Myocardial Reperfusion in Acute Experimental Ischemia

Beneficial Effects of Prior Treatment with Steroids

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SUMMARY To test the hypothesis that prior steroid administration may enhance the mechanical and metabolic response to myocardial reperfusion, regional myocardial function (Hg-in-silastic length gauges), transmyocardial lactate balance and K⁺ difference were measured in 12 control and 13 treated (30 mg/kg methylprednisolone, 30 to 60 min postocclusion) dogs. At three hours of ischemia, systolic shortening in the ischemic segment was greater in treated dogs (40.6% vs. 12%, P < 0.05), while both lactate balance and K⁺ arteriovenous difference became positive. Lactate balance and K⁺ difference remained negative in the untreated animals. After three hours of occlusion and one hour of reperfusion, recovery of shortening was significantly greater in the treated animals (75.9 vs. 31.6%, P < 0.05). In addition, while lactate balance remained negative among the control dogs, it further improved in the treated dogs.

Thus, steroid administration during experimental coronary occlusion impedes the progression of ischemia and is additive to reperfusion in reversing ischemic dysfunction.

IT HAS BEEN DEMONSTRATED that myocardial reperfusion is an effective means of reversing mechanical dysfunction due to ischemic injury. However, the routine use of myocardial reperfusion for the treatment of acute myocardial infarction faces two major problems: 1) its effectiveness decreases as the period of ischemia increases, and 2) reperfusion itself may be associated with both hemodynamic deterioration and cell death. Ineffectiveness of reperfusion has been attributed to the fact that after one hour of coronary occlusion, irreversible cellular damage occurs in the infarcted area. Hemodynamic deterioration and death are in large part attributable to intramyocardial hemorrhage and severe ventricular arrhythmias following reperfusion.

Although currently available data are not entirely consistent, the preponderance of evidence suggests that progression of myocardial ischemia to infarction can be substantially delayed by steroid administration. Libby and coworkers have shown that steroid administration decreases the size of an acute myocardial infarction in dogs at 24 hours postocclusion. If the effects of reperfusion are contingent on the degree of cellular injury and if agents exist which do in fact alter the rate of progression of ischemia to infarction, then it could be hypothesized that the administration of steroids shortly after occlusion should influence positively the subsequent effects of reperfusion. The purpose of this study, therefore, was to evaluate this hypothesis.

Methods

Studies were carried out in 25 (12 control, 13 treated) healthy mongrel dogs weighing from 22 to 28 kg. The animals received morphine sulfate (2.2 mg/kg, i.m.) 20 minutes prior to anesthesia with chloralose (100 mg/kg i.v.). After endotracheal intubation, respiration was maintained with a Harvard ventilator. A left thoracotomy was performed via the fifth interspace, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was isolated for subsequent occlusion at an average of 3.5 cm from its origin. Systemic arterial pressure was continuously monitored with a P23Db pressure transducer (Statham Instruments, Hato Rey, Puerto Rico) by a transfemoral catheter advanced to the thoracic aorta. Left ventricular pressure was recorded by a transducer (Model BT-70, Bio-Tech, Pasadena, California), connected to a 10F catheter inserted in the ventricle through the apex. A large atrial catheter connected to a variable height reservoir was inserted into the left atrium, thus allowing manipulation of preload.

For assessment of regional function, methods previously described in this laboratory were employed.
mercury-in-silastic length gauge (0.31 mm I.D.; 0.62 mm O.D., Parks Electronics, Beaverton, Oregon) was sutured to the epicardial surface of the left ventricle parallel to the fibers perfused by the coronary artery to be occluded. The stiffness of this gauge is 1 gram of force per 5% elongation. The length gauge was prestressed for 30 min before each experiment; calibration was performed by attaching the end of the gauge to the jaws of a vernier caliper and extending the gauge by fixed increments. Resting length of the gauge was 10 mm; when in use this length varied from 10 to 20 mm. Within such a range the calibration of the gauge is linear, ±5%.13 The output of the gauge was recorded on paper (Visicorder Model 1505, Honeywell, Inc., Denver, Colorado), and the systolic shortening during the ejection period of the left ventricle was measured.

For studies of potassium arteriovenous difference and lactate balance, blood samples were obtained simultaneously from the femoral artery and the vein accompanying the artery chosen for occlusion. The epicardial vein was cannulated using a 2 inch, 20 gauge, thin-walled Teflon catheter (Beckton-Dickinson and Company, Rutherford, New Jersey). To avoid aspiration of blood from nonischemic zones, the blood from the ischemic zone was withdrawn through a Y system, allowing the operator to monitor continuously the aspirating pressure, which was not allowed to exceed 1 cm water. After blood withdrawal, samples for lactate and potassium measurements were prepared immediately.

Blood lactate concentration was measured in duplicate by a modification of the enzymatic method of Marbach and Weil,17 using a semiautomated Gilford spectrophotometer (Model 300N). Duplicate measurements agreed within ±4%. Lactate balance in the regional ischemic myocardium was calculated by the formula [(A-V)/A] × 100, in which A = arterial lactate concentration and V = venous lactate concentration (regional vein). Serum potassium was also measured in duplicate in a flame photometer (IL, Boston, Massachusetts, Model 143). Duplicate measurements agreed within ±1.1%. Regional potassium metabolism was expressed as the difference between arterial and regional vein concentrations.

Procedure and Data Analysis

During the control period blood from a donor dog was infused through the left atrial cannula to obtain a left ventricular function curve over a wide range of end-diastolic segmental lengths. During the period of ischemia and reperfusion, preload was again manipulated for matching segmental length in the control state.

After obtaining control measurements, the left anterior descending coronary artery was occluded by a snare occluder. A continuous infusion of lidocaine at a rate of 2.0 mg/min was maintained from the time of occlusion until 30 min before release in all dogs. In the steroid treated group, methylprednisolone was infused between 30 to 60 min after occlusion, over a period of approximately 15 min to a total dose of 30 mg/kg. A possible effect of the diluent was excluded by injecting it in three of the control dogs. No detectable hemodynamic or metabolic effects was observed. The snare was released after three hours of occlusion. At one hour following reperfusion, when blood pressure and cardiac rhythm were stable, the final measurements were obtained and the animals were sacrificed. The heart was placed in formalin and later serially sliced in approximately 1 cm sections perpendicular to the long axis and examined macroscopically for evidence of hemorrhage.

Segmental systolic shortening of the ischemic segment is reported as percent of the preocclusion control value at approximately the same end-diastolic length (±10%). There were 12 dogs in the control group, six of which underwent simultaneous hemodynamic and metabolic studies; three dogs were included for metabolic studies only. In the treated group there were 13 dogs, six of which had simultaneous hemodynamic and metabolic studies; four dogs were included for metabolic studies only. Both in control and treated groups nine animals were studied hemodynamically and three additional dogs in the control group and four in the treated group were included only for metabolic studies. The unpaired Student’s t-test was used for comparison of the data between the treated and untreated groups, and the paired t-test was used for comparisons within the same group.

Results

Effect of Steroids During Coronary Occlusion

Control and steroid-treated dogs did not differ prior to occlusion in mean arterial pressure, heart rate, regional systolic shortening, potassium arteriovenous difference or lactate balance (table 1). Coronary occlusion produced comparable reduction in mean arterial pressure in both control and treated groups. Heart rate did not change significantly. In the untreated animals arterial pressure declined slightly but significantly during ischemia, averaging 72.2 mm Hg at the end of three hours. In the treated animals, however, mean arterial pressure was unchanged during the ischemic period and averaged 87.5 mm Hg. Heart rate was not significantly altered by steroid administration.

Regional Myocardial Function During Ischemia

Following coronary occlusion significant reduction of the systolic shortening and paradoxical lengthening in late systole in the ischemic segment was observed in all the animals. Segmental shortening was reduced to 16% of its control value in the untreated group and to 27% among the treated dogs; the difference between the two groups was not statistically significant. Segmental shortening in the untreated group exhibited a trend toward progressive deterioration and averaged 12% of control at three hours postocclusion; this change was not statistically significant. In contrast, following steroid administration treated animals exhibited slight increase in segmental shortening; as in the control group this change was not statistically significant. Segmental shortening averaged 40.6% of the control value at three hours of occlusion. Consequently, at the end of three hours the treated animals had a significantly greater systolic shortening in the ischemic segment than those in the control group (fig. 1).

Regional Metabolism During Occlusion

Following coronary occlusion, potassium arteriovenous difference and lactate balance became negative in the two
groups (table 2). In the control group small, but not significant trends toward reversal of potassium loss were observed. At the end of three hours, potassium arteriovenous difference remained negative (fig. 2). In contrast, the treated animals exhibited reversal of potassium loss following steroid administration, such that the arteriovenous potassium difference became normal at the end of the observation period ($P < 0.05$). Lactate balance improved spontaneously in the control dogs, but evident production persisted, averaging $-27.8 \pm 12.5\%$ (fig. 3, table 2). A more significant improvement in lactate balance, however, occurred following steroid administration and in five of nine dogs lactate production returned to extraction; the mean value at three hours postocclusion was $0.9 \pm 5.7\%$ (fig. 3). This difference in lactate balance between the two groups is statistically significant ($P < 0.05$).

Effects of Reperfusion

Although no evidence of macroscopic hemorrhage was found in either control or treated dogs following reperfusion, dramatic differences were observed in the response of regional ischemic myocardium. All dogs in both groups responded to reperfusion with an increase in systolic shortening. The magnitude of response was quite variable in both groups. In the untreated dogs, systolic shortening was found to vary from 9.7 to 72.9% of the preocclusion control value, and postreperfusion function exceeded 50% of preocclusion control in only two dogs. The mean reperfusion value averaged 31.6 $\pm$ 8.3% of the control value. The steroid treated animals also exhibited a wide range of return in function, varying from 13 to 200% of the preocclusion control value. In contrast to the untreated group, the magnitude of return exceeded 50% in all except two dogs, and the average was 75.9 $\pm$ 23.0% in the treated animals. This difference of return in function between the two groups was statistically significant ($P < 0.05$).

In figures 4 and 5 typical examples of the effects of occlusion and reperfusion in both untreated and treated dogs are shown. A comparison of segmental systolic shortening during reperfusion with shortening in the preocclusion period in each dog indicates that recovery was substantially greater in the treated than in the control animal. In each group one

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**Table 1. Hemodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Treated group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ischemia</td>
<td>Ischemia</td>
</tr>
<tr>
<td></td>
<td>Control (9)</td>
<td>Control (9)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>119.7 $\pm$ 8.7</td>
<td>121.9 $\pm$ 8.0</td>
</tr>
<tr>
<td>(beats/min)</td>
<td>111.1 $\pm$ 6.8</td>
<td>110.1 $\pm$ 6.2</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>118.0 $\pm$ 6.8</td>
<td>111.9 $\pm$ 6.2</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td>88.6 $\pm$ 6.8</td>
<td>87.3 $\pm$ 6.2</td>
</tr>
<tr>
<td>Segmental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>end-diastolic length (mm)</td>
<td>$\pm$ 0.8</td>
<td>$\pm$ 0.8</td>
</tr>
<tr>
<td>(%) control</td>
<td>16.0 $\pm$ 5.0</td>
<td>27.2 $\pm$ 8.2</td>
</tr>
</tbody>
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All values are mean $\pm$ standard error of the mean.

* Different from control ($P < 0.05$).

$P < 0.05$ compared to 30 minutes ischemia.

$\ddagger P < 0.05$ compared to 3 hours ischemia.

$\$ $P < 0.05$ compared to control group.

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**Figure 1. Segmental systolic shortening ($\Delta L$) is shown as percent of preocclusion control. Cross-hatched bars represent mean values during ischemia and reperfusion for the control group. Open bars represent mean values for the treated group. $P$ values indicate the difference between control and treated groups; there was no significant difference between the groups at 30 minutes of ischemia.**

**Figure 2. Effects of methylprednisolone upon potassium arteriovenous difference across ischemic myocardium in individual dogs. Art. = potassium arterial concentration in mEq/L; Region. = potassium concentration (mEq/L) in the vein draining the ischemic zone; MP = methylprednisolone. Duration of ischemia was equal in the two groups.**
animal was followed for four hours following reperfusion, and no further changes occurred in segmental function in this period.

The metabolic effects of reperfusion were similar to those observed in regional function. Due to hemolysis, potassium data was obtained in only four dogs in each group and thus no statistical analysis was carried out. Control animals showed a trend toward improvement in lactate balance, but this change was not statistically significant; lactate balance remained negative after one hour of reperfusion, averaging \(-14.2 \pm 10.5\%\) (fig. 3). The steroid treated dogs, however, exhibited a more significant improvement and lactate balance became positive following reperfusion, averaging \(19.2 \pm 8.7\%\). This difference in lactate balance between the two groups is statistically significant \((P < 0.05)\).

**Discussion**

The most significant finding in this investigation is that reperfusion following acute coronary occlusion produces superior results in dogs pretreated with steroids than in non-treated animals. A significantly greater recovery of segmental myocardial function and improvement in lactate balance supports this conclusion. A better understanding of the processes underlying these results is gained by analyzing separately the effects of steroids during the occlusion and reperfusion periods.

**The Effects of Steroids During Coronary Occlusion**

The improvement in lactate balance during continuing coronary occlusion suggests a decrease in the magnitude of anaerobic metabolism. In light of the subsequent improved response to reperfusion, this finding provides indirect support to the hypothesis that steroid administration served to preserve the viability of jeopardized cells.
Potassium loss paralleled the changes in lactate levels. Loss of intramyocardial potassium is an early sign of myocardial ischemia and is probably due to increase in membrane permeability secondary to ischemia. While in the control dogs potassium loss continued unchanged throughout the occlusion period, steroid treated animals showed a complete reversal. This reversal of potassium loss, like the improvement in lactate balance, suggests maintenance of cellular integrity in the presence of ischemia, which in turn could result in prolongation of the period of viability of the ischemic cells. This conclusion is supported by the studies of Libby, in which reduction in infarct size at 24 hours after occlusion was observed following administration of hydrocortisone, and Spath et al. in which methylprednisolone prevented the decline in creatine phosphokinase (CPK), β-glucuronidase, and cathespine D activity in the ischemic myocardium during five hours of coronary occlusion in cats. More recently, Morrison and coworkers reported a reduction in infarct size in man following administration of methylprednisolone, as measured by the CPK infarct-sizing technique.

The effect of methylprednisolone during coronary occlusion, however, was not limited to lactate and potassium balance. A beneficial effect upon the mechanical performance of the ischemic segment was also documented. The effects of steroids upon mechanical function during occlusion parallel the regional metabolic changes. It is noteworthy, however, that steroid administration produced preservation of function rather than significant enhancement; control dogs exhibited reduction in systolic shortening during occlusion, whereas the treated animals experienced slight improvement.

Although improved mechanical performance during the occlusion period might be logically related to the improved metabolic state, other possibilities need to be considered. Since the measurements of systolic shortening were made at similar end-diastolic lengths, preload changes are not likely to account for the observed improvement in segmental mechanical performance. Afterload and heart rate were unchanged by steroid administration. There is no evidence that steroids exert any consistent positive inotropic effect. Thus, it seems likely that a major factor in improved performance was the reduction in the degree of ischemia, as indicated by the lactate and potassium data.

Influence of Steroids on Myocardial Reperfusion

Following reperfusion, treated and untreated animals also behaved differently. Although significant recovery of segmental shortening occurred in both treated and control animals, the treated group reached 76% of the control value, whereas untreated animals reached only 32% (P < 0.05) of control.

Previous investigations in animals have shown that reversal of mechanical dysfunction by reperfusion following coronary occlusion depends on the duration of the period of ischemia, i.e., the longer the period of ischemia the less probability of recovery; and after some hours, reperfusion actually results in further metabolic and functional deterioration. In our study, however, a significant improvement in regional segmental shortening following reperfusion was observed. The data is consistent with the findings of Maroko et al., who documented reduction in infarct size when reperfusion was performed three hours after coronary occlusion.

The higher level of postreperfusion regional mechanical performance among the steroid-treated dogs in this study appeared to reflect greater prereperfusion function, rather than a greater magnitude of recovery of function following reperfusion. These findings suggest that the effectiveness of reperfusion will be found to be a function not only of the duration of but also of the intensity of ischemia.

The substantial difference in postreperfusion regional myocardial performance between treated and untreated dogs was paralleled by changes in lactate balance. In the control group lactate balance improved slightly but remained negative despite reperfusion, a finding previously reported by Lang et al. in closed chest dogs submitted to a three hour occlusion. As with the recovery of mechanical function, the lactate data indicate that steroid administration allowed a more significant reversal of ischemia following reperfusion.

Hypotheses for the Mechanism of Action

Although this study was not designed to elucidate the mechanism of action of steroids in protecting ischemic myocardium or in improving the response to reperfusion, some hypotheses can be made. The reduction of the degree of ischemia could be due to a favorable effect upon the oxygen supply-demand ratio. An increase in coronary blood flow and a shift to the right of the oxygen-hemoglobin equilibrium curves have been observed when steroids were administered. These two effects would increase oxygen availability. Furthermore, steroids may reduce myocardial oxygen consumption, as indicated by experiments in rat myocardium. Finally, steroids are known to have a stabilizing effect upon the lysosomal membranes. In the presence of hypoxia and acidosis, lysosome rupture occurs and proteolytic enzymes are liberated; the activity of such enzymes probably contributes to the irreversibility of cellular damage. The data on potassium balance are indirect evidence that such protective effect of steroids upon cellular membrane did occur.

Implications

Despite obvious differences in species, this study may have significant clinical relevance. The data imply that ad-
ministration of steroids early in the course of an ischemic episode sufficient to cause an acute myocardial infarction substantially impedes the progression of ischemia, and by so doing may reduce ultimate infarct size. Furthermore, since emergency surgical revascularization in patients is limited by the rapid rate of development of infarction, steroids could prolong this period of time sufficiently that revascularization may become feasible in some cases. In the absence of supporting clinical experience, however, several important differences beyond the usual species differences should be noted. This study utilized acute total occlusion in the presence of otherwise normal vessels, a situation extremely rare in clinical cardiology. Second, steroids were administered within the first hour of ischemia, and presumably would have been less effective if administered at a later time. Nevertheless, the improved metabolism and function during occlusion and following reperfusion suggest that, in the absence of contraindications or subsequently demonstrated deleterious effects, steroid administration should be considered in rapidly progressing myocardial ischemia in which developing infarction may increase the risk of surgical intervention.

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References

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