Pharmacologic Modification of Myocardial Ischemia

LEWIS WETSTEIN, M.D., MICHAEL B. SIMSON, M.D., PETER D. FELDMAN, B.A., AND ALDEN H. HARKEN, M.D.

SUMMARY The value of three agents in reducing the area of myocardial ischemia in rabbit hearts perfused with crystalloid solution was examined. Ten hearts received crystalloid solution with methylprednisolone (M), 0.25 mg/ml; 18 with hyaluronidase (H), 4 U/ml; and 10 with propranolol (P), 1 μg/ml. Thirty-six hearts served as controls. The mitral valves were excised, the hearts were paced at 240 beats/min and a coronary artery was ligated. The ischemic area was evaluated by nicotinamide adenine dinucleotide autofluorescence photography, an intrinsic, high-resolution display of anoxic tissue. The ischemic area was determined by computer from standardized photographs. Myocardial oxygen consumption (MVO₂) was determined and photographs were taken before and at 10-minute intervals after ligation. At 60 minutes, each heart was perfused with rhodamine dye and quick-frozen. In hearts treated with M and H, coronary blood flow increased by 151% (51.7 ± 3 to 77.9 ± 3 ml/min) and 150% (48.3 ± 2 to 72.3 ± 2 ml/min), respectively (p < 0.001), whereas in hearts treated with U and P, coronary flow decreased at 60 minutes. In the control hearts, the ischemic area did not change between 5 and 40 minutes of ischemia. The ischemic area of H-treated hearts decreased from 136 ± 4 mm² to 110 ± 9 mm² between the postligation control and the end of the experiment (p < 0.01). The ischemic area of M-treated hearts decreased from 131 ± 5 mm² to 113 ± 5 mm² (p < 0.05). P produced no change in ischemic area (p > 0.4). There was no change in the oxygen-diffusion zone of P-treated or control hearts (439 ± 13 vs 383 ± 12 μ, p > 0.1). The oxygen-diffusion zone between perfused and anoxic tissue in the M and H hearts increased from 383 ± 12 μ to 861 ± 76 μ and 681 ± 62 μ, respectively (p < 0.001). We conclude that significant volumes of myocardium remain normoxic within nonperfused areas of M-, P- and H-treated hearts.

LIMITING myocardial necrosis during an evolving myocardial infarction is of major clinical significance because the extent of damage after coronary artery occlusion is a strong determinant of patient survival.1,2 Numerous studies have investigated the character, extent and reversibility of myocardial ischemic injury, but the nature of the transition from reversible to irreversible damage remains obscure. The continuum of metabolic, biophysical and electrophysiologic changes between the center of an ischemic area and the adjacent normal tissue provides a conceptual framework for the presence of a critical oxygen-diffusion zone in tissue.3 We defined this zone as the area of unperfused, but not anoxic, tissue surrounding a myocardial infarction. Effective therapy of a border zone might salvage a substantial volume of reversibly injured myocardial tissue. Many histochemical,4 metabolic,5,6 and radioisotopic7 techniques have been used to measure this border zone. The reported widths are highly dependent on the measurement technique and have varied from 2 to 15 mm.8

Infarct size can be influenced by pharmacologic intervention, but numerous studies of similar agents have produced conflicting results. Further, not only are the therapies controversial, but also the methods used to evaluate them have been disputed because of the inability to accurately differentiate ischemic from normoxic myocardium.

In this study, we used nicotinamide adenine dinucleotide (NADH) fluorophotography9 to evaluate the influence of three pharmacologic agents on limiting infarct size. Hyaluronidase, an oxygen-diffusion facilitator; methylprednisolone, a steroidal anti-inflammatory agent; and propranolol, an agent for reducing oxygen demand, were administered after acute coronary ligation in the isolated, perfused rabbit heart.

Methods and Materials

Seventy-four New Zealand rabbits that weighed 2–3 kg were anesthetised with sodium pentobarbitol, 30 mg/kg. The hearts were rapidly excised and perfused with a blood-free crystalloid solution at 37°C. The perfusate consisted of Krebs-Ringer’s bicarbonate solution containing 5 mM glucose and 2.5 mM calcium and saturated with 95% oxygen and 5% carbon dioxide. The perfusion circuit included an overflow reservoir 104 cm above the level of the aortic cannula to maintain perfusion pressure at 80 mm Hg.

The venae cavae and pulmonary veins were ligated, and a soft plastic cannula was secured in the pulmonary artery. Pulmonary arterial flow was directed through a Clark-type oxygen electrode and coronary venous oxygen tension (PO₂) was thus recorded and temperature corrected on an oxygen electrode amplifier (Johnson Research Foundation).

The hearts were paced at 240 beats/min with bipolar leads placed on the right atrial appendage; the pacing stimulus was provided by a stimulator that synchronized the flash to end-diastole (Murray Bloom). The left atrium was opened and the left ventricle was decompressed by excising a portion of the mitral valve.

Five minutes after establishment of stable atrial pacing, epicardial NADH fluorescent photographs were taken and myocardial oxygen consumption (MVO₂) was recorded. Flows were measured by collecting the
coronary venous and thebesian effluent flow that dripped from the heart. Epicardial NADH fluorescence photographs were taken with a Hasselblad camera fitted with a 75-mm focal lens reversed to provide an image 1.5 times the actual size. Two 400-J xenon flash tubes (E.G. & G. Corp., FX-47C2) covered with Corning 5840 filters yielded exciting light in the 330–380-nm region. The camera lens was filtered with Wratten #2E and #4 filters, which allowed transmission of fluorescence in the 430–510-nm region. The xenon flash tubes were triggered from the pacemaker 10 msec before the onset of the electrical stimulus. By this technique, photographs were taken at end-diastole.

At 10 minutes, the coronary sinus Po2 (PcO2), MVO2 and coronary flow were again recorded, and a coronary artery supplying the anteroapical portion of the left ventricle was ligated. After 5 minutes of ischemia, an epicardial NADH fluorophotograph was taken and PcO2, MVO2 and coronary flow were recorded.

Next, 18 rabbits were treated with hyaluronidase (4 U/ml of crystalloid perfusate), 10 with methylprednisolone (0.25 mg/ml) and 10 with propranolol (1 μg/ml); 36 hearts received no treatment. Once the perfusate was altered to include one of these pharmacologic agents, the hearts were continuously perfused with the modified perfusate for the remainder of the experiment (40 minutes). Three liters of perfusate were used after pharmacologic modifications. Therefore, the total doses of each agent were hyaluronidase, 12,000 U (3000 × 4); methylprednisolone, 0.750 mg; and propranolol, 3 mg. PcO2, MVO2 and coronary flow were recorded and photographs were taken at 20, 30, 40 and 50 minutes (5, 10, 20, 30 and 40 minutes after treatment).

Forty minutes after ligation, each heart was perfused with rhodamine. As soon as the dye emerged from the pulmonary artery catheter (2–3 seconds), the heart was quick-frozen between large metal tongs and plunged into liquid nitrogen. After the heart was completely frozen, the epicardium was trimmed to a depth of 0.5 mm to remove the epicardial vessels and surface ice, and NADH fluorophotographs were taken. The heart was then removed from the aluminum block and weighed.

The photographs were enlarged × 15 and the area not perfused with rhodamine and the anoxic zone of NADH fluorescence were digitized by computer. In addition, the distance between rhodamine and NADH fluorescence was measured at 20 points around the ischemic border.

MVO2 was calculated and expressed as microliters of oxygen per minute per gram wet weight of heart. Calculations were based on the standard formula: MVO2 = (PAO2 – PVO2) × 0.00029 μl O2/ml perfusate × flow/g wet weight of heart, where 0.00029 ml O2/mm Hg is the solubility of oxygen in perfusate at 37°C.

Statistical significance within a treatment group was assessed using a repeated-measures analysis of variance. Comparison between treatment groups was performed using one-way analysis of variance and the Newman-Keuls test.

Results

After coronary artery ligation, flow decreased in the treated as well as the untreated groups (fig. 1). There was no difference between groups before or immediately after ligation. After treatment, flow increased by 150% (48.3 ± 3 to 72.3 ± 2 ml/min) in the hyaluronidase group and by 151% (51.7 ± 3 to 77.9 ± 3) in the methylprednisolone group (p < 0.001) (table 1). The untreated and the propranolol-treated hearts had a further decline in coronary flow to 85% (50 ± 2 to 42.3 ± 2 ml/min) and 86% (54.2 ± 3 to 46.8 ± 3 ml/min), respectively (p < 0.01). The change in flow in the hyaluronidase- and methylprednisolone-treated hearts was different from that in the untreated hearts (p < 0.05). There was no difference in the propranolol-treated hearts and the untreated hearts (p > 0.1).

There was no difference in PcO2 between groups before or after ligation (fig. 2). PcO2 increased to 153% in the hyaluronidase group (277.5 ± 19 to 425.8 ± 18 mm Hg) and to 134% (217.0 ± 18 to 291.0 ± 19 mm Hg) in the methylprednisolone group (p < 0.001) (table 2). With time, the propranolol-treated hearts showed no further change in PcO2 (215.6 ± 13 to 231.4 ± 11 mm Hg, p > 0.1). The untreated hearts declined to 79% of control during the experiment (236.9 ± 11 to 186.5 ± 10 mm Hg, p < 0.001). The increase in coronary sinus Po2 in the hyaluronidase and methylprednisolone groups was significant compared with the untreated hearts (p < 0.05). The postligation Pco2 of the control hearts was not different from that of the propranolol-treated hearts.

After ligation, MVO2 decreased in all groups except
TABLE 1. Coronary Flow

<table>
<thead>
<tr>
<th></th>
<th>Before ligation (5 min)</th>
<th>After ligation (5 min)</th>
<th>After treatment (40 min)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.3 ± 2</td>
<td>50.0 ± 2</td>
<td>47.8 ± 2</td>
<td>42.3 ± 2</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>58.6 ± 2</td>
<td>48.3 ± 2</td>
<td>61.2 ± 2</td>
<td>72.3 ± 2</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>55.9 ± 4</td>
<td>51.7 ± 3</td>
<td>70.9 ± 4</td>
<td>77.9 ± 3</td>
</tr>
<tr>
<td>Propranolol</td>
<td>60.6 ± 4</td>
<td>54.2 ± 3</td>
<td>51.4 ± 3</td>
<td>46.8 ± 4</td>
</tr>
</tbody>
</table>

Values are expressed as ml/min.

*p value represents one-way analysis of variance comparing treated and control groups.

The methylprednisolone group (fig. 3). After treatment, MVO₂ declined in the hyaluronidase-treated heart to 63% (58.4 ± 4 to 37.0 ± 3 μl/O₂/min, p < 0.001) of postligation control (table 3). The methylprednisolone, propranolol and untreated hearts demonstrated no further changes in MVO₂ during the procedure.

During the course of the experiment, there was no change in the NADH-determined epicardial ischemic area between the postischemic control and 40 minutes postligation in the untreated hearts (132 ± 2 mm² vs 127 ± 2 mm²) or in the propranolol-treated hearts (129 ± 5 mm² vs 123 ± 9 mm², p > 0.4). However, the epicardial ischemic area decreased between the postligation control and the end of the experiment in the hyaluronidase- and methylprednisolone-treated hearts (136 ± 4 mm² vs 110 ± 9 mm² and 131 ± 5 mm² vs 113 ± 5 mm², respectively) (fig. 4). The difference in the hyaluronidase and methylprednisolone groups was significant (p < 0.05 vs untreated hearts) (fig. 5).

In the untreated hearts after 40 minutes, the distance between perfused and anoxic tissue in the mid-myocardium (frozen hearts) was 383 ± 12 μ (fig. 6). In the treated hearts, the distances were 861 ± 76 μ for hyaluronidase, 681 ± 62 μ for methylprednisolone and 439 ± 13 μ for propranolol. The distance between perfused and anoxic tissue was larger in hyaluronidase and methylprednisolone (p < 0.05) than in the propranolol or untreated hearts.

In the untreated hearts, 82 ± 9% of the nonperfused area was anoxic and NADH fluorescent (fig. 7). In the propranolol-treated hearts, 78.4% ± 2% of the nonperfused area was anoxic. In contrast, 55 ± 6% of the nonperfused area was anoxic in the hyaluronidase-treated hearts and 67% ± 8% in the methylprednisolone group. The hyaluronidase- and methylprednisolone-treated hearts were different (p < 0.05) from the untreated group, and had a greater amount of normoxic tissue in the nonperfused area. The hearts treated with propranolol were not different from the untreated hearts (p > 0.1).

Discussion

The ability to modify myocardial infarct size is an important clinical goal. The delineation of a periischemic oxygen-diffusion zone has stimulated efforts directed at saving it and thereby reducing infarct size. Methods of quantitating the extent of myocardial ischemia suffer from a lack of spatial resolution and discrimination between ischemic and normoxic myocardium.

In this study, we exploited the ability of NADH fluorophotography to locate areas of cellular anoxia.

TABLE 2. Coronary Sinus Oxygen Tension

<table>
<thead>
<tr>
<th></th>
<th>Before ligation (95 min)</th>
<th>After ligation (5 min)</th>
<th>After treatment (40 min)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>255.9 ± 12</td>
<td>236.9 ± 11</td>
<td>216.8 ± 10</td>
<td>186.5 ± 10</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>277.6 ± 18</td>
<td>277.5 ± 19</td>
<td>363.8 ± 26</td>
<td>425.8 ± 18</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>226.9 ± 14</td>
<td>217.0 ± 16</td>
<td>283.8 ± 18</td>
<td>291.0 ± 19</td>
</tr>
<tr>
<td>Propranolol</td>
<td>222.0 ± 12</td>
<td>215.6 ± 13</td>
<td>237.7 ± 9</td>
<td>231.4 ± 11</td>
</tr>
</tbody>
</table>

Values are expressed as mm Hg.

*p value represents one-way analysis of variance comparing treated and control groups.
NADH is a component of the mitochondrial electron transport chain that transfers electrons from substrate to oxygen while generating ATP. When cells become anoxic, electron transport stops and NADH rapidly accumulates. Reduced NADH represents anoxic tissue and fluoresces when excited with ultraviolet light; oxidized NADH does not fluoresce. Chance and colleagues showed that NADH fluorescence is a reproducible marker of intramitochondrial anoxia and reliably indicates a loss of energy-linked functions such as oxidative phosphorylation, mitochondrial calcium uptake and the maintenance of the mitochondrial membrane potential. NADH fluorescence correlates with other indicators of myocardial ischemia, such as epicardial ST-segment changes and ultrastructural damage.

In the isolated, perfused rabbit heart, ligation of a coronary artery produces a discrete zone of transmural ischemia that is demonstrated as a uniform area of NADH fluorescence. In untreated hearts, there was no change in epicardial ischemia area after 60 minutes (p > 0.4). During this period, the ischemic cells in a normothermic, working heart undergo irreversible injury.

Using a combination of NADH fluorescence photography, rhodamine angiography and a freeze clamp technique, we delineated a dark narrow zone between perfused (rhodamine) and anoxic (NADH fluorescent) tissue (fig. 8). This dark band corresponds to tissue that is not perfused (and therefore not stained with rhodamine) and is not anoxic (and therefore not NADH fluorescent). This "oxygen-diffusion zone" was observed in all hearts.

By assessing both two- and three-dimensional distribution of ischemic anoxia after coronary artery ligation in isolated rabbit hearts, we confirmed that hyaluronidase and methylprednisolone can reduce the zone of anoxic tissue within an ischemic area. We could not confirm the ability of propranolol to reduce the extent of myocardial ischemia in the present model.

Our results show that in the isolated, perfused rabbit heart, ligation of a coronary artery produces a discrete zone of transmural ischemia. The ischemia is uniform, nonheterogeneous and has a small (383 ± 12 μ) identifiable border zone of intermediate ischemia. Unlike dog hearts, rabbit hearts are devoid of interarterial intercoronary collateral anastomoses. NADH fluorescence (anoxia) was homogeneous in the ischemic areas of the rabbit hearts (fig. 3).

Hyaluronidase and methylprednisolone reportedly increased coronary collateral flow to the ischemic myocardium. By rhodamine angiography we found no evidence for increased collateral flow into the ischemic zone. We could not demonstrate any areas of perfused tissue within the ischemic area. Hyaluronidase and methylprednisolone both caused an increase in total coronary flow (p < 0.001).

Our data confirm an increase in total coronary flow and a decrease in MVO₂ in hearts treated with hyaluronidase. Further, we delineated an increase in the distance between perfused and anoxic tissue at the ischemic border and a decrease in the epicardial ischemic area after ligation. Hyaluronidase therefore increases the amount of non–NADH fluorescent tissue in nonperfused areas. These therapeutic responses were repeated during subacute ischemia and therefore appear to have more clinical relevance.

\[ P_{O_2} \] increased in the face of fixed arterial \( P_{O_2} \) in hyaluronidase-treated hearts. In the absence of shunting, the coronary capillary \( P_{O_2} \) therefore must have increased. In addition, coronary flow increased and MVO₂ decreased. Each of these factors would increase the distance that oxygen could diffuse into ischemic areas.

**Table 3. Myocardial Oxygen Consumption**

<table>
<thead>
<tr>
<th></th>
<th>Before ligation</th>
<th>After ligation (5 min)</th>
<th>After treatment (5 min)</th>
<th>After ligation (40 min)</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.2 ± 3</td>
<td>54.5 ± 3</td>
<td>54.2 ± 2</td>
<td>54.7 ± 3</td>
<td></td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>67.1 ± 4</td>
<td>58.4 ± 4</td>
<td>50.4 ± 4</td>
<td>37.0 ± 3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>52.4 ± 4</td>
<td>53.0 ± 5</td>
<td>56.6 ± 4</td>
<td>63.0 ± 6.5</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Propranolol</td>
<td>59.4 ± 6</td>
<td>56.7 ± 6</td>
<td>54.1 ± 5</td>
<td>47.0 ± 5</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Values are expressed as μl/min · g.

\( *p \) value represents one-way analysis of variance comparing treated and control groups.
FIGURE 5. The ratio of anoxic areas 40 minutes after and before treatment. Hyaluronidase (H) decreased the total anoxic area by 20%; methylprednisolone decreased it by 14%. The untreated (U) hearts and those treated with propranolol (P) remained unchanged (96% and 95% of control).

tissue from the perfused edge. In fact, the oxygen diffusion zone did increase ($p < 0.001$), which reduced the anoxic area. Although we have not excluded the possibility that increased arteriovenous shunting in hyaluronidase-treated hearts caused the elevated $P_{cO_2}$, this seems unlikely because of the clear increase in oxygen diffusion (wider oxygen-diffusion zone) into the ischemic zone from the perfused edge. Using a model of oxygen diffusion across a flat surface and assuming that coronary venous $P_O_2$ reflects capillary $P_O_2$, we calculate that the diffusion distance should increase 1.6-fold in hyaluronidase-treated hearts. We measured an increase, relative to control, of 1.9-fold. Although other postulated mechanisms, such as facilitative oxygen transport, may be operative, a wider oxygen-diffusion zone appears to be explained by increased diffusion due to higher oxygen tension at the capillaries and lower $MV_O_2$.

At first glance it might appear that hyaluronidase is a direct tissue metabolic depressant, as it produced an
increase in coronary sinus Po2, a decrease in MVO2 and perhaps a compensatory increase in coronary sinus Po2. This cannot have been the mechanism of action, however, because the myocardial tissue remained oxidized and was not NADH fluorescent. Indeed, the anoxic tissue area decreased 20% in hyaluronidase-treated hearts. The present results support previous clinical work16, 24, 25 suggesting a decrease in the volume of myocardium that eventually becomes necrotic in patients treated with hyaluronidase during documented myocardial infarction. The determinants of MVO2 — heart rate, contractility and left ventricular wall tension — were not examined in this study. We have no explanation for the observed and reproducible decrease in MVO2.

Our results demonstrate that methylprednisolone increases in total coronary flow (p < 0.001) and coronary sinus Po2 (p < 0.5). Unlike hyaluronidase, methylprednisolone did not change MVO2 (p > 0.1). The epicardial ischemic area was reduced compared with postligation control (p < 0.05). Our results confirm previous reports that steroids permit an increase in coronary flow,26, 27 perhaps by preventing the endothelial swelling associated with ischemia.28, 29 This increase in coronary flow and the increase in capillary oxygen tension without a corresponding increase in myocardial oxygen demand allow oxygen to diffuse farther into the ischemic zone. This was documented by an increase in the oxygen-diffusion zone (p < 0.001). Since the anoxic area was encroached upon by an increased oxygen gradient, there was also a decrease in the ratio of the anoxic to the nonperfused area (p < 0.001).

The major criticism of using steroids to treat acute myocardial infarction is their deleterious effect on the healing process.30-33 Other studies34, 35 have shown that hydrocortisone (2–10 mg/kg daily) had no significant effect on healing 2–60 days after acute experimental coronary arterial ligation.

Our finding that propranolol failed to reduce the extent of epicardial or transmural ischemia requires further consideration. None of the variables examined demonstrated an advantageous effect on the ischemic myocardium or on the oxygen-diffusion zone. Propranolol can salvage reversibly damaged myocardial tissue in vivo.36-37 In our model, the hearts were neurohormonally isolated, the heart rate was fixed and the ventricles did not generate pressure. Therefore, decreasing MVO2 in the face of maximal catecholamine output would be of no benefit in our preparation. We did verify observations that propranolol does not increase collateral flow to ischemic myocardium. We could not corroborate evidence that propranolol may provide protection for the ischemic myocardium by mechanisms other than its β-blocking activity and myocardial oxygen balance.38, 39

Acknowledgment

We thank Linda Gautier and Nancy Wells for secretarial assistance.

References


5. Opie LH, Owen P: Effect of glucose-insulin-potassium infusions on arteriovenous differences of glucose and of free fatty acids and
on tissue metabolic changes in dogs with developing myocardial infarction. Am J Cardiol 38: 310, 1976
15. Simson MB, Harden WR, Barlow CH, Harken AH: Visualization of the distance between perfusion and anoxia along an ischemic border. Circulation 60: 1151, 1979
18. Brachfeld N: Metabolic evaluation of agents designed to protect the ischemic myocardium and to reduce infarct size. Am J Cardiol 37: 528, 1976
Pharmacologic modification of myocardial ischemia.
L Wetstein, M B Simson, P D Feldman and A H Harken

Circulation. 1982;66:548-554
doi: 10.1161/01.CIR.66.3.548
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/66/3/548

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/