Effect of angioplasty-induced endothelial denudation compared with medial injury on regional coronary blood flow

ERIC R. BATES, M.D., MARK J. MCGILLEM, B.S., TED F. BEALS, M.D., SCOTT F. DEBOE, B.S., JUDITH K. MIKELSON, M.D., G. B. JOHN MANCINI, M.D., AND ROBERT A. VOGEL, M.D.

ABSTRACT To determine the effect of angioplasty-induced arterial injury on regional coronary blood flow, resting and postocclusion reactive hyperemic flows were measured in the left anterior descending (LAD) and circumflex (LCx) coronary arteries of 32 dogs after one of five interventions in the LAD with a balloon angioplasty catheter: group A, no injury; group B, endothelial denudation; group C, medial injury; group D, pretreatment with 325 mg of aspirin 2 hr before medial injury. Resting flows did not change in any group. In group C, hyperemic flow decreased in both the LAD and LCx by 15% to 20% (p < .001) over 30 to 90 min, suggesting that a circulating substance changed coronary resistance. Histologic and ultrastructural studies of the LADs demonstrated an intact endothelial cell layer in group A, endothelial disruption with a few adherent platelets in group B, medial injury with a dense layer of adherent platelets in group C, and medial injury with a few adherent platelets in group D. Thus endothelial denudation results in relatively mild platelet deposition and no change in resting or hyperemic coronary blood flow. In contrast, medial injury results in relatively marked platelet deposition and a significant decrease in hyperemic flow, both of which are prevented by platelet inhibition with aspirin.


THE MORPHOLOGIC CONSEQUENCES of coronary angioplasty consistently include splitting of the atheromatous plaque and stretching of the media and adventitia. These have been described in both animal preparations1–5 and in human postmortem arteries.6–8 Electron microscopic examinations have also demonstrated that angioplasty produces endothelial denudation followed by rapid platelet deposition.1, 9–15 Occasionally, local arterial injury results in vasospasm or thrombosis.15–17 Possible contributing mechanisms include decreased production of prostacyclin18, 19 or endothelin-derived relaxing factor,20, 21 by endothelial cells or increased local release of serotonin,22, 23 thromboxane,24, 25 or other factors by activated platelets. Although it has been shown that endothelial denudation potentiates vasoconstrictor responses26, 27 and that medial injury produces macroscopic mural thrombus formation,11, 12, 14 little is known about the effect of angioplasty on regional coronary blood flow. The purpose of this study was to determine the effect of angioplasty-induced endothelial denudation vs medial injury on regional basal and hyperemic coronary blood flow.

Methods

Protocol. Mongrel dogs of either sex weighing 25 to 35 kg were anesthetized with intravenous sodium pentobarbital (35 mg/kg) and ventilated with room air via a Harvard respirator. Electrocardiographic limb leads were attached to monitor heart rate and rhythm. The left carotid artery and jugular vein were dissected free for 2 to 3 cm. An intravenous catheter was placed in the vein for fluid and drug administration. A No. 9F angiographic introducer sheath was placed in the artery for introduction of angiographic catheters. A left thoracotomy was performed and the heart was suspended in a pericardial cradle. Then the proximal left anterior descending (LAD) and left circumflex (LCx) coronary arteries were dissected free for 2 cm, and appropriately sized and calibrated electromagnetic flow probes (Model EP200; Carolina Medical Electronics, King, NC) were placed on each artery. A pneumatic cuff occluder was placed distal to each flow probe. A high-fidelity micromanometer catheter (Millar Instruments, Houston) was passed through the apex of the heart into the left ventricular cavity to measure systolic blood pressure, left ventricular end-diastolic pressure, and the first derivative of left ventricular pressure (dP/dt).

Continuous recordings of heart rate, systolic pressure, left...
ventricular end-diastolic pressure, dP/dt and LAD and LCx blood flow were obtained on an eight-channel physiologic recorder (Model 2800S; Gould Electronics, Cleveland). Three to five measurements of basal coronary blood flow and hyperemic coronary blood flow after a 20 sec occlusion were made in the LAD and LCx. One of four protocols was then performed. Group A (n = 8) had no intervention made and served as the control group to confirm hemodynamic and coronary blood flow stability in the experimental preparation. Group B (n = 8) underwent endothelial denudation of the LAD. A No. 8F Amplatz angioplasty guiding catheter (USCI, Billerica, MA) was inserted through the arterial sheath and positioned in the LAD. Through it, a 3.0 × 20 mm balloon angioplasty catheter (USCI) was advanced into the LAD until the balloon was just distal to the flow probe. Endothelial denudation was accomplished by inflating the balloon to 1 atm (ACS indeflator, Mountain View, CA) and gently passing the catheter down and up the artery for 15 sec. Group C (n = 8) underwent medial injury of the LAD. The balloon catheter was positioned as above, and the balloon was inflated until the catheter was immobile (2 to 5 atm). Two 60 sec inflations separated by 60 sec were performed. Group D (n = 8) was pretreated with 325 mg of aspirin 2 hr before undergoing medial injury as described for group C.

Baseline measurements were made immediately before balloon inflation. All hemodynamic and flow measurements were repeated at 30, 60, and 90 min after the intervention. At the conclusion of each experiment, 400 mg of Evans blue dye dissolved in 20 ml of isotonic saline was injected intravenously. Five minutes later the dog was killed by injecting intravenously 20 meq of potassium chloride, and the heart was extirpated. The coronary tree was flushed with buffer to remove nonadherent cells and serum proteins. Then the LAD and LCx were opened longitudinally and inspected for blue staining, which signifies endothelial trauma.20 The section of the LAD distal to the flow probe was excised and fixed in 2% glutaraldehyde.

Pathologic examination. All arteries were patent without visible evidence for thrombus formation. Blue staining of the subendothelium was easily seen in each artery where the flow probes and pneumatic occluders were placed. Light staining of the LAD was present in group B arteries at the site of endothelial denudation. Darker staining was present in group C and D arteries at the site of medial injury. No staining was present where arterial trauma was not inflicted.

Light microscopy confirmed that the appropriate depth of injury was accomplished in each group (figure 2). Group A arteries had an intact endothelial cell layer. The internal elastic lamina and media were normal. Endothelial cells were absent in group B arteries, but the internal elastic lamina and media were normal. In group C and D arteries, endothelial cells were absent, the internal elastic lamina was disrupted or absent, and the media showed interstitial edema and smooth muscle cell swelling consistent with acute injury. In addition, focal hemorrhage and accumulation of acute inflammatory cells were occasionally seen.

Scanning electron microscopic examination in group A arteries demonstrated intact endothelial cells with surface microvilli (figure 3). Disruption of the endothelial cell layer was present in group B arteries. A few adherent platelets were seen on both endothelial cells and exposed basal lamina. In group C and D arteries, portions of fragmented elastic lamina, strands of fibrin, and exposed collagen and cells within the media were seen. The luminal surfaces in group C arteries were coated with a dense layer of adherent platelets, whereas the number of adherent platelets in group D arteries was considerably decreased.

Discussion

The platelet–vessel wall interaction has been an area of intense investigation for several years. Most investigators have not employed preparations in vivo with intact circulations. Those who have usually have uti-
TABLE 1
Hemodynamic data (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No intervention (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>146 ± 7</td>
<td>142 ± 7</td>
<td>143 ± 8</td>
<td>143 ± 7</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>126 ± 5</td>
<td>129 ± 6</td>
<td>128 ± 5</td>
<td>125 ± 5</td>
</tr>
<tr>
<td>RPP (×10⁻²)</td>
<td>185 ± 13</td>
<td>185 ± 16</td>
<td>185 ± 16</td>
<td>185 ± 15</td>
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<tr>
<td>LVEDP (mm Hg)</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Peak (+) dP/dt (mm Hg/sec)</td>
<td>2075 ± 72</td>
<td>2125 ± 100</td>
<td>2138 ± 93</td>
<td>2175 ± 103</td>
</tr>
<tr>
<td>Peak (−) dP/dt (mm Hg/sec)</td>
<td>2288 ± 81</td>
<td>2350 ± 144</td>
<td>2313 ± 86</td>
<td>2250 ± 96</td>
</tr>
<tr>
<td>B. Endothelial denudation (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>150 ± 6</td>
<td>152 ± 6</td>
<td>150 ± 6</td>
<td>149 ± 7</td>
</tr>
<tr>
<td>SBP</td>
<td>141 ± 5</td>
<td>140 ± 6</td>
<td>139 ± 5</td>
<td>140 ± 4</td>
</tr>
<tr>
<td>RPP</td>
<td>214 ± 8</td>
<td>211 ± 7</td>
<td>207 ± 29</td>
<td>208 ± 30</td>
</tr>
<tr>
<td>LVEDP</td>
<td>10 ± 1</td>
<td>10 ± 2</td>
<td>10 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Peak (+) dP/dt</td>
<td>2263 ± 212</td>
<td>2250 ± 198</td>
<td>2275 ± 196</td>
<td>2337 ± 191</td>
</tr>
<tr>
<td>Peak (−) dP/dt</td>
<td>2600 ± 140</td>
<td>2613 ± 139</td>
<td>2619 ± 149</td>
<td>2600 ± 131</td>
</tr>
<tr>
<td>C. Medial injury (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>151 ± 9</td>
<td>150 ± 9</td>
<td>148 ± 11</td>
<td>149 ± 12</td>
</tr>
<tr>
<td>SBP</td>
<td>134 ± 5</td>
<td>134 ± 5</td>
<td>139 ± 6</td>
<td>140 ± 5</td>
</tr>
<tr>
<td>RPP</td>
<td>205 ± 18</td>
<td>203 ± 18</td>
<td>211 ± 23</td>
<td>211 ± 22</td>
</tr>
<tr>
<td>LVEDP</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>10 ± 2</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Peak (+) dP/dt</td>
<td>2263 ± 167</td>
<td>2025 ± 165^b</td>
<td>2163 ± 181</td>
<td>2025 ± 188^b</td>
</tr>
<tr>
<td>Peak (−) dP/dt</td>
<td>2425 ± 161</td>
<td>2344 ± 117</td>
<td>2500 ± 144</td>
<td>2513 ± 148</td>
</tr>
<tr>
<td>D. Aspirin/medial injury (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>147 ± 10</td>
<td>147 ± 10</td>
<td>148 ± 10</td>
<td>146 ± 11</td>
</tr>
<tr>
<td>SBP</td>
<td>140 ± 19</td>
<td>139 ± 5</td>
<td>139 ± 5</td>
<td>140 ± 5</td>
</tr>
<tr>
<td>RPP</td>
<td>208 ± 19</td>
<td>206 ± 19</td>
<td>207 ± 20</td>
<td>206 ± 21</td>
</tr>
<tr>
<td>LVEDP</td>
<td>9 ± 2</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Peak (+) dP/dt</td>
<td>2119 ± 135</td>
<td>1914 ± 95^a</td>
<td>1981 ± 87</td>
<td>1875 ± 80^a</td>
</tr>
<tr>
<td>Peak (−) dP/dt</td>
<td>2481 ± 134</td>
<td>2436 ± 140</td>
<td>2475 ± 133</td>
<td>2419 ± 151</td>
</tr>
</tbody>
</table>

HR = heart rate; LVEDP = left ventricular end-diastolic pressure; RPP = rate-pressure product; SBP = systolic blood pressure.

^ap < .05; ^bp < .01; all p values indicate differences from baseline.

![LAD ARTERY](image1)

![LCX ARTERY](image2)

FIGURE 1. Percent change in hyperemic coronary blood flow after LAD intervention. ● = group A; △ = group B; ■ = group C; □ = group D. *p < .05; **p < .01.
lized pharmacologic stimuli in their protocols to evaluate endothelial cell and platelet function. Coronary blood flow changes after arterial injury, such as those that occur after angioplasty, have not been well described. This study is important because it demonstrates the effect of arterial injury on regional coronary blood flow in an intact circulation without pharmacologic stimuli.

**Endothelial denudation.** Vascular endothelial cells actively mediate vascular tone in part by producing prostacyclin, a vasodilator that also inhibits platelet aggregation, and endothelium-derived relaxing factor, a substance that potentiates vasodilator responses and inhibits vasoconstrictor responses of vascular smooth muscle cells to various neurohumoral stimuli. Potentiation of vasoconstrictor responses by nonphysiologic doses of pharmacologic stimuli after balloon endothelial denudation has been demonstrated. The absence of basal or hyperemic coronary blood flow changes in this study suggests that changes in the release of prostacyclin and endothelium-
derived relaxing factor and the degree of platelet activation induced by endothelial denudation alone have little physiologic effect on blood flow in an intact circulation without coronary stenoses or atherosclerosis.

**Medial injury.** The vascular injury resulting from angioplasty consistently includes splitting and stretching of the media with subsequent smooth muscle cell necrosis. Quantitative platelet deposition after medial injury is increased at least 10-fold compared with that stimulated by endothelial denudation alone. Qualitative evidence of this phenomenon is demonstrated in the present study. Furthermore, this study demonstrates that LAD medial injury results in a significant decrease in hyperemic coronary blood flow in both the LAD and LCx. Prevention of platelet deposition and hyperemic flow changes by aspirin implicates a platelet-associated mechanism as the major stimulus for the blood flow perturbation.

The exact mechanism by which hyperemic blood flow was decreased after medial injury remains under investigation. Arterial thrombosis did not occur. The cyclic reductions in coronary blood flow attributed to episodic embolization of platelet-fibrin microthrombi in stenotic arteries was not seen. Although arterial spasm can occur after angioplasty, even the 40% reduction in diameter noted by Lam et al. in a similar arterial injury model is not sufficient to change hyperemic blood flow in nonatherosclerotic arteries. The parallel decrease in hyperemic blood flow in both the LAD and LCx suggests that a circulating substance may have changed coronary arteriolar resistance. Since intercoronary collaterals are numerous in the dog, and usually epicardial in location, it is likely that cross-circulation occurs between the LAD and LCx. Prevention of the decrease in hyperemic blood flow by pretreatment with aspirin suggests that products of the cyclooxygenase pathway of prostanoid metabolism are involved. Prostaglandins play an important role in vessel wall interactions after vascular injury, and preliminary data exist regarding their response to angioplasty. Cragg et al. have reported that prostacyclin production was reduced in the dog carotid arterial wall after angioplasty, whereas vasoconstrictor hydroperoxy acids were increased. The same group also demonstrated at 20-fold increase in blood flow through the mural vasa vasorum after angioplasty that was significantly attenuated by aspirin. No short-term increase in coronary venous thromboxane production was demonstrated in two clinical studies after uncomplicated angioplasty, although increased levels were noted by Peterson et al. in two
patients in whom angioplasty was complicated by arterial occlusion. In contrast, Mehta et al.\textsuperscript{35} recently demonstrated thromboxane release immediately after uncomplicated angioplasty. No data exist regarding prostaglandin release more than 5 min after angioplasty. Serotonin is another vasoactive substance released by activated platelets, but its response to angioplasty-mediated arterial injury is not known.

\textbf{Clinical implications.} Thrombosis, arterial spasm, and restenosis are platelet-mediated responses that have limited the immediate and long-term efficacy of angioplasty.\textsuperscript{16, 36} The influence of platelet inhibitor drugs on these complications has been incompletely studied, in part because there are no reliable indexes by which to measure interference of platelet function in man.\textsuperscript{36} Aspirin, heparin, and dextran have been given empirically to patients, although animal studies using electron microscopy and indium-111 platelet scintigraphy to evaluate platelet deposition have not conclusively demonstrated efficacy.\textsuperscript{9, 12, 37-39} However, Cunningham et al.\textsuperscript{40} studied men who had received aspirin 12 to 96 hr before angioplasty and demonstrated significantly reduced platelet deposition by indium-111 platelet scintigraphy.

This study adds perspective to previous studies showing platelet-thrombus deposition after medial injury\textsuperscript{11, 12, 14, 15} by demonstrating an associated decrease in hyperemic blood flow that was inhibited by aspirin. Previous studies attempting to evaluate platelet inhibition therapy during angioplasty in which only endothelial denudation was accomplished\textsuperscript{37} did not reproduce the deep arterial injury resulting from angioplasty and therefore were inconclusive, probably because platelet deposition was not adequately stimulated. The conclusion by O'Gara et al.\textsuperscript{37} that platelet function after angioplasty could not be studied in the canine preparation is disputed by our study, which reproduced the arterial injury caused by angioplasty, showed both platelet activation and reactive hyperemic flow changes, and demonstrated inhibition of platelet activation and flow changes with aspirin.

\textbf{Limitations.} In this study we used electron microscopy to evaluate platelet deposition qualitatively in the dog coronary artery instead of using the quantitative

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Light microscopy. \textit{A}, Group A, intact endothelial cell layer. \textit{B}, Group B, absent endothelial cell layer with normal internal elastic lamina and media. \textit{C} and \textit{D}, Groups C and D, absent endothelial cell layers and internal elastic lamina; interstitial edema in the media with smooth cell swelling consistent with acute injury.}
\end{figure}
techniques employed by the investigators evaluating deep arterial injury in the pig carotid artery. Since both preparations are able to produce medial injury and to cause a several-fold increase in platelet deposition, the qualitative technique used in this study is probably sufficient. The angioplasty technique in this study is actually more reproducible in producing medial injury because balloon inflation pressure was adjusted in each artery rather than held constant regardless of arterial size. Also, the degree of injury is probably less significant, since no total or near thrombotic occlusions occurred, as were reported for the other preparation. Consistent changes in hyperemic coronary blood flow were obtained in each artery, which allowed the relatively small change in hyperemic flow of 15% to 20% to reach statistical significance.

The problem of extrapolating results in normal animal arteries to human atherosclerotic arteries has been recently summarized. It is likely, however, that arterial injury and platelet activation after clinical angioplasty are greater than they are after angioplasty in a nonatherosclerotic animal artery. Therefore, the decrease in hyperemic coronary blood flow described in this report might be less than it would be in patients not pretreated with aspirin before angioplasty.

In summary, endothelial denudation results in relatively mild platelet deposition and no change in resting or hyperemic coronary blood flow. In contrast, medial injury results in relatively marked platelet deposition and a significant decrease in hyperemic blood flow, both of which are inhibited by antiplatelet therapy with aspirin. This study suggests that this canine preparation can be used to test the efficacy of antithrombotic or other platelet inhibitor drugs in angioplasty.

We thank Diane Bauer for her excellent assistance in the preparation of this manuscript.

References


12. Lam J, Chesebro JH, Steele PM, Badimon L, Fuster V: Production of tears into the media during arterial angioplasty predisposes to platelet-thrombus deposition. J Am Coll Cardiol 5: 520, 1985 (abst)


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