The Effects of Hyaluronidase on Coronary Blood Flow following Coronary Artery Occlusion in the Dog

JOSEPH ASKENAZI, L. DAVID HILLIS, PABLO E. DIAZ, MICHAEL A. DAVIS, EUGENE BRAUNWALD, AND PETER R. MAROKO

SUMMARY. In an attempt to determine the mechanism by which hyaluronidase reduces myocardial injury following coronary artery occlusion, myocardial blood flow was studied in 20 open-chest dogs with occlusion of the left anterior descending coronary artery. Ten dogs served as controls, and 10 received hyaluronidase (500 NF units/kg) intravenously 20 minutes after occlusion. At 15 minutes and at 6 hours after occlusion, regional myocardial blood flow in the epicardial and endocardial halves of both ischemic and nonischemic zones were determined with radionabeled microspheres. Mean arterial pressure, heart rate, and cardiac output were similar in the untreated and treated dogs through the 6 hours of the experiment. Moreover, regional blood flow to nonischemic myocardium (areas without epicardial S-T segment elevation) 15 minutes after occlusion was similar in the two groups 15 minutes and 6 hours after occlusion. Fifteen minutes after occlusion, the flow to the ischemic myocardium subjacent to sites with S-T segment elevation exceeding 2 mV) in the untreated group was: transmural, 28.1 ± 2.2 (mean ± se) ml/min per 100 g; endocardial, 20.7 ± 1.8; and epicardial, 38.5 ± 3.1. The endocardial-epicardial flow ratio was 0.56 ± 0.04. Six hours after occlusion, the untreated group demonstrated a further decrease in blood flow to the ischemic myocardium: transmural, 15.2 ± 1.4 ml/min per 100 g; endocardial, 6.8 ± 1.1; and epicardial, 24.3 ± 1.9. The endocardial-epicardial flow ratio fell to 0.28 ± 0.04. In contrast, the hyaluronidase-treated dogs showed no further reduction in blood flow to ischemic myocardium 6 hours after occlusion: transmural, 30.3 ± 3.1 ml/min per 100 g; endocardial, 21.3 ± 2.5; and epicardial, 38.8 ± 3.8. These regional myocardial flows were significantly higher than those of the untreated dogs 6 hours after occlusion. Thus, salvage of damaged myocardium by hyaluronidase might be explained by its beneficial effect on collateral blood flow to the ischemic tissue, though this effect on collateral flow could be the consequence rather than the cause of this salvage.

IN 1959 IT WAS reported that intravenous hyaluronidase reduced the magnitude of S-T segment elevation both in animals with coronary artery ligation and in patients with acute myocardial infarction. This effect was attributed to

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occlusion can be modified by a variety of interventions.\(^3,4\) Subsequently, this concept was extended to patients with acute myocardial infarction.\(^5\) The effectiveness of hyaluronidase as one of these interventions has been demonstrated both in dogs\(^6,7\) and in patients\(^8\) using the fall in S-T segment elevation\(^6,8\) and better preservation of the myocardium using light microscopy and myocardial enzymatic activity\(^6,7\) as indices of a reduction in myocardial damage. In the rat with experimentally produced coronary occlusion, hyaluronidase has been shown by direct measurement to reduce infarct size.\(^9\) The mechanism by which hyaluronidase exerts its salutary effect on ischemic myocardium remains unknown. The present study was performed in an attempt to elucidate this mechanism of action.

**Methods**

Twenty mongrel dogs of both sexes weighing between 17 and 40 kg were anesthetized with sodium thiamylal (25 mg/kg, iv) and artificially ventilated with a volume respirator (Harvard Apparatus). A left thoracotomy was performed in the 5th intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free from adjacent tissue and ligated. Ten to 16 sites on the anterior surface of the left ventricle were selected for the recording of unipolar electrograms. Each site selected for electrocardiographic recording was recognized by its specific relationship to the branching of the coronary arteries and veins. Sites were chosen from within the area supplied by the occluded vessel, from distant regions (and, therefore, presumably normal), and from the border zone. The input impedance of the amplifier was 100 MΩ, and the frequency response of the system was ±0.5 dB from 0.14 to 70 Hz. The impedance of the electrode was maintained constant, as reflected in the reproducibility of the tracings. The electrode employed was a 15-mm\(^2\) copper cylinder with a saline-soaked wick connected to the precordial V lead and held by a cable perpendicular to the electrode to minimize mechanical stress. Because of the large area of the electrode, small variations in location did not change the configuration of the recordings. The electrograms obtained with this system are reproducible in repetitive coronary artery occlusions.\(^4\) Electrocardiographic lead aVF and systemic arterial pressure recorded through a catheter in the carotid artery (Statham P23Db pressure transducers) were recorded continuously for the duration of the experiments on a Brush polygraph. A polyethylene catheter was placed in the left atrium through its appendage for injection of radiolabeled microspheres, and another catheter was positioned in the femoral artery for the simultaneous withdrawal of reference blood samples.

In 10 dogs the left anterior descending coronary artery was occluded for 6 hours, and no interventions were employed. Epicardial electrograms were recorded before and 15 minutes after occlusion. Fifteen minutes after occlusion, \(2 \times 10^6\) microspheres, 7-10 \(\mu\m\) in diameter (in 4 ml of solution), labeled with either \(^113\)Ce or \(^85\)Sr (3M Company) were injected into the left atrium over a period of 15 seconds. During the next 15 seconds, the catheter was flushed with 4 ml of normal saline. Simultaneously, blood was withdrawn from the femoral artery at a constant rate of 15.3 ml/min for 60 seconds. The microspheres were suspended in a 50% sucrose solution to which 2 drops of polysorbate 50 (Tween 50) had been added. Prior to injection they were ultrasonicated for 30 minutes, then vigorously shaken by hand. Six hours after occlusion, microspheres labeled with the other isotope were injected in a similar manner, and blood was again collected from the femoral artery. Immediately thereafter, the dogs were killed, the hearts removed, and transmural myocardial specimens obtained, as described below, for analysis of radioactivity.

In another 10 dogs coronary artery occlusion, epicardial electrograms at 15 minutes, and injection of radiolabeled microspheres at 15 minutes and 6 hours were performed exactly as in the first group. In addition, hyaluronidase (Alidase, Searle) (500 NF units/kg) was administered intravenously 20 minutes after occlusion. The dogs were killed shortly after the injection of microspheres at 6 hours, and the hearts were removed.

The term hyaluronidase (hyaluronate glucanohydrolase) has been applied to a group of dissimilar enzymes that depolymerize certain acid mucopolysaccharides such as hyaluronic acid. Testicular hyaluronidase (Alidase, Searle) is a typical glucosidase isolated from bovine testes and which has both endohexosaminidase and transglycosidase activity. Substrates include hyaluronic acid and chondroitin sulfates A and C. Molecular weight of the glycol protein is 61,000. The preparation used in this study showed four electrophoretic peaks of which the two larger exhibited hyaluronidase activity and were 75% of the total area under the curves.

From each heart six to eight transmural specimens (average weight, 2.20 ± 0.07 g) were obtained, two or three from areas without epicardial S-T segment elevation 15 minutes after occlusion and three to five from areas subjacent to sites with at least 3 mV of S-T segment elevation 15 minutes after occlusion. Each specimen was divided into an endocardial and an epicardial half. The radioactivity of these specimens and that of the arterial blood samples was then determined in a well scintillation counter (Nuclear-Chicago, model 4233). Cardiac output and regional myocardial blood flow were calculated as described previously.\(^10,11\)

To compare myocardial blood flow within each group of dogs, the paired \(t\) test was used. To compare the untreated with the treated group, Student’s \(t\) test for group analysis was used.\(^12\)

**Results**

Heart rate, mean arterial pressure, and cardiac output (expressed in ml/min per kg of body wt) were similar in the two groups both 15 minutes and 6 hours after occlusion (Table 1).

**REGIONAL MYOCARDIAL BLOOD FLOW**

**Occlusion Alone**

In the 10 dogs 28 specimens were obtained from nonischemic areas, i.e., those regions not demonstrating S-T segment elevation 15 minutes after occlusion. The transmural flow to these areas was 98.1 ± 2.6 (mean ± se) ml/
min per 100 g 15 minutes after occlusion and 106.5 ± 3.9 ml/min per 100 g 6 hours after occlusion (Table 1). The endocardial-epicardial flow ratio was 1.10 ± 0.05 at 15 minutes after occlusion and 0.96 ± 0.05 at 6 hours after occlusion (Table 1).

Forty-one specimens were obtained from areas subjacent to sites demonstrating S-T segment elevation of at least 3 mV 15 minutes after occlusion. The transmural blood flow to these areas was 28.1 ± 2.2 ml/min per 100 g 15 minutes after occlusion, falling by an average of 46% to 15.2 ± 1.4 ml/min per 100 g 6 hours after occlusion (P < 0.001) (Figs. 1 and 2). Over the 6 hours after occlusion the flow to the endocardial layer fell by an average of 67%, from 20.7 ± 1.8 ml/min per 100 g 15 minutes after occlusion to 6.8 ± 1.1 ml/min per 100 g 6 hours after occlusion (P < 0.001) (Fig. 2), while the epicardial flows fell by an average of 37% from 38.5 ± 3.1 ml/min per 100 g 15 minutes following occlusion to 24.3 ± 1.9 ml/min per 100 g 6 hours after occlusion (P < 0.001) (Fig. 2). The ratio between the endocardial and epicardial flows was 0.56 ± 0.04 at 15 minutes after occlusion and 0.28 ± 0.04 at 6 hours after occlusion (P < 0.001), reflecting a redistribution of flow in the ischemic zone in addition to the absolute decline already described (Figs. 1 and 3).

**Occlusion with Hyaluronidase**

In the 10 dogs that received hyaluronidase intravenously 20 minutes after occlusion, 23 specimens were obtained from areas without S-T segment elevation 15 minutes after occlusion. As expected, the regional flows were similar to those in the dogs with simple occlusions. The transmural blood flow to these sites was 106.3 ± 5.7

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**Table 1 Hemodynamics and Flow to the Normal Myocardium**

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Cardiac output (ml/min/kg)</th>
<th>Normal RMBF* (ml/min/100 g)</th>
<th>Endocardial-epicardial ratio in nonischemic sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated dogs</td>
<td>15 min†</td>
<td>141 ± 5</td>
<td>115 ± 4</td>
<td>100.6 ± 7.4</td>
<td>98.1 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>6 hr†</td>
<td>148 ± 4</td>
<td>122 ± 4</td>
<td>95.8 ± 5.3</td>
<td>106.5 ± 3.9</td>
</tr>
<tr>
<td>Hyaluronidase-treated dogs</td>
<td>15 min</td>
<td>147 ± 5</td>
<td>121 ± 5</td>
<td>102.1 ± 9.2</td>
<td>106.3 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>146 ± 4</td>
<td>112 ± 3</td>
<td>86.1 ± 6.1</td>
<td>115.3 ± 0.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.

* Normal RMBF = regional myocardial blood flow in the nonischemic myocardium.
† Time after coronary artery occlusion.

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**Figure 1** Left: a schematic representation of the heart and its arteries. The left anterior descending artery (LAD) was occluded at its midportion (OCCL). The shaded area represents the zone of S-T segment elevation 15 minutes after occlusion. A polyethylene catheter was placed in the left atrium (LA) for the injection of radiolabeled microspheres. Right: transmural myocardial blood flows and endocardial/epicardial (endo/epi) flow ratios at 15 minutes and at 6 hours after occlusion in an untreated dog. The letters correspond to the biopsy sites in the left panel. Note that the blood flow and endo/epi ratio did not change from 15 minutes to 6 hours after occlusion in sites A and F, both of which were taken from areas remote from the ischemic region. In contrast, sites B, C, D, and E, all located within the area of ischemia, demonstrated a decline in transmural flow and in endo/epi ratios from 15 minutes to 6 hours after occlusion.

**Figure 2** Mean values of regional myocardial blood flow (RMBF) for all sites with S-T segment elevation of at least 3 mV 15 minutes after occlusion. The upper panel represents the untreated dogs, the lower panel the hyaluronidase-treated ones. Note that in the untreated group, RMBF fell significantly from 15 minutes after occlusion to 6 hours after occlusion (P < 0.001). The hyaluronidase-treated dogs demonstrated no such decline. Consequently, RMBF 6 hours after occlusion was higher in the hyaluronidase-treated than in the untreated group. († = P < 0.001 in comparison to blood flows 15 minutes after occlusion in the untreated dogs; † = P < 0.001 in comparison to blood flows 6 hours after occlusion in the untreated dogs.)
ml/min per 100 g 15 minutes after occlusion and 115.3 ± 6.8 ml/min per 100 g 6 hours after occlusion. The endocardial-epicardial ratio was 1.05 ± 0.05 at 15 minutes after occlusion and 0.95 ± 0.04 at 6 hours after occlusion (Table 1).

Forty-seven specimens were obtained from areas subjacent to sites with S-T segment elevation of at least 3 mV 15 minutes after occlusion. The transmural blood flow to the ischemic areas was 28.3 ± 3.2 ml/min per 100 g 15 minutes after occlusion and was maintained at 30.3 ± 3.1 ml/min per 100 g 6 hours after occlusion [not significant (NS)] (Figs. 2 and 4). The flow to the endocardial layer was 20.4 ± 2.4 ml/min per 100 g 15 minutes after occlusion and 21.3 ± 2.5 ml/min per 100 g at 6 hours (NS); while in the epicardium it was 36.3 ± 4.2 ml/min per 100 g 15 minutes after occlusion and 38.8 ± 3.8 ml/min per 100 g 6 hours later. The ratio between endocardial and epicardial flow was 0.56 ± 0.06 15 minutes after occlusion and 0.61 ± 0.06 at 6 hours (NS) (Figs. 3 and 4).

Fifteen minutes after occlusion (before the administration of hyaluronidase), the blood flow to the ischemic areas (transmural, endocardial, and epicardial) and the ratio of endocardial to epicardial flow were similar in the untreated and treated dogs; this demonstrates that the magnitude of ischemia was similar. However, 6 hours after occlusion, transmural, endocardial, and epicardial blood flows, as well as the ratio between endocardial and epicardial flows, were significantly higher in the hyaluronidase-treated dogs than in the untreated ones (Figs. 2 and 3).

Discussion

Limitation of the extent of myocardial infarction following coronary artery occlusion is potentially of great clinical importance, since the salvage of contractile tissue otherwise destined to undergo necrosis may be expected to reduce the incidence of heart failure and ultimately, of the mortality secondary to myocardial infarction. Various inter-ventions—pharmacological, hemodynamic, and metabolic—have been shown to reduce ischemic injury in its reversible phase and thus diminish the extent of infarction.\(^2^\)\(^4\) It has been observed previously that for any level of S-T segment elevation 15 minutes after occlusion, hyaluronidase reduces necrosis at the same site 24 hours later, as measured biochemically [by myocardial creatine phosphokinase (CPK) activity], histologically, and electrocardiographically (by an analysis of epicardial QRS changes).\(^5\)\(^6\) Recently, the size of myocardial infarction following coronary artery occlusion in the rat has been quantified by measuring CPK activity in homogenized left ventricles and by planimetry of the infarcted area in histological sections of the left ventricles. In these experiments, the administration of hyaluronidase resulted in smaller infarcts than in the untreated rats.\(^9\) The beneficial effects of hyaluronidase also have been demonstrated in a small number of patients.\(^8\) Despite these consistent observations, the mechanism by which hyaluronidase exerts its beneficial effect remains unknown.

Studies in experimental animals have shown that ischemia rapidly leads to the accumulation of intracellular water.\(^13\)\(^15\) In both the brain and kidney, swelling of ischemic cells is an important factor in the development of irreversible damage.\(^16\)\(^17\) More recently this concept has been applied to the ischemic myocardium.\(^18\) It has been...
shown that ischemic myocardial cells are swollen and that the administration of hyperosmotic mannitol reduces the extent of cell swelling. As a consequence, collateral flow to the ischemic myocardium is improved and decreases the extent of myocardial necrosis.19

The present study suggests that hyaluronidase also may exert its salutary effect on ischemic myocardium by enhancing collateral blood flow. In this study regional myocardial blood flow was determined with radiolabeled microspheres 15 minutes and 6 hours after coronary artery occlusion in two groups of dogs: in the first group there was no intervention but in the second hyaluronidase was administered intravenously 20 minutes after occlusion. In both groups the blood flow to the ischemic myocardium was similar 15 minutes after occlusion (prior to the administration of hyaluronidase); this demonstrates that the severity of ischemia was similar in the two groups. Over the succeeding 6 hours, the untreated group demonstrated a significant decline in blood flow to the ischemic areas: endocardial flow fell by 67%, epicardial flow by 37%, transmural flow by 46%, and the endocardial-epicardial flow ratio by 50%. In contrast, the hyaluronidase-treated dogs showed no further decline in blood flow to the ischemic myocardium over the 6 hours after occlusion, nor did the distribution between endocardial and epicardial flows change (Figs. 2 and 4). Since heart rate, mean arterial pressure, and cardiac output were similar in the two groups both 15 minutes and 6 hours after occlusion (Table 1), this beneficial effect of hyaluronidase cannot be attributed to hemodynamic changes.

Numerous studies have attempted to quantify regional myocardial blood flow during the hours to days following coronary artery occlusion in the experimental animal. Studies with 133Xe washout20-22 as well as direct anatomical evaluations of coronary collateral channels23-25 have demonstrated consistently that collateral flow to the ischemic myocardium is increased 3-4 days after coronary artery occlusion as compared to that measured immediately after occlusion. Recently, Bishop et al.26 have shown in the conscious, closed-chest dog that regional myocardial blood flow 6 hours after occlusion is not significantly different from that measured 10 minutes after occlusion. In contrast, the present study demonstrates an unequivocal decline in regional myocardial blood flow from 15 minutes to 6 hours after occlusion in those dogs not given hyaluronidase. This disparity of results is most likely due to the striking differences in experimental design (conscious, closed-chest dogs as opposed to anesthetized, open-chest dogs). Although hyaluronidase has not been evaluated definitively in the conscious experimental animal, it has been shown by indirect techniques to exert a beneficial effect on ischemic myocardial damage in conscious patients with acute myocardial infarction.37 Although blood flow to the ischemic area may indeed remain unchanged from 10 minutes to 6 hours after occlusion in the closed-chest, conscious dog not treated with hyaluronidase,28 it is possible that the hyaluronidase-treated conscious dog may show an improvement in collateral blood flow during the 6 hours after coronary artery occlusion, leading to the salvage of ischemic tissue.

Hyaluronidase is known to decrease local edema and has been used successfully to promote the absorption of saline administered by dermalysis.28 In addition, it has been suggested that hyaluronidase diminishes myocardial edema after coronary artery occlusion, although this decrease in edema may be either the cause or the consequence of a smaller infarct.8 The present study demonstrates that hyaluronidase prevents the reduction of collateral blood flow following coronary artery occlusion, perhaps by diminishing the extent of edema formation. Although this beneficial effect on collateral flow is an attractive hypothesis to explain the limitation of infarct size, the possibility remains that salvage of ischemic myocardium by hyaluronidase may be due to the depolymerization of polynucleotides that was demonstrated histochemically even in the central zone of the infarction 24 hours after occlusion when hyaluronidase was administered in a single dose 20 minutes after occlusion.25 It was postulated that this may be responsible for increasing the supply of nutrients to the myocardium and for increasing the washout of damaging metabolites.17 It is conceivable that, if these are the primary mechanisms of action of hyaluronidase, the increase in flow is secondary rather than primary. Therefore, it is not certain that the maintenance of blood flow with hyaluronidase contributes to the limitation of infarct size.

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References

Reflex Regulation of Sympathetic Activity in the Spontaneously Hypertensive Rat

JOHN H. COOTE AND YUKO SATO

SUMMARY The influence that the pressure-sensitive receptors in the cardiovascular system have on renal nerve activity and on heart rate was compared in normotensive rats (NTR) and spontaneously hypertensive rats (SHR). The cardiovascular receptors were stimulated by raising the blood pressure (BP) with intravenous phenylephrine. The duration of silence in the record of renal sympathetic nerve activity produced by a number of different rises in BP was measured. We found that the pressure that was just able to produce a silence in the nerve activity (threshold pressure) was higher in the SHR (170 mm Hg) than in the NTR. It is suggested that this is most likely due to a high sympathetic activity in the SHR.

Periphera l factors localized to the blood vessels, such as changes in the reactivity of smooth muscle to transmitter1 or a decrease in the wall to lumen ratio of the vessels2 may contribute to the development of hypertension in the spontaneously hypertensive rat. However, there is also evidence that an increase in sympathetic activity plays an important role in the development of hypertension.3,4 This is somewhat surprising because it has long been thought that the activity in sympathetic nerves to blood vessels is normally regulated quite efficiently by inhibitory feedback from mechanoreceptors sensitive to pressure changes in the circulatory system.5,6 The question arises, how is it that in the potentially hypertensive animal an increase in sympathetic activity could occur without invoking inhibitory feedback from the receptors sufficient to restore it to its original level? One possibility is that suggested by Foklow et al.4 These authors believe that sympathetic activity is only periodically or spasmodically increased and that during these episodes there is some abrogation of the baroreceptor reflex.5 If this occurs often enough it may lead to morphological changes in the arterial walls which themselves sustain the hypertension.5 There is some evidence that weak, repeated stimulation of the hypothalamic defense region in rats leads to hypertension and that hypertensive rats are more responsive than normal rats to acute mental stress, and this might support such a hypothesis.5,6

Another possibility is that the ability of the barorece-
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