Effects of Coronary Artery Reperfusion on Myocardial Infarct Size and Survival in Conscious Dogs

KENNETH L. BAUGHMAN, M.D., PETER R. MAROKO, M.D., AND STEPHEN F. VATNER, M.D.

SUMMARY The effects of coronary artery reperfusion at 1 and 3 hours after coronary artery occlusion were examined on myocardial infarct size and survival in conscious dogs. Left circumflex coronary artery occlusion was induced by inflating an hydraulic occluder and confirmed thereafter by measuring the absence of coronary blood flow. Of the 77 dogs that underwent coronary artery occlusion, 18 died within 1 hour. Of the 59 remaining dogs, permanent coronary artery occlusion was carried out in 31 dogs, 12 underwent reperfusion after 1 hour and 16 underwent reperfusion after 3 hours. Survival at 1 week was enhanced significantly ($p < 0.01$) by reperfusion carried out at either 1 or 3 hours; only 29% of dogs with permanent coronary artery occlusion survived, whereas 83% and 75% of dogs survived 1 week with reperfusion at 1 hour and 3 hours, respectively. Average infarct size at 1 week was smaller in dogs with reperfusion (NS). The inability to reach statistical significance was most likely the result of two factors: (1) There was a marked variation in infarct size in dogs with permanent coronary artery occlusion — infarcts averaged 21.3 ± 7.5% and ranged from 0.7-72.6% of the left ventricle. (2) Dogs that died 1-7 days after coronary artery occlusion had significantly ($p < 0.05$) larger infarcts (40 ± 4% of left ventricle) than those that survived 1 week in any of the three groups. Thus, if all dogs had survived 1 week, a beneficial effect on infarct size could have been demonstrated. Nevertheless, coronary artery reperfusion at either 1 or 3 hours after coronary artery occlusion induces a striking beneficial effect on survival, which is of the utmost clinical significance.

CORONARY ARTERY REPERFUSION in the form of coronary artery bypass has become one of the most important therapeutic interventions in medical practice. This procedure is most frequently carried out in patients with chronic coronary artery disease, but is also used in patients with acute myocardial ischemia and infarction.1,2 The physiologic basis for the value of this intervention is controversial, particularly in the acute phase of myocardial infarction. The results from experimental studies in anesthetized animal models have conflicted, showing either considerable improve-


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to occur after coronary artery reperfusion in conscious dogs. Two criteria were used to determine the beneficial effects of coronary artery reperfusion: (1) reduction in myocardial infarct size and (2) increase in survival at 1 week after coronary artery occlusion and reperfusion.

Methods

Seventy-seven mongrel dogs (20-35 kg) underwent left thoracotomy using general anesthesia with sodium pentobarbital, 30 mg/kg, and sterile surgical technique. Heparin-filled tygon catheters (Norton Co.) were implanted in the aorta, and a Doppler ultrasonic flow transducer and a hydraulic occluding cuff were placed around the left circumflex coronary artery 3-5 cm from the origin but proximal to the first marginal branch.

Arterial pressure was measured with a Statham P23Db strain-gauge manometer. Coronary blood flow was measured continuously over 24 hours with a Doppler ultrasonic flowmeter to confirm complete occlusion and reperfusion and to ensure that occlusion remained complete throughout the 24-hour period after occlusion. If partial release of the occlusion occurred, it was corrected immediately. Partial release occurred most frequently at 8-24 hours after occlusion. Mean values of arterial pressure and coronary blood flow were determined using electronic integrators. Arterial blood gases were measured with a Radiometer acid-base analyzer (PHM 71 Mk2) and blood microsystem (BHS Mk5).

Experiments were conducted 3-6 weeks after operation. After recording baseline values of left circumflex coronary artery blood flow, arterial pressure, heart rate and arterial blood gases, the coronary artery was occluded. Coronary blood flow, arterial pressure and heart rate were recorded after occlusion, immediately after reperfusion and at 24 hours and 1 week after to the initial occlusion. It was predicted that mortality would be highest in dogs with permanent coronary artery occlusion. Accordingly, while dogs were randomly allocated to the three groups 1 hour after coronary occlusion, twice as many dogs were assigned to the permanent occlusion group in order to attempt to arrive at approximately equal numbers of surviving dogs in the three groups at the end of 1 week. No dogs were defibrillated or treated with drugs. After final recordings were made 1 week later, all surviving dogs were sacrificed with an overdose of sodium pentobarbital. The hearts were excised immediately and washed repeatedly in iced saline. The left and right ventricles were removed from atria, valvular apparatus, aorta, pulmonary artery and pericardial fat. The free wall of the right ventricle was dissected from the left ventricle and interventricular septum, weighed and immersed in homogenizing medium (25 ml/g of myocardium in 0.25 M sucrose, 0.001 M neutralized sodium EDTA and 1 mM mercaptoethanol). The left ventricle was sliced in 1-cm serial sections in a plane parallel to the atrioventricular groove, and the infarcted area, which was clearly demarcated, was dissected from normal myocardium. The normal and infarcted tissues were weighed to determine gross infarct size (ISp). Thereafter, the ventricular myocardium was minced with scissors and homogenized with the above-described solution in a Waring blender. The solution was centrifuged initially at half speed, two 15-second bursts, then at 10,000 rpm for 10 minutes at 0°C and the supernatant fraction was removed and centrifuged at 16,000 rpm for 10 minutes.

In all dogs that survived 1 week, infarct size was determined by myocardial creatine kinase (CK) depletion (ISm). CK was assayed using CK n-1 reagent (Worthington Biochemical Corporation). Protein contents of homogenates and of tissue fractions were determined by the biuret procedure. Results were expressed as 1U/mg protein, or 1U/ml/g myocardium. The techniques and formulas described by Kjekshus, Sobel and Shell were used to calculate ISm. This amount of CK depleted by the myocardium was calculated as the difference between CK concentration measured in normal myocardium and CK concentration in homogenized entire left ventricular mass. In separate experiments it was found that the difference in CK concentrations between normal and the center of the infarct was 74.6%. This value, i.e., 25.4%, was used to represent the nondepletable CK in calculating infarct size.

The effects of coronary occlusion on infarct size and hemodynamics in the three groups were compared using analysis of variance; the results for survival were compared statistically using the chi-square test.

Results

Hemodynamics

Baseline values for arterial blood gases and hematocrits were in the normal range for conscious dogs and did not differ in the three groups studied. Values for heart rate, mean arterial pressure and coronary blood flow did not differ in the three groups before or 1 hour after occlusion (table 1). At 24 hours after occlusion, only coronary blood flow in dogs with permanently occluded coronary arteries was significantly different from control or from the values that were observed in the 1-hour or 3-hour reperfusion groups.

Infarct Size

Infarct size was determined in all dogs that survived 1 week by myocardial CK depletion and by gross pathology. Infarct size tended to be greater in dogs with permanent coronary artery occlusion, but these changes were not statistically significant, probably because of the disparity of size of infarcts in dogs with permanently occluded coronary arteries, ranging from 0.7 to 72.6% of left ventricle (fig. 1). There was a good correlation between ISm and ISp (table 2) except in the 1-hour reperfusion group. The dogs with reperfusion at 1 hour had less demarcated areas of infarction; actually, the infarcted myocardium was characterized by
TABLE 1. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion control</th>
<th>Time after initial coronary occlusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour 24 hours</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-hour reperfusion group</td>
<td>107.8 ± 3.5</td>
<td>108.0 ± 3.9</td>
</tr>
<tr>
<td>3-hour reperfusion group</td>
<td>97.7 ± 4.7</td>
<td>107.0 ± 4.9</td>
</tr>
<tr>
<td>Permanent occlusion group</td>
<td>93.8 ± 3.5</td>
<td>98.6 ± 5.4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-hour reperfusion group</td>
<td>101.0 ± 3</td>
<td>127.0 ± 10*</td>
</tr>
<tr>
<td>3-hour reperfusion group</td>
<td>103.0 ± 12</td>
<td>137.0 ± 8*</td>
</tr>
<tr>
<td>Permanent occlusion group</td>
<td>86.0 ± 4</td>
<td>117.0 ± 7*</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-hour reperfusion group</td>
<td>43.0 ± 2.3</td>
<td>0*</td>
</tr>
<tr>
<td>3-hour reperfusion group</td>
<td>43.0 ± 3.3</td>
<td>0*</td>
</tr>
<tr>
<td>Permanent occlusion group</td>
<td>41.0 ± 2.9</td>
<td>0*</td>
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</tbody>
</table>

*SSignificantly different from preocclusion control (p < 0.01).
†Significantly different from permanently occluded group (p < 0.01).

Figure 1. Average (± SEM) infarct sizes, determined by myocardial CK depletion, are compared for the three groups of dogs studied and surviving 1 week (shaded bars) and with dogs that died 1–7 days after coronary artery occlusion, where infarct size was determined by gross pathology (solid bars). In dogs that survived 1 week, infarct size tended to be larger (NS) in the dogs with permanent coronary artery occlusion than in those in which reperfusion was carried out. However, dogs that died 1–7 days after coronary artery occlusion had significantly larger infarcts than those in any of the groups that survived for 1 week. LV = left ventricle.

TABLE 2. Myocardial Infarction Expressed As Percent of Left Ventricle and Determined by Gross Pathology and Myocardial CK Depletion

<table>
<thead>
<tr>
<th></th>
<th>ISp</th>
<th>ISm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-hour reperfusion group</td>
<td>17.4 ± 2.9</td>
<td>13.8 ± 1.9</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-hour reperfusion group</td>
<td>14.7 ± 3.5</td>
<td>15.4 ± 3.4</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent occlusion group</td>
<td>23.4 ± 8.2</td>
<td>21.3 ± 7.5</td>
</tr>
<tr>
<td>(n = 9)</td>
<td></td>
<td></td>
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</table>

Abbreviations: ISp = infarct size determined by gross pathology; ISm = infarct size determined by myocardial CK depletion.
Survival significantly less different in occlusion than death. These either of permanent with occlusion, 18 of the three groups of dogs. Survival was not significantly different in dogs with reperfusion at 1 and 3 hours, but it was significantly less in dogs with permanent coronary artery occlusion than in either of the groups with reperfusion.

Survival (fig. 2)

Before assignment in any group, i.e., before 1-hour occlusion, 18 of the 77 dogs died suddenly. Arterial pressure was well maintained in these dogs, so we assumed that ventricular fibrillation was the cause of death. These dogs were excluded from further analysis. Of the 59 remaining dogs, 31 were assigned to the permanently occluded group, whereas 16 were assigned to the 3-hour reperfusion group and 12 were assigned to the 1-hour reperfusion group. There was a clear-cut difference in the survival of these remaining dogs. Eighty-three percent (10 of 12 dogs) survived in the 1-hour reperfusion group. Seventy-five percent (12 of 16 dogs) survived in the 3-hour reperfusion group. Only 29% (nine of 31 dogs) survived in the permanently occluded group. The survival in the permanently occluded group was significantly less ($p < 0.01$) than in either the 1-hour or 3-hour reperfusion groups. Arrhythmias were often observed in the permanently occluded group, but occurred more frequently in dogs after reperfusion. Surprisingly, no dogs died immediately after reperfusion. The time of death for the 28 dogs that died 1–7 days after coronary artery occlusion is shown in figure 3.

Discussion

Blumgart et al. demonstrated in 1941 that periods of coronary artery occlusion of as long as 20 minutes resulted in no histologic evidence of myocardial necrosis. They also observed that the extent of myocardial infarction correlated with the duration of occlusion, and that arrhythmia and death occurred frequently with reperfusion. Yabuki et al. reported hemorrhage and inflammatory cell infiltrate in myocardium reperfused after 30 minutes of occlusion. Jennings confirmed that cell damage begins as soon as 20 minutes after coronary artery occlusion. Thus, little permanent damage is incurred by a coronary artery occlusion of less than 30 minutes. However, the extent to which coronary artery reperfusion is beneficial after this time period is controversial.

Most studies showing marked positive effects of coronary artery reperfusion have been carried out in anesthetized animals. Maroko et al. demonstrated myocardial preservation by means of coronary artery...
reperfusion 3 hours after occlusion. The myocardial area at risk was identified in an open-chest dog model by electrocardiographic ST-segment elevation 15 minutes after occlusion. Reperfusion resulted in less myocardial CK depletion, less histologic necrosis, and an improved left ventricular wall motion compared with permanently occluded dogs. Ginks et al. extended these observations and evaluated infarct size 1 week after either permanent occlusion or reperfusion after 3 hours. Reperfusion resulted in a sixfold reduction in myocardial infarct size. Beneficial effects of reperfusion on infarct size have also been reported by Reimer et al. and Costantini et al. Reimer et al. evaluated the percent transmural myocardial necrosis in an anesthetized animal model after either permanent occlusion or 40 minutes, 3 or 6 hours of occlusion, followed by reperfusion. In the group with occlusion of 40 minutes, 55% of the transmural area at risk remained viable, compared with 33% viable in the 3-hour group and 16% in the 6-hour group. Costantini et al. examined hemodynamics, wall motion and regional metabolism acutely and after 7 days in anesthetized dogs with either 3-hour or permanent occlusions of the left anterior descending coronary arteries. The reperfused dogs displayed a mean infarct size 50% less than those of the permanently occluded group. In addition, although all hemodynamic and functional parameters deteriorated during the period of occlusion and for 1 hour after reperfusion, by 1 week these values had returned to nearly preocclusion levels. Puric evaluated contractility, ATP and CK acutely or at 2 weeks, with variable periods of reperfusion after coronary artery occlusion in an open-chest model. Reperfusion after 1 hour failed to return contractility to normal immediately. However, 2 weeks later, myocardial enzymes and contractility returned to normal. The recovery of reperfused myocardium was less marked with reperfusion at 3 hours.

Other investigators have not always observed beneficial effects of reperfusion even 1–3 hours after coronary artery occlusion. Lang et al. reported significant arrhythmias, hypotension, abnormal lactate and potassium metabolism and accelerated necrosis and hemorrhage in anesthetized dogs reperfused for 5 hours after 3 hours of occlusion. Banka et al. observed an accentuation of myocardial dysfunction induced by reperfusion at 2 hours and in some animals by reperfusion as early as 1 hour after occlusion. In conscious dogs reperfused after 5 hours of occlusion, Bresnahan et al. reported an extension of myocardial necrosis greater than infarct size predicted by CK release in 44% of dogs studied 24 hours after occlusion. This extension was associated with intramyocardial hemorrhage. In the other 56% of the dogs, infarct size decreased. Mathur et al. in a conscious but sedated dog model, found that reperfusion at 5 hours failed to improve left ventricular function, determined angiographically, or infarct size when compared to dogs with permanent coronary artery occlusion. Finally, coronary artery reperfusion at 1 and 3 hours was not associated with reduced infarct size in a recent study conducted in this laboratory in conscious dogs sacrificed 24 hours after occlusion.

Fewer studies have been concerned with the effects of reperfusion on survival. However, neither Reimer et al. nor Mathur et al. noted a significant difference in survival in dogs with permanent coronary artery occlusion as opposed to those in which coronary artery reperfusion was carried out. In the present investigation a significantly improved survival at 1 week was observed in conscious dogs reperfused at either 1 or 3 hours after left circumflex coronary artery occlusion as compared with dogs with permanent coronary occlusion. There are important differences in the present investigation compared with those of Reimer et al. and Mathur et al. As noted previously, the study by Reimer et al. was conducted in anesthetized animals. However, Mathur et al. studied conscious dogs. The major difference between the latter study and ours was the lower mortality in the permanently occluded group of Mathur et al. In our study, coronary blood flow in the occluded vessel was monitored for the entire 24-hour period after occlusion. As noted earlier, partial reperfusion occurred in some dogs, most characteristically between 8 and 24 hours after occlusion. If this also occurred in the study by Mathur et al. their permanently occluded control dogs could have actually been partially reperfused.

In the surviving dogs in this study, myocardial infarct size as determined by left ventricular CK depletion or by gross pathology tended to be diminished by reperfusion, but these effects were not statistically significant unless dogs with very small infarcts were excluded from analysis. This supports the position that the inability to reach statistical significance in the series was probably due to the large variability in infarct size in permanently occluded dogs. The large variability in infarct size contrasts strikingly with the data of Reimer et al., who studied anesthetized animals and found remarkably consistent sizes of infarcts, i.e., 85 ± 2% of the posterior papillary muscle. It may be that anesthesia and the open chest reduces compensatory reflex mechanisms. Therefore, any occlusion of the left circumflex coronary artery in the anesthetized dogs will tend to induce a significant myocardial infarction, thereby eliminating dogs that show almost no infarction despite permanent coronary occlusion. In support of this hypothesis is that in anesthetized preparations, coronary artery occlusion invariably leads to a reduction in left ventricular systolic or arterial pressure, whereas this is not generally observed in conscious animals. Thus, the reduction in arterial pressure could act to intensify the extent of infarction.

While the differences in infarct size between all the dogs with permanent coronary artery occlusion and all the dogs with coronary artery reperfusion were not statistically significant using analysis of variance as a test for significance, we cannot conclude that reperfusion does not have a beneficial effect on infarct size. The dogs that died had significantly larger infarctions, i.e., in seven dogs who died 1–7 days, infarct size was 40 ± 4% of the left ventricle. Therefore, if all dogs had survived, a beneficial effect on infarct size could have
been demonstrated. This conclusion is based on the combined observations that infarct size was larger in dogs that died and that many more dogs died after permanent coronary artery occlusion than when coronary reperfusion was carried out. Thus, this study clearly shows that because infarct size and mortality are correlated, an intervention can express a beneficial effect either by reducing mortality or by reducing infarct size in the surviving dogs. In the case of a reduced mortality, as was observed in this study, the surviving population is skewed because the dogs with larger infarcts, which were more frequent in the control group, died and therefore were not included in the statistical analysis of infarct size. Studies in man have also shown that mortality is correlated with infarct size, further supporting the contention that infarct size would have been significantly greater in the permanently occluded group if all dogs had survived.

The large variability in infarct size was partially why we could not demonstrate a significant effect of coronary artery reperfusion on infarct size in this investigation. It might be assumed that those dogs in the permanently occluded group with small infarcts had little myocardium at risk. Excluding those dogs from analysis, as well as the dogs in the reperfusion groups with small infarcts (which in this case could have been due either to the beneficial effects of the intervention or to the possibility that little myocardium was at risk), significantly different infarct sizes were found among the three groups. This type of analysis does not bias the results in favor of demonstrating a beneficial effect from reperfusion because animals in which reperfusion reduced infarct size to less than 10% of the left ventricle were excluded. These data support the hypothesis that coronary artery reperfusion is beneficial in terms of infarct size and survival and that the inability to demonstrate statistical significance was due to those dogs that did not demonstrate substantial infarction despite prolonged coronary artery occlusion.

Occlusion of the left circumflex coronary artery for only 1 hour results in a significant amount of permanent myocardial damage. In our study, average infarct size was found to be 14% of the left ventricle in dogs that survived 1 week after coronary artery occlusion and reperfusion at 1 hour. It is particularly important in this instance to use the data for infarct size derived from the technique of myocardial CK depletion, because by using that technique, it is not important if islands of viable myocardium exist within an infarcted region. Thus, these data are consistent with those of Reimer et al., who found a significant amount of necrosis in dogs in which reperfusion was carried out 40 minutes after coronary occlusion. The data from both studies point out the critical initial period between 20 minutes and 1 hour after coronary artery occlusion, where complete salvage of myocardial tissue is no longer possible by coronary artery reperfusion.

It has been well documented that acute coronary artery reperfusion is frequently associated with ventricular tachycardia and fibrillation. Therefore, it is surprising that no dogs died in this study immediately upon coronary artery reperfusion, although this period was frequently characterized by arrhythmias. Most studies demonstrating ventricular fibrillation upon release of coronary artery occlusion have used occlusions of 10-40 minutes duration and then rapid release of occlusion. It could be that coronary artery reperfusion at 1 or 3 hours after occlusion is not as deleterious in terms of inducing ventricular fibrillation as is reperfusion after 10-40 minutes of coronary occlusion. The rate of release of the occlusion could also have been less rapid in the present investigation.

Our results show that coronary artery reperfusion induces clear-cut beneficial effects on survival; clinically, this finding is of the utmost importance.

References
EFFECT OF COUNTERSHOCKS ON CONTRACTILITY/Kerber et al. 323

Effect of Direct-current Countershocks on Regional Myocardial Contractility and Perfusion

Richard E. Kerber, M.D., James B. Martins, M.D., Joseph A. Gascho, M.D., Melvin L. Marcus, M.D., and Joseph Grayzel, M.D.

SUMMARY Very high energy electrical countershocks can cause morphologic damage to the myocardium. In this study we searched for functional correlates of these shock-induced morphologic changes. We used ultrasonic sonomicrometers to measure myocardial contractility and radiolabeled microspheres to assess perfusion. Acute and chronic experiments were conducted in 45 dogs, assessing the effect of both direct (epicardial) and transthoracic shocks on beating and fibrillating hearts. High-energy or rapidly repeated epicardial shocks caused subepicardial contraction abnormalities. This indicates that electrical current delivered to the myocardium in sufficient high amounts and concentration can cause functional damage. Thus, in open-chest defibrillation during cardiac surgery, low energies (10–20 J) should be used initially and higher energies resorted to only if lower-energy shocks fail. However, single and multiple transthoracic shocks up to 460 J delivered energy caused no detectable contraction abnormalities. Myocardial perfusion did not fall after shocks. Thus, high-energy transthoracic shocks may have no deleterious effects on the contraction and perfusion of normal myocardium.

ALTHOUGH ELECTRICAL DEFIBRILLATION of the heart has been an accepted clinical therapy for many years, some important features of this lifesaving maneuver remain undefined. Very high levels of electrical energy and current can cause gross and microscopic myocardial damage, and this may be an argument for lower-energy defibrillation. However, the electrical doses used to cause such damage are far in excess of the amounts necessary to defibrillate. Lower-energy shocks result in less histologic damage. The effect of shocks on myocardial function has not been well characterized.

The purpose of these studies was to determine the effects of both low- and high-energy countershocks on two sensitive measurements of myocardial function—regional myocardial contractility and perfusion. We conducted experiments in both open- and closed-chest dogs, using ultrasonic sonomicrometers to register myocardial contractility and radiolabeled microspheres to measure perfusion.

Methods

Forty-five mongrel dogs that weighed 17–45 kg were studied. Dogs undergoing acute experiments were anesthetized with chloralose, 100 mg/kg, plus urethane, 1000 mg/kg i.v., with supplemental amounts as needed. Dogs that underwent sterile surgery for chronic experiments were anesthetized with
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