Improvement in the Relationship between Flow to Ischemic Myocardium and the Extent of Necrosis with Glycolytic Intermediates that Decrease Blood Oxygen Affinity in Dogs

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SUMMARY Reducing blood oxygen affinity may enhance myocardial oxygen delivery during ischemia. We evaluated this hypothesis in awake, previously instrumented dogs that received a 20 ml/kg infusion of a solution of dihydroxyacetone, phosphate, and pyruvate after acute occlusion of either the left anterior descending or circumflex coronary artery. This infusion reduced blood oxygen affinity (BOA) after 2 hours; the Pm increased from 29.9 ± 0.7 torr (mean ± sd) to 32.1 ± 0.6 torr; P < 0.01 (BOA group). Four dogs received 20 ml/kg of phosphate and pyruvate solution to assess volume effects (V group), and five dogs were controls (C group). The 2-hour Pm values in V and C were unchanged. Regional flow (15-μm spheres) reduction 2 hours postocclusion was compared to the percent tissue infarcted determined by histology 7-9 days after occlusion for multiple samples from the endocardial layer of the left ventricle. When flow was less than 40% of normal, V and C had 55% infarction while 1 BOA had 37% (P < 0.05); at flow less than 20% of normal, V and C had 79% infarction while 1 BOA had 38% (P < 0.001); and at less than 10% of normal, V and C had 87% and 94% infarction, respectively, while 1 BOA had 58% (P < 0.001). Reducing blood oxygen affinity after coronary artery occlusion significantly decreased the extent of myocardial necrosis for the same degree of ischemia. Reducing BOA may increase oxygen delivery to ischemic myocardium when flow is restricted. Circ Res 49: 395-404, 1981

THE heart requires a constant supply of oxygen sufficient to meet its metabolic needs. It preferentially adjusts to increased metabolic demands by increasing blood flow, whereas increasing oxygen extraction plays a secondary role. When severe coronary artery stenosis or occlusion occurs, the metabolic needs of a region of the heart cannot be met, since adequate flow is not maintained. In this setting, enhancement of oxygen extraction might improve oxygen delivery. Previous work from this laboratory showed that, in normal resting dogs, an infusion of dihydroxyacetone, phosphate and pyruvate solution stimulated 2,3-diphosphoglycerate (2,3-DPG) production, leading to a significant reduction in blood oxygen affinity and enhanced oxygen extraction (Bristow et al, 1977a; Krall et al., 1978). In this study, we investigated whether a rapid reduction of blood oxygen affinity would decrease the extent of myocardial necrosis observed at specific levels of ischemia.

Methods

Surgical Preparation

Our methods are similar to the published techniques of Rivas et al. (1976). Mongrel dogs of either sex weighing between 17 and 26 kg were anesthetized with nitrous oxide and halothane. Through a left thoracotomy, the heart was suspended in a pericardial cradle. A polyvinyl catheter (o.d. = 1.9 mm and i.d. = 1.1 mm) was inserted into the left atrium through the appendage and secured with a purse-string ligature of 5-0 silk. It was filled with 1:100 aqueous heparin and the free end was knotted. The left anterior descending (LAD) coronary artery was dissected free just below the origin of the circumflex (CX) artery. A #3 monofilament suture was looped loosely around the artery and both ends were fed through a polyethylene catheter (o.d. = 1.7 mm and i.d. = 1.2 mm). This catheter was anchored adjacent to the artery to prevent rotation and was tied at its free end to prevent inadvertent occlusion of the artery. In later experiments, we placed the snares around the proximal circumflex because the LAD occlusion frequently resulted in a small infarction or none at all. The two catheters were brought out through the chest wall at a site above the thoracotomy incision and left with their free ends in the subcutaneous tissue.
Coronary Artery Occlusion

Experiments were performed 7–8 days after surgery with the dogs awake and lying on their right side. One hour after subcutaneous administration of 15 mg of morphine sulfate, the left atrial and coronary artery catheters were exposed under local lidocaine anesthesia. An additional catheter was inserted percutaneously into a femoral artery under local anesthesia and advanced to the abdominal aorta. Left atrial and aortic pressures and electrocardiogram (ECG) were monitored throughout the experiment. After a 45-minute stabilization period, lidocaine, 2 mg/kg, was given iv over several minutes followed by an infusion of 2 mg/min which was continued for the duration of the experiment. Fifteen minutes after the lidocaine infusion had started, the snare was tightened permanently, occluding the coronary artery. During the next 2-hour period, pressures and ECG were monitored continuously and recorded at 15-minute intervals.

Microsphere Injections

Fifteen minutes prior to and 2 hours after coronary artery occlusion, radiouclide spheres (15 ± 5 μm in diameter) (3M Company), labeled with either cerium-141 (141Ce) or strontium-85 (85Sr), were injected into the left atrium during a period of 15 seconds. A reference blood sample was withdrawn from the aorta at a constant rate for 1 minute, starting 5 seconds before the injection of microspheres. The number of spheres injected (between 1.5 and 2.5 × 10^6) was calculated for each animal so that a 1-g sample of myocardium (the smallest sample counted) contained approximately 600 spheres (Buckberg et al., 1971).

Experimental Groups

Beginning 15 minutes after the coronary artery occlusion, the dog received one of three possible therapies.

1. Reduced Blood Oxygen Affinity Group. As previously described (Bristow et al., 1977a), dogs received a solution of phosphate, pyruvate, and dihydroxyacetone at 20 ml/kg (maximum of 400 ml) over 90 minutes. This solution is described in Table 1; it significantly reduced blood oxygen affinity 2 hours after starting the infusion by stimulating erythrocyte 2,3-DPG production (Table 2).

2. Volume Group. These dogs received a solution of phosphate and pyruvate (Table 1) at 20 ml/kg (maximum of 400 ml) over 90 minutes. This solution did not alter blood oxygen affinity (Table 2) and was used to separate the effects of volume and pH from the effects of a reduced blood oxygen affinity.

3. Control Group. A control group of dogs received no therapy during the 2-hour period post-infarction.

Microsphere Counting

After the completion of the protocol, the dogs were returned to their quarters. Six to 9 days after the study, the dogs were killed with intravenous barbiturate. Immediately after the heart was removed, the coronary arteries were injected with cardiogreen dye to confirm the occlusion. The heart was then placed in 10% buffered formalin for 48 hours. The great vessels, atria, right ventricle, large epicardial vessels, and epicardial fat were removed. The left ventricle was weighed and cut into 24–36 pieces (Rivas et al., 1976); the exact number depended on the weight of the left ventricle. Each of these was divided into endocardial and epicardial halves, weighed, labeled, and placed in a counting vial with 10% buffered formalin. Each section weighed between 1 and 3 g. Each section was counted for 4 minutes in a Nuclear-Chicago Automatic Gamma Counting System equipped with a 3-inch crystal and a dual channel analyzer. The window for 141Ce was 50-400 KeV and for 85Sr was 400-650 KeV. Coronary blood flow to each section of tissue was expressed as ml/min per g (Heymann et al., 1977).

After samples had been counted, four vertical sections were cut from different levels of each piece of tissue and stained with hematoxylin and eosin. The percentage of infarcted tissue was determined for each piece of myocardium by the point-counting method (Weidel, 1963). A clear sheet with a hexagonal lattice of points was placed over the slide. The number of points overlying infarcted tissue was divided by the total number of points over the entire section, giving an estimate of the fraction of that section of tissue that was infarcted. The slides were evaluated independently by two individuals who had no knowledge of the calculated flow or the treatment the dog received. If agreement to within 10% between the two individuals did not occur, the slide was reviewed by both and concordance reached. This occurred for less than 5% of the sections. The percent infarction for each piece was the average of the two observers' determinations.

Blood Oxygen Affinity

Blood samples were drawn 15 minutes prior to and 2 hours after coronary artery occlusion, and the following variables were measured. Blood oxygen

Table 1 Description of Solutions used in Reduced Blood Oxygen Affinity and Volume Groups

<table>
<thead>
<tr>
<th></th>
<th>Reduced blood oxygen affinity group</th>
<th>Volume group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydroxyacetone</td>
<td>0.175 M</td>
<td>None</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>0.10 M</td>
<td>0.10 M</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.10 M</td>
<td>0.10 M</td>
</tr>
<tr>
<td>pH</td>
<td>8.1</td>
<td>8.6</td>
</tr>
<tr>
<td>Osmolality (mOsm/liter)</td>
<td>355</td>
<td>374</td>
</tr>
</tbody>
</table>

Both groups received 20 ml/kg (up to 400 ml) infused over 90 minutes with a Harvard pump.
Table 2  Erythrocyte 2,3-Diphosphoglycerate Concentration and \( P_\text{O}_2 \) for each Group

<table>
<thead>
<tr>
<th>Dog no</th>
<th>2,3-DPG (( \mu M/\text{g Hb} ))</th>
<th>( P_\text{O}_2 ) (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-ocl.</td>
<td>2 hr Post-ocl.</td>
</tr>
<tr>
<td>1</td>
<td>32.8</td>
<td>36.5</td>
</tr>
<tr>
<td>2</td>
<td>30.0</td>
<td>33.3</td>
</tr>
<tr>
<td>3</td>
<td>24.3</td>
<td>29.8</td>
</tr>
<tr>
<td>4</td>
<td>24.8</td>
<td>29.3</td>
</tr>
<tr>
<td>5</td>
<td>27.1</td>
<td>31.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>27.8 ± 3.6</td>
<td>32.1 ± 2.9*</td>
</tr>
</tbody>
</table>

Volume Group

<table>
<thead>
<tr>
<th>Dog no</th>
<th>2,3-DPG (( \mu M/\text{g Hb} ))</th>
<th>( P_\text{O}_2 ) (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.1</td>
<td>30.7</td>
</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>26.5</td>
</tr>
<tr>
<td>3</td>
<td>27.3</td>
<td>24.0</td>
</tr>
<tr>
<td>4</td>
<td>24.8</td>
<td>24.5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.8 ± 2.5</td>
<td>26.4 ± 3.0†</td>
</tr>
</tbody>
</table>

Control Group

<table>
<thead>
<tr>
<th>Dog no</th>
<th>2,3-DPG (( \mu M/\text{g Hb} ))</th>
<th>( P_\text{O}_2 ) (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4†</td>
<td>31.9</td>
</tr>
<tr>
<td>2</td>
<td>21.3</td>
<td>21.7</td>
</tr>
<tr>
<td>3</td>
<td>25.6</td>
<td>25.0</td>
</tr>
<tr>
<td>4</td>
<td>29.1</td>
<td>30.4</td>
</tr>
<tr>
<td>5</td>
<td>22.6</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>24.7 ± 3.5</td>
<td>24.9 ± 4.8†</td>
</tr>
</tbody>
</table>

Pre-ocl. = Pre-occlusion value; 2 hr Post-ocl. = 2 hours post-occlusion value
* \( P < 0.01 \).
† Not significant (\( P > 0.06 \)).
‡ Samples not obtained.

Blood oxygen affinity was measured as blood \( P_\text{O}_2 \) (the partial pressure of oxygen in torr required for 50% saturation of hemoglobin at plasma pH 7.40 and 38°C) by the mixing technique (Edwards and Martin, 1966). A decrease in blood oxygen affinity is defined by an increase in \( P_\text{O}_2 \). Hemoglobin concentration was measured as the absorbance of cyanmethemoglobin at wave length 450 nm on a Coleman spectrophotometer, model 6/20. 2,3-DPG was assayed by its activity in converting 3-phosphoglycerate to 2-phosphoglycerate in the presence of 2,3-DPG mutase (Atkinson, 1972).

Statistical Analysis

Results are reported as mean ± sd. Statistical significance was evaluated using an analysis of variance. Significance was \( P < 0.05 \).

Results

Surgical Procedure

Fourteen dogs were successfully studied and are reported on in this paper. Five were in the reduced blood oxygen affinity group, four in the volume group, and five in the control group. Fifteen dogs were excluded from analysis. Seven dogs (three with reduced blood oxygen affinity and four control dogs) did not have any histological infarction despite occlusion of the proximal coronary artery and ECG changes of acute ischemia. All these dogs had LAD occlusion, and this led us to change to circumflex artery occlusion. Seven dogs (four with reduced blood oxygen affinity, two volume, and one control) died within 18 hours of coronary artery occlusion. No cause of death could be identified at autopsy and, though the dogs were not monitored at the time, an arrhythmia was felt to be the most likely cause of death. These seven dogs were excluded because infarct size could not be determined accurately by our histological methods this early after infarction. One control dog was excluded because pre-occlusion flow and lack of ECG changes after tightening the snare indicated that the artery had become occluded sometime during the week prior to the experiment.

Blood Oxygen Affinity

Table 2 confirms that the dogs treated with phosphate, pyruvate, and dihydroxyacetonate had a statistically significant increase in 2,3-DPG concentration and in \( P_\text{O}_2 \) (decrease in blood oxygen affinity) at 2 hours post-occlusion. These changes are similar to our previously published results (Bristow et al., 1977a; Krall et al., 1978). The pre-occlusion and 2-hour post-occlusion values did not differ significantly in the volume and control dogs.

Hemodynamic Data

Pre-occlusion and 2-hour post-occlusion hemodynamic data are shown in Table 3. Hemoglobin concentration at 2 hours post-occlusion was not different from the pre-occlusion value in any group. Oxygen need was estimated by the heart rate and
mean arterial pressure product (Kitamura et al., 1972). Within each group, there was a significant increase 2 hours post-occlusion in this product compared to the pre-occlusion value; however, there was no significant difference in the rate-pressure product between the groups prior to or 2 hours after occlusion.

Table 4A shows that pre-occlusion and 2 hour post-occlusion flows were not significantly different between the groups. Two-hour post-occlusion endocardial flow to normal tissue increased to about the same level in all three groups (Table 4B).

Infarction Size

The initial experiments were done with left anterior descending artery occlusion, but this resulted in small areas of infarction or, in many dogs, none at all. We felt this was due to extensive collateral flow, especially through the septal perforator artery branch which was likely proximal to the snare. This led us to place the snare around the circumflex artery and, in general, the percent of the left ventricle infarcted was larger than with the left anterior descending artery occlusion, but considerable variation still occurred. Four dogs had LAD occlusion (one each in the control and reduced oxygen affinity groups and two in the volume group), and 10 had circumflex occlusion. The percentage of the left ventricle infarcted in each group (Table 5) was not significantly different.

Infarction vs. Blood Flow

The relationship between flow and the extent of necrosis for each group is shown in Figure 1. Most of the infarct was confined to the endocardial half of the ventricular wall, so results are presented for only that half. Each dot represents a 1- to 3-g (average 1.5-g) section of endocardium. The vertical axis shows the percent necrosis in a given section; the horizontal axis represents the mean flow to non-infarcted tissue. The number of points in a graph is determined by infarct size and left ventricular weight. Larger ventricles were cut into more pieces; this is why the reduced blood oxygen affinity group has more points than the control group. The volume group has the least points because it has one less dog than the other two groups. To deter-

Table 3 Hemodynamic Data Pre-occlusion and 2 Hours Post-occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Hemoglobin (g)</th>
<th>Heart rate (beats/min)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Rate-pressure product (x 10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 2 hr</td>
<td>P Value</td>
<td>Pre 2 hr</td>
<td>P Value</td>
</tr>
<tr>
<td>L BOA</td>
<td>15.0 ± 1.3</td>
<td>NS*</td>
<td>69 ± 136</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Volume</td>
<td>16.0 ± 1.7</td>
<td>NS</td>
<td>77 ± 126</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>13.7 ± 1.4</td>
<td>NS</td>
<td>75 ± 112</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NS = not significant (P > 0.05)

Table 4 Left Ventricular Blood Flow Measurements for Each Group

A. Pre-occlusion flow (ml/min per g) | B. 2 Hours post-occlusion flow (ml/min per g)

<table>
<thead>
<tr>
<th>Endocardium</th>
<th>Epicardium</th>
<th>Endo/Epi</th>
<th>Endocardium</th>
<th>Normal tissue</th>
<th>Necrotic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>L BOA</td>
<td>0.79 ± 0.26</td>
<td>0.65 ± 0.22</td>
<td>1.23 ± 0.09</td>
<td>1.14 ± 0.16</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>Volume</td>
<td>0.76 ± 0.25</td>
<td>0.63 ± 0.20</td>
<td>1.21 ± 0.06</td>
<td>1.05 ± 0.17</td>
<td>0.42 ± 0.10</td>
</tr>
<tr>
<td>Control</td>
<td>0.77 ± 0.26</td>
<td>0.63 ± 0.22</td>
<td>1.24 ± 0.06</td>
<td>1.09 ± 0.46</td>
<td>0.39 ± 0.23</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Two Hours Post-occlusion Flow

| L BOA       | 0.73 ± 0.16 | 0.66 ± 0.10 | 1.09 ± 0.09 |
| Volume      | 0.61 ± 0.16 | 0.75 ± 0.18 | 1.10 ± 0.13 |
| Control     | 0.77 ± 0.25 | 0.72 ± 0.34 | 1.06 ± 0.11 |
| P value     | NS          | NS          | NS          |

Pre-occlusion and 2 hours post-occlusion blood flow to endocardium and epicardium and the ratio of endocardial to epicardial flow were not significantly different between the groups (A). At 2 hours post-occlusion, the mean flow to non-infarcted endocardial tissue increased while the mean flow to infarcted endocardial tissue decreased about the same in each group (B). Only scattered areas of necrosis were present in the epicardial layer. NS = not significant (P > 0.05).
TABLE 5  Percent of Left Ventricle Infarcted in Each Group

<table>
<thead>
<tr>
<th>No./group</th>
<th>1 BOA</th>
<th>Volume</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5%</td>
<td>9.1%</td>
<td>12.6%</td>
</tr>
<tr>
<td>2</td>
<td>11.3%</td>
<td>13.0%</td>
<td>15.0%</td>
</tr>
<tr>
<td>3</td>
<td>13.7%</td>
<td>15.8%</td>
<td>16.9%</td>
</tr>
<tr>
<td>4</td>
<td>5.5%</td>
<td>10.6%</td>
<td>17.7%</td>
</tr>
<tr>
<td>5</td>
<td>26.6%</td>
<td></td>
<td>24.9%</td>
</tr>
</tbody>
</table>

Means ± SD 12.1 ± 9.1 12.1 ± 2.9 17.4 ± 4.2

1 BOA = reduced blood oxygen affinity

mine whether blood with low oxygen affinity reduced the extent of infarction resulting from a given reduction in blood flow, the extent of necrosis in sections of myocardium with similar reductions in flow were compared between the groups. The results are shown in Figure 2. The X-axis is divided into 10% flow ranges, and the height of the bar represents the mean percent necrosis to all sections within that flow range for each of the three groups. For comparable reductions in flow, there was less necrosis in myocardial tissue from dogs with reduced blood oxygen affinity than in the volume or control dogs. This difference became statistically significant when flow was reduced to below 40% of normal tissue flow.

Microsphere Technique

The mean pre-occlusion blood flow for the endocardial and epicardial layers of the left ventricle and the endocardial to epicardial ratio are shown for each group in Table 4. These results are similar to those reported by other workers using awake, closed-chest dogs (Neill et al., 1973; Cobb et al., 1974). To determine whether the presence of infarction affected the calculated pre-occlusion flow (i.e., the concentration of microspheres), the relationship between pre-occlusion flow and the percent necrosis in a section of myocardium was compared to the pre-occlusion flow to normal tissue for each group. Figure 3 shows this relationship. The extent of necrosis in a given section of tissue is shown on the X-axis, while the pre-occlusion ratio of the flow to infarcted areas vs. flow to normal areas is shown on the Y-axis. In two different regions of the heart, pre-occlusion flow should be similar and the ratio should be 1. In the dogs with reduced blood oxygen affinity and in control dogs, there was no significant change in this ratio as the percent of infarction increased. Thus, in these two groups, the extent of necrosis did not significantly affect the calculated pre-occlusion flow. In the volume group, the ratio was significantly lower than unity in tissue with greater than 20% infarction. In this group, the microsphere technique systematically underestimated preocclusion flow to tissue which subsequently suffered more than 20% necrosis.

Discussion

Blood Oxygen Affinity and Myocardial Oxygenation

It has been shown that an increase in blood oxygen affinity (leftward shift of the oxy-hemoglobin dissociation curve) causes adverse circulatory effects. Woodson et al (1973) showed that when P50 was decreased from 36 to 23 torr, the exercise capacity of rats decreased by 10%. Holsinger et al. (1973) infused blood depleted of 2,3-diphosphoglycerate into the circumflex coronary artery of a dog with an anterior infarction previously induced by occlusion of the left anterior descending artery. During the infusion of this high-affinity blood, the left ventricular end-diastolic pressure increased and ST segment elevation was noted, a response that

REGIONAL ENDOCARDIAL FLOW VS EXTENT OF NECROSIS

Figure 1 The percent necrosis in a sample of endocardium is plotted as a function of its blood flow (relative to that of normal myocardium) at 2 hours post-occlusion for each group. n = the number of points in each graph.
Figure 2 Using the data for Figure 1, the mean percent infarction is compared to its flow (over 10% flow ranges) in each group. For comparable reductions in flow, the group with reduced blood oxygen affinity (iBOA) had significantly less necrosis than the control and volume groups.

also occurred when the circumflex artery was transiently occluded. Moores et al. (1978) reported that when the hearts of pigs were perfused with blood having increased oxygen affinity, the stroke volume decreased significantly.

These adverse effects of an increased blood oxygen affinity suggest that a decrease in blood oxygen affinity might have beneficial circulatory effects. Work performed at this institution (Krall et al., 1978) showed that when blood oxygen affinity was reduced in healthy dogs by the solution used in this study, the animals responded with increased oxygen extraction by peripheral tissues (from 4.4 to 4.9 ml/kg per min) without a change in mixed venous Po2, and a concomitant, although not statistically significant, decrease in cardiac output (from 111 to 89 ml/kg per min). Oxygen consumption was the same in the two conditions.

Although coronary flow is the major determinant of oxygen delivery, myocardial oxygen extraction can be increased in a variety of conditions (Lombaro et al., 1953; Trenouth et al., 1976). Restorff et al. (1977) reported that an increase of myocardial oxygen extraction was an important mechanism to meet increased myocardial oxygen needs during exercise in dogs. During ischemia, oxygen extraction can increase to 80-85% (Shea et al., 1962; Daniell, 1973), and if anemia is also imposed, oxygen extraction can exceed 90% (Weber and Janicki, 1979). The advantage to the myocardium in using an increase in P50 to enhance oxygen extraction is that the increase in arteriovenous oxygen difference across the myocardium should occur without a change in the coronary sinus Po2. This would preserve the oxygen gradient between capillary blood and mitochondria, which is an important determinant of the diffusion rate of oxygen from blood to tissue. Thus, an increase in P50 could deliver additional oxygen to tissue without a change in coronary flow or in capillary Po2.

The 2- to 3-torr increase in P50 achieved in this study theoretically could increase oxygen extraction by 10-15% at a coronary sinus Po2 of 20 torr; that means an additional 1-2 ml of oxygen/100 ml of
A decreased blood oxygen affinity decreases infarction. Pantely et al. 401

The relationship between extent of necrosis and pre-occlusion flow is shown in Figure 3. The extent of necrosis in a given section of tissue is shown on the X-axis, while the pre-occlusion ratio of flow to necrotic areas vs. normal areas is shown on the Y-axis. The number of pieces of myocardium in each bin is indicated at the base of each column. The dashed horizontal line is the line of unity. Ratios significantly less than unity indicate "loss" of spheres from necrotic tissue. This occurred only in the volume group in sections with greater than 20% necrosis. 'Different from unity at \( P < 0.001; + \) Different from unity at \( P < 0.01; \downarrow \text{BOA} = \) reduced blood oxygen affinity.

Flow. This is supported by a report that indicated a decrease in blood oxygen affinity occurs across the myocardial vascular bed as a compensatory response to improve oxygen delivery during transient ischemia (Shappell et al., 1970); in one patient a 2.9-torr increase in \( P_{so} \) delivered an additional 1.7 volumes of oxygen per 100 ml of flow without a change in coronary flow or in coronary sinus \( P_{O_2} \). The increase in \( P_{so} \) was not mediated by 2,3-DPG. We did not measure the \( P_{so} \) of the coronary sinus blood to determine whether blood oxygen affinity changed across the myocardium during ischemia. If it did occur, our results would indicate that our method of rapidly stimulating 2,3-DPG production was additive to the spontaneous mechanism operating to reduce blood oxygen affinity during ischemia. This would be similar to the report that the greatest oxygen extraction during myocardial ischemia occurred when anemia was present (Weber and Janicki, 1979); anemia enhances oxygen delivery by increasing erythrocyte 2,3-DPG concentration.

In our study, we used a dog preparation and experimental protocol similar to one reported by Rivas et al. (1976). They showed that there was no significant change in endocardial flow between measurements made 2 and 6 hours after occlusion, and that the flow to a section of myocardium 2 hours after occlusion was an excellent predictor of the extent of necrosis found histologically 6-9 days later. The relationship they reported between the extent of infarction and flow in the endocardial layer at 2 hours post-occlusion is very similar to our results in the control and volume groups; in addition, we have shown that this relationship can be altered favorably by a reduction in blood oxygen affinity (Fig. 2). A difference between the reduced blood oxygen affinity group compared to the control and volume groups first appeared when flow was reduced to below 70% of normal tissue flow and was consistently noted when flow was reduced to below 40% of normal.

The reason for the lack of consistent benefit with lesser amounts of ischemia (Fig. 3) is not certain. It is probably due, in part, to the insensitivity of the techniques used; a beneficial effect becomes increasingly difficult to demonstrate as the amount of necrosis and the degree of ischemia diminish. Rivas et al. (1976) noted a similar result when they compared the extent of infarction and flow in the epicardial and endocardial layers of the left ventricle. They reported that when samples in the same range of flow were compared, the extent of infarction in endocardial samples exceeded that in epicardial
samples. This was most apparent with severe reductions in flow, whereas little difference was noted with slight reductions in flow.

Some sections of severely ischemic myocardium from each dog with reduced blood oxygen affinity did not show a decrease in the extent of infarction (Fig. 2). Studies have shown that if a coronary artery is occluded for at least 40 minutes and then released, a portion of the myocardium in the center of the ischemic region will be irreversibly damaged. The size of this region increases the longer the artery is occluded (Reimer et al., 1977; Darsee and Kloner, 1980). Since our solution was infused between 15 and 90 minutes post-occlusion, some tissue already had been irreversibly damaged. All the sections of myocardium from dogs with decreased blood oxygen affinity that did not show a decrease in the extent of infarction, on histological examination, contained relatively preserved necrotic muscle without hemorrhage or inflammation; these histological findings indicate that these sections represent the central ischemic core of the infarction which is the region of earliest cell death (Bishop et al., 1976; Reimer et al., 1977). However, our intervention did salvage a portion of the myocardium that was viable, despite 1–2 hours of ischemia.

We can only assume that less necrosis was present at similar reductions in flow because blood with reduced oxygen affinity delivered more oxygen. The \( P_{50} \) is measured under standard laboratory conditions and measures only a potential for enhanced oxygen delivery; we did not measure oxygen consumption of the entire heart or of the ischemic region. The major determinants of oxygen supply and demand that were measured (hemoglobin, rate-pressure product, and flow) were not significantly different between the groups. The only significant difference noted was the reduced blood oxygen affinity in the dogs treated with dihydroxycetone, phosphate, and pyruvate. Thus, our assumption seems reasonably justified. Two preliminary reports support our assumption. Apstein et al. (1980) used an isolated rabbit heart preparation and reported improved myocardial contractile function and enhanced myocardial oxygen consumption when the heart was perfused with blood having reduced oxygen affinity. Rude et al. (1980) used ortho-iodosodium benzolate (OISB) to decrease blood oxygen affinity and reported a reduction in infarct size in dogs treated with OISB compared to controls.

The components of our solution were selected for their ability to increase 2,3-DPG concentration and \( P_{50} \); our purpose was to determine whether a decrease in blood oxygen affinity was beneficial during myocardial ischemia. Other effects of the solution which we did not measure could contribute to the benefit observed. (1) The phosphate in the solution may have transiently lowered serum calcium. Hypercalcemia does reduce inotrophy and myocardial oxygen consumption (Seifen et al., 1964; Bristow et al., 1977b). (2) The components of the solution are glycolytic intermediates which could enhance aerobic and anaerobic metabolism. Liedtke and coworkers (Liedtke et al., 1976; Liedtke and Nellis, 1978) have shown that buffer pyruvate increased aerobic and anaerobic metabolism and improved function in the working ischemic swine heart. The two possibilities listed above could decrease the extent of infarction without an increase in flow. (3) The solution is hypertonic. Leaf et al. (1972) have reported that an infusion of mannitol improved left ventricular function and increased collateral flow to the ischemic region. (4) There is a gradual increase in flow to the severely ischemic myocardium beginning 6 hours post-occlusion, too late to be of benefit (Bishop et al., 1976; Schaper and Pasyk, 1976; Reimer et al., 1977). It is possible that our solution enhanced the degree and rapidity with which flow returned to the ischemic region. Decreased inotropy, enhanced metabolic function, and benefits due to hypertonicity may be less likely possibilities because the solution of pyruvate and phosphate infused in the volume group did not decrease the extent of infarction.

Although we would have liked to show a decrease in infarct size between the groups, the finding of no significant difference in infarct size in each group was not unexpected. A high ligation of the left anterior descending or circumflex coronary artery (within 1 cm of its bifurcation) has been reported to result in damage to 4–35% of the left ventricle (Bishop et al., 1976; Rivas et al., 1976). This variability is felt to be due to differences in coronary anatomy and the extent of collateral flow between dogs. Our experience confirms these observations. This variability makes it very difficult to determine whether an intervention decreased infarct size, especially with small groups. The occlusion of the LAD in four dogs and the circumflex in 10 dogs in our study probably increased the variability. Despite all this, a trend toward smaller infarcts was present in four dogs in the reduced blood oxygen affinity group; this is not evident in the group mean because the fifth dog had a large infarction. Nevertheless, no conclusions can be drawn from our study about the effect of a reduction of blood oxygen affinity on left ventricular infarct size.

Errors in Microsphere Technique

Several groups have presented evidence of a significant error when microspheres were used to measure pre-occlusion flow to myocardium that subsequently was made ischemic or became necrotic. Capurro et al. (1979) used 15-µm spheres and noted a 20% decrease in pre-occlusion flow to ischemic tissue as early as 24 hours and as late as 8 days post-infarction. Jugdutt et al. (1979) used 10-µm spheres and reported an average 19% decrease in pre-occlusion flow when dogs were killed at 12–14, 48, and 96 hours post-occlusion. They estimated that 40% of the decrease in calculated flow was due
to edema, whereas 60% was due to physical loss. A report by Reimer and Jennings (1979) indicates that the time after infarction at which spheres were counted will affect the microsphere method of measuring flow to necrotic tissue. At 4 days post-infarction, calculated preocclusion flow to ischemic tissue was 34% below normal tissue flow, but this increased to greater than 300% of normal tissue flow at 28 days post-infarction. They did not attribute these changes to sphere loss, but suggested that "expansion" of the infarct by edema, cellular infiltrate, and hemorrhage was responsible for the initial decrease in calculated flow after tissue became ischemic; at 28 days, scar formation led to "contracture" of the tissue and an increase of calculated flow to necrotic tissue compared to normal tissue.

We found no evidence of "sphere loss" in tissue that became necrotic in either the control or reduced blood oxygen affinity group (Fig. 3). There was some "sphere loss" in the volume dogs. When the extent of infarction vs. flow is plotted in this group, this "sphere loss" would tend to show an erroneous beneficial effect in the volume group; that is, it would show less infarction for a given blood flow compared to the control group. The amount of "sphere loss" was not sufficient, however, to make the relationship between infarction and flow in the volume group significantly different from the control group. It does not appear that the benefit observed in the dogs with reduced blood oxygen affinity was due to inaccuracies (sphere loss or infarct expansion/contraction) in the microsphere technique of measuring flow to tissue that subsequently became necrotic.

The results shown in Figure 3 fit best with the infarct expansion and contraction concept (Reimer and Jennings, 1979). At 7-9 days post-infarction, tissue edema had apparently resolved in the control and reduced blood oxygen affinity groups, giving the same pre-occlusion flow to necrotic and normal tissue. In the volume group, sections of myocardium with greater than 20% necrosis may still have contained edema fluid, giving a slightly reduced pre-occlusion flow.

In conclusion, this study has shown that, for the same degree of endocardial ischemia, an infusion of pyruvate, phosphate, and dihydroxyacetone after coronary artery occlusion significantly decreased the extent of myocardial necrosis. This probably was due, in part, to the reduction of blood oxygen affinity that allowed enhanced oxygen delivery to and extraction by the ischemic myocardium. Other association effects of the solution also may have been involved. An acute reduction in blood oxygen affinity may be useful to increase oxygen delivery when flow is restricted and oxygen demand exceeds supply.

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