Original Contributions

Effects of Low Dose Aspirin On Platelet Function In Patients With Recent Cerebral Ischemia

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SUMMARY We tested the antiplatelet effects of low-dose aspirin in patients with occlusive cerebrovascular disease, because conventional dosage aspirin inhibits vascular synthesis of prostacyclin at the same time that it inhibits platelets. The effects on platelet function and thromboxane A2 synthesis of 40 mg of aspirin daily or 40 mg aspirin plus dipyridamole were measured in 23 patients starting within a week after the onset of cerebral ischemia. All patients had normal baseline platelet aggregation responses to four stimuli: arachidonate, epinephrine, adenosine diphosphate and collagen. The generation of thromboxane A2 by platelets, measured as serum thromboxane B2, was also normal. After 3 to 7 days of low dose aspirin therapy, platelet aggregation responses were suppressed to the extent observed with higher dosage aspirin. Serotonin release during platelet aggregation was inhibited by more than 95% and thromboxane B2 levels in clotted blood fell by more than 95%. Responses to aspirin treatment were similar in patients with transient ischemic attacks and in those with stroke and were also similar in both sexes. No differences in platelet responses were observed between patients receiving aspirin alone and aspirin plus dipyridamole. Thus 40 mg aspirin daily inhibited platelet responses as effectively as higher doses of aspirin in patients who had recent cerebral ischemia and showed a cumulative antiplatelet effect.

Recent advances in understanding that aspirin inhibits prostaglandin production in tissues other than platelets and further elucidation of its actions on platelet function have, however, raised doubts as to the specificity for platelets of the drug as used in these clinical trials. For example, very low doses of aspirin suffice to inhibit platelet aggregation, prevent platelet release of vasoactive mediators, and prolong the bleeding time in normal subjects to an extent similar to that observed after much higher doses of aspirin. Even 20–40 mg of oral aspirin daily has a cumulative effect on platelet function resulting in 95% inhibition of thromboxane A2 formation. Moreover, our own as well as other studies show that vascular production of prostacyclin (PGI2) is readily inhibited by a single dose of 325 mg aspirin or less.

Aspirin has a protracted inhibitory effect upon platelets because it irreversibly inactivates fatty acid cyclooxygenase, the enzyme which oxygenates arachidonic acid to prostaglandin endoperoxides, key intermediates in platelet aggregation and thromboxane production. This prolonged inhibition results from the inability of platelets to resynthesize new cyclooxygenase or any other protein during the week to ten days that platelets circulate. Since vascular and other tissues are capable of protein synthesis, they recover quickly from aspirin inhibition of cyclooxygenase by synthesizing new, active enzyme. Hence, although vascular cyclooxygenase is substantially inhibited by the doses of aspirin used in the clinical trials of cerebral ischemia, its more rapid resynthesis compared to that of platelets suggests that a differential dosage or schedule of aspirin could be found which would be more selective for platelet inhibition while sparing vascular prostacyclin synthesis. In addition, it has been observed that low dose aspirin spares renal prostaglandin synthesis; reducing the dose of aspirin may thus help maintain renal function in patients with renal disease, in whom customary doses of anti-inflammatory drugs can worsen renal function.

Patients with recent episodes of cerebral ischemia may show transient or persistent platelet hyperactivity; it is not clear whether their platelet function would be readily inhibited by the same low doses of aspirin which inhibit normal platelets. Moreover, atherosclerotic vessels produce less PGI2 than normal vessels, but no data yet address the question if cyclooxygenase in such arteries is more sensitive to aspirin than in normal vessels. Should this be so, use of...
a low dose of aspirin might better preserve vascular prostacyclin production than would higher dosage aspirin. We therefore examined whether low dose aspirin could inhibit platelet function in patients recently admitted to hospital with cerebral ischemia. This feasibility study was based on the hypothesis that a low, platelet-selective dose of aspirin might be beneficial to patients with atherosclerosis. Twenty-three patients were studied within seven days of acute stroke or transient ischemic attack. We observed that platelet function in these patients was inhibited by low dose aspirin to an extent similar to that found during treatment with the conventional dosage of 1–1.5 g/day. The addition of dipyridamole to the aspirin therapy did not alter the results observed with administration of aspirin alone.

Materials and Methods

Adult patients of both sexes admitted to the Neurology or Internal Medicine services of The New York Hospital-Cornell Medical Center who had not ingested an aspirin-containing drug for two weeks were eligible for study. Diagnosis of stroke or transient ischemic attack was based on formal examination by the Stroke Team and was confirmed by angiography and/or computerized tomography. Patients were only excluded if they were allergic to aspirin, had gastrointestinal bleeding, or were currently receiving anticoagulants. After informed consent was obtained, baseline evaluations of platelet aggregation responses, serum thromboxane formation and bleeding time were performed, and each patient received either 40 mg of aspirin daily or 40 mg aspirin daily plus 75 mg dipyridamole three times a day for one week. Platelet function was restested after three and seven days of treatment, each patient serving as his own control. Some patients were further tested after weeks to months of this therapy.

Platelet aggregation was tested in platelet rich plasma prepared from citrated (0.32% trisodium citrate final concentration) blood drawn by free-flowing venipuncture with minimal stasis. The platelet rich plasma was kept tightly covered under 5% CO2 in air at room temperature until use and was tested within two hours of being drawn. Aggregation was carried out using a Payton dual channel aggregation module (Payton, Inc., Buffalo, NY) equipped with a Soltec recorder (Soltec Corp, Sun Valley, CA). Four agonists were tested over a dose range which gave full responses at baseline; the same doses were compared in the follow-up studies after administration of aspirin. The following compounds were used: Adenosine-5'-diphosphate (ADP, Sigma Diagnostics, St. Louis, MO), 2.5–10.0 μM, sodium arachidonate, 125–500 μM (NuChek, Elysian, MN), collagen 0.1–2.0 μg/ml (Hormon-Chemie, Munich, West Germany), and epinephrine, 0.5–2.0 μM (Parke Davis, Chicago, IL). All concentrations of stimuli are expressed as the final concentration of 1–1.5 g/day. The addition of dipyridamole to the aspirin therapy did not alter the results observed with administration of aspirin alone.

Confirmation of a low dose of aspirin might better preserve vascular prostacyclin production than would higher dosage aspirin. We therefore examined whether low dose aspirin could inhibit platelet function in patients recently admitted to hospital with cerebral ischemia. This feasibility study was based on the hypothesis that a low, platelet-selective dose of aspirin might be beneficial to patients with atherosclerosis. Twenty-three patients were studied within seven days of acute stroke or transient ischemic attack. We observed that platelet function in these patients was inhibited by low dose aspirin to an extent similar to that found during treatment with the conventional dosage of 1–1.5 g/day. The addition of dipyridamole to the aspirin therapy did not alter the results observed with administration of aspirin alone.

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mg/ml. No difference was observed between values of patients with TIA and those with stroke, nor between men and women (data not shown). Following administration of aspirin for 3 or 4 days, the average serum thromboxane fell to 8 ± 2 ng/ml and after aspirin for 7 days, to 3 ± 1 ng/ml, representing a 98% inhibition of the pre-aspirin response (p < 0.001 for difference between baseline and 3 or 7 days therapy and p < 0.001 for difference between 3 and 7 days therapy).

Release of serotonin from PRP prelabelled with 14C-serotonin during aggregation ranged from 39 to 54% for the four stimuli tested (table 3) at baseline examination of six subjects. Following the daily administration for one week of 40 mg of aspirin or 40 mg aspirin plus dipyridamole, serotonin release fell to 0–2% (p < 0.01). No further depression in serotonin release was observed in PRP from five other patients treated with 80 mg/day aspirin.

Serial bleeding time measurements were made in a subgroup of 10 patients. Baseline bleeding times were normal, averaging 4.0 ± 0.5 minutes. After treatment with 40 mg aspirin/day there was a modest but significant prolongation in bleeding time to 6.0 ± 0.8 min (p < 0.01).

Patients who received dipyridamole in addition to aspirin showed inhibition of platelet aggregation and a decrease in serum thromboxane comparable to patients who received aspirin alone (table 4). Indeed, the fall in serum thromboxane level was slightly less than that of patients receiving aspirin alone. No significant differences in prolongation of bleeding time or inhibition of serotonin release was noted in patients receiving both drugs rather than aspirin alone.

Discussion

Hyperactive platelet responses have been associated with acute vascular events such as cerebral ischemia and myocardial infarction, probably representing an acute phase reaction.14–16 In some groups of patients

### Table 1: Effect of Treatment of Patients with Stroke or Transient Ischemic Attack

<table>
<thead>
<tr>
<th>Therapeutic status</th>
<th>Men (n = 12)</th>
<th>Women (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.7 ± 3.5</td>
<td>15.6 ± 2.5</td>
</tr>
<tr>
<td>Aggregation score after 7 days therapy</td>
<td>4.4 ± 1.1</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>Inhibition of thromboxane A2</td>
<td>95 ± 7%</td>
<td>97 ± 2%</td>
</tr>
</tbody>
</table>

### Table 2: Effect of Aspirin Therapy on Platelet Responses of Men and Women

<table>
<thead>
<tr>
<th>Men (n = 12)</th>
<th>Women (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.4 ± 2.0</td>
</tr>
<tr>
<td>Aggregation score after 7 days therapy</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>Inhibition of thromboxane A2</td>
<td>94 ± 4%</td>
</tr>
</tbody>
</table>

### Table 3: Release of 14C-Serotonin by Platelets

<table>
<thead>
<tr>
<th>Therapeutic status</th>
<th>Percent Serotonin Release upon Stimulation with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arach</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>ADP</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Epi</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Collide</td>
<td>16 ± 2</td>
</tr>
</tbody>
</table>

### Table 4: Effect of Therapy with Aspirin or Aspirin and Dipyridamole on Platelet Aggregation and Thromboxane Formation

<table>
<thead>
<tr>
<th>Therapeutic status</th>
<th>Aspirin (40 mg/d) (n = 13)</th>
<th>Aspirin and dipyridamole (75 mg tid) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.8 ± 3.4</td>
<td>15.0 ± 2.3</td>
</tr>
<tr>
<td>Aggregation score after 7 days therapy</td>
<td>4.5 ± 1.0</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>Inhibition of thromboxane A2</td>
<td>96 ± 5%</td>
<td>91 ± 8%</td>
</tr>
</tbody>
</table>
with vascular disease, increased platelet sensitivity to aggregating agents, raised levels of platelet release products, or shortened platelet survival have been found to persist in the absence of acute ischemic events. Examples of these include young patients with stroke, hypercholesterolemic patients, and patients with mitral valve prolapse.21-26 Elderly persons, whether demonstrating clinical atherosclerosis or not, appear to have platelets which are more reactive than those of younger persons.

Clinical trials of aspirin therapy for prevention of cerebral ischemia or myocardial infarction have generally employed aspirin dosage in the range of 1-1.5 g daily,1-4, 28-29 although one trial of aspirin to prevent myocardial infarction in patients with unstable angina utilized 325 mg daily.30 Such doses of aspirin alter platelet function in a well established pattern, resulting in abolition of the platelet aggregation response to arachidonic acid, decrease in response to collagen, and prevention of second phase aggregation responses to epinephrine and ADP without any effect on the primary aggregation response; the aggregation response to thrombin is unaltered. Thromboxane synthesis by platelets is inhibited by 95-100%, and bleeding time is generally doubled from baseline values.

It is clear that this dose of aspirin is far greater than that needed to inhibit platelet aggregation similarly in normal subjects, where doses of 20 to 50 mg daily inhibit platelet aggregation and TXA2 synthesis.8,9 This indicates first, that normal platelets are very sensitive to aspirin and second, that the drug has a cumulative effect on platelet function, since a single dose of 40 mg only partially inhibits platelet aggregation and thromboxane formation.8,9 It is not well established that maximal inhibition of platelet function in a population of patients might require a higher standard dosage, or individualization of dose. Svensson and Samuelsson reported that 3/9 patients with prior cerebral infarction or TIA given 50 mg aspirin daily failed to achieve suppression of arachidonate induced platelet aggregation, although aggregation to ADP and thromboxane formation were inhibited in all subjects.27 Byosen et al found "satisfactory" inhibition of platelet aggregation in 19 out of 23 patients given 50–75 mg of aspirin daily following carotid endarterectomy or completed stroke, suggesting that some patients may require slightly higher dosage.28 The possible causes of individual variation in response to aspirin include differences in absorption of the drug, rate of deacetylation of aspirin in the plasma and liver, and the turnover rate of platelets.

The present study confirms the feasibility of a low dose of aspirin to suppress platelet aggregation and thromboxane formation in patients with recent cerebral ischemia, and, moreover, indicates that serotonin release which accompanies platelet aggregation is almost completely inhibited by 40 mg of aspirin daily. The suppression of serotonin release is of particular interest since serotonin has enhanced vasospastic effects at atherosclerotic sites in blood vessels, and platelet activation appears to occur at such sites.34, 35

A cumulative effect on platelet function of this low dose of aspirin has been demonstrated, and it may be expected that a partial inhibition of vascular prostacyclin formation also takes place.8-10 Within the scope of this study, the addition of dipyridamole to aspirin did not augment the inhibition of platelet function as tested. Since dipyridamole also has effects upon platelet adhesion to the vascular surface and on adenosine uptake,36 not evaluable by current laboratory testing, the contribution of dipyridamole to antiplatelet efficacy of a combination drug approach is difficult to evaluate. It now remains to be demonstrated whether the antiplatelet effects achieved using low dose aspirin are responsible for the antithrombotic efficacy of this drug, and whether the sparing of vascular prostacyclin formation
and of renal function associated with low dose aspirin use play clinically relevant roles. Such demonstration will require a large-scale prospective, randomized clinical trial.

References
Effects of low dose aspirin on platelet function in patients with recent cerebral ischemia.

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