Long-Term Preservation of Ischemic Myocardium in the Dog by Hyaluronidase

ROBERT A. KLONER, M.D., PH.D., EUGENE BRAUNWALD, M.D., AND PETER R. MAROKO, M.D.

SUMMARY The administration of hyaluronidase is a promising intervention to protect the ischemic myocardium in man, but evidence of the extent to which it may reduce the ultimate size of an infarct is not well-defined. Hence, open chest, anesthetized dogs were randomized into 10 control dogs which received saline and eight treated dogs which received three doses of hyaluronidase (500 NF units/kg I.V.) at 15 minutes, 2 hours and 24 hours after occlusion of the left anterior descending coronary artery (CAO). Regional myocardial blood flow (RMBF) assessed by the microsphere technique was measured 12 minutes after CAO. The chest was then closed and the dogs were allowed to recover. Twenty-one days after CAO, the hearts were excised, divided into 1 cm thick slices and incubated in triphenyl tetrazolium chloride. Infarct size was then determined by planimetry. The left ventricular myocardium was divided into multiple samples for RMBF analysis. In control dogs 23.2 ± 2% of the left ventricle was infarcted, compared to only 9 ± 2.8% (P < 0.001) in hyaluronidase-treated dogs. RMBF in noninfarcted myocardium directly adjacent to the infarct was similar to that in the normal zone remote from the infarct in the control dogs; however, in the hyaluronidase-treated dogs, blood flow in the myocardium adjacent to the infarct was significantly reduced to 68% of normal (P < 0.01) in the outer myocardial wall and to 86% of normal (P < 0.02) in the inner myocardial wall, which indicates that this tissue, at least in some part, was in jeopardy, but was salvaged by hyaluronidase.

Epipical electrocardiographic data showed that three weeks after CAO, Q waves were less frequent and smaller in hyaluronidase compared to untreated dogs. Preservation of the frequency and magnitude of R waves was greater in the hyaluronidase-treated group at three weeks. We conclude that hyaluronidase resulted in long-term preservation of the ischemic myocardium.

HYALURONIDASE HAS BEEN SHOWN to be potentially beneficial in decreasing myocardial infarct size in man, as assessed by electrocardiographic changes,¹ and is soon to be tested in a multi-center clinical trial designed to limit necrosis in patients with myocardial infarctions. Hyaluronidase has also been shown to have a beneficial effect in dogs after 24 hours of occlusion.² Whether its effect persists in the dog after weeks of occlusion is unknown. In a previous study in rats, hyaluronidase was shown to decrease infarct size measured either 48 hours or three weeks after occlusion.³ Hyaluronidase exerts this favorable effect presumably by depolymerizing interstitial glycoproteins and enhancing diffusion of oxygen and substrate into and of metabolically active products out of the ischemic zone.³ In view of the small size of the rodent heart with its limited diffusion distance, these results may not be applicable to the much larger human heart. The objective of this study was to determine whether in dogs hyaluronidase results in long-term preservation of ischemic myocardium after several weeks of coronary occlusion. In addition, regional myocardial blood flow (RMBF) was measured after occlusion but before hyaluronidase administration to determine whether hyaluronidase salvaged underperfused myocardium. We also tried to determine whether the electrocardiographic method used to determine the efficacy of interventions designed to limit infarct size⁴ correlates with pathological results in a long-term ischemia model, since this correlation was previously studied only in a 24-hour model of myocardial infarction.⁴

Methods

Mongrel dogs weighing 14–30 kg were anesthetized with sodium thiamylal (25 mg/kg I.V.), intubated and ventilated with a Harvard respirator. Thoracotomies were performed under sterile conditions through the fifth left intercostal space. The lungs were retracted and the pericardium was incised. The left anterior descending coronary artery was isolated from the adjacent tissues just above the apical branch. A catheter was placed into the left atrium for injections of radioactive microspheres. A second catheter was placed into the left femoral artery via the left saphenous arterial artery for withdrawal of a reference blood sample during the injection of microspheres and for monitoring arterial pressure. A third catheter was placed into the left femoral vein and was used for intravenous injections.

Epipical electrocardiograms from 10 sites on the surface of the left ventricular wall were obtained before and 10 minutes after ligation of the coronary artery, as previously described.⁵ Briefly, each site selected was chosen before occlusion and recognized by its specific relationship to the coronary anatomy. Two of these sites were remote from the distribution of the left anterior descending artery; the remaining eight were selected to fall within its distribution. The electrode employed was a 15 mm² copper cylinder with a saline-soaked wick connected to the precordial lead and hand-held with a cable attached perpendicular to the electrode to minimize mechanical stress.
The height of the ST segment 10 minutes after coronary occlusion was measured at the J point, using the TQ segment as a baseline. The amplitude of R and Q waves at each site was measured before and three weeks after coronary occlusion. Careful diagrams of the coronary anatomy of each heart, and the location of each epicardial site in relation to the anatomy, were constructed so that three weeks after occlusion the same epicardial sites could be used. The coronary anatomy on the surface of the heart remained identifiable so as to permit electrode repositioning after three weeks.

To determine RMBF before treatment, approximately $1.5 \times 10^6$ radioactive microspheres labeled with either $^{141}$Ce or $^{113}$Sn (8–10 μm in diameter) were injected 12 minutes after coronary artery occlusion as described in detail previously. Dogs were randomized into untreated or hyaluronidase-treated groups by the toss of a coin 14 minutes after coronary artery occlusion. They received either saline (10 dogs) or hyaluronidase (eight dogs, 500 NF units/kg i.v.) 15 minutes, 2 hours, and 24 hours after occlusion. The chest was then closed and the dogs were allowed to recover for three weeks. All dogs received benzathine penicillin (2.4 million units intramuscularly) 24 hours and 48 hours following the operation.

Three weeks later, the dogs were reanesthetized with thiamylal, intubated, and placed on a Harvard respirator. The chest was reopened and epicardial electrocardiograms obtained at the same loci as previously. The hearts were then excised and the distance from the aorta to the site of occlusion was measured. The hearts were "breadloafed" from apex to base into 1 cm thick slices. Slices were examined for the presence of infarcted tissue, which was easily identified as a pale yellow area. In order to enhance the contrast between viable and necrotic tissue, the slices were then incubated for 30 minutes in triphenyl tetrazolium chloride, which stains viable lactate dehydrogenase (LDH)-containing tissue dark red but does not stain necrotic tissue. The use of this agent to distinguish infarcted from noninfarcted tissue has been described in detail previously.

Two methods were used to determine the percentage of the left ventricle which was infarcted. In the first, a clear glass plate was placed over each slice and the infarcted and noninfarcted portions of tissue traced directly onto clear plastic sheets. The tracings were transferred to bond paper and the portions of the paper representing infarcted and noninfarcted tissue were cut out and weighed. The second method of determining infarct size was by directly summing the weight of all the infarcted and noninfarcted portions of tissue separately. This was done following dissection of the heart for blood flow analysis.

The 1 cm thick slices of the left ventricle were dissected for analysis of RMBF. Slices were dissected radially into approximately 1 cm sections from the following four zones of the heart: 1) the center of the infarct, 2) the peripheral portion of the infarcted myocardium which was termed the "infarct border," 3) normal tissue adjacent to the infarction either in the lateral or in the epicardial direction, which was referred to as "normal adjacent" tissue, and 4) normal myocardium distant from the area of the infarct. Each of these sections was further dissected into inner (subendocardial-half) and outer (subepicardial-half) portions of myocardium. RMBF and cardiac output were calculated as previously described. Groups were compared using Student t test for group observation.

### Results

#### General Observations

The randomization of the dogs into control and hyaluronidase groups resulted in general similarity between the two groups. The mean weight of dogs in the untreated group was $23 \pm 2$ kg (SEM), and that in the hyaluronidase-treated group was $25 \pm 1$ kg (NS). The average distance from the aorta to the site of occlusion was similar between the two groups ($2.5 \pm 0.2$ cm and $2.2 \pm 0.2$ cm in the control and hyaluronidase-treated dogs, respectively). The initial 10-minute epicardial electrocardiograms showed that both groups had a similar number of sites with ST segment elevation greater than 2 mV among the eight sites within the distribution of the left anterior descending coronary artery (6.7 ± 0.4 and 6.0 ± 0.6 (NS) in control and hyaluronidase-treated dogs, respectively). The mean sum of R wave voltage per dog was $104 \pm 19$ mV in control and $105 \pm 18$ mV in hyaluronidase-treated animals 10 minutes after coronary occlusion. Hemodynamics were similar in both groups 10 minutes after coronary artery occlusion as well; heart rate was $149 \pm 6$ and $141 \pm 5$ beats/min in the control and hyaluronidase groups, respectively, while the systolic and diastolic systemic arterial pressures were $148 \pm 6/103 \pm 5$ and $162 \pm 12/103 \pm 9$ mm Hg (NS) in control and hyaluronidase groups. Cardiac output 12 minutes after occlusion was $2.4 \pm 0.3$ l/min in control and $2.3 \pm 0.2$ l/min (NS) in treated dogs.

RMBF was similar in the central region of ischemia in untreated dogs ($22.1 \pm 4.3%$ of RMBF in the normal myocardium distant from the infarct in the inner half, and $42.3 \pm 8.5%$ of normal flow in the outer half) compared to hyaluronidase-treated dogs ($20.6 \pm 5.8%$ of normal flow in the inner half (NS); and $45.2 \pm 13.2%$ of normal flow in the outer half (NS)).

Six of the 10 untreated dogs and five of the eight hyaluronidase-treated dogs exhibited multiple premature ventricular contractions within the first 15 minutes of coronary artery occlusion. Hence, the two groups of dogs were comparable before treatment with respect to body weight, site of occlusion, epicardial ST segment changes, regional myocardial blood flow and the occurrence of premature ventricular contractions.

Seven dogs which originally were part of the study were eliminated, since they died within 21 days. Two dogs developed ventricular fibrillation within the first 10 minutes, and thus were not randomized into either group. Five untreated dogs died and were eliminated.
from the study. Four of these died within 24 hours of occlusion of unknown causes, presumably arrhythmias. The fifth died after 10 days with severe pneumonia. This left 10 dogs in the untreated group. All of the eight hyaluronidase-treated animals survived.

Pathological Observations

The infarcts appeared as pale yellow or grey areas three weeks after coronary occlusion. They were located on the anterior free wall of the heart, and usually involved the apex. When tissue slices were incubated in triphenyl tetrazolium chloride, normal tissue stained dark red, while infarcted tissue remained pale yellow or grey, enhancing the contrast between normal and infarcted tissue. In the control group nine of 10 dogs had transmural infarcts, while in the hyaluronidase-treated group, a significantly smaller number, three of eight ($\chi^2 = 5.5, P < 0.05$) dogs had transmural infarcts; in the other dogs the infarcts were confined to the inner myocardial wall (fig. 1). In no instance was thinning of the myocardial wall noted. Infarct size, expressed as a percentage of infarcted tissue of the left ventricle and determined by the paper-tracing technique, was 23.2 ± 2% ($n = 10$) in the control dogs and 9 ± 2.8% ($n = 8, P < 0.001$) in the hyaluronidase-treated dogs (figs. 1 and 2); determined by the tissue weight technique, these values were 21.6 ± 3.3% and 8.7 ± 3.2% ($P < 0.02$) in untreated and hyaluronidase-treated dogs, respectively. These two techniques of expressing the percent of necrotic myocardium correlated closely ($r = 0.89$).

Regional Blood Flow

Microsphere injections were performed in nine control and seven hyaluronidase-treated dogs. RMBF in the “normal distant” tissue was similar in the inner (1.08 ± 0.09 ml/min/g and 1.05 ± 0.10 ml/min/g) and outer (1.05 ± 0.10 ml/min/g and 1 ± 0.10 ml/min/g) walls of both the untreated and hyaluronidase-treated animals, respectively.

When blood flow to the entire infarct before treatment was calculated, flow was significantly lower (0.24 ± 0.04 ml/min/g) in infarcts of the hyaluronidase-treated dogs than in the untreated dogs (0.41 ± 0.05 ml/min/g, $P < 0.05$). This demonstrates

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Original tracings of ventricular slices in an average control dog (top) and hyaluronidase dog (bottom). The hatched areas represent infarcted tissue. Note that in the control dog the infarct extended transmurally, while in hyaluronidase-treated dog it did not.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Myocardial infarct size expressed as a percent of infarcted left ventricle as determined by the paper-tracing technique. Each point represents data obtained from one dog. The open circles represent the mean and the bars represent the SEM for the two groups of dogs.
that in the hyaluronidase-treated animals, only areas with lower flow became necrotic.

RMBF in the normal zone adjacent to the infarct also differed between the two groups. In the control dogs RMBF in the normal zone adjacent to the infarct was similar to that in normal distant myocardium, both in the inner and outer halves of the myocardium. In contrast, RMBF in the normal zone adjacent to the infarct in the hyaluronidase-treated dogs was reduced significantly to an average of 68 ± 7% (P < 0.01) in the outer portion and to 86 ± 2% (P < 0.02) in the inner portion of the myocardium, as compared to the normal distant myocardium (fig. 3). In the subepicardial half of the myocardium of the normal zone adjacent to the infarct, blood flow was often markedly diminished in the hyaluronidase-treated group, especially when the infarct was confined to the subendocardial wall. Only five of the 77 specimens obtained from the subepicardial half of myocardium adjacent to the infarct of the nine untreated dogs, had flows lower than 0.45 ml/min/g, compared to 25 of the 69 specimens obtained from the seven hyaluronidase-treated dogs (χ² = 19.7; P < 0.01). This indicates that regions with low flow survived more frequently in the hyaluronidase-treated dogs.

Electricdiograms

The number of sites in the distribution of the occluded left anterior descending coronary artery which developed Q waves deeper than 2 mV, three weeks after occlusion, averaged 4.4 ± 0.2 in the 10 control dogs and was significantly less, 2.2 ± 0.8 (P < 0.01), in the eight hyaluronidase-treated dogs (figs. 4 and 5). Of the eight sites in the distribution of the occluded left anterior descending coronary artery, 3.8 ± 0.7 in the control and a significantly larger number, 7.2 ± 0.5 (P < 0.01) in the hyaluronidase-treated animals, retained R waves. The average depth of the Q waves was 4.7 ± 0.8 mV in the control dogs and 1.6 ± 0.5 mV (P < 0.01) in hyaluronidase-treated animals, while the average height of the R waves per site (from the eight sites in the distribution of the occluded left anterior descending artery) was 3.2 ± 0.8 in the control and 6.6 ± 1.4 mV (P < 0.05) in the hyaluronidase-treated animals. The mean percent fall in R wave between electrocardiograms recorded before coronary artery occlusion and after three weeks was 67.6 ± 5.8% in control dogs and 45.3 ± 8.7% (P < 0.05) in the hyaluronidase-treated dogs. These results indicate that the electrocardiographic evidence of necrosis was more circumscribed in the hyaluronidase-treated animals. In nonischemic tissue remote from the infarct, there was no change in the QRS complex between the initial electrocardiogram and the electrocardiogram taken at three weeks, in either group.

Discussion

In previous studies in the dog, hyaluronidase was shown to decrease myocardial infarct size 24 hours after coronary occlusion, as determined by the relation between epicardial ST segment elevations and myocardial creatine kinase activity, histologic studies and QRS complex analysis. It was also shown to reduce infarct size significantly, but by decreasing amounts when its administration was begun 20 minutes, 3 hours and 6 hours after coronary artery occlusion, but to be ineffective when it was delayed for 9 hours after occlusion. In addition, hyaluronidase has shown by enzymatic and histologic techniques to decrease infarct size in the rat, measured 48 hours and three weeks after occlusion when the drug was administered twice during the first 24 hours after coronary occlusion. Precordial mapping techniques in man have shown that hyaluronidase reduces electrocardiographic evidence of myocardial ischemic injury (i.e., ST segment elevations) when administered during the first 8 hours after the presumed onset of

Figure 3. Regional myocardial blood flow expressed as a percentage of blood flow in the distant normal zone in 1) the infarct center, 2) infarct border and 3) normal-adjacent zones of the left ventricle from nine untreated and seven hyaluronidase-treated dogs. Blood flow was significantly less in the normal adjacent zone of hyaluronidase-treated compared to untreated dogs.
infarction.\textsuperscript{14} Moreover, electrocardiographic evidence of necrosis (i.e., alterations of the QRS complex) was significantly less in patients with acute myocardial infarction treated with hyaluronidase than in controls.\textsuperscript{1}

The question of whether hyaluronidase, when administered to a patient with acute myocardial infarction, actually leaves him with a larger quantity of viable myocardium several weeks after the event, is still unsettled. This study in the dog indicates that the administration of hyaluronidase during the first 24 hours after coronary artery occlusion resulted in long-term preservation of substantial amounts of myocardium, and not merely a delay in necrosis in a larger animal as well. After three weeks of coronary occlusion, the infarcts were significantly smaller (by 60\%) in the hyaluronidase-treated dogs. In addition, there was no thinning of scars as has been observed in the rat model of myocardial infarction when multiple doses of glucocorticoids were used.\textsuperscript{15}

The electrocardiographic studies in these dogs lend further support to the analysis of the QRS complex for evaluating the efficacy of an intervention designed to protect ischemic myocardium.\textsuperscript{1, 4, 17} Q waves occurred less frequently and were smaller at the time of study three weeks after coronary occlusion in the hyaluronidase-treated compared to the untreated

**Figure 4.** Epicardial electrocardiographic changes in a control and a hyaluronidase-treated dog from eight sites in the distribution of the left anterior descending artery. While there is significant ST segment elevation at 10 minutes after occlusion (before treatment) in both dogs, at three weeks there is less loss of R wave voltage in the hyaluronidase-treated dog than in the control dog. In addition, the hyaluronidase-treated dog is without the deep QS complexes which were present in the untreated dog.
groups. In addition, R wave voltage was preserved to a greater extent in hyaluronidase-treated animals. These results correlate well with the direct measurements of infarct size; they lend support to the previous findings that directional changes in the QRS pattern parallel changes in infarct size at 24 hours after occlusion\(^1\)\(^–\)\(^13\) and suggest that the method remains valid for as long as three weeks after the myocardial infarction.

Recently, there has been discussion of whether a border zone of ischemic tissue exists around a central necrotic zone. The term "border zone" has been variously defined. Some investigators have used it to denote an area where a gradient of flow between normal and ischemic tissue exists.\(^16\) Some,\(^17\) but not all,\(^18\) investigators have detected such an area. Others have referred to a border zone of histologic changes, including contraction bands or lipid droplets surrounding the infarct.\(^19\) or have defined it on the basis of histochemical changes, such as alterations in dehydrogenase and glycogen staining.\(^20\),\(^21\) Williamson et al.
\(^22\) found sharp demarcations between hypoxic and nonhypoxic tissue using xenon flash photographs of pyridine nucleotide fluorescence in rat hearts. In studies of preservation of ischemic myocardium, the border zone can be considered to be that region of the myocardium in which the cells remain viable as a result of a treatment, despite reductions of blood flow which lead to necrosis in untreated animals.

In this study a border zone defined in this manner did exist. Anatomically, it was most prominent in the epicardial wall of the ischemic myocardium overlying infarcts confined to the inner wall, although present to a lesser extent laterally. Flow in the outer half of the myocardial wall of the normal zone adjacent to the infarct was in many instances markedly depressed shortly after coronary occlusion. The fact that many such sites were ultimately viable in treated dogs, while a significantly smaller number occurred in control dogs, indicates that myocardial salvage was most prominent in the outer wall of the ischemic myocardium. Myocardial salvage also was most prominent in the outer ischemic myocardial wall in a study in which propranolol was administered to dogs with 24 hours of occlusion of the left circumflex coronary artery.\(^24\)

The present study also showed that the region which is ischemic 12 minutes after coronary artery occlusion will infarct in the absence of an interposed intervention. This suggests that changes in collateral blood flow occurring after the initial flow measurements at 12 minutes were insufficient to salvage any of the ischemic zone in untreated animals.

It can be hypothesized that the regional myocardial blood flow in the "normal adjacent" tissue in the hyaluronidase-treated group should be identical to the flow in the "infarct border" in the control animals. This could not be demonstrated in this study since the salvaged "infarct border" and the "normal adjacent" zone have the same gross appearance (i.e., that of normal myocardium) in the treated dogs and therefore, the flow reported for this zone which is identified as "normal adjacent" represents a mixture of that which occurs in the "infarct border" and the "normal adjacent" myocardium in the control groups.

Recently, two studies indicated that microspheres deposited in areas of infarction may leave these zones after 24 hours of occlusion, resulting in falsely depressed flow values.\(^24\),\(^25\) However, in the present study, values of RMBF noted in tissue which had been necrotic for three weeks generally were in the range of 5–50% normal, values similar to those obtained in canine myocardium during the first few hours after coronary occlusion.\(^26\) Even if the values of RMBF in these dogs were falsely low, the possibility that microspheres are lost from an area of infarction does
not alter the fact that in the present study, in the hyaluronidase-treated dogs there were areas of normal tissue adjacent to the infarct with low flow values which did not become necrotic.

Acknowledgments

The authors wish to thank Joseph Gannon and Alice Carmel for their technical assistance, and Deidre Bernard for secretarial assistance.

References


