Reduction by Hyaluronidase of Myocardial Necrosis following Coronary Artery Occlusion

By Peter R. Maroko, M.D., Peter Libby, M.D., Colin M. Bloor, M.D., Burton E. Sobel, M.D., and Eugene Braunwald, M.D.

SUMMARY
Electrocardiographic, enzymatic, and morphologic signs of myocardial ischemic injury following coronary occlusion have previously been shown to be ameliorated by reducing myocardial oxygen requirements, and/or by increasing the availability of oxygen as well as of substrates for anaerobic ATP production. Since hyaluronidase increases diffusion through the extracellular space and may facilitate delivery of substrates to ischemic cells, the influence of its administration on the size of experimentally produced infarcts was studied. In 14 control dogs epicardial electrocardiograms were taken in 10–15 sites on the anterior surface of the left ventricle before and after occlusion of the left anterior descending coronary artery. Twenty-four hours later, transmural specimens were obtained from the same sites from which electrocardiograms had been recorded, and were analyzed for creatine phosphokinase (CPK) activity, for histologic changes, and glycogen content. In control dogs, sites exhibiting S-T-segment elevation 15 min after occlusion showed early structural signs of necrosis and glycogen depletion in 97% of specimens taken after 24 hours. The relationship between S-T-segment elevation at 15 min (mv) and CPK activity 24 hours later (IU/mg protein) was log CPK = −0.061 S-T + 1.26. Hyaluronidase (225 u/kg) was given to 15 dogs; no hemodynamic changes occurred but the depression of CPK activity was reduced following occlusion; log CPK = −0.024 S-T + 1.28. Similarly, only 55% of sites that showed S-T-segment elevation prior to hyaluronidase administration exhibited histologic signs of early infarcts and glycogen depletion 24 hours later. It is concluded that hyaluronidase diminished myocardial necrosis following acute coronary occlusion.

Additional Indexing Words:
Myocardial ischemic injury Epicardial electrocardiography S-T-segment elevation
Myocardial creatine phosphokinase Left ventricular function
Myocardial infarction

In 1959, Oliveira et al. described reductions in S-T-segment elevation in experimental infarcts as well as in patients with acute myocardial infarction following the administration of hyaluronidase, attributing these effects to the decrease in myocardial edema. Other investigators subsequently confirmed this observation. Recently we described methods for examining the extent and severity of myocardial ischemic injury following coronary occlusion and demonstrated that various pharmacologic and hemodynamic interventions may alter infarct size substantially.

From the School of Medicine, Departments of Medicine and Pathology, University of California, San Diego, La Jolla, California.
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Address for reprints: Peter R. Maroko, M.D., Department of Medicine, Peter Bent Brigham Hospital, Boston, Massachusetts 02115.
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The purpose of this investigation was to utilize these methods in order to study the effects of hyaluronidase on the size of an experimentally produced myocardial infarct.

Methods

Studies were carried out in 35 dogs weighing between 17 and 25 kg. They were anesthetized with sodium thiamyl (25 mg/kg) with respiration maintained by a Harvard respirator. The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) or its apical branch was dissected free from the adjacent tissue and occluded permanently with a ligature or intermittently with a Schwartz intracranial arterial clamp. As previously described,\textsuperscript{4, 5} epicardial electrocardiograms were obtained from 10–15 sites on the anterior surface of the left ventricle distributed in areas supplied by the occluded artery as well as in areas remote to it and presumably adequately perfused. The animals were divided into two groups.

Control Group I: Occlusion Alone

Occlusion alone, without any drug intervention, was performed in 14 dogs. Epicardial electrocardiograms were taken prior to and 5, 10, 15, 20, 30 and 45 min following occlusion. The thorax was then closed and the dogs were allowed to awaken. Twenty-four hours later they were reanesthetized and the heart was excised. Immediately after the excision transmural specimens were taken for analysis of creatine phosphokinase (CPK) activity and histologic structure at the sites from which electrocardiograms had previously been recorded. During the 24-hour experimental period the animals received 40 ml/kg normal saline intravenously. Aortic pressure (Statham model P23Db pressure gauge) and the electrocardiogram (lead aV\textsubscript{F}) were monitored in all animals, and in five of them left atrial pressure was also constantly monitored.

Group II: Occlusion with Hyaluronidase

The 21 dogs in which the effects of hyaluronidase were studied were divided into three subgroups:

Subgroup A

In six dogs a permanent occlusion was performed and epicardial electrocardiograms obtained prior to and 5, 10, 15, 20, and 30 min after occlusion; at this time, 30 min after occlusion, 225 u hyaluronidase*\textsuperscript{5} /kg were injected as a bolus intravenously. Forty-five minutes after the occlusion, epicardial electrocardiograms were repeated. Subsequently, the procedure was identical to that in group 1, i.e. the chest was closed, the animal allowed to recover, and 24 hours later myocardial specimens were taken for CPK analysis and histology at the same sites from which electrocardiograms had previously been recorded.

Subgroup B

In nine dogs two occlusions were performed. The first was a 20-min occlusion without any drug intervention, during which epicardial electrocardiograms were recorded at 5-min intervals. After 20 min the occlusion was released and the dogs allowed to recover for 60 min; then another epicardial electrocardiographic map was taken to ensure the return of S-T segments to control levels. Subsequently, hyaluronidase (225 u/kg) was injected intravenously as a bolus. Ten minutes later the coronary artery was reoccluded at the same site and epicardial electrocardiograms were taken as before for 20 min. In seven of these dogs the thorax was closed, and subsequently the procedure was identical to that in groups I and II A.

Subgroup C

Six dogs were instrumented with a stainless-steel intraventricular cannula introduced through the apex of the left ventricle and catheters in the left atrium and the aorta. Left ventricular peak systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), aortic mean (AP) and phasic pressures, were recorded. The first derivative of left ventricular pressure (dP/dt) was obtained by electrical differentiation.

Myocardial CPK analysis was carried out as previously described\textsuperscript{4} by spectrophotometric assay of CPK activity. Hyaluronidase did not influence the CPK assay system itself as judged by experiments with purified CPK, tissue slices, and homogenates with and without added hyaluronidase.

Histological studies were carried out using absolute alcohol fixation and hematoxylin eosin (H&E) and Best-Carmine stains. The sections were classified as normal or showing signs of early myocardial necrosis as described in detail previously.\textsuperscript{5} The results were then analyzed statistically using the chi-square test for fit.

In four dogs (two from the control group and two from the hyaluronidase-treated group) specimens were taken 24 hours after occlusion, fixed in 10% neutral formaldehyde, and stained with Alcian green to demonstrate the presence of hyaluronic acid.

Results

Group I: Occlusion Alone

The predictive value of S-T-segment elevation to CPK activity and histologic structure 24 hours later was established in this group. Sites remote from the occluded region with normal S-T segments (0–2 mV elevation) showed normal CPK activity, 18.8 ± 0.4 (mean ± SEM) IU/mg of protein (14 dogs, 47 specimens). Log CPK activity 24 hours after ligation was inversely proportional to S-T-segment elevation 15 min after occlusion: log CPK = −0.061 S-T + 1.26 (r = 0.79; N = 102 specimens). Thus, the higher the S-T segment 15 min after occlusion, the lower the CPK activity 24 hours later (figs. 1, 2).

Of the sites with S-T-segment elevations exceeding 2 mV 15 min after occlusion, 97% (51 of 53) exhibited early signs of myocardial necrosis. These findings included a more pronounced eosinophilic appearance, loss of muscular cross striations, karyolysis, karyorrhexis, and leukocyte cell infiltration (fig. 2; table 1). On the other hand, 96% (27 of 28 specimens) of sites with normal S-T segments (0–2 mV elevation) showed normal myocardial structure (fig. 2; table 1). Also, the sites with normal S-T segments showed preservation of glycogen granules in 96% (27 of 28 sites), whereas 97% (51 of 53) of sites with S-T segment elevation exceeding 2 mV showed glycogen depletion.

Group II: Occlusion + Hyaluronidase Treatment

Subgroup A

In this group in which hyaluronidase was injected intravenously 30 min after occlusion, the sum of S-T-segment elevations (Σ S-T) at all sites at which epicardial electrocardiograms were taken just prior to and 15 min following hyaluronidase administration decreased from 59.6 ± 2.8 to 29.0 ± 8.1 mV (P < 0.05) (fig. 3, left). The number of sites that showed S-T-segment elevation exceeding 2 mV (NS-T) decreased from 8.4 ± 0.5 to

![Figure 1](http://circ.ahajournals.org/)

**Figure 1**

Relationship between epicardial S-T-segment elevation 15 min after occlusion and myocardial creatine phosphokinase activity 24 hours later in the same sites. Line A: Control group I (14 dogs), log CPK = −0.061 S-T + 1.26 (r = 0.79; N = 102 specimens). Line B: Group IIA (maintained occlusion, hyaluronidase administered 15 min after epicardial electrocardiographic mapping, seven dogs), log CPK = −0.025 S-T + 1.29 (r = 0.6; N = 47 specimens). Line C: Group IIB (two occlusions, hyaluronidase given prior to the second occlusion, six dogs). The line compares S-T-segment elevation 15 min after the first occlusion, i.e. prior to hyaluronidase administration, to CPK 24 hours later: log CPK = −0.024 S-T + 1.27 (r = 0.6; N = 54 specimens).

![Figure 2](http://circ.ahajournals.org/)

**Figure 2**

The relationship of S-T-segment elevation 15 min after occlusion to CPK activity and histologic structure 24 hours later in an experiment in group I (occlusion alone). (Left) A schematic representation of the anterior surface of the heart and its arteries. The shaded area represents the area of S-T-segment elevations 15 min after occlusion. The circles represent sites where biopsies were taken. L.A. = left atrial appendage; L.A.D. = left anterior descending coronary artery; SITE OF OCCL. = site of occlusion. (Right) Comparison between S-T segment elevation, CPK, and histologic analysis 24 hours later in the same sites.
Table 1

Relationship of S-T Segment to Necrosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal S-T-segment elevation* (0-2 mv)</th>
<th>Abnormal S-T-segment elevation* (≥ 3 mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specimens/animals (no.)</td>
<td>Normal (%)</td>
</tr>
<tr>
<td>Group I</td>
<td>28/8</td>
<td>96</td>
</tr>
<tr>
<td>Group II</td>
<td>24/12</td>
<td>100</td>
</tr>
<tr>
<td>Subgroup IIA</td>
<td>13/6</td>
<td>100</td>
</tr>
<tr>
<td>Subgroup IIB</td>
<td>11/6</td>
<td>100</td>
</tr>
</tbody>
</table>

*15 min after a simple coronary occlusion, i.e. before administration of hyaluronidase.
†24 hours after coronary occlusion.
‡Significantly greater than group I (P < 0.005).

4.8 ± 1.3 (P < 0.05) (fig. 3, right). In comparison, in the control dogs there was no statistical change in ΣS-T and NS-T during the same time interval, i.e. between 30 and 45 min after occlusion (ΣS-T from 52.7 ± 11.8 to 57.6 ± 13.1 mv and NS-T from 5.8 ± 0.8 to 6.0 ± 1.0). Heart rate, systemic arterial pressure, and left atrial pressure remained unchanged in all cases following hyaluronidase administration.

Twenty-four hours later CPK activity in sites with normal S-T segments in dogs that were treated with hyaluronidase was not significantly different from the normal group (19.6 ± 0.8, N = 26 as compared to 18.8 ± 0.4, N = 47 in group I). However, CPK activity was less depressed than expected (from the results in group I) in sites which were elevated prior to hyaluronidase administration. Moreover, some border-zone sites which had S-T-segment elevations prior to hyaluronidase administration subsequently showed completely normal CPK activity and histology (fig. 4; table I). The reduced depression of CPK activity subsequent to the administration of hyaluronidase 30 min following occlusion is reflected in the slope of the line relating S-T-segment elevation 15 min after occlusion and prior to hyaluronidase administration to the CPK activity 24 hours later; log CPK = −0.025 S-T + 1.29 (r = 0.6; N = 47; fig. 1). Histologically, 100% (13 of 13) sites with normal S-T segments exhibited normal structure (table I). The sites that showed S-T-segment elevations after occlusion showed abnormal structure in only 44% (15 of 34 specimens). Thus, 56% of specimens which, on the basis of control observations, would have been expected to exhibit necrosis were protected in the hyaluronidase-treated group (table I). Similarly, all sites with normal S-T segments (13 of 13) showed preservation of glycogen granules, while only 44% of sites with

Figure 3

The effect of hyaluronidase on myocardial ischemic injury. (Left) Sum of S-T-segment elevations (Σ ST) before (open symbols) and after (closed symbols) hyaluronidase treatment. Each continuous line connecting two symbols represents one animal. Triangles = group IIA (nine animals); circles = group IIB (six animals); squares = mean ± standard error of all 15 dogs. (Right) Number of sites showing S-T-segment elevation over 2 mv (NST), before (open symbols) and after (closed symbols) hyaluronidase administration. Each continuous line represents one experiment. Triangles = group IIA; circles = group IIB; squares = mean ± standard error.
The effect of hyaluronidase on the relationship of S-T-segment elevation (prior to drug administration) to CPK activity and histologic structure 24 hours later in an experiment from group IIA. (Left) Schematic representation of the anterior surface of the heart and its arteries. L.A. = left atrial appendage; L.A.D. = left anterior descending coronary artery; SITE OF OCCL. = site of occlusion; shaded area = area of S-T-segment elevation 15 min after coronary occlusion (prior to hyaluronidase administration). (Right) Comparison between S-T-segment elevation 15 min after occlusion, i.e., prior to hyaluronidase administration and CPK activity and histlogic structure 24 hours later.

pathologic S-T-segment elevations showed glycogen depletion.

Subgroup B

In this subgroup two successive occlusions were carried out, the first without drug intervention and the second after hyaluronidase pretreatment. $\Sigma$S-T was significantly lower during the second occlusion than the first, the average $\Sigma$S-T increasing from $61.4 \pm 13.3$ mv 15 min after the first occlusion to $36.1 \pm 11.1$ mv ($P < 0.01$) 15 min after the second occlusion (fig. 3, left). Similarly, NS-T decreased from $7.9 \pm 0.7$ after the first occlusion to $5.1 \pm 1.1$ ($P < 0.05$) after the second occlusion (fig. 3, right). An example showing the decrease in $\Sigma$S-T and the area of S-T-segment elevation is illustrated in figure 5. Heart rate, systemic arterial pressure, and left atrial pressure were not altered by hyaluronidase administration. At each site CPK activity 24 hours after coronary occlusion was compared to the S-T-segment elevation 15 min after occlusion alone (first occlusion). Figure 1 shows that, just as in subgroup A, there was less CPK depression than that expected from the observations in the control group. Histologically, 100% (11 of 11) sites with normal S-T segments showed normal histology and preservation of glycogen. However, 35% (12 of 35) of sites with S-T-segment elevations exceeding 2 mv after the first occlusion were protected from undergoing necrosis and their glycogen stores were maintained (table 1). In the four hearts (two from group I and two from group II) examined by Alcian green staining, the specimens from untreated animals showed a considerable amount of Alcian-positive material in the arterial walls and perivascular spaces. In the sections obtained from comparable sites in the hyaluronidase-treated group the amount of Alcian-positive material was substantially less.

Cardiovascular Function. In the normal heart (six dogs), 225 u/kg hyaluronidase did not alter heart rate, aortic pressure, or left atrial pressure. Similarly, in the dogs with
ischemic hearts this dose did not alter heart rate (N = 15), systemic arterial blood pressure (N = 15), left atrial pressure (N = 8), left ventricular systolic and end-diastolic pressures (N = 6), and peak dP/dt (N = 6). In four additional animals with ischemic hearts, 500 u/kg also failed to alter any of these variables.

Discussion

The mortality of patients with acute myocardial infarction who develop cardiogenic shock is prohibitive. Autopsy studies have revealed that cardiogenic shock is uniformly associated with large infarcts and, since it has been shown that infarct size can be influenced markedly by pharmacologic interventions as well as by the hemodynamic state following coronary occlusion, efforts have been made to establish the conditions which can decrease the size of the potential infarct. It has been found that interventions which improve myocardial oxygen balance such as propranolol and practolol, elevation of arterial pressure, and intracoronary balloon counterpulsation do reduce the extent and magnitude of myocardial ischemic injury and/or necrosis. Moreover, the infusion of a combination of glucose, insulin, and potassium, and to a lesser extent glucose alone, also limit the development of myocardial necrosis, probably by enhancing anaerobic energy production.

The administration of hyaluronidase offers several potential advantages compared to the above-mentioned interventions: (1) Its application is simple and does not require any special equipment. (2) It does not depress cardiac contractility. (3) It does not cause systemic arterial hypotension. (4) It does not have the intrinsic property of changing S-T segments, as does potassium, and thus the electrocardiographic signs of ischemic injury may be used for monitoring the extent and severity of ischemic injury atraumatically. (5) Hyaluronidase has been used widely clinically and it was found that its toxicity is extremely low. Allergic reactions are rather rare clinically (0.05%), generally occur only after frequent exposure, and may be avoided completely if a skin test is performed. Finally, in terms of effectiveness in reducing myocardial necrosis following coronary ligation, hyaluronidase compares favorably with other interventions such as propranolol, glucose, and glucose-insulin-potassium. This decrease in damage to the myocardium following coronary occlusion due to hyaluronidase is reflected in the substantial reduction in the magnitude and extent of the area of S-T-segment elevation (SS-T decreased by 41% and NS-T by 46%), by strikingly reduced depression of CPK activity, by a much smaller area of histologic damage, and by preservation of myocardial glycogen granules in sites which were expected to undergo necrosis on the basis of observations in control dogs.

The mechanism of action of hyaluronidase in reducing infarct size is not known. Oliveira postulated that it produced a decrease in interstitial myocardial edema and subsequently showed a decrease in total myocardial water after hyaluronidase administration. However, the decrease in edema may not be the cause but the consequence of less extensive myocardial injury.

It is suggested that the action of hyaluronidase is based on its postulated effect on the transport of energy-producing nutrients from the bloodstream to the myocardial cells. It has been shown previously that increasing coronary perfusion pressure reduces the area of ischemic injury. This action may be explained by postulating that this intervention increases the supply of nutrients through the collateral vessels as well as the pressure and concentration gradients across the wall of the capillaries, thus augmenting the transport of oxygen and substrates to the interstitial space. The infusion of the combination of glucose-insulin-potassium, in addition to increasing the quantity of substrate available for anaerobic glycolysis, also accelerates the transport of glucose into the cell through the action of insulin. It is known that hyaluronidase depolymerizes hyaluronic acid and that it thereby facilitates the transport of a variety of substances through the interstitial spaces.
and increases capillary permeability. This action may be particularly important in the presence of coronary occlusion in which nutrients have to be transported through longer extravascular pathways than when the coronary arteries are patent. Using a histologic staining method for hyaluronic acid (Alcian green) we have shown that 24 hours after occlusion the quantity of positively stained material in the interstitial tissue of the heart is clearly reduced by the administration of hyaluronidase. This observation is consistent with the hypothesis that hyaluronidase acts through its depolymerizing property.

In conclusion, though its mechanism of action is open to speculation, hyaluronidase was shown to protect the myocardium from undergoing extensive ischemic injury and consequent necrosis following coronary occlusion. This action is possible without apparent deleterious side effects and suggests that this substance may be of value in the treatment of patients with impending myocardial infarction, or early after coronary occlusion.

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PETER R. MAROKO, PETER LIBBY, COLIN M. BLOOR, BURTON E. SOBEL and EUGENE BRAUNWALD

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