Aspirin-Induced Increase in Collateral Flow After Acute Coronary Occlusion in Dogs

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SUMMARY Aspirin (acetylsalicylic acid) has inhibitory effects on platelet function and prostaglandin synthesis. Since alterations in either platelet function or prostaglandin-mediated vascular responses could influence blood flow to ischemic myocardium, we tested the effects of aspirin on coronary collateral flow after acute occlusion of the left anterior descending coronary artery in dogs. Aspirin dose (600 mg i.v.) consistently inhibited in vitro ADP-induced platelet aggregation. In 13 open-chest dogs, regional myocardial blood flows (radioactive microsphere technique) were determined at 5 minutes and 4 hours after occlusion. In seven of these dogs, aspirin (600 mg i.v.) was administered 1 hour before occlusion. In the aspirin-treated dogs, collateral flow increased significantly ($p < 0.05$), from $0.09 \pm 0.02$ ml/min/g at 5 minutes to 0.15 and 0.02 ml/min/g 4 hours after occlusion. Collateral flow was not significantly altered over 4 hours in control dogs. The aspirin-induced increase in collateral flow was confined to epicardium (12 ± 4% of normal zone flow at 5 minutes to 18 ± 4% at 4 hours after occlusion).

ASPIRIN-INDUCED INHIBITION of platelet aggregation may be beneficial after acute myocardial infarction. Accumulation of platelets in the area of ischemia, particularly at the periphery of the infarct, has been demonstrated after acute coronary ligation in primates and after induced coronary thrombotic obstruction in dogs. Aspirin also has been shown to decrease platelet trapping in the dog and concomittantly reduce electrocardiographic evidence of ischemia.

By inhibiting platelet aggregation, aspirin may prevent platelet-mediated vascular obstruction after coronary occlusion, thus improving flow to the ischemic region. However, aspirin also interrupts the synthesis of prostacyclin and other prostaglandins with vasodilator influence, which may decrease flow to ischemic myocardium. The present investigation was undertaken to determine the influence of a platelet-inhibitory dose of aspirin on coronary collateral flow after acute coronary occlusion in the dog.

Methods

Dogs of either sex, weighing 20–27 kg, were anesthetized with sodium pentobarbital, 40 mg/kg. The animals were intubated and artificially respirated with room air. The heart was exposed through a left thoracotomy and the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissues at a point just distal to the first major diagonal branch. An electromagnetic flow probe (Carolina Medical Electronics Inc, King, North Carolina) of appropriate size was placed around the LAD. A snare was also placed around the artery just distal to the flow probe. Femoral artery pressure, LAD flow and ECG were continuously recorded.

The experimental protocol follows: The LAD was ligated and microspheres injected at 5 minutes after occlusion. At 8 minutes, which was the end of the 3-minute blood withdrawal period, the LAD was reperfused. Occlusion was verified by zero flow recorded by the flowmeter; release was verified by reactive hyperemia. The purpose of this first occlusion was to determine baseline collateral flow before the administration of aspirin. After successful reperfusion the dogs were treated. Seven dogs each received 600 mg aspirin (acetylsalicylic acid). The aspirin was dissolved in 100 ml saline at 37°C and was administered as an intravenous infusion over 7 minutes. Six control dogs each received a 100 ml saline (37°C) infusion during the same 7-minute period. Blood samples for salicylate determinations were obtained 10 minutes after the end of the aspirin infusion. (Serum salicylate levels were determined by MetPath, Rockville, Md.) The hearts were allowed to stabilize for 1 hour after reperfusion. The LAD was occluded for 4 hours and microspheres were injected at 5 minutes, 30 minutes and 4 hours after occlusion.

The radioactive microsphere technique for determination of regional flow has been described previously. In brief, approximately 1 million microspheres (15 ± 5 μM Co) labeled with either Ce-141, Yb-169, or Sr-85 were injected through a left atrial catheter and reference samples were obtained from the femoral artery. At the end of the study, the hearts were excised and full thickness samples (1–2 g) of myocardium were obtained from the normal zone (four samples) and from the ischemic zone (four to seven samples). Ischemic zone was defined anatomically as that portion of the left ventricle bounded by LAD and by the LAD branch distal to the occlusive cuff. Endocardial and epicardial halves of each sample were counted separately. Radioactivity was measured with counting windows spanning the main photopeaks of each isotope; simultaneous

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equations, to correct for overlap, were used in computing myocardial blood flow values.

The effect of in vivo aspirin, 600 mg, on in vitro platelet aggregation was determined in a separate series of eight dogs. After sedation with morphine (1.5 mg/kg) and pentobarbital (350 mg), 600 mg aspirin in 100 ml saline was administered intravenously over 7 minutes. Blood samples were taken for platelet aggregation studies before aspirin administration and 10 min after aspirin infusion. Anticoagulation was achieved with citrate, 18.2 mM (final concentration). Blood samples were spun successively at 800, 1200 and 4000 rpm for 15 minutes to collect two platelet-rich plasma fractions (PRP-1 and PRP-2) and platelet poor plasma (PPP). PRP-1 and PRP-2 were mixed in proportions such that each PRP contributed an equal number of platelets. This mixture was diluted as needed with PPP to give a final platelet count of 300,000/ml; final pH was adjusted to 7.75 with small amounts of 0.1 M NaOH. Two different PRP fractions were prepared separately and recombined in fixed proportions to minimize variation resulting from differences in platelet size distribution in individual animals. Aggregation (measured in a Payton Aggregometer) was induced by the addition of various amounts of ADP to give final ADP concentrations of $2.2 \times 10^{-6}$M to $4.4 \times 10^{-6}$M.

Statistical analyses (t test for paired data where appropriate) were performed with the individual mean for each dog contributing one sample. Values are reported as mean ± SEM.

Results

Pretreatment Occlusion

During an initial 8-minute occlusion, collateral flow was determined in each dog by microsphere injection 5 minutes after occlusion. In the seven dogs later treated with aspirin, collateral flow during the first (pretreatment) occlusion averaged 0.10 ± 0.02 ml/min/g. This value was not different from mean collateral flow, 0.07 ± 0.02 ml/min/g, in the six control dogs. Similarly, normal zone flows were equivalent in the two groups of dogs, averaging 1.06 ± 0.06 ml/min/g in the treatment group and 1.36 ± 0.24 ml/min/g in the control group.

Post-treatment Occlusion

Regional transmural myocardial flows determined at 5 minutes and at 4 hours after the second, sustained occlusion are shown in figure 1. At 5 minutes, collateral flow averaged 0.09 ± 0.02 in the treated group (unchanged from the flow measured during the initial pretreatment occlusion) and 0.06 ± 0.02 ml/min/g in the control group. Normal zone flows averaged 1.28 ± 0.17 ml/min/g in the treated and 1.27 ± 0.20 ml/min/g in the control group. At 4 hours after occlusion, normal zone flow was not significantly altered from the 5-minute value in either group of dogs. In the ischemic zone, however, flow significantly increased by 67% ($p < 0.05$) in the aspirin-treated group. In contrast, flow in the ischemic zone of the control group was not significantly altered 4 hours after occlusion. Thus, while pretreatment with aspirin did not improve collateral flow immediately after occlusion, it did significantly augment flow by 4 hours after occlusion.

In figure 2, both epicardial and endocardial collateral flow are expressed as a percent of the 5-minute normal zone values. Epicardial flow at 5 minutes averaged 0.15 ± 0.03 ml/min/g (12 ± 4% of normal zone epicardial flow) in the treated dogs and 0.10 ± 0.03 ml/min/g (6.2%) in control dogs (NS). Epicardial flow did not change significantly at 4 hours in control dogs; however, epicardial flow significantly increased ($p < 0.05$) to 23.4% in the aspirin-treated dogs. This 4-hour value present in the treated dogs was also significantly greater than the comparable value in the control dogs. In contrast, endocardial flow, which averaged 0.06 ± 0.01 ml/min/g (3.4 ± 1% of normal zone endocardial flow) and 0.04 ± 0.02 ml/min/g (2.4
Heart rate and blood pressure values for the 4 hours of occlusion are shown in Table 1. Heart rate did not change with occlusion or with aspirin administration. Blood pressure decreased comparably in both groups with occlusion but was not further changed at 4 hours.

Serum salicylate levels averaged 11.1 ± 0.7 mg/100 ml in the aspirin-treated dogs. Salicylates were not detected in the serum of control animals.

Platelet Aggregation

In vitro platelet aggregation was induced by ADP in a separate series of dogs. Minimum ADP concentration inducing aggregation in the baseline (pre-aspirin) samples ranged from $2.2 \times 10^{-4} \text{M}$ to $4.4 \times 10^{-4} \text{M}$. Four of the eight baseline samples aggregated with a minimum of $2.2 \times 10^{-4} \text{M}$ ADP. None of the post-aspirin platelet samples aggregated in response to ADP in concentrations as high as $4.4 \times 10^{-4} \text{M}$ (the maximum tested). In vitro aggregation was also performed on platelets from one of the above open-chest dogs treated with aspirin. The baseline samples aggregated with $2.2 \times 10^{-4} \text{M}$ ADP; after aspirin, platelets did not aggregate with the maximum ADP concentration ($4.4 \times 10^{-4} \text{M}$).

Discussion

By inhibition of cyclooxygenase, aspirin blocks synthesis of prostaglandins with vasodilator influence (e.g., prostacyclin) as well as prostaglandins that favor vasoconstriction and platelet aggregation such as thromboxane. Thus, the net influence of aspirin and other inhibitors of cyclooxygenase on tissue perfusion may depend on the relative activity of prostaglandin synthetic pathways within the tissue.

This ambivalence is particularly evident when considering blood flow to ischemic myocardium. Prostaglandins of the E series are known to be released during myocardial ischemia.4-6 Although the influence of these endogenous prostaglandins on perfusion of ischemic tissue is unknown, it is possible that blockage of synthesis of prostaglandins with vasodilator activity may decrease coronary collateral flow. Recent studies have suggested that inhibition of cyclooxygenase by indomethacin increases ST-segment elevation and decreases coronary collateral flow after acute occlusion in the dog.7-8 Other studies, however, have suggested a beneficial role for aspirin in myocardial infarction: Aspirin significantly reduced ST-segment elevation after acute coronary occlusion in dogs.8

Our data favor the concept that the net effect of

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**Table 1. Hemodynamic Data During Acute Coronary Occlusion**

<table>
<thead>
<tr>
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<th>Heart rate (beats/min)</th>
<th>Blood pressure (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline 5 min* 4 hrs</td>
<td>Baseline 5 min* 4 hrs</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>166 ± 8 168 ± 10 169 ± 6</td>
<td>120 ± 4 113 ± 4 113 ± 4</td>
</tr>
<tr>
<td>Aspirin-treated (n = 7)</td>
<td>175 ± 4 170 ± 9 164 ± 6</td>
<td>126 ± 3 116 ± 4† 113 ± 3</td>
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*Numbers indicate time from occlusion.
†p <0.05 different from baseline.
aspirin on perfusion to acutely ischemic canine myocardium is favorable. Collateral flow to acutely ischemic epicardium nearly doubled in dogs pretreated with aspirin, while no consistent change occurred in ischemic epicardium of control animals. Although the absolute magnitude of flow increase was small, the rise might be important in preserving myocardial viability.

The basis for the salutary effect of aspirin may rest in its platelet inhibitory action. Platelet trapping in acutely ischemic myocardium, evidenced by accumulation of Cr51-tagged platelets, has been shown in dogs and in primates. Restriction of blood flow through the coronary vasculature by deposition of platelet aggregates in certain pathological states is suggested by a number of studies. Platelet aggregation has been associated with myocardial infarction after ADP infusion in pigs and administration of epinephrine in dogs. Pretreatment with aspirin abolished epinephrine-induced necrosis in canine myocardium. Further, aspirin abolished spontaneous, cyclical reductions in flow that were associated with platelet aggregation in dogs with partial coronary occlusion.

The increase in coronary collateral flow in dogs pretreated with a platelet-inhibitory dose of aspirin in this study may be due to the prevention of formation of platelet aggregates that would otherwise block the microcirculation of the heart. However, aspirin's beneficial effect may be the result of some action unrelated to platelet aggregation. In a study by Moschos et al., coronary occlusion produced by an intracoronary balloon-tipped catheter was not accompanied by accumulation of platelets in the infarct area, yet aspirin administration reduced the incidence of ventricular fibrillation. Treatment with aspirin was associated with inhibition of the rise in plasma-free fatty acids, and with reduction of water and sodium accumulation and potassium loss seen in control animals. The beneficial effect of aspirin on collateral flow in the present study may be the result of such effects or may be related to anti-inflammatory properties of the drug.

The increase in collateral flow which we observed in our study was confined to epicardium. The reason for this restriction in aspirin-induced rise in flow is unknown. However, during acute coronary occlusion, flow to endocardium is consistently less than flow to epicardium. Platelet aggregates may offer significant flow limitation in the epicardium, while flow to the endocardium has additional limitation due to hemodynamic factors. Moreover, epicardial (but not endocardial) collateral flow begins to increase in untreated dogs 6–12 hours after acute coronary occlusion, with the greatest increase 12–24 hours afterward. It may be that abolishing platelet aggregates could accelerate this natural process responsible for increasing coronary collateral flow.

Definition of the net influence of aspirin during acute myocardial ischemia is important, since this drug is being used more frequently in patients with advanced coronary occlusive disease. Our results suggest that aspirin does not reduce perfusion during acute myocardial ischemia. On the contrary, collateral flow to the epicardium is significantly augmented 4 hours after the onset of occlusion.

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References

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