Relative Effects of Aspirin on Platelet Aggregation and Prostaglandin-mediated Coronary Vasodilatation in the Dog

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SUMMARY Aspirin, as an inhibitor of platelet aggregation, may be of benefit in ischemic heart disease. However, aspirin blocks not only platelet aggregation but also synthesis of prostacyclin, a vasodilator and platelet deaggregator. The relative sensitivity of prostacyclin-mediated coronary vasodilatation and platelet aggregation to inhibition by aspirin remains uncertain. We therefore investigated the relative dose-response relationship of aspirin on arachidonic acid–induced increments in coronary blood flow and on ADP-induced aggregation of platelets. In 11 open-chest dogs, intracoronary arachidonic acid, 0.1–3.0 mg, produced dose-related increases in coronary blood flow that were inhibited progressively by i.v. aspirin over the dose range 0.3–3.0 mg/kg. Aspirin at 3 mg/kg almost completely obliterated the response to 3 mg of arachidonic acid. Similarly, aspirin doses of 0.3–3.0 mg/kg progressively raised the minimal concentration of ADP necessary for platelet aggregation. The threshold concentration of ADP that produced aggregation of platelets from 10 control dogs ranged from $2.3 \times 10^{-4} M$ to $1.2 \times 10^{-4} M$. Aspirin at 3 mg/kg completely inhibited aggregation of platelets from 11 of 12 dogs, even with ADP at $2.3 \times 10^{-4} M$ concentration, the maximum tested. Aspirin at 0.1 mg/kg failed to inhibit either ADP-induced platelet aggregation or arachidonic acid–induced increments in coronary blood flow. Thus, the two test systems showed similar sensitivity to inhibition by aspirin with respect to threshold dose and maximal effect. These results show that very low doses of aspirin inhibit arachidonic acid–induced coronary vasodilatation and that aspirin at low doses does not appear to selectively inhibit platelet activity relative to coronary vasodilatation.

THE SUGGESTION that frequent use of aspirin is associated with a decreased incidence of acute myocardial infarction has led to extensive clinical trials of the role of aspirin and other antiplatelet agents in the prevention of infarction. Aspirin’s antiplatelet activity is a possible mechanism for benefit in coronary artery disease. However, aspirin also inhibits synthesis of prostacyclin, a potent vasodilator and platelet deaggregator generated from arachidonic acid in vascular tissues. The aspirin-induced defect occurs early in the prostaglandin pathway through inhibition of cyclooxygenase, the enzyme that converts arachidonic acid to prostaglandin endoperoxides. In vitro studies with human platelets and cultured human arterial smooth muscle cells suggest that human aortas and coronary arteries suggest that platelet cyclooxygenase is more sensitive to inhibition by the aspirin than is the cyclooxygenase of vascular tissue. Additionally, platelet aggregation was suppressed at lower oral doses of aspirin than was production of prostacyclin by incubated mesenteric artery slices from rabbits.

Selective inhibition of platelet aggregation without inhibition of synthesis of vasodilator prostaglandins might maximize potential for benefit in ischemic heart disease. If possible, treatment of ischemic heart disease should use doses of aspirin that produce such selective inhibition. Other investigators assessed inhibition of cyclooxygenase or inhibition of prostacyclin production in vitro vascular preparations but did not evaluate the ability of aspirin to alter prostaglandin-mediated changes in coronary blood flow in the intact animal. We therefore tested the relative sensitivity of platelet aggregation and prostaglandin-mediated coronary vasodilatation to inhibition by aspirin in dogs.

Methods

Coronary Flow Measurements

Dogs of either sex weighing 21–30 kg were anesthetized with 40 mg/kg of i.v. sodium pentobarbital, intubated and ventilated with a respirator. Arterial oxygen saturation above 88% and pH of 7.35–7.45 were maintained by adjustments in ventilation. The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. An electromagnetic flow probe (Carolina Medical Electronics) was placed around the left anterior descending coronary artery (LAD). Zero flow was determined by brief occlusion of the artery just distal to the flow probe. A 27-gauge lymphangiographic needle was inserted in the LAD distal to the probe for intracoronary (i.c.) administration of arachidonic acid. Clotting within the lumen of the needle was prevented by a slow but constant infusion (<0.2 ml/min) of heparin 10 U/ml in normal
Arachidonic acid (> 99%; NuChek) was dissolved in 0.1 M carbonate under nitrogen atmosphere. The pH was adjusted to 9.3 with HCl. Final dilutions in saline were made immediately before each injection. Arachidonic acid was injected directly into the coronary vessels through an intracoronary needle in doses of 0.1, 0.3 and 1.0 mg (1 mg/ml) and 3 mg (5 mg/ml). Each drug injection was made over approximately 15 seconds and was followed by a 1-ml saline flush over the next 15 seconds. Intra coronary injection of the vehicle alone (0.1 M carbonate, pH adjusted to 9.3 with HCl) produced a transient increase in coronary flow in some dogs; however, the increase was always less than 5% of the response to arachidonic acid in an equal amount of vehicle.

Aspirin (acetylsalicylic acid, crystalline, Sigma) was dissolved in saline by adding 1 M sodium bicarbonate in sufficient amounts to solubilize the aspirin but maintains the pH below 8. Aspirin was administered in doses of 0.1–3.0 mg/kg i.v.

For each of 11 dogs, the experimental protocol consisted of (1) two baseline determinations of the response to 0.1, 0.3 and 1.0 mg of i.c. arachidonic acid, (2) administration of aspirin in increasing cumulative doses of 0.1, 0.3, 1.0 and 3.0 mg/kg i.v., and (3) repetition of the arachidonic acid dose-response evaluation 12 minutes after each dose of aspirin. When inhibition of response was evident after any particular dose of aspirin, an additional 3 mg of arachidonic acid was administered. The 3-mg dose of arachidonic acid was not administered during baseline determinations, as it often resulted in a marked and prolonged decrease in systemic arterial pressure. The time between first administration of arachidonic acid and the end of the study was 3 hours.

In a series of five control dogs given no aspirin, arachidonic acid dose-response evaluations were repeated hourly to determine the reproducibility of the response with time.

Platelet Aggregation

Blood samples for platelet studies were obtained before administration of drugs (baseline) and 10 minutes after each dose of aspirin from seven of the drug-treated dogs and from eight additional dogs that received i.v. aspirin but no arachidonic acid. Because platelet reactivity did not differ between the two groups, the data from all 15 dogs were pooled.

Blood from leg veins was drawn into plastic by a two-syringe technique using as anticoagulant a mixture of trisodium citrate and citric acid with 120 mM total citrate concentration and pH 5.0. For each sample, 33 ml of blood were drawn into 5 ml of anticoagulant. Samples were centrifuged in plastic tubes successively at 300 g and 2000 g for 15 minutes to collect, respectively, platelet-rich plasma (PRP) and platelet-poor plasma (PPP). PRP was diluted with PPP as needed to give a final platelet count of 300,000/mm³. Adjusted PRP had a pH of 7.1–7.3 and was maintained continuously in contact with an atmosphere of 5% CO₂ in air. Aggregation was measured in a Payton Aggregometer at 37°C with a stirring rate of 1000 rpm. Aggregation was induced in 0.45-ml aliquots of adjusted PRP by addition of small volumes (10–50 μl) of ADP (Sigma) in saline to give final ADP concentrations of 2.3 × 10⁻⁴ to 2.3 × 10⁻³ M. The minimal concentration of ADP that induced full platelet aggregation was determined for each sample. Full aggregation was defined as a sustained increase in optical transmission in excess of 50%, on a linear scale adjusted so that PRP gave 0% transmission and PPP gave 90% transmission.

Statistical Analyses

For coronary flow data, values are reported as mean ± sem. Statistical analyses were performed using the t test for paired data. For platelet data, the Wilcoxon rank-sum test was used to determine significant differences between groups.

Results

Coronary Blood Flow

Hemodynamic data for aspirin-treated and control dogs are summarized in table 1. Systemic arterial pressure, heart rate and coronary flow did not vary with time and were not significantly altered by i.v. aspirin, up to 3 mg/kg.

Arachidonic acid, 0.1–1.0 mg i.c., produced dose-dependent increments in coronary blood flow (fig. 1). For each injection, total volume increment was determined by planimeter. Although the magnitude of the volume response varied considerably from dog to dog, each dog exhibited progressive increases in the flow response with increasing doses of arachidonic acid (fig. 2). To normalize values, each response was subsequently expressed as a percentage of the average initial volume response to 1 mg of arachidonic acid in the same dog.

The arachidonic acid–induced increments in coronary blood flow were inhibited by aspirin in a dose-dependent fashion (fig. 3). Aspirin, 0.1 mg/kg, did not

<table>
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<tr>
<th>Table 1. Hemodynamic Variables in Aspirin-treated and Control Dogs</th>
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<tbody>
<tr>
<td>SAP (mm Hg)</td>
</tr>
<tr>
<td>Aspirin-treated dogs (n = 11)</td>
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<tr>
<td>Baseline</td>
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<td>Aspirin 3 mg/kg</td>
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<td>Control dogs (n = 5)</td>
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<td>Baseline</td>
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<td>3 hours*</td>
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<td>Values are mean ± sem.</td>
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<td>*Time from first administration of arachidonic acid.</td>
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<td>Abbreviations: SAP = systemic arterial pressure; HR = heart rate; CF = blood flow through the left anterior descending coronary artery.</td>
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ASPIRIN INHIBITION OF PLATELET AGGREGATION/Capurro et al.

Figure 1. Effects of arachidonic acid (AA) on coronary blood flow. In each panel are tracings of mean coronary blood flow recorded over 10 minutes after intracoronary injection of AA (arrow). Response was expressed as the total volume increment in coronary flow determined by planimetering the area above baseline flow. Volume responses = 89 ml (11.7%), 290 ml (38.1%) and 763 ml (100%) for 0.1 mg, 0.3 mg and 1.0 mg of AA, respectively.

alter the arachidonic acid-induced coronary flow response. However, 0.3 mg/kg of aspirin significantly (p < 0.01) reduced the response to 1.0 mg of arachidonic acid. At an aspirin dose of 3 mg/kg, the response to as much as 3 mg of arachidonic acid was almost completely obliterated.

In five control dogs not given aspirin, arachidonic acid dose-response evaluations were repeated at hourly intervals. The arachidonic acid response did not diminish over 3 hours (fig. 4).

Platelet Aggregation

ADP-induced platelet aggregation was also inhibited by aspirin in a dose-dependent fashion. Tracings

Figure 2. Arachidonic acid-induced increases in coronary blood flow. The total integrated increment in coronary flow, determined by planimetering the area above baseline flow, is plotted as a function of the arachidonic acid dose in individual dogs. i.c. = intracoronary.

Figure 3. Inhibition by aspirin (ASA) of arachidonic acid (AA)-induced increments in coronary blood flow. Points are means of 11 dogs. For each dose of AA in each dog, the coronary flow response was determined as a percentage of the average initial volume response to 1.0 mg of AA. The average initial volume responses to 1.0 mg of AA (100%) ranged from 89–846 ml (mean 336 ± 70 ml). * p < 0.01; compared with own control value by paired t test.
from a single dog showing the progressively diminishing aggregation response with increasing doses of aspirin are given in figure 5. Values of the minimum dose of ADP required to produce full platelet aggregation in individual samples are plotted in figure 6. The minimum ADP dose necessary to produce platelet aggregation in control samples ranged from $2.3 \times 10^{-4} \text{ M}$ to $1.2 \times 10^{-4} \text{ M}$. After aspirin, 0.1 mg/kg, minimum ADP doses were not different from control. After aspirin, 1 mg/kg, the minimum dose of ADP required to produce full aggregation was significantly higher ($p < 0.05$). Platelet samples obtained after administration of 3.0 mg/kg of aspirin did not aggregate fully even in the presence of the highest ADP concentration used, $2.3 \times 10^{-4} \text{ M}$.

Figure 7 shows the percentage of dogs whose platelets required at least a twofold increase in ADP concentration to achieve full aggregation or failed to aggregate fully even at maximal ADP concentrations after a given i.v. dose of aspirin. Aspirin, 0.1 mg/kg, failed to inhibit aggregation of platelets from any of the dogs. However, 0.3 mg/kg of aspirin produced partial inhibition of platelet aggregation, i.e., raised the minimum dose of ADP necessary to induce full aggregation, in five of eight dogs. At aspirin doses of 1.0 and 3.0 mg/kg, platelets from all dogs exhibited at least partial inhibition of ADP-induced aggregation. At 3.0 mg/kg of aspirin, platelets from 11 of 12 dogs were fully inhibited, i.e., failed to aggregate with even the highest ADP concentration, and platelets from the one dog were partially inhibited.

Thus, the two test systems exhibited similar sensitivity to inhibition by aspirin with respect to threshold dose and maximum effect.
Aspirin is known to impair the hemostatic properties of human platelets. In experimentally produced partial coronary obstruction in dogs, aspirin has been shown to abolish the spontaneous, cyclical reductions in flow that are presumably due to platelet aggregation in vivo and are associated occasionally with arrhythmias and death. Thus, aspirin’s ability to suppress platelet activity might prove beneficial in ischemic heart disease, and may be the basis for the suggested negative association between aspirin use and incidence of myocardial infarction in retrospective as well as in prospective studies. Early results from the Anturane Reinfarction Trial, a prospective study, indicate that salicylic acid, another drug that suppresses platelet function, reduces the incidence of cardiac death in the first year after myocardial infarction.

Aspirin, however, interrupts prostaglandin synthesis early in the synthetic pathway through inhibition of cyclooxygenase, the enzyme that converts arachidonic acid into cyclic endoperoxides. Because this action also interferes with vascular synthesis of prostacyclin, a potent vasodilator and platelet deaggregator, it is important to discern whether a dose-related dissociation between aspirin’s effects on platelet aggregation and on prostacyclin production in the vessel walls is possible.

It has been shown that the arachidonic acid-induced decrease in systemic arterial pressure in the dog is inhibited by aspirin and is independent of the presence of platelets. Arachidonic acid injected directly into a coronary artery in small doses produces an increase in coronary flow, with little or no systemic effect. Hitz et al. demonstrated that meclofenamic acid, an inhibitor of prostaglandin synthesis, blocked arachidonic acid–induced coronary vasodilation but not coronary responses to a vasodilator prostaglandin.

These data indicate that vasodilatation results from arachidonic acid conversion to a vasodilator prostaglandin, probably prostacyclin. Our results support this conclusion and also show that a substantial inhibition of conversion, as assessed by in vivo vasodilator response, is produced by extremely low doses of aspirin. These low doses correspond to minimum doses that fully inhibit platelet aggregation not only in the dog (fig. 6) but also in man and other species.

Our results appear to contradict a report by Limas and Cohn, who found that canine myocardial prostaglandin synthase is insensitive to aspirin. However, these workers used an isolated, broken-cell preparation of the entire myocardium, and it may be that this in vitro preparation contained relatively little enzyme from coronary vasculature.

After we demonstrated that aspirin could inhibit arachidonic acid–induced coronary dilatation, we compared the sensitivity of aspirin in inhibiting this action with the sensitivity of aspirin in inhibiting platelet aggregation. Aggregation of platelets by ADP was studied because aspirin inhibits the second wave of ADP-induced aggregation. Our results indicate that the dosage of aspirin for inhibition of arachidonic acid–induced coronary vasodilatation is similar to that for inhibition of ADP-induced platelet aggregation.

Aspirin’s antiplatelet activity may also depend on its ability to interrupt prostaglandin synthesis. Because aspirin inhibits prostaglandin production early in the pathway, it blocks synthesis not only of prostacyclin but also of thromboxane A2, a potent vasoconstrictor and platelet aggregator generated in platelets from arachidonic acid. The nature of the involvement of the prostaglandin system in aggregation of canine platelets is not fully understood, as dog platelets are unique in that addition of arachidonic acid results in production of thromboxane A2 but not in platelet aggregation. Nonetheless, we found that aspirin consistently inhibited ADP-induced in vitro aggregation of canine platelets in a dose-related fashion. This would suggest that, for canine platelets, thromboxane A2 production is a necessary but not sufficient cause of aggregation. Very recent data strongly support this concept. In an appropriate biochemical environment (i.e., low levels of platelet cyclic AMP), arachidonic acid will indeed aggregate dog platelets, but this effect of arachidonic acid is blocked by low concentrations of aspirin. Thus, dog platelets do not appear to exhibit primary unresponsiveness to thromboxane. Rather, the levels of cyclic AMP in dog platelets seem to exert a uniquely important modulating influence on arachidonate-induced aggregation responses.

Although canine platelets differ from human platelets with respect to sensitivity to arachidonic acid, the minimum aspirin doses that inhibit aggregation of dog platelets are comparable to those that inhibit aggregation of human and rabbit platelets. Thus, although there may be some ambiguity in comparing the relative effects of aspirin on dog platelets and coronary vessels, our most important finding was
the exquisite sensitivity of the vasodilator response of the coronary vasculature to the inhibiting effects of aspirin.

These data conflict with the hypothesis that low doses of aspirin selectively inhibit platelet function, while much higher doses of aspirin are necessary to interfere with generation of prostacyclin by vessel walls. This hypothesis is based primarily on studies by Baenziger et al., Burch et al., and who used human platelets and vascular tissues, and by Basista et al., who compared aspirin sensitivity of rabbit vascular tissues and platelets. However, our results are in agreement with the more recent study of Jaffe and Weksler, who found that low concentrations of aspirin inhibited prostacyclin production by cultured human endothelial cells. They concluded that the cyclooxygenase of endothelial cells and the cyclooxygenase of platelets are equipotent to aspirin.

Applicability of our findings to the clinical setting is restricted by several factors. First, species differences cannot be discounted. Second, differences in route of aspirin administration might compound the species difference. Clinically, aspirin would most likely be administered orally; we gave aspirin intravenously. When aspirin is administered orally, the platelets may be exposed to a much higher concentration of drug than is the vasculature. Third, we evaluated platelet and vascular activity 10 minutes after aspirin administration, but did not address a differential sensitivity that may occur with time. Jaffe and Weksler found that once aspirin is removed from the environment, endothelial cells are able to regenerate cyclooxygenase completely within about 36 hours. This contrasts sharply with platelets, which cannot resynthesize the enzyme. Fourth, in the setting of ischemia, the beneficial effect of aspirin in inhibiting platelet aggregation may outweigh the potentially harmful influence of interruption of prostacyclin synthesis. Although prostaglandins of the E and F series are released during experimental myocardial ischemia, it is not known whether prostacyclin is released or whether any of these endogenous prostaglandins have a role in the maintenance of vascular tone during ischemia. In diseased human coronary vessels, prostacyclin generation may be impaired even in the absence of aspirin. Further, platelet behavior within ischemic tissue may differ substantially from that observed during in vitro testing.

Nonetheless, our results suggest that we cannot assume that low-dose aspirin will inhibit platelet aggregation while allowing continued prostacyclin production in the coronary arteries. Drugs exerting a more selective platelet-inhibitory action than aspirin may be more effective in the prevention and treatment of coronary artery disease.

Acknowledgment

The authors are grateful for the valuable technical assistance of Lilly Lee, Richard McGill, and William Parker.
The Effects of Intravenous Nitroglycerin on Hemodynamics, Coronary Blood Flow and Morphologically and Enzymatically Estimated Infarct Size in Conscious Dogs

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SUMMARY Nitroglycerin (TNG) decreases ST-segment elevation accompanying myocardial ischemia, but its effect on morphometrically and enzymatically estimated infarct size (IS) has not been defined. Accordingly, coronary occlusion was produced in 92 conscious dogs; 65 survived for 24 hours. Thirty-three received TNG (200–300 μg/min i.v. for 8 hours) and the results were compared with those in 32 untreated dogs. Coronary blood flow (CBF) was measured with tracer microspheres (125I, 14C and 51Cr) 5 minutes after occlusion before TNG, 20 minutes after TNG and again at 8 hours. Mean blood pressure decreased from 103 to 84 mm Hg with TNG, vs 99 to 94 mm Hg in controls (p < 0.02). Nitroglycerin increased CBF in the subendocardial areas by 45% (0.09 to 0.13 ml/min/g). The dogs were sacrificed after 24 hours and IS was estimated morphometrically (25 ± 1% vs 27 ± 1% of left ventricular weight) and from myocardial CK depletion (23 ± 1% vs 24 ± 1%) were similar for the two groups. Thus, despite increased subendocardial CBF, prolonged i.v. TNG did not decrease infarct size, although a 15% difference would have been detected with this sample size. TNG may relieve coronary spasm but does not appear to be beneficial with sustained coronary occlusion.

Nitroglycerin has long been used clinically for the treatment of angina pectoris, although the basis of relief of angina is controversial. Nitroglycerin reduces ventricular afterload and preload by venular and arteriolar dilatation and has been proposed as the predominant mechanisms for relief of angina. Whether nitroglycerin increases coronary flow as a result of direct coronary vasodilatation is controversial, but redistribution of flow to the ischemic subendocardium is well documented and may play a role in its beneficial effect. Recently, nitroglycerin was found to be beneficial in patients with heart failure that occurred in association with acute myocardial infarction. The recent use and availability of i.v. nitroglycerin and the demonstration of a beneficial effect on myocardial ischemia in experimental animals have encouraged its use in patients with acute myocardial infarction, despite the possible reduction of coronary perfusion pressure.

Smith et al. showed that nitroglycerin administered intravenously to dogs with evolving myocardial infarction was associated with decreased ST-segment elevation. Flaherty et al. observed similar effects in patients with acute myocardial infarction. When methoxamine or phenylephrine was administered with nitroglycerin to prevent the associated drop in blood flow, changes in ST-segment elevation were consistent with a reduction in myocardial perfusion and with occurrence of ischemia.

From the Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri.

Supported in part by SCOR in Ischemic Heart Disease grant HL 17646, NIH.

Presented in part at the Scientific Session of the American Heart Association, Dallas, November 1978.

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Received June 29, 1979; revision accepted May 8, 1980.

Circulation 62, No. 6, 1980.
Relative effects of aspirin on platelet aggregation and prostaglandin-mediated coronary vasodilatation in the dog.

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Circulation. 1980;62:1221-1227
doi: 10.1161/01.CIR.62.6.1221

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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