Salvage of Ischemic Myocardium by Dipyridamole in the Conscious Dog

DAVID S. BLUMENTHAL, M.D., GROVER M. HUTCHINS, M.D.,
BODH I. JUGDUTT, M.B., AND LEWIS C. BECKER, M.D.

SUMMARY We investigated the effect of i.v. dipyridamole, a potent small-vessel coronary vasodilator, on myocardial infarct size in conscious dogs. Dipyridamole, 7-9.7 μg/kg (15 dogs) or saline (15 dogs) was infused for 6 hours beginning 10 minutes after acute permanent occlusion of the mid-circumflex coronary artery. After sacrifice, 48 hours after occlusion, stereoscopic postmortem angiography was used to define the mass of the occluded coronary bed. Infarct size was determined by planimetry of weighed, unstained left ventricular slices. Dipyridamole produced a striking reduction in mean infarct mass compared with control (3.1 g vs 13.2 g, p < 0.001), while mean occluded bed mass was similar (30.3 g vs 32.7 g, NS). As a percentage of the occluded bed, mean infarct size was reduced from 36.8% to 8.6% (p < 0.001). Mean arterial blood pressure declined approximately 10% after dipyridamole. Heart rate and left atrial pressure did not change significantly. Collateral blood flow, measured with 8-μ radioactive microspheres, increased in all regions during dipyridamole infusion. The infarct center and border regions had sustained increases over 6 hours of 23-80%, while nonischemic regions demonstrated a diminishing response over time, with a large (98-125%) increase 10 minutes after infusion and a smaller (22-25%) increase 6 hours later. Although antplatelet or local metabolic effects cannot be excluded, the myocardial salvage produced by dipyridamole was most likely due to the increase in collateral blood flow.

DRUGS that dilate the coronary arteries are often categorized by their predominant site of action, the large epicardial conductance arteries or the small, pre- capillary (arteriolar) resistance vessels. Large-vessel dilators, such as nitrates or slow-channel calcium blockers, have been shown to reduce ischemic injury during coronary artery occlusion by increasing collateral flow,1-4 reducing myocardial oxygen demands5,7 or both; small-vessel dilators have produced variable results. In some studies these agents have produced a deleterious effect by diverting flow away from ischemic myocardium toward nonischemic regions — a so-called coronary steal. This problem has been associated in the dog with the presence of occlusive lesions in more than one coronary artery.8 In other reports, hypotension resulting from the systemic effects of these small-vessel dilators has led to reduced coronary perfusion pressure and diminished collateral flow.

This study was designed to determine whether dipyridamole, a prototype coronary arteriolar dilator, could augment collateral blood flow and reduce ultimate infarct size in conscious dogs with permanent coronary artery occlusion. By using a model with a single coronary artery occlusion and by using dipyridamole in small doses that produced minimal decreases in blood pressure, we hypothesized that beneficial effects on myocardial salvage would be found.

Methods

Instrumentation

Thirty-eight dogs, 18-24 kg, were instrumented under pentobarbital general anesthesia. A left thoracotomy was performed and an occluder snare placed around the left circumflex coronary artery just past the first large marginal branch, 2-3 cm from the aorta. The snare consisted of a silk thread that was attached to a plastic tube at one end and looped around the coronary artery before exiting through the same plastic tube. Polyethylene catheters were placed in the right external jugular vein, right common carotid artery and left atrium. The distal ends of these catheters and the tube containing the snare thread were externalized at the back of the neck through a subcutaneous tunnel. Penicillin (1 million units) and Streptomycin (1 g) were given intramuscularly after surgery and the catheters were filled with heparinized saline.

Experiment

The experimental protocol was performed 1 week later on the 36 surgical survivors. The dogs stood in a restraining sling and morphine, 0.25 mg/kg i.v., was given for sedation and analgesia. Continuous recording of left atrial and aortic pressures (Statham P23Db transducers) and lead II of the ECG were made on a direct-writing recorder. Myocardial blood flow measurements were made using 7-10-μ diameter radioactive microspheres8 with Tween-80 added, labeled with 125I, 141Ce, 99Sr, 99Nb, or 48Sc (3M Company). Microsphere vials were agitated for 3-5 minutes. Approximately 2 million microspheres, followed by a 5-ml saline flush, were injected into the left atrium for each measurement. Starting just before injection and con-
continuing for 2 minutes, reference blood samples were withdrawn at the constant rate of 2.17 ml/min by a calibrated Harvard pump. The first myocardial blood flow measurement was made 30 minutes after the morphine was given. Lidocaine (1 mg/kg i.v.) was then administered. Five minutes later, coronary occlusion was established by tightly pulling the free external end of the silk thread and then clamping the thread to the plastic tube. Six dogs died from ventricular fibrillation within the first 5 minutes. A second microsphere measurement was made 5 minutes after occlusion in the 30 survivors. The dogs were then randomly allocated to receive dipyridamole or saline. In the experimental group an initial bolus was given of 5 mg of dipyridamole (obtained in 2-ml ampules containing 10 mg dipyridamole, 4 mg tartaric acid, and 100 mg polyethylene glycol 600 [Boehringer Ingelheim]). This dose was based on pilot studies in four conscious dogs with acute coronary occlusions and represented the amount required to decrease mean arterial blood pressure by about 10%. Fifteen minutes after the bolus injection, corresponding to the peak action of i.v. dipyridamole, a third flow measurement was made. Then, a continuous infusion of dipyridamole was begun to deliver 5 mg i.v. every 30 minutes, based on observations that the duration of action of i.v. dipyridamole is 30-40 minutes. The dipyridamole was mixed with normal saline to a volume of 30 ml, all of which was infused over 6 hours. Control dogs received a similar volume of saline. Microsphere measurements were made again at 1 hour and 6 hours after occlusion. Phasic and mean arterial and left atrial pressures and six-lead ECGs were made before and after each microsphere injection. No attempt was made to suppress ventricular ectopy after coronary occlusion. At the end of the 6-hour experiment the dogs were returned to their cages. Two control dogs died during the next 2 days. At 48 hours after occlusion, the 28 survivors were brought back for electrocardiographic and hemodynamic recordings in the fully conscious state. A lethal dose of anesthetic was given, and the hearts were removed, washed free of blood and weighed.

Measurement of Infarct Size, Mass of Occluded Bed, and Regional Myocardial Blood Flow

The size of the occluded coronary bed was measured by postmortem angiography. Cannulas were placed in the origins of the right, left anterior descending and circumflex coronary arteries and simultaneous injections were made of a barium sulfate-gelatin mass at a controlled pressure of 160 mm Hg. After packing with gauze to maintain diastolic relationships, the hearts were fixed in formalin and radiographed stereoscopically. Completeness of occlusion was confirmed by an abrupt interruption of the barium column between the proximal circumflex filled by antegrade injection and the distal circumflex filling retrograde via collaterals. Each heart was sliced into four or five transverse sections 1.2-1.4 cm thick. Pairs of wire markers were placed at opposite points through the wall of the ventricle in each section and paired stereoscopic radiographs were made. Using the whole-heart images and the radiographs for each slice, an independent observer marked the boundaries of the occluded coronary bed by following the course of each major coronary branch from ring to ring and examining the patterns of interdigitation of terminal branches. Retrograde filling of the occluded bed via collaterals from the nonoccluded vessels enabled definition of the border between coronary branches. We have previously shown good interobserver reproducibility with this technique. The formalin-fixed heart slices were dissected free of the atria, right ventricle, large epicardial vessels and fat. After weighing, the top and bottom surfaces of each slice were traced on plastic transparencies, thereby outlining the left ventricle. Areas of infarction were identified by gross inspection and marked on each tracing. In general, the infarct consisted of a relatively pale area, surrounded by an area of hemorrhage 1-4 mm wide. The latter frequently had islands of normal-appearing myocardium intermixed. The outer margin of the infarct was marked along the border of hemorrhage, recognizing that small amounts of normal myocardium would necessarily be included, but insuring that border regions adjacent to the infarct would be free of necrosis. The tracing of each ring was superimposed on the corresponding radiograph to permit precise transfer of the marked boundaries of the occluded bed. Tracings and radiographs were aligned using both natural markers (cavity and wall shape) and the wire markers. Areas of left ventricle (whole ring), occluded coronary bed, and infarct were measured by electronic planimetry for each ring, and top and bottom surface areas were averaged for left ventricle and infarct. Masses of infarct and occluded coronary bed were calculated by multiplying the weight of each ring by the ratio of infarct and occluded bed area to the area of the corresponding whole ring.

Twenty-three dogs (10 dipyridamole-treated and 13 control) had visible infarcts, in each case centered around the posterior papillary muscle. Surrounding each infarct was a region of myocardium that lay within the boundary of the occluded bed but was visibly normal. The spatial distribution of infarct within the occluded coronary bed was determined in these control and dipyridamole-treated animals by creating average ring-by-ring maps for the two groups. For each ring, the center of the infarct on the endocardial surface was marked. Distances along the endocardium from the infarct center to the edges of the occluded bed and the edges of the infarct were measured to the nearest millimeter. At each of these points the width of the myocardial ring was determined. The thickness of the infarct was measured at the infarct center and at points on the endocardial surface midway between the infarct center and each edge. Corresponding measurements were then averaged and areas of occluded coronary bed and infarct were electronically planimetered for each of these average rings.
in each treatment group. Four rings were constructed in each group. In dogs with five rather than four left ventricular rings, the apical one was discarded for this analysis.

Sampling for regional myocardial blood flow was made transmurally (0.5–3.0-g specimens) in the center of the infarct, in the normal tissue within the occluded bed on either side of the infarct (border zone) and in the region of the nonischemic anterior papillary muscle. Seven dogs (five dipyridamole-treated and two control) had no grossly visible infarct in any ring. Samples in these cases were taken transmurally through the anterior and posterior papillary muscle in each ring. Samples were divided into inner and outer halves and each of these was weighed. Samples were placed in vials of formalin and counted for radioactivity along with reference blood samples in a well-type gamma scintillation counter (Packard model 5986) at energy windows adjusted to the peak emission from each of the five nuclides. Regional myocardial blood flow (F) was calculated using the formula: $F = R \times Cm / Cr$ (ml/min/g), where $R$ = reference blood flow pump withdrawal rate, $Cm$ = counts per gram in the myocardial tissue sample and $Cr$ = counts in the reference blood sample. Flows for corresponding regions in each ring were combined (weighted averaging) to yield single values for infarct, border and nonischemic regions. Flows were then corrected for true and apparent microsphere loss. Flow values in each region for each time interval were corrected using a factor specific for the individual dog and region. The preocclusion content of microspheres in each ischemic region expressed relative to that in the nonischemic area was used as a quantitative measure of the combined effects of microsphere loss, local edema, hemorrhage and inflammatory cell infiltrate. Inner flow values were corrected using preocclusion subendocardial anterior wall flows and outer flow values were corrected using subepicardial flows. Thus, the following formula was used: 

$$F_e = F \times \frac{A}{P},$$

where $F_e$ = corrected flow, $F$ = uncorrected flow, $P$ = preocclusion flow in the sampling region and $A$ = preocclusion flow in the corresponding nonischemic region.

### Histology

The entire occluded bed of the middle left ventricular ring was excised and embedded in paraffin. Histologic sections (average four per animal) were stained with hematoxylin-eosin to correlate microscopic and gross estimates of necrosis.

### Statistics

Differences within and between groups for hemodynamic data and measurements of infarct, left ventricular, and occluded coronary bed masses were calculated using paired and unpaired $t$ tests. Linear regression analysis for comparison of infarct mass and occluded coronary bed mass was done by the least-squares method and the significance of $r$ values, slopes and intercepts was calculated. The significance of changes in sequential flow measurement was determined by analysis of variance with orthogonal contrast within groups, while groups were compared by trend analysis. Values are mean ± sem.

### Results

Of the 36 dogs that underwent coronary occlusion, six developed ventricular fibrillation within the first 5 minutes and were not studied. The remaining 30 dogs were allocated to dipyridamole or saline treatment (15 in each group). In the next 2 days two other dogs died, both controls. These two dogs survived more than 24 hours after occlusion and had clearly defined infarcts, and were therefore included in the analysis. The difference in survival to 48 hours (100% vs 87%) was not statistically significant.

### Effect of Dipyridamole on Infarct Size

Dipyridamole treatment resulted in a 77% reduction in infarct size compared with controls (table 1). Myocardial salvage was significant when results were expressed in terms of absolute infarct mass, infarct mass normalized to left ventricular mass, or infarct mass normalized to occluded bed size. Control and dipyridamole treated groups were similar with respect to mean left ventricular mass and occluded coronary bed size.

Although occlusions were made at the same anatomic site in all dogs, the masses of occluded bed and infarct in control animals were variable. Figure 1 shows the relationship between developed infarct mass and mass of the occluded coronary bed for control and dipyridamole-treated dogs. The slope of the linear regression was less for the dipyridamole group than for the control group (0.37 ± 0.08 vs 0.95 ± 0.14, $p < 0.005$), indicating less infarction for a given size of occluded bed. The horizontal-axis intercept for the dipyridamole group was slightly displaced to the right compared with controls (22.4 ± 0.9 vs 18.8 ± 2.3 g; NS). Because the intercepts were forced to a similar value by inclusion of animals with small occluded bed

| Table 1. Masses of Infarct, Occluded Coronary Bed, and Left Ventricle |
|-------------|-------------|-------------|-------------|-------------|-------------|
|             | Control     | Dipyridamole |             |             |             |
|             | (n = 15)    | (n = 15)     |             |             |             |
| Infarct mass (g) | 13.2 ± 2.2 | 3.1 ± 0.9 | < 0.001    |             |             |
| Mass of occluded coronary bed (g) | 32.7 ± 2.1 | 30.3 ± 1.9 | NS          |             |             |
| Left ventricular mass (g) | 90.2 ± 4.4 | 89.5 ± 4.8 | NS          |             |             |
| Infarct/LV (%) | 16.4 ± 3.7 | 3.7 ± 1.1 | < 0.005    |             |             |
| Occluded coronary bed/LV (%) | 34.7 ± 1.9 | 33.9 ± 1.4 | NS          |             |             |
| Infarct/occluded coronary bed (%) | 36.8 ± 5.5 | 8.6 ± 2.3 | < 0.001    |             |             |

Abbreviation: LV = left ventricle.
mass, the data were reexamined for dogs with occluded bed mass greater than 25 g. The slope of the linear regression was still less for the dipyridamole-treated group (0.53 ± 0.13 vs 0.88 ± 0.18), although the difference was no longer statistically significant. The extrapolated horizontal-axis intercepts, however, were now significantly different (25.7 ± 1.0 g vs 17.2 ± 2.0 g, p < 0.001), implying that a greater minimum mass of occluded coronary bed was required for infarction to occur in dipyridamole-treated dogs.

Figure 2 shows the average spatial distribution of infarction within the occluded bed in the 10 dipyridamole-treated and 13 control dogs that developed infarcts after coronary occlusion. Both groups show a tapering of infarct and occluded bed from base to apex. Although the areas of the occluded bed in each ring were comparable, the extent of infarct was much less in the dipyridamole group. Myocardial salvage was both subepicardial and lateral and occurred to a comparable extent in each ring.

Seven dogs had no visible infarction, five from the dipyridamole group and two from the control group. Occluded bed mass was smaller in these dogs than in the 23 dogs with infarcts (23.0 ± 2.4 g vs 34.0 ± 1.7 g, p < 0.005). Left ventricular mass was also smaller, although the difference was not statistically significant (77.4 ± 9.1 g vs 99.5 ± 4.8 g, NS). Both dogs in the control group without infarct had small occluded bed masses (19.0 and 24.3 g), but this was true for only three of the five dipyridamole-treated dogs (16.0, 21.2, and 21.4 g). The other two dogs had occluded bed masses of 27.9 and 28.8 g, values which invariably have been associated with infarction in the absence of treatment.

**Hemodynamic Changes (table 2)**

Heart rate, mean arterial pressure and left atrial pressure were similar before coronary occlusion in the two groups. Occlusion caused a significant increase in mean heart rate, a small rise in mean left atrial pressure and no change in mean arterial pressure. The changes 5 minutes after occlusion were similar in control and dipyridamole groups (pretreatment values). During the remainder of the experiment, the control group showed no significant hemodynamic changes, although heart rate, mean arterial pressure and mean left atrial pressure decreased slightly. In contrast, the dipyridamole-treated group showed a decrease in blood pressure immediately after the initial i.v. bolus that progressed throughout the experiment. The fall in mean arterial pressure averaged 10% at 15 minutes after treatment (123 ± 5 to 111 ± 5 mm Hg; NS), while at 1 hour after treatment the mean arterial pressure was reduced by 12% (to 108 ± 5 mm Hg; p < 0.05) and at 6 hours by 16% (to 103 ± 4 mm Hg; p < 0.05). The 6-hour mean arterial pressure in the dipyridamole group was 12% less than that in the control group (103 ± 4 vs 117 ± 4 mm Hg, p < 0.005). Heart rate and left atrial pressure showed slight decreases comparable to those in the control group.

Compared with the 23 dogs with infarcts, the seven without infarction tended to have slower heart rates and lower mean arterial and left atrial pressures, both before occlusion and 5 minutes after occlusion (before treatment was begun). None of the differences were statistically significant except for postocclusion mean.
## TABLE 2. Hemodynamic Changes

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Left atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dipyridamole</td>
<td>Control</td>
</tr>
<tr>
<td>Before occlusion</td>
<td>100 ± 6*</td>
<td>94 ± 7*</td>
<td>120 ± 4</td>
</tr>
<tr>
<td>5 minutes after</td>
<td>120 ± 7</td>
<td>114 ± 7</td>
<td>121 ± 4</td>
</tr>
<tr>
<td>occlusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes after</td>
<td>118 ± 7</td>
<td>116 ± 9</td>
<td>121 ± 5</td>
</tr>
<tr>
<td>dipyridamole or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>114 ± 5</td>
<td>110 ± 6</td>
<td>119 ± 5</td>
</tr>
<tr>
<td>6 hours</td>
<td>113 ± 4</td>
<td>110 ± 9</td>
<td>117 ± 4†</td>
</tr>
</tbody>
</table>

All values are mean ± SEM.

* *p < 0.05; †p < 0.01 vs value 5 minutes after occlusion (paired t test).

Regional Myocardial Blood Flow

Coronary occlusion decreased blood flow throughout the occluded bed. These changes were significant in all regions except the outer border zone in control dogs (table 3). The reduction in flow was greatest in the infarct center, with the subendocardium showing a greater reduction than the subepicardium. Similarly, the border region reductions were less than those for the infarct center, again with greater subendocardial than subepicardial reductions. The dipyridamole and control groups had similar flows in the infarct center after occlusion (before treatment), but border region flows after occlusion were lower in the dipyridamole group. Tissue for this region was taken from the edge of the infarct in both groups. Dipyridamole produced smaller infarcts; thus, the border zone in this group was located closer to the center of the occluded bed.

Over the 6 hours after occlusion, flow did not change in the control dogs. In contrast, dipyridamole

## TABLE 3. Regional Myocardial Blood Flow in All Dogs Developing Infarction

<table>
<thead>
<tr>
<th></th>
<th>Dipyridamole (n = 10)</th>
<th>Infarct</th>
<th>Before occlusion</th>
<th>5 minutes after occlusion</th>
<th>15 minutes after bolus</th>
<th>1 hour after infusion</th>
<th>6 hours after infusion</th>
<th>p‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner</td>
<td>1.10 ± 0.18</td>
<td>0.10 ± 0.02†</td>
<td>0.17 ± 0.05</td>
<td>0.18 ± 0.06</td>
<td>0.18 ± 0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>0.96 ± 0.17</td>
<td>0.35 ± 0.03*</td>
<td>0.43 ± 0.07</td>
<td>0.46 ± 0.07</td>
<td>0.55 ± 0.06</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Border</td>
<td>Inner</td>
<td>1.13 ± 0.18</td>
<td>0.53 ± 0.03*</td>
<td>0.84 ± 0.17</td>
<td>0.68 ± 0.10</td>
<td>0.86 ± 0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>0.94 ± 0.17</td>
<td>0.57 ± 0.03*</td>
<td>0.91 ± 0.16</td>
<td>0.83 ± 0.10</td>
<td>0.92 ± 0.06</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>Inner</td>
<td>1.10 ± 0.18</td>
<td>1.02 ± 0.12</td>
<td>2.30 ± 0.74</td>
<td>1.07 ± 0.17</td>
<td>1.27 ± 0.12</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>0.95 ± 0.16</td>
<td>0.91 ± 0.08</td>
<td>1.94 ± 0.69</td>
<td>1.03 ± 0.14</td>
<td>1.11 ± 0.09</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>Infarct</td>
<td>Inner</td>
<td>0.99 ± 0.08</td>
<td>0.09 ± 0.05†</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>1.00 ± 0.12</td>
<td>0.32 ± 0.04†</td>
<td>0.36 ± 0.06</td>
<td>0.36 ± 0.06</td>
<td>0.37 ± 0.07</td>
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<td></td>
</tr>
<tr>
<td>Border</td>
<td>Inner</td>
<td>0.99 ± 0.08</td>
<td>0.73 ± 0.09†</td>
<td>0.75 ± 0.09</td>
<td>0.72 ± 0.08</td>
<td>0.76 ± 0.10</td>
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</tr>
<tr>
<td></td>
<td>Outer</td>
<td>1.00 ± 0.12</td>
<td>1.09 ± 0.18</td>
<td>1.12 ± 0.17</td>
<td>1.06 ± 0.15</td>
<td>1.00 ± 0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>Inner</td>
<td>0.99 ± 0.08</td>
<td>1.07 ± 0.08</td>
<td>1.10 ± 0.08</td>
<td>1.03 ± 0.04</td>
<td>1.02 ± 0.10</td>
<td>NS</td>
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<tr>
<td></td>
<td>Outer</td>
<td>1.00 ± 0.11</td>
<td>1.16 ± 0.13</td>
<td>1.18 ± 13</td>
<td>1.03 ± 0.06</td>
<td>1.05 ± 0.11</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

* *p < 0.05; †p < 0.001 vs preocclusion (paired t test).

Significance of changes over time (5 minutes–6 hours) using analysis of variance with orthogonal contrast.
caused an increase in flow in the infarct center and in
the border region 15 minutes after the i.v. bolus, which
continued at 1 and 6 hours during the infusion. The
eyearly increase in flow was 23–70% and the 6-hour in-
crease 50–90% in the various regions within the
occluded bed. Analysis of variance revealed sta-
tistically significant flow increases in the infarct outer
zone, the border inner zone and the border outer
zones. The infarct inner zone flow changes did not
reach statistical significance by analysis of variance
\( (p < 0.09) \). Compared with control dogs, dipyri-
damole-treated dogs had significantly higher flows in
the infarct center, while border region flows in the
dipyridamole group increased to levels similar to those
in controls.

In dipyridamole-treated dogs without visible infarction
(table 4), postocclusion flows in the occluded bed
( pretreatment) were significantly higher than in dogs
with infarction (0.39 ± 0.06 vs 0.10 ± 0.2 ml/min/g, \( p < 0.05 \); and 0.62 ± 0.08 vs 0.35 ± 0.03 ml/min/g, \( p < 0.05 \), for inner and outer zones of occluded bed,
respectively). However, postocclusion subendocardial
flow in the center of the occluded bed was at a level
frequently associated with necrosis. \(^{11} \) Treatment with
dipyridamole increased flow in that region to
0.65 ± 0.13 ml/min/g 15 minutes after the initial i.v.
bolus and to 0.84 ml/min/g 6 hours after infusion,
values that have been correlated with tissue survival. \(^{11} \)
In the nonoccluded region flow did not change in
controls. In the dipyridamole-treated dogs, two- to
threefold increases in flow were seen at 15 minutes,
although the increases at 1 and 6 hours were smaller.

Figure 3 is a comparison of flow vs infarct size in
control and dipyridamole-treated dogs. Transmural
blood flow in the center of the occluded coronary bed 1
hour after saline or dipyridamole infusion was com-
pared with the mass of infarct normalized for the mass
of occluded coronary bed. Flows greater than 0.5
ml/min/g were associated with little or no necrosis.
For flows less than 0.5 ml/min/g, infarct size in-
creased approximately linearly as flow decreased.
These data suggest that dipyridamole produced myo-
cardial salvage by increasing collateral flow. If there
had been a flow-independent effect, some points
should have appeared in the low flow–small infarct
region.

Table 4. Regional Myocardial Blood Flow in Five Dogs Receiving Dipyridamole That Did Not Develop Infarction

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>5 minutes after occlusion</th>
<th>15 minutes after bolus</th>
<th>1 hour after infusion</th>
<th>6 hours after infusion</th>
<th>( p ) value for time trend†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center of occluded bed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner</td>
<td>1.01 ± 0.23</td>
<td>0.39 ± 0.06*</td>
<td>0.65 ± 0.13</td>
<td>0.64 ± 0.13</td>
<td>0.84 ± 0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>Outer</td>
<td>0.97 ± 0.25</td>
<td>0.62 ± 0.08</td>
<td>1.23 ± 0.39</td>
<td>0.85 ± 0.12</td>
<td>1.10 ± 0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>LAD region</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inner</td>
<td>1.01 ± 0.23</td>
<td>0.84 ± 0.15</td>
<td>2.43 ± 1.08*</td>
<td>1.05 ± 0.11</td>
<td>1.80 ± 0.42</td>
<td>0.05</td>
</tr>
<tr>
<td>Outer</td>
<td>0.97 ± 0.25</td>
<td>0.85 ± 0.14</td>
<td>2.17 ± 0.95</td>
<td>1.07 ± 0.06</td>
<td>1.59 ± 0.27</td>
<td>0.05</td>
</tr>
</tbody>
</table>

All values are means ± SEM.

* \( p < 0.05 \) vs pre-occlusion by paired \( t \) test.
† Using analysis of variance.

Abbreviation: LAD = left anterior descending coronary artery.

FIGURE 3. Transmural blood flow in the center of the occluded coronary bed 1 hour after occlusion is compared to mass of infarct normalized for the mass of the occluded coronary bed. The flow vs necrosis relationship appears similar in each group except that dipyridamole-treated dogs generally had higher flows and less necrosis.

Histology

Representative sections from the infarct center and
border zone of the middle ring were examined. The
infarct center had 100% histlogic necrosis, while the
degree of epicardial necrosis was variable. All border
zone samples had less than 1% necrosis, confirming
the gross observation that these were infarct-free
regions lying within the occluded coronary bed.

Discussion

Dipyridamole, a potent small-vessel coronary vas-
dilator, produced a marked reduction in infarct size
and a large increase in collateral blood flow in con-
scious dogs with permanent coronary artery occlusion.
In this study, dipyridamole was associated with a 77%
reduction in the extent of infarction.

Roberts et al. \(^{14} \) recently reported a 56% reduction in
infarct mass when dipyridamole, 3 mg/kg, was in-
flushed i.v. for 45 minutes in anesthetized dogs subjected
to permanent left anterior descending coronary artery
occlusion. Although the mass of occluded coronary
bed was not determined, control and treatment groups
were similar with respect to the amount of ST-
segment elevation 30 minutes after occlusion and just before treatment. Mean arterial pressure decreased 20% with dipyridamole infusion. Dogs were killed at 24 hours and infarct mass was measured using nitroblue tetrazolium staining and planimetry. Regional myocardial blood flow was not determined.

Other authors have used changes in ST-segment elevation as a measure of dipyridamole's effect on ischemic myocardium in anesthetized animals. The variable results may be explained by overriding blood pressure changes. Watanabe et al. found that ischemic region ST-segment elevation increased as blood pressure fell 28% during dipyridamole infusion, and concluded that the adverse effect was directly related to reduced coronary perfusion pressure. In contrast, Becker found that ischemic ST-segment elevation decreased during dipyridamole infusion when blood pressure was maintained.

We found that dipyridamole increased collateral blood flow throughout the occluded bed. The percent increase was largest in the subendocardium of the infarct center, although the absolute flow increase was least in this region. All other regions within the occluded bed demonstrated substantial flow increases, which were present 15 minutes after i.v. dipyridamole was begun and were sustained at 6 hours. In the anesthetized dog with one-vessel coronary occlusion, dipyridamole has been shown to increase, or have no effect on regional myocardial blood flow measured by a variety of techniques. Authors who found regional myocardial blood flow to decrease with dipyridamole attributed the adverse effect to decreases in mean arterial pressure of 27-28%. In contrast, in the current study with conscious dogs, mean arterial blood pressure in the dipyridamole-treated group was, at most, 12% reduced compared with controls. Pasyk et al. also used a conscious dog model and found dipyridamole had no effect on collateral blood flow measured by Xe clearance. However, only four dogs were studied, and relatively small doses of dipyridamole (3-5 mg) were used.

Sphere Loss
We used radioactive microspheres to estimate regional myocardial blood flow. Recent work suggests certain limitations of this technique in myocardial infarction because of true microsphere dropout and apparent sphere loss caused by local edema and inflammatory reaction. We attempted to circumvent this problem by correcting all flow values for sphere loss using differences in flow values before occlusion as a measure of combined true and apparent loss. We made the assumption that sphere loss was equal for each microsphere since all spheres were injected over 6 hours and the dogs were not sacrificed until 42 hours later. The amount of true plus apparent sphere loss ranged from 16% in the subendocardium of the infarct center to 4% in the outer part of the border zone. The amount of loss was somewhat less in the dipyridamole-treated dogs than in the controls, although collateral flow was higher and more sphere washout might have been expected. This was probably related to the myocardial salvage produced by dipyridamole.

Mechanism of Myocardial Salvage
Myocardial salvage by dipyridamole most likely resulted from an increase in collateral blood flow, although the mild reduction in afterload, with concomitant reduction in myocardial oxygen demand, may also have contributed. Dipyridamole blocks erythrocyte uptake of adenosine, leading to increased tissue adenosine levels. Like adenosine, it dilates primarily smaller resistance vessels in the coronary circulation, although larger conductance vessels are probably less affected. During maximal small-vessel dilatation produced by myocardial ischemia, large-vessel effects of dipyridamole can be observed. A reduction in collateral resistance by dipyridamole could be accounted for by dilatation of arteries within nonischemic regions supplying the collateral flow, large vessels within ischemic areas (which do not dilate during ischemia), or the collateral vessels themselves, but it would be difficult to attribute to small-vessel dilatation on the arteriolar level. Small-vessel effects, on the other hand, probably explain the increases in flow in nonischemic regions and possibly also in less ischemic areas located peripherally within the occluded bed.

Effects on prostaglandins could also have helped to produce myocardial salvage. Dipyridamole inhibits thromboxane synthetase and may potentiate the effects of prostacyclin, leading to decreased platelet aggregation. Because platelet trapping in ischemic regions has been demonstrated and could result in reduced collateral flow and further necrosis, thromboxane A inhibition by dipyridamole could be beneficial. In support of this hypothesis, Kraikitch et al. found that dipyridamole decreased epinephrine-induced myocardial injury that is attributed to platelet aggregation.

Another theoretical mechanism for salvage is a possible effect of dipyridamole on the decrease in myocardial ATP during ischemia and the hypothesis that loss of high-energy phosphates may contribute to irreversible myocardial injury. Prolonged infusions of adenosine increase myocardial ATP levels in a variety of species under nonischemic conditions. In addition, inosine, another product of ATP breakdown, has been shown to ameliorate ATP loss during ischemia. By increasing myocardial levels of adenosine, dipyridamole might reduce myocardial ATP loss and thereby exert a favorable metabolic effect upon ischemic myocardium.

Difficulties in the Use of Dipyridamole
The net effect on regional myocardial blood flow of a potent small-vessel dilator such as dipyridamole depends on the balance between its effect on peripheral, large coronary vessel, and small-coronary-vessel effects. Beneficial effects upon blood flow
to ischemic areas could be entirely negated by either systemic blood pressure reductions or by redistribution of myocardial blood flow resulting in a so-called myocardial steal. Thus, although a mild reduction in mean arterial pressure, with reduction in afterload, might be beneficial, an excessive fall in coronary perfusion pressure could be detrimental by decreasing collateral blood flow. In one-vessel stenosis, excessive small-vessel dilatation in the distribution of the narrowed artery could increase the pressure drop across the narrowing and reduce myocardial perfusion to the ischemic region, particularly the subendocardium. Similarly, if myocardium in the distribution of an occluded artery were dependent on collateral flow from an adjacent stenotic vessel, then vasodilatation in the distribution of the stenotic vessel might decrease the driving pressure for collateral flow to the occluded bed and thereby decrease blood flow in the ischemic region.

Previous work with dipyridamole in the anesthetized dog subjected to acute coronary occlusion supports these considerations. A dose of 1 mg/kg dipyridamole given to dogs with one-vessel coronary occlusion decreased blood pressure 31% and had no effect on regional flow in the ischemic zone. When blood pressure was supported with methoxamine, flow increased 72% above baseline. In contrast, when arteries adjacent to the occlusion were stenosed, 1-1.5 mg/kg i.v. dipyridamole decreased collateral flow in the ischemic region, even with blood pressure maintained constant.

In conclusion, dipyridamole increases regional myocardial blood flow and decreases infarct size when given intravenously to conscious dogs subjected to acute coronary artery occlusion. Extrapolation from this experimental result to man should be made with caution. Because of theoretical considerations, we used an experimental design with one-vessel coronary occlusion, normal adjacent coronary arteries, and a dose of dipyridamole producing minor hypotension. The presence of stenoses in adjacent vessels or the development of significant hypotension might have eliminated the observed myocardial salvage. However, as the trend toward early cardiac catheterization during acute myocardial infarction continues, patients with isolated one-vessel stenoses may be identified. In these subjects, carefully titrated dipyridamole should be most effective.

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