High-Dose Aspirin Inhibits Shear-Induced Platelet Reaction Involving Thrombin Generation

C.P. Ratnatunga, MS, FRCS; S.F. Edmondson, FRCS; G.M. Rees, MS, FRCS; and I.B. Kovacs, MD, PhD, FRC Path

Background. A unifying concept of explaining all pharmacological actions of aspirin by the irreversible blockage of the enzyme cyclooxygenase and therefore the inhibition of prostaglandin biosynthesis has left many unanswered questions.

Methods and Results. Two hundred ninety-four patients taking 75 mg/day aspirin were tested 3 months after coronary artery bypass surgery. Platelet thromboxane formation (whole blood aggregation to arachidonate) was completely prevented in 80% of patients. Compared with matched healthy controls (n=95), a significant platelet hyperreactivity was observed in patients (p<0.0001 versus <0.002). Ninety patients were advised to increase their daily dose of aspirin from 75 mg to 300 mg. Platelet reactivity retested 1 month after increasing the dose has significantly decreased (p=0.0008; <0.0001), whereas it remained unchanged in those patients (n=84) who continued with the same dose regimens. In normal subjects, ingestion of a single 600-mg aspirin significantly inhibited shear-induced platelet reaction.

Conclusions. It is concluded that aspirin does affect the platelet response to shear forces, but this requires higher dosage (>300 mg/day), suggesting a mechanism probably different from that of interference with thromboxane formation. (Circulation 1992;85:1077–1082)

Key Words • platelets • thrombosis • acetylsalicylic acid

Ever since the discovery of the inhibitory effect on platelets, the mechanism of action of aspirin is an unresolved issue.1 A unifying concept of explaining all pharmacological actions of aspirin by the irreversible blockage of the enzyme cyclooxygenase and therefore the inhibition of prostaglandin biosynthesis2 has left many unanswered questions. To resolve the contradictions, it has been suggested that aspirin exerts its analgesic,3 anti-inflammatory,4 and antithrombotic effects5 independent from or not entirely through the effect on cyclooxygenase.

The unequivocal antithrombotic effect of aspirin in patients with ischemic heart disease and cerebrovascular disease presents a dilemma concerning both the mechanism of action of the drug and the effective dose regimen.6,7 The dosage of aspirin that almost completely inhibits thromboxane A2 (TXA2) synthesis is 20–40 mg/day.8,9 However, the minimal dose in which a significant antithrombotic effect was observed was 160 mg/day (ISIS-2 trial), whereas in the majority of trials aspirin was administered in the 300–600 mg/day dose range. An attractive hypothesis for the selective inhibition of platelet TXA2 formation without influencing the antiaggregatory prostacyclin production of the vascular endothelium failed both in theory and in practice.10 Low-dose aspirin (<150 mg/day) showed no clinical antithrombotic effect11,12 and failed to inhibit in vivo variables of platelet reactivity.13 Furthermore, the view of different sensitivity of platelet and endothelial cyclooxygenase to the inhibitory effect of low-dose aspirin has been challenged.14 Even 75 mg aspirin had a very substantial inhibitory effect on prostacyclin production by the vascular wall.15

Thrombosis on a ruptured atheromatous plaque is now generally regarded as the pathomechanism of coronary16 and carotid thrombosis.17 In this mechanism, platelet activation by hemodynamic (shear) forces and thrombin generation by the activated platelets play a decisive role. Aspirin was shown to have no effect either on shear18,19 or thrombin-induced platelet reaction.20

We have tested the thrombotic status of patients taking 75 mg/day aspirin 3 months after coronary artery bypass surgery. A novel technique was used that allows the in vitro measurement of shear-induced platelet thrombus formation in which thrombin generation plays a crucial role. Our present findings provide evidence that at a higher dose aspirin does inhibit these important contributors of arterial thrombogenesis.

Methods

Study Population

Two hundred ninety-four patients were studied at 3 months after coronary artery bypass graft (CABG). Their mean age was 58.0±0.5 years and ranged from 34 to 76 years. Of this population 86% were men and 14%
women. None had recurrence of symptoms at the time of measurement. One hundred ninety-two patients were taking aspirin alone (75 mg/day) and 102 patients were taking both aspirin (75 mg/day) and dipyridamole (100 mg t.i.d.). Patients were tested 3 months after CABG, thus avoiding the effect of surgery, angiography, and early occlusion by acute thrombosis because of mechanical and surgical injury to vein grafts.

The study population was compared with 95 healthy volunteers who were not taking any regular medication, had not taken antiplatelet drugs, and had no history of chronic illness. The mean age was 55.0±1.7 years, ranging from 43 to 78 years. Of these controls 78% were men and 22% women.

The effect of a single dose of 600 mg of aspirin on thrombosis variables was tested on 12 male volunteers (age range, 20–40 years) who had no positive medical history and had refrained from taking any medication in the previous 10 days.

Shear-Induced Hemostasis Test

The instrument and the analysis of the recordings have been described in detail elsewhere. Evidence for the relevance of these measurements to pathogenesis of coronary thrombosis has been described.

Nonanticoagulated blood was perfused through polyethylene tubing at a constant pressure, the latter being continuously monitored (Figure 1). Once a steady pressure had been attained (2 minutes after withdrawal of blood), holes were punched in the tube with a fine needle, which resulted in “bleeding” into saline (estimated initial shear stress, 320 dynes/cm²). This led to an immediate fall in the pressure within the system. The pattern of pressure recovery represented the hemostatic reaction, which was computer analyzed into two arbitrarily chosen phases. Recovery of pressure to 30% of its original (prepunch) level represents the initial phase of the hemostatic reaction (H1) and is because of the activation/aggregation of platelets by physical (shear) forces and chemical factors (mainly adenosine diphosphate released from the sheared-damaged cells). The later recovery of pressure to its original level coincides with the arrest of bleeding and represents overall hemostasis; that is, completed hemostatic plug formation (H2). It has been demonstrated that thrombin generation by the aggregating platelets plays a central role in this later phase of hemostasis. Thrombin concludes platelet aggregation and stabilizes the thrombus, thus enabling it to withstand the pressure rise within the system. The pattern of pressure changes (H1, H2) was analyzed by calculating the integrated areas (mm Hg · sec) under the respective curves. After the hemostasis measurement, because the blood was nonanticoagulated, coagulation occurred. The time at both the beginning of coagulation (a pressure drop >10 mm Hg from the baseline of 60 mm Hg; CT1) and the completion of coagulation, when the perfusion pressure fell below 10 mm Hg for >1 minute (CT2) were recorded. The difference (CT2–CT1, clot formation time) shows the fibrin crosslinking, the formation of a clot stiff enough to withstand the back-pressure.

Coefficients of variation of repeated measurements (n = 18) of thrombosis variables H1, H2, and CT2 in one healthy individual over 2 years were 19%, 14%, and 11%, respectively.

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Upper panel: Schematic diagram of shear-induced hemostasis. Blood is perfused by paraffin oil displacement through polyethylene tubing from a syringe (right) into an oil-filled reservoir (left). Tubing is pierced by a needle to induce “bleeding” and hemostatic platelet plug formation in holes. Reaction is monitored by pressure changes in reservoir. Lower panel: Definition of hemostasis variables (H1 [initial phase of hemostatic reaction] and H2 [completed hemostatic plug formation]; mm Hg · sec) and analysis of the recording. Beginning (CT1) and the completed coagulation (CT2) are indicated.

Platelet Aggregation Measurement

Blood was drawn into 3.8% trisodium citrate and diluted 1:2 with phosphate-buffered saline. Aggregation of platelets induced by arachidonate (1.5 mmol, Sigma) was measured in whole blood by an impedance aggregometer (Chrono-Log Corporation, Havertown, Pa.). Measurements were performed 30 minutes after venipuncture. Both the maximum and the rate of aggregation were calculated 6 minutes after addition of the aggregating agent.

Statistical Analysis

Values are expressed as mean±SEM. Statistical analysis for differences between groups was performed using Student’s t test.

**Results**

Reactivity of platelets to shear stress and dynamic coagulation of blood obtained from patients on aspirin and from matched controls are shown in Table 1. Although taking 75 mg aspirin daily, a significant platelet hyperreactivity to shear stress was demonstrated in
patients. No significant difference was observed between patients who were taking aspirin alone or in combination with dipyridamole. Additionally, coagulation or clot stabilization did not differ between patients and controls.

Of the 10 patients taking 75 mg/day aspirin and tested for platelet aggregation to arachidonate, eight showed no response, one showed minimal aggregation, and only one patient had platelet aggregation comparable to the controls (n=5).

Ninety patients with significantly enhanced platelet reactivity were advised to increase their daily aspirin dose from 75 to 300 mg and were retested 1 month later. As shown in Table 2, increasing the daily dose of aspirin significantly increased the antiplatelet effect assessed by the shear-induced test. Distribution of data is shown in Figure 2. Furthermore, a significant prolongation of (dynamic) coagulation time was observed after increasing the aspirin dose. In contrast, patients who continued with the same dose of aspirin and were retested 1 to 3 months later showed no significant difference in any of the measured thrombosis parameters (Table 3).

The effect of a single dose of 600 mg aspirin on thrombosis variables in healthy volunteers is shown in Table 4. A single aspirin ingested significantly inhibited shear-induced hemostatic plug formation ex vivo.

Typical recordings demonstrating the effect of specific thrombin-inhibitors (recombinant hirudin and low molecular weight peptide PPACK [D-Phe-Pro-Arg-CH2Cl]) is shown in Figure 3. Antagonism of thrombin practically prevented shear-induced hemostasis.

**Discussion**

The importance of hemodynamic forces and thrombin generation by the activated intrinsic clotting system in the pathogenesis of thrombosis at the site of a disrupted atherosclerotic plaque is well documented. The technique used in this study used only shear forces to induce hemostatic aggregation of platelets in native blood. The shear rate in the system corresponded to the

<table>
<thead>
<tr>
<th>Table 1. Platelet Function and Coagulation Measurements in Healthy Controls and Patients on Continuous Antiplatelet Medication 3 Months After Coronary Artery Bypass Grafting</th>
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</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Controls (n=95)</td>
</tr>
<tr>
<td>All patients (n=294)</td>
</tr>
<tr>
<td><em>p</em></td>
</tr>
</tbody>
</table>

**Medication**

| Aspirin (75 mg/day) (n=192) | 477±34 | 3,362±219 | 14.1±0.4 | 18.7±0.5 | 4.60±0.3 |
| Aspirin+dipyridamole (100 mg t.i.d.) (n=102) | 431±40 | 3,039±257 | 14.7±0.5 | 19.8±0.3 | 5.10±0.4 |
| *p* | NS | NS | NS | NS | NS |

H1, initial phase of hemostatic reaction; H2, completed hemostatic plug formation; CT1, time at the beginning of coagulation; CT2, time at completion of coagulation; mm Hg·s, integrated areas of the hemostasis recordings; figures are inversely related to platelet reactivity.

See “Methods” for definition of variables.
TABLE 3. Variation of Thrombosis Parameters in Patients on Same Dose of Aspirin, Tested on Two Separate Occasions of 1–3-Month Intervals

<table>
<thead>
<tr>
<th>n</th>
<th>Aspirin (mg/day)</th>
<th>Hemostasis (mm Hg·sec)</th>
<th>Time at completion of coagulation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H1</td>
<td>H2</td>
</tr>
<tr>
<td>40</td>
<td>75</td>
<td>336</td>
<td>2,021</td>
</tr>
<tr>
<td></td>
<td></td>
<td>296</td>
<td>1,981</td>
</tr>
<tr>
<td>Difference (%)</td>
<td>-11.9</td>
<td>-2.0</td>
<td>+2.3</td>
</tr>
<tr>
<td>p ½</td>
<td>0.22</td>
<td>0.71</td>
<td>0.82</td>
</tr>
<tr>
<td>44</td>
<td>300</td>
<td>502</td>
<td>3,006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>513</td>
<td>3,165</td>
</tr>
<tr>
<td>Difference (%)</td>
<td>+2.2</td>
<td>+5.3</td>
<td>-2.1</td>
</tr>
<tr>
<td>p ½</td>
<td>0.92</td>
<td>0.68</td>
<td>0.88</td>
</tr>
</tbody>
</table>

H1, initial phase of hemostatic reaction; H2, completed hemostatic plug formation.

A hemodynamic situation that occurs in plaque rupture when the vascular lumen is already greatly reduced by stenosis.

When platelets are exposed to shear forces, the most significant alteration is the exposure of procoagulant phospholipids from the cytoplasmic face of the plasma-lemma. The shear-induced hemostatic reaction is initiated probably by the release of von Willebrand factor from platelets and adenosine diphosphate from the shear-damaged (lysed) cells. This concept is in agreement with our earlier findings demonstrating delay in the onset of the hemostatic reaction by antagonizing adenosine diphosphate and complete inhibition of hemostasis by monoclonal antibody against von Willebrand factor or its receptor, platelet membrane GPIIb/IIIa. However, it is the large amount of thrombin generated that seems to play the major role in the shear-induced hemostatic reaction. Aspirin acetylates not just the cyclooxygenase but also other proteins such as albumin, hemoglobin, or antithrombin III. Whether inhibition of shear-induced platelet reaction by aspirin involves the preservation of membrane surface proteins so that less procoagulant activity is expressed or the interference with the release or effect of von Willebrand factor and adenosine diphosphate is not clear.

Our findings are in conflict with a study using a viscometer to induce aggregation of platelets in a washed suspension by shear forces. Shear-induced platelet aggregation required von Willebrand factor but aspirinization of platelets had no effect on the reaction.

The important role of thrombin in the pathogenesis of platelet-rich arterial occlusion has been documented by the dramatic effect of selective thrombin inhibitors completely preventing the occurrence of any thrombotic reaction. At deep-vessel wall injury, the intrinsic coagulation pathway is initiated by the contact of blood with the exposed subendothelial layers and then is markedly accelerated by the large amount of thrombin generated principally by the activated platelets.

Aspirin has no inhibitory effect on (exogenous) thrombin-induced aggregation of platelets and release of granule contents. In citrated plasma, aspirin may inhibit platelet aggregation in response to agonists other than TXA2. It has been shown, however, that in a low-Ca2+ medium (citrate), platelet aggregation and release of adenosine diphosphate, collagen, and probably shear stress (stirring) are the result primarily of the formed TXA2 and its effect on its receptor. The possibility that some thrombokane is formed during shear activation of platelets in the hemostatometer cannot be ignored. However, completed hemostasis entirely depends on the large amount of thrombin generated by the shear-activated platelets. This was shown earlier and documented here again by the profound inhibitory effect of selective thrombin antagonists on the reaction.

As opposed to the hemodynamic forces and thrombin, the role of TXA2 formation in the pathogenesis of acute coronary thrombotic event is probably secondary. The limited clinical effectiveness of aspirin is in conflict with the observed, almost complete inhibition of TXA2 formation (aggregation response to arachidonate) by 75 mg/day aspirin. Clinical trials with thromboxane synthetase inhibitors have been disappointing. Therefore, our finding that aspirin inhibits important contributors of arterial thrombogenesis other than TXA2 has both theoretical and practical implications.

In a smaller population earlier we have provided evidence for platelet hyperreactivity (to shear stress) in patients with coronary artery disease. Additionally, platelet hyperreactivity to shear stress (spontaneous aggregation) proved to be a useful predictor of coronary events and mortality. This study demonstrates that aspirin can inhibit the effect of shear stress on platelets and thrombin generation.

TABLE 4. Thrombosis and Coagulation Variables in 12 Healthy Volunteers Before and After Ingestion of Single 600 mg Aspirin

<table>
<thead>
<tr>
<th>Aspirin</th>
<th>Shear-induced hemostasis (mm Hg·sec)</th>
<th>Dynamic coagulation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1</td>
<td>H2</td>
</tr>
<tr>
<td>Before</td>
<td>582±92</td>
<td>3,262±428</td>
</tr>
<tr>
<td>After</td>
<td>827±92</td>
<td>5,385±1,002</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>

H1, initial phase of hemostatic reaction; H2, completed hemostatic plug formation.
after platelet activation, both in healthy controls and in patients with coronary artery disease. Clearly these effects require an aspirin dose of at least 300 mg/day. If our present observations are confirmed in a much greater and also in different patient populations, then the dose regime for aspirin needs serious reevaluation.

References

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High-dose aspirin inhibits shear-induced platelet reaction involving thrombin generation.
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