Brief Antecedent Ischemia Attenuates Platelet-Mediated Thrombosis in Damaged and Stenotic Canine Coronary Arteries

Role of Adenosine

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**Background**—Recent studies suggest that patients with angina before myocardial infarction exhibit improved recovery of coronary perfusion after thrombolysis by an as-yet-unknown mechanism. We therefore proposed that brief antecedent ischemia/reperfusion may, via release of adenosine, improve vessel patency in damaged and stenotic coronary arteries.

**Methods and Results**—Anesthetized dogs underwent coronary injury + stenosis, resulting in repeated cyclic variations in coronary blood flow (CFVs) caused by the formation/dislodgment of platelet-rich thrombi. Vessel patency was assessed for 3 hours after stenosis by quantification of the nadir of the CFVs, duration of total thrombotic occlusion (flow = 0), and area of the flow-time profile (expressed as percent of baseline flow \( \times 180 \) minutes). In protocol 1, dogs received 10 minutes of coronary occlusion +10 minutes of reflow or a comparable 20-minute control period before injury + stenosis. The median nadir of the CFVs was higher (4.0 versus 0.3 mL/min), median zero flow duration per 30-minute time interval was shorter (0.4 versus 15.1 minutes), and mean percent flow-time area was greater (54±8% versus 28±9%) in dogs that received antecedent ischemia versus controls (\( P < .05 \)). These benefits of antecedent ischemia/reperfusion were largely mimicked by a 10-minute intracoronary adenosine infusion (400 \( \mu \)g/min) in lieu of brief ischemia (protocol 2) and were abolished by administration of the adenosine \( \text{A}_1/\text{A}_2 \) receptor antagonist PD 115,199 (3 mg/kg IV) before brief antecedent coronary occlusion (protocol 3).

**Conclusions**—Brief antecedent ischemia attenuates subsequent platelet-mediated thrombosis in damaged and stenotic canine coronary arteries, due, in large part, to an adenosine-mediated mechanism. ([Circulation. 1998;97:692-702.]

**Key Words:** adenosine ■ ischemia ■ stenosis ■ thrombosis ■ platelets

Numerous laboratory studies have documented that brief episodes of ischemia protect or “precondition” the myocardium and reduce infarct size caused by a subsequent more prolonged ischemic insult. Brief antecedent ischemia also has, in some instances, been reported to confer protection in the clinical setting. For example, analysis of data from the TIMI-4 and TIMI-9B trials revealed that patients with myocardial infarction preceded by angina had smaller infarct sizes and better in-hospital outcome after thrombolytic therapy than patients without preinfarction angina.

Although the mechanism(s) responsible for these clinical observations remain unresolved, Andreotti et al recently reported that in patients with acute myocardial infarction preceded by unstable angina, thrombolytic therapy resulted in smaller infarct sizes and accelerated reperfusion compared with patients without preinfarction angina. This suggests that, despite the unquestionable beneficial effects of PC on myocyte viability per se, the improved myocardial salvage seen in patients with preinfarct angina may be due, at least in part, to a favorable association between antecedent ischemia and more rapid restoration of myocardial blood flow. However, in contrast to the intensive investigation aimed at infarct size reduction with PC, the consequences of brief ischemia on subsequent coronary patency have, to date, been largely unexplored. Using a well-established canine model of spontaneous, platelet-mediated coronary thrombosis, we therefore sought to determine whether (1) brief episodes of antecedent “PC” ischemia improve vessel patency in damaged and stenotic coronary arteries and (2) release of adenosine (a potent inhibitor of platelet aggregation) during brief ischemia/reperfusion plays a role in this phenomenon.
Methods

This study was approved by the Institutional Animal Care and Use Committee of the Hospital of the Good Samaritan (an institution accredited by the American Association for the Accreditation of Laboratory Animal Care) and conforms to the principles endorsed in the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

Surgical Preparation

Fifty-two mongrel dogs weighing between 14 and 27 kg were anesthetized with sodium pentobarbital (30 mg/kg IV), intubated, and ventilated with room air. After the left jugular vein (for administration of fluids and drugs) and the left carotid artery (for measurement of heart rate and arterial pressure) were cannulated, the heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. Two adjacent segments of the LAD were isolated, usually midway along its course. The distal segment was instrumented with a Doppler flow probe (Transonic Systems Inc) for continuous measurement of mean CBF, and the proximal segment served as the site of later injury+stenosis. Arterial pressure and CBF were continuously recorded throughout each experiment on a Gould recorder (Gould Inc).

Initiation of Platelet-Mediated Coronary Thrombosis

After 10 minutes of stabilization, baseline measurement of hemodynamics and CBF, and a 20- to 30-minute intervention period (described in detail below and illustrated in Fig 1), the isolated LAD segment was gently squeezed with a hemostat to induce arterial injury. A plastic micromanometer constrictor was then positioned around the site of injury and tightened such that mean CBF was reduced to \( \approx 30\% \) of its baseline value. This procedure, first described by Folts et al, triggers the development of CFVs caused by the repeated spontaneous accumulation and dislodgment of platelet-rich thrombi at the site of injury+stenosis (Fig 2) and mimics many of the fundamental pathophysiologic components of both thrombotic occlusion resulting in acute myocardial infarction, and repeated ischemia in instances of unstable angina after primary angioplasty and after initial thrombolysis. If progressive and spontaneous reduction in CBF was not observed within \( \approx 5\) minutes after application of the stenosis, the constrictor was slightly tightened: this process was repeated until a gradual decrease in CBF was obtained. Once initial spontaneous platelet aggregation was established, CBF was monitored for 3 hours without further intervention.

At the end of the 3-hour observation period, cardiac arrest was produced under deep anesthesia by intracardiac injection of KCl. Because the severity of arterial injury is recognized to be a crucial determinant of platelet-mediated thrombosis and generation of CFVs, the damaged LAD segment was collected from all dogs and stored in 10% neutral buffered formalin for later histological evaluation.

Experimental Design

The study consisted of three separate protocols (Fig 1).

**Protocol 1: Effect of Brief Antecedent Ischemia on Platelet-Mediated Thrombosis**

To address our first objective (ie, to determine whether brief antecedent PC ischemia attenuates platelet-mediated thrombosis and improves subsequent vessel patency), the first 20 dogs enrolled in the study were randomly assigned to receive either 10 minutes of total mechanically induced coronary artery occlusion (achieved by traumatic vascular clamps placed at the site at which the stenosis would later be applied) followed by 10 minutes of reperfusion or a comparable 20-minute control period before the onset of injury+stenosis. To limit the incidence of lethal VF during the PC stimulus, all dogs in protocol 1 received a prophylactic dose of lidocaine (\( \approx 1.5\) mg/kg IV) before brief ischemia/no intervention. No lidocaine was administered before or during the 3-hour observation period.

**Protocol 2: Effect of IC Adenosine Infusion on Platelet-Mediated Thrombosis**

As an initial test of our second hypothesis, ie, that adenosine may inhibit subsequent platelet-mediated thrombosis, we evaluated whether brief antecedent infusion of adenosine, in lieu of brief ischemia/reflow, would improve vessel patency after injury+stenosis. In the next 19 animals entered into the study, the proximal LAD was cannulated with a 24-gauge catheter, and each dog was randomized to receive a 10-minute IC infusion of either adenosine (Sigma Chemical Co; 400 \( \mu \)g/min dissolved in saline) or saline alone at a rate of 1.0 mL/min. The infusion was then terminated, and 10 minutes was allowed to elapse before injury+stenosis was initiated. This treatment regimen was based on the observation of Yao and Gross that adenosine administered in this manner exhibited the same efficacy as

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**Figure 1. Experimental protocols.**

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**Selected Abbreviations and Acronyms**

- CBF = coronary blood flow
- CFV = cyclic variations in CBF
- IC = intracoronary
- LAD = left anterior descending coronary artery
- PC = preconditioning, preconditioned
- PD = adenosine A1/A2 receptor antagonist PD 115,199
- VF = ventricular fibrillation

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ischemic PC in reducing myocardial necrosis in dogs. The remainder of protocol 2 was identical to that of protocol 1, except that no lidocaine was administered and, in an effort to modestly attenuate the severity of arterial injury (see “Results”), the hemostat was lightly coated in wax before the isolated LAD segment was squeezed.

Protocol 3: Effect of Adenosine Receptor Antagonist on Platelet-Mediated Thrombosis

Finally, to identify a causal relationship between adenosine release during the PC stimulus and attenuation of subsequent platelet-mediated thrombosis, the final 13 animals entered into the study received a 3-mg/kg IV bolus of PD, a potent antagonist of both adenosine A1 and A2 receptors in rat (Ki of 14.0 and 4.1 nmol/L, respectively) shown to effectively block coronary A2 receptors at this dose in the dog.18–20 Specifically, the agent (obtained as a gift, through the efforts of Kim Gallagher, PhD, from Parke-Davis Pharmaceuticals, Ann Arbor, Mich) was suspended in 0.5 mL polyethylene glycol, mixed with 0.5 mL of 0.5N NaOH, sonicated until clear, and diluted in saline to a final volume of 20 mL. Ten minutes after administration of the antagonist, all dogs received a prophylactic dose of lidocaine and were randomized, as in protocol 1, to receive either 10 minutes of total coronary artery occlusion and 10 minutes of reperfusion or a comparable 20-minute control period. Coronary injury was induced with a wax-coated hemostat, and the protocol was completed as described for the first two limbs of the study.

Exclusion Criteria

Dogs were excluded from analysis according to the following prospective criteria: (1) the absence of spontaneous platelet aggregation after injury + repeated tightening of the micromanometer constrictor (all protocols); (2) VF during episodes of thrombotic occlusion (all protocols), ie, dogs that fibrillated during the 3-hour observation period were not resuscitated; (3) intractable VF during brief, mechanically induced ischemia/reperfusion requiring more than three attempts at cardioversion with low-energy (20-j) DC pulses applied directly to the heart (protocols 1 and 3); and (4) technical failures (all protocols), including failed LAD cannulation (protocol 2).

Analysis

Heart rate and arterial pressure were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. Initial platelet aggregation and dislodgment were assessed by measurement of the time (in minutes) from the onset of injury + stenosis to the first nadir in CBF and the time (in minutes) from the first nadir to the first episode of spontaneous reperfusion.

CFVs during the 3 hours after injury + stenosis (example shown in Fig 2) were analyzed by measurement of both the number of CFVs and the mean nadir of the CFVs per 30-minute time interval. A CFV was specifically defined in our study as a slow decrease followed by an abrupt (within 20 seconds) increase in CBF with an amplitude of ≥50% of the poststenotic CBF value.
Vessel patency in each 30-minute time interval was assessed by measurement of two variables: the duration (in minutes) of total thrombotic occlusion (CBF < 50) and percent flow-time area, defined as the area of the flow-time tracing (measured by computerized planimetry) normalized for each dog to the baseline flow 30 minutes.

Histological analysis of all damaged and stenotic LAD segments was performed by one investigator (P.W.), blinded with regard to both the treatment group and vessel patency data. Four to six cross sections (5 μm thick) were cut from each sample, stained with hematoxylin-eosin and picrosirius red (to facilitate specific visualization of collagen fibers), and viewed at magnifications of ×10 to ×25. For each artery, the severity of injury was assigned a semiquantitative score according to the following criteria: 1, endothelial denudation with little or no damage to the tunica media; 2, tears and dissections in the media without exposure of tunica adventitia; 3, loss of media and/or deep tears, with focal points of adventitial exposure totaling <10% of the arterial circumference; and 4, loss of media with confluent and extensive (>10%) adventitial exposure.

Statistics
Because protocols 1, 2, and 3 were conducted sequentially and not concurrently, statistical analyses were performed separately for each limb of the study.

### Hemodynamics

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Baseline</th>
<th>Prestenosis</th>
<th>Poststenosis</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Control</td>
<td>133±8</td>
<td>ND</td>
<td>134±8</td>
<td>141±8</td>
</tr>
<tr>
<td>PC</td>
<td>137±5</td>
<td>ND</td>
<td>138±5</td>
<td>136±7</td>
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<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
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<td></td>
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<tr>
<td>Control</td>
<td>111±7</td>
<td>ND</td>
<td>114±5</td>
<td>117±4</td>
</tr>
<tr>
<td>PC</td>
<td>115±8</td>
<td>ND</td>
<td>116±9</td>
<td>118±6</td>
</tr>
<tr>
<td><strong>CBF, mL/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.9±1.9 (100%)</td>
<td>11.3±1.6 (99±7%)</td>
<td>3.2±0.6† (28±3%)</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>10.8±1.2 (100%)</td>
<td>10.2±1.2 (93±4%)</td>
<td>2.9±0.5† (26±2%)</td>
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</tr>
</tbody>
</table>

### During IC Infusion

<table>
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<th>Protocol 2</th>
<th>Baseline</th>
<th>Prestenosis</th>
<th>Poststenosis</th>
<th>3 h</th>
</tr>
</thead>
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<td><strong>Heart rate, bpm</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Saline</td>
<td>136±12</td>
<td>136±12</td>
<td>134±12</td>
<td>136±12</td>
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<tr>
<td>Adenosine</td>
<td>158±5</td>
<td>155±5</td>
<td>154±5</td>
<td>156±5</td>
</tr>
<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
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<td></td>
</tr>
<tr>
<td>Saline</td>
<td>114±10</td>
<td>116±9</td>
<td>116±9</td>
<td>115±9</td>
</tr>
<tr>
<td>Adenosine</td>
<td>99±6</td>
<td>98±6</td>
<td>101±8</td>
<td>99±6</td>
</tr>
<tr>
<td><strong>CBF, mL/min</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>10.2±1.2 (100%)</td>
<td>9.9±1.1 (97±2%)</td>
<td>10.1±1.0 (101±7%)</td>
<td>3.1±0.3* (32±4%)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>8.0±1.1 (100%)</td>
<td>40.6±8.8†‡ (508±32%)</td>
<td>8.7±1.5 (107±8%)</td>
<td>2.3±0.3* (31±4%)</td>
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</table>

### 9 min After PD

<table>
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<tr>
<th>Protocol 3</th>
<th>Baseline</th>
<th>Prestenosis</th>
<th>Poststenosis</th>
<th>3 h</th>
</tr>
</thead>
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<td><strong>Heart rate, bpm</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD+Control</td>
<td>153±5</td>
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<td>152±6</td>
<td>153±6</td>
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<tr>
<td>PD+PC</td>
<td>154±8</td>
<td>153±7</td>
<td>144±5</td>
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<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PD+Control</td>
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<td>134±11</td>
<td>138±11</td>
<td>136±10</td>
</tr>
<tr>
<td>PD+PC</td>
<td>114±12</td>
<td>118±12</td>
<td>120±10</td>
<td>121±11</td>
</tr>
<tr>
<td><strong>CBF, mL/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD+Control</td>
<td>8.2±1.3 (100%)</td>
<td>7.5±1.5 (89±5%)</td>
<td>7.0±1.2 (85±4%)</td>
<td>2.3±0.3† (30±3%)</td>
</tr>
<tr>
<td>PD+PC</td>
<td>7.6±1.6 (100%)</td>
<td>7.5±1.5 (98±4%)</td>
<td>8.9±3.7 (102±17%)</td>
<td>2.8±0.5† (38±4%)</td>
</tr>
</tbody>
</table>

ND indicates not determined.
*P<.05, †P<.01 vs corresponding baseline value.
‡P<.01 vs saline group.
In each protocol, hemodynamics and CBF were measured at three to five time points: baseline (all protocols); in response to drug treatment (5 minutes into adenosine infusion in protocol 2 and 9 minutes after injection of PD in protocol 3); immediately before application of the stenosis (ie, 10 minutes after relief of PC ischemia [protocols 1 and 3] or termination of adenosine infusion [protocol 2]); immediately after injury-stenosis (all protocols); and at the end of the 3-hour observation (heart rate and arterial pressure only; all protocols). Comparisons between matched control and treated groups were made by two-factor ANOVA for group and time with repeated measures across the second factor, and if significant F ratios were obtained, further pairwise comparisons were made by Tukey’s test.

For indices of arterial injury, initial platelet aggregation/dislodgment, CFVs, and overall vessel patency during the 3-hour observation periods, Lilliefors’ test was first applied to determine whether the data were normally distributed. If \( P > 0.05 \) by Lilliefors’ test, results from matched control and treated groups were compared by Student’s \( t \) tests and expressed as mean \( \pm \) SEM. If the criteria for parametric testing were not met, Mann-Whitney tests were applied, and results were reported as the median and 25th and 75th percentiles. In addition, for parameters measured continuously throughout the 3-hour observation period (CFVs, total thrombotic occlusion, and percent flow-time area), temporal variations were assessed by comparison of data obtained at 0 to 30 minutes, 30 to 60 minutes, and 2.5 to 3 hours after stenosis by either two-factor ANOVA with repeated measures (parametric) or the Friedman test (nonparametric).

**Results**

**Protocol 1**

**Exclusions**

Of the 20 dogs enrolled in protocol 1, 10 were randomized to receive PC ischemia and 10 were assigned to the control group. No animals were excluded from analysis.

**Hemodynamics**

Heart rate and arterial pressure did not differ between groups and did not vary significantly during the protocol (Table).

CBF was comparable at baseline in the control and PC cohorts. As expected, dogs in the PC group exhibited myocardial cyanosis and dyskinesis during the 10-minute episode of antecedent ischemia, and all were hyperemic (with a maximum CBF of 82±11 mL/min, or \( \approx 800\% \) of baseline) on reflow. CBF was similar between groups both before and after application of the stenosis, with stenotic flow averaging 3.2 and 2.9 mL/min (28% and 26% of baseline) in control and PC dogs, respectively.

**Initial Platelet Aggregation and Dislodgment**

The first thrombotic episode (ie, first nadir in CBF) occurred within 1 to 3 minutes after placement of the stenosis, with no difference between control and PC groups. However, the time from the first nadir in CBF to the first episode of spontaneous reperfusion was significantly accelerated in the PC group versus controls (median of 1.4 versus 4.5 minutes; \( P = .03 \), Fig 3).
Comparison of CFVs and Vessel Patency Between Groups

Both groups exhibited 2 to 4 CFVs per 30-minute time interval throughout the protocol ($P=NS$). However, the median nadir of the CFVs during the 3-hour observation period was significantly higher in dogs that received antecedent PC ischemia (4.0 mL/min) compared with controls (0.3 mL/min; $P<.02$; Fig 4A). In addition, vessel patency, reflected in terms of both the duration of total thrombotic occlusion (CBF=0) and area of the flow-time profile throughout the 3-hour observation period, was significantly enhanced with antecedent PC ischemia compared with time-matched controls. Specifically, median zero flow duration was reduced from 15.1 to 0.4 minutes ($P<.03$; Fig 4B), and mean percent flow-time area was increased from 28% to 54% ($P<.04$; Fig 4C) in control versus PC groups, respectively.

Temporal Changes in CFVs and Vessel Patency During the 3-Hour Observation Period

In addition to the overall differences in CFVs and vessel patency between control and PC groups throughout the 3 hours after injury, both groups also exhibited temporal improvements during the course of the protocol. For example, between 0 and 30 minutes and 2.5 and 3 hours after stenosis, mean percent flow-time area increased from 18% to 40% in controls and from 38% to 62% in the PC group ($P<.01$ over time by two-factor ANOVA; Fig 5).

Histology

The severity of arterial injury was comparable in control and PC groups, with mean injury scores averaging 2.3±0.3 and 2.0±0.3, respectively. That is, arterial injury induced in protocol 1 was typically characterized by medial tearing and dissection (Fig 2B). However, 30% of all dogs had evidence of adventitial collagen exposure, and in 2 animals (1 per group), >50% of the arterial circumference was devoid of media (score of 4). Not surprisingly, extensive adventitial exposure was associated in both groups with prolonged periods of total thrombotic occlusion and the presence of fibrin (in addition to platelets and red blood cells) within the thrombi. Indeed, it was largely the influence of these animals with severe arterial injury that skewed the distributions of many of the study end points and mandated the use of nonparametric statistics. Thus, in an effort to maintain a high incidence of medial injury yet minimize the incidence of extensive adventitial exposure, arterial injury in protocols 2 and 3 was induced with hemostats lightly coated in wax.

Protocol 2

Exclusions

Of the 19 dogs enrolled in protocol 2, 10 were assigned to receive adenosine and 9 were randomized to the saline control group. Seven animals were excluded from analysis: 4 because of technical failures (3 adenosine and 1 saline control), 2 controls because of the complete absence of spontaneous platelet aggregation after injury, and 1 adenosine-treated dog because of lethal VF during thrombotic occlusion. Data are therefore reported for the 6 saline- and 6 adenosine-treated dogs that successfully completed protocol 2.
Baseline values of hemodynamics and CBF were similar between groups (Table).

As expected, IC infusion of adenosine markedly increased CBF to a mean of 500% of baseline (P < .01 versus baseline and P < .01 versus saline infusion), with no effect on heart rate or arterial pressure. However, by 10 minutes after infusion (prestenosis), CBF had returned to control values. In both groups, application of the stenosis reduced CBF to 31% to 32% of baseline.

Comparison of CFVs and Vessel Patency Between Groups
Antecedent IC infusion of adenosine significantly improved subsequent vessel patency during the 3 hours after injury + stenosis: zero flow duration was reduced from a median of 8.4 to 2.1 minutes (P = .04; Fig 6B) and percent flow-time area was increased from 24% to 70% (P = .003; Fig 6C) in saline- versus adenosine-treated groups, respectively. This was accompanied by trends toward an increase in both the frequency and median nadir of the CFVs in adenosine-treated dogs compared with saline controls (5 ± 2 versus 2 ± 1 CFVs per 30-minute interval, P = .18, and 4.0 versus 0.6 mL/min, P = .13, Fig 6A, respectively).

Temporal Changes in CFVs and Vessel Patency During the 3-Hour Observation Period
No significant temporal differences were observed during the 3 hours after injury + stenosis in either the saline- or adenosine-treated group. For example, percent flow-time area remained unchanged at 23% to 24% in controls and 65% to 69% in adenosine-treated dogs between the first and final 30 minutes after stenosis.

Figure 7. Summary of vessel patency throughout 3 hours after injury + stenosis for control vs PC groups in protocol 3. All animals received PD before PC/no intervention period. Nadir of CFVs (A) and zero flow duration per 30-minute interval (B) are presented as median and 25th and 75th percentiles (middle, lower, and upper lines, respectively), and percent flow-time area (C) is expressed as mean ± SEM.

Histology
Mean semiquantitative injury score was 1.7 ± 0.3 in adenosine-treated dogs and 2.0 ± 0.4 in the saline controls (P = NS between groups) and, as anticipated, extensive adventitial exposure (score of 4) was prevented by use of wax-coated hemostats.

Protocol 3
Exclusions
Of the 13 dogs enrolled in protocol 3, 6 were assigned to the PD control group and 7 to the PD + PC group. One animal (PD + PC) was excluded because of technical difficulties. Data are therefore reported for 6 animals in each of the PD + control and PD + PC cohorts.

Hemodynamics
Heart rate, arterial pressure, and CBF were similar between groups at baseline (Table).

All dogs in the PD + PC group exhibited cyanosis and dyskinesis during antecedent ischemia and hyperemia on reflow (maximum CBF was 35 ± 7 mL/min, or ~450% of baseline). CBF, however, was similar between groups both immediately before and after injury + stenosis. Although PD had no immediate hemodynamic consequences, mean arterial pressure at the end of the 3-hour protocol was significantly increased (by ~16 to 19 mm Hg compared with baseline) in both the PD + control and PD + PC groups, similar to the modest hypertension described previously with this compound in rabbits.

Initial Platelet Aggregation and Dislodgment
The first thrombotic episode occurred in all animals within 1 to 3 minutes of injury + stenosis. In addition, there was no
difference between PD-treated cohorts in the time from the first nadir to the first episode of reflow (2.4±0.9 versus 1.9±0.6 minutes in the PD+control and PD+PC groups, respectively).

Comparison of CFVs and Vessel Patency Between Groups

The frequency of CFVs was comparable between groups, averaging 3 to 4 per 30-minute time interval. Moreover, the median nadir of the CFVs (Fig 7A), zero flow duration (Fig 7B), and percent flow-time area (Fig 7C) were similar in PC and control dogs that received PD.

Temporal Changes in CFVs and Vessel Patency During the 3-Hour Observation Period

No temporal improvements in vessel patency or the nadir of CFVs were observed in control or PC dogs treated with PD; ie, percent flow-time area in both groups was 34% to 36% during the initial 30 minutes after stenosis and 33% to 38% during the final 30 minutes of the protocol (P=NS for both groups over time).

Histology

The severity of arterial injury was comparable in control and PC dogs that received PD, with injury scores averaging 2.0±0.4 and 1.6±0.3, respectively.

Discussion

In the present study, we have demonstrated that brief episodes of antecedent ischemia attenuate platelet-mediated thrombosis and improve subsequent vessel patency in the anesthetized open-chest dog. In addition, brief IC adenosine infusion in large part mimicked, and the adenosine receptor antagonist PD abolished, the benefits of antecedent ischemia. These results support our hypothesis that brief antecedent ischemia attenuates subsequent platelet-mediated thrombosis via an adenosine-mediated mechanism.

Effect of Brief Antecedent Ischemia on Platelet-Mediated Thrombosis

Andreotti et al and others have recently shown that in patients with acute myocardial infarction preceded by unstable angina, thrombolytic therapy resulted in more rapid reperfusion than in patients without preinfarction angina. These results suggest a favorable association between antecedent ischemia and accelerated recovery of CBF and lead us to propose that factor(s) released during brief antecedent ischemia (preinfarct angina) may contribute to this improved vessel patency. We evaluated this concept using a well-established canine model of in vivo platelet-mediated thrombosis. Indeed, we found that in the PC group in protocol 1, the first episode of spontaneous reperfusion occurred earlier, the nadir of CFVs was higher, the duration of total thrombotic occlusion was shorter, and percent flow-time area was greater than in controls. Thus, the results of protocol 1 are consistent with our hypothesis that brief antecedent ischemia attenuates subsequent platelet-mediated thrombosis and improves vessel patency in injured and stenotic canine coronary arteries.

Role of Adenosine

The obvious question arising from protocol 1 is, what specific factors associated with brief myocardial ischemia/reperfusion are responsible for this later preservation of coronary flow? Brief transient ischemia triggers the release of several compounds and metabolites known to attenuate platelet aggregation, including adenosine, NO, and prostaglandins. As the benefits of brief antecedent ischemia were manifest throughout the 3-hour protocol (ie, well beyond the 10-minute PC stimulus per se), we inferred that the later sustained improvement in vessel patency was in all likelihood a receptor-mediated phenomenon. Release of adenosine during brief ischemia/reflow and resultant activation of platelet adenosine A2 receptors might therefore provide one logical explanation for the results obtained in protocol 1.

As a first test of this theory, we evaluated whether transient IC infusion of adenosine, in lieu of brief ischemia, would improve later vessel patency. Protocol 2 revealed that brief IC adenosine infusion mimicked, in many aspects, the benefits of brief antecedent ischemia: although the first episode of spontaneous reflow was not significantly accelerated with adenosine treatment, the nadir of the CFVs tended to be increased, the duration of total thrombotic occlusion was reduced, and percent flow-time area was greater throughout the later protocol in adenosine-treated dogs versus matched saline-treated controls. Given the short half-life (minutes) of adenosine in whole blood (and thus its expected transient effect on CBF), the results imply that, as was the case with brief ischemia/reflow, the benefits of brief adenosine infusion are receptor-mediated.

These data do not, however, conclusively identify adenosine as the factor responsible for the improved vessel patency seen in either protocol 1 or 2; for example, formation of NO triggered by transient alterations in shear stress during postischemic hyperemia or adenosine infusion may conceivably also play a role. For this reason, the contribution of adenosine was further evaluated in protocol 3 by administration of the potent adenosine receptor antagonist PD. We found that pretreatment with PD blocked the benefits of brief PC occlusion: ie, the nadir of CFVs, the duration of total thrombotic occlusion, and percent flow-time area were similar between the matched control and PC groups.

Must PD be administered before the PC stimulus or does delayed administration of the adenosine receptor antagonist also attenuate a subsequent antplatelet effect? To obtain preliminary insight into this question, vessel patency was assessed in an additional 3 nonrandomized dogs that received a 3-mg/kg IV bolus of PD after the 10-minute period of antecedent ischemia. Flow-time area after injury/stenosis averaged 66% in these “posttreated” animals, greater than the values of 34% to 37% seen in control and PC cohorts of protocol 3 pretreated with PD and comparable to the mean flow-time area of 54% seen in PC dogs.
in protocol 1. These results obtained with PD provide more substantive evidence in support of the concept that release of adenosine (and stimulation of adenosine receptors) during brief ischemia/reperfusion plays an important role in the improved vessel patency seen with PC.

Comparisons Among Protocols
Although all protocols involved application of a stenosis at a site of arterial injury, qualitative inspection of Figs 4 through 7 shows variations in vessel patency among the three control cohorts. For example, median zero flow duration per 30-minute interval was 15.1, 8.4, and 3.5 minutes, respectively, in the three limbs of the study.

This variation among the three protocols may be explained by at least three methodological components of the studies. First, as discussed previously, is the issue of arterial injury: we made a conscious effort in protocols 2 and 3 to damage the media but minimize the incidence of extensive adventitial exposure. Adventitial collagen is recognized to be a highly thrombogenic substrate,9,16,27 and there is no doubt that, in protocol 1, dogs with extensive loss of media exhibited prolonged periods of total thrombotic occlusion. Indeed, post hoc analysis revealed that for the subgroup of control animals in protocol 1 with injury scores of 1 and 2 (endothelial denudation and medial injury with no adventitial exposure), median zero flow duration per 30-minute time interval was 8.1 (25th and 75th percentiles, 0.1 and 17.2) minutes, more consistent with the values seen in protocols 2 and 3. Importantly, improved vessel patency with PC was still manifest when analysis was confined to this subgroup: ie, zero flow duration for PC dogs with injury scores of 1 and 2 was 0.1 (0; 0.7) minutes; \( P < .05 \) versus the comparable subgroup of controls. Second, vessel patency in all dogs in protocol 2 (IC infusion of adenosine versus saline) was no doubt influenced by the IC catheter. Brief ischemia (<1 minute) during initial placement of the catheter was unavoidable, but, perhaps more importantly, the continued presence of the cannula throughout the protocol would effectively reduce proximal lumen area of the LAD, increase the Reynolds number (an index of fluid mechanics and flow pattern),28,29 and alter shear stress along the arterial wall, factors identified in in vitro studies as significant determinants of platelet activation and deposition.27,28 Finally, all dogs in protocol 3 were pretreated with PD suspended in polyethylene glycol and dissolved in basic solvent. We cannot exclude the possibility that either the drug or the vehicle alone may have contributed to the somewhat better vessel patency (perhaps via the modest increase in arterial pressure) seen in the control cohort of protocol 3. The potential consequences of these seemingly minor variations among protocols underscore the importance of basing our conclusions regarding vessel patency on matched control versus treated groups.

A second difference among the protocols is that only dogs in protocol 1 exhibited a temporal improvement in vessel patency over the 3-hour observation period. It is tempting to surmise that, in the first limb of the study, episodes of thrombotic occlusion early during the 3-hour observation period may have contributed to a later attenuation of platelet-mediated occlusion. In contrast, in protocols 2 and 3, the initial episodes of thrombotic occlusion may have been too short (because of the intentional reduction in the severity of arterial injury and/or the influence of the IC catheter and PD treatment) to trigger a later antiplatelet effect. This explanation, however, is speculative: further prospective studies will be needed to definitively establish whether brief ischemia caused by thrombotic occlusion indeed attenuates later platelet-mediated occlusion and, if so, to define the undoubtedly complex relationship between the severity of arterial injury and resultant severity and duration of thrombosis-induced ischemia needed to elicit a subsequent antiplatelet effect.

Choice of End Points
In the majority of previous studies using the in vivo canine model of injury + stenosis, the frequency, amplitude, and nadir of CFVs have been used as the primary indices of platelet-mediated thrombosis, with abolition of CFVs often considered the hallmark of attenuated platelet aggregation and improved vessel patency.8,9,12–14,30,31 The definition of a CFV, however, is subjective and variable among studies, ranging from no formal definition, to a reduction followed by a spontaneous return of CBF to near control values,9 to the more quantitative descriptor of a slow decrescendo followed by an abrupt increase in CBF with an amplitude reaching \( \geq 25\% \) of the poststenotic CBF value.10 For this reason, we considered that the combined measurement of zero flow duration and percent flow-time area, together with the nadir of the CFVs, would provide an objective and comprehensive assessment of vessel patency in our model. Indeed, according to our prospective criteria, we observed no significant difference in the frequency of CFVs with either antecedent ischemia or brief adenosine infusion. That is, in contrast to the results obtained with some pharmacological antiplatelet agents,9 spontaneous platelet aggregation was not prevented with either intervention. However, coronary flow was clearly better maintained after injury + stenosis with both PC ischemia and adenosine infusion.

Limitations and Unanswered Questions
Although our results implicate adenosine released during brief antecedent ischemia/reperfusion as the mediator of the improved vessel patency seen after injury + stenosis, important limitations with regard to both clinical implications and mechanistic issues must be acknowledged. First, it would be tempting to conclude that the results of the present study confirm the accelerated restoration of CBF seen by Andreotti et al10 in patients with antecedent angina and identify (as later speculated by these authors15) adenosine as the mechanism responsible for this clinical observation. Although this clinical report provided, in part, the impetus for our protocols and there is no doubt that the pathogenesis of unstable angina and myocardial infarction involves platelet activation and aggregation at sites of coronary
artery injury and stenosis. Further studies using models of myocardial infarction induced by thrombotic occlusion will be needed to establish whether the adenosine-mediated attenuation of platelet thrombosis described with brief antecedent ischemia in the present study also results in accelerated reflow and enhanced vessel patency after thrombolysis. Indeed, any extrapolation of our results obtained with brief ischemia, adenosine, and PD to clinical instances of recurrent platelet-mediated thrombosis must be made with caution.

Second, although the well-established antiplatelet effects of adenosine are attributed to A2 receptor activation on the platelet surface, our use of the nonspecific antagonist PD precludes the precise confirmation of the receptor subtype involved. Similarly, we cannot specify the site of action of adenosine: rather than activating platelet A2 receptors per se, adenosine may have attenuated later platelet-mediated thrombosis in an indirect manner by, for example, reducing the release of superoxide anions (known to promote platelet aggregation and adhesion) from neutrophils. That is, platelet activation and aggregation is a complex and multifactorial process, and the direct or indirect contribution of other factors (attenuation of superoxide anion production, release of NO and/or other potential mediators, etc) in our model remain to be investigated. Nonetheless, our results strongly suggest that brief antecedent ischemia attenuates subsequent platelet-mediated thrombosis in damaged and stenotic canine coronary arteries due, in large part, to an adenosine-mediated mechanism.

References


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