricular function, particularly in the first few minutes after reperfusion. These changes returned to prereperfusion values within 30 minutes. These results are consistent with the findings of other investigators who observed a decrease in global contractility after reperfusion. 3, 27, 28 Despite the transient depression in global contractility after reperfusion, a return in regional contractility may be significantly more prolonged. Banka et al. 29 reported that reperfusion after 1 or 2 hours of coronary occlusion in dogs resulted in an immediate, significant depression in regional contractility within the ischemic zone that lasted for 3 hours after reperfusion. Further, Heyndrickx et al. 28 reported that in dogs subjected to 15-minute periods of occlusion followed by reperfusion, global left ventricular...
function may return to normal within 30 minutes, although regional ischemic zone function may take up to 6 hours to return to normal. The mechanism by which this depression in contractility occurs has been well clarified. Nonetheless, it has been consistently demonstrated that reperfusion results in an initial decrease in regional and global left ventricular function, even though ultimate infarct size may be reduced.

In conclusion, our results showed that in baboons, a primate model in which minimal collateral support is available to the perfusion bed of an occluded coronary artery, reperfusion after 2 hours of coronary occlusion can produce substantial salvage of ischemic myocardium. These results may help clarify the time constraints during which coronary revascularization in man may be expected to result in significant salvage of ischemic myocardium in the absence of a well-established collateral circulation.

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