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 $A_2$ 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 $A_2$ by platelets, measured as serum thromboxane $B_2$, 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 $B_2$ 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.

THE ACCEPTANCE of aspirin therapy for prevention of cerebral ischemia is based on the positive results of several large clinical trials, reinforced by the simplicity, safety, and apparent selectivity of this antiplatelet treatment. Benefits representing a 40–50% reduction in subsequent stroke or death were observed in four major studies utilizing aspirin prophylaxis for patients who incurred at least one signal transient ischemic attack or prior stroke. The usual dose of aspirin was 1–1.5 g/day.

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 $A_2$ formation. Moreover, our own as well as other studies show that vascular production of prostacyclin (PGI$_2$) 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 PGI$_2$ 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 concentrations attained in the platelet rich plasma. A platelet stimulus which acts independently of cyclooxygenase, endoperoxide analog U46619, 2.9 μM (a gift from Dr. John Pike, The Upjohn Co., Kalamazoo, MI), was also used. Aggregation induced by U46619 served to confirm any aspirin effect, since this stimulus bypasses the aspirin block; it also provided a standard maximum response to which the other responses could be compared. In addition, the occurrence of spontaneous aggregation in stirred PRP without a stimulus was evaluated.

The areas under the aggregation curves generated during five minutes following addition of the stimulus were measured by planimetry; these areas were compared to that of the curve obtained with the endoperoxide analog, and a scoring system of 0-5 was established for each stimulus to yield a total aggregation score for each patient at each time point tested. Each response was expressed as a percent of that patient’s response to U46619. No aggregation response was scored as 0; 1-25% as 1; 26-50% as 2; 51-75% as 3; 76-100% as 4; spontaneous aggregation or a full aggregation response to the lowest test dose of stimulus, suggesting platelet hyperactivity, was scored 5. Thus, with four agonists, the total possible score was 20 and a “normal” response was 16.

Inhibition of Thromboxane Synthesis by Platelets

was assessed by measurement of thromboxane B₂ (TXB₂), the hydrolytic product of thromboxane A₂ (TXA₂), generated during blood coagulation using a specific radioimmunoassay for TXB₂. Venous blood was allowed to clot in plain glass tubes at 37 deg C for one hour in a water bath. Serum was then separated by centrifugation at 4 deg C. Serums were stored frozen until testing. It has previously been shown that this preparation of serum permits maximal formation of TXB₂ from endogenous arachidonate during blood coagulation via the action of released thrombin.19

Serotonin Release During Aggregation was assayed by labelling platelets in platelet rich plasma with [³⁵S]serotonin (New England Nuclear, Cambridge, MA) for 30 minutes at 37 deg C, then carrying out platelet aggregation studies. Plasma aliquots were removed at 5 minutes after adding the aggregating agent, diluted 1/10 in ice cold EDTA-saline, and residual platelets were removed by centrifugation at 4 deg C. Serotonin released to the supernatant plasma was counted in a liquid scintillation counter. Released serotonin was calculated according to a standard method.20

Bleeding Time was measured by a modified Ivy technique using a Simplate II device (General Diagnostics, Morris Plains, NJ) on the volar surface of the forearm.

Statistical Analysis was carried out by two-tailed Student’s t test for paired values and the Wilcoxon signed ranks test for comparison between patient groups. Values for test results are presented as mean ± sd.

Results

Twelve patients (aged 62 ± 10 yrs) had transient ischemic attacks as the reason for admission to hospital and thirteen patients (aged 65 ± 10 yrs) had ischemic strokes. Thirteen patients were women, 12 men. Eight had a history of previous atherosclerotic cardiovascular disease, 12 had hypertension, and 6 had diabetes
Table 1: Effect of Treatment of Patients with Stroke or Transient Ischemic Attack

<table>
<thead>
<tr>
<th></th>
<th>Transient ischemic attack (n = 12)</th>
<th>Stroke (n = 11)</th>
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<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.7 ± 3.5</td>
<td>15.6 ± 2.5</td>
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<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>
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Table 2: Effect of Aspirin Therapy on Platelet Responses of Men and Women

<table>
<thead>
<tr>
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<th>Men (n = 12)</th>
<th>Women (n = 11)</th>
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<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.4 ± 2.0</td>
<td>15.3 ± 3.9</td>
</tr>
<tr>
<td>Aggregation score after 7 days therapy</td>
<td>5.0 ± 1.1</td>
<td>3.9 ± 1.3</td>
</tr>
<tr>
<td>Inhibition of thromboxane A2</td>
<td>94 ± 4%</td>
<td>96 ± 6%</td>
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Table 3: Release of 14C-Serotonin by Platelets

<table>
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<tr>
<th>Therapeutic status</th>
<th>Percent Serotonin Release upon Stimulation with</th>
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<tbody>
<tr>
<td>Arach ADP Epi Coll</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Baseline</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>Aspirin 40 mg/d</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Aspirin 80 mg/d</td>
<td>1 ± 1</td>
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Table 4: Effect of Therapy with Aspirin or Aspirin and Dipyridamole on Platelet Aggregation and Thromboxane Formation

<table>
<thead>
<tr>
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<th>Aspirin (40 mg/d) and dipyridamole (75 mg tid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aggregation score</td>
<td>15.8 ± 3.4</td>
</tr>
<tr>
<td>Aggregation score after 7 days therapy</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Inhibition of thromboxane A2</td>
<td>96 ± 5%</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. 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, although one trial of aspirin to prevent myocardial infarction in patients with unstable angina utilized 325 mg daily. 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 andTXA2 synthesis. 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. It is not well established whether such low doses of aspirin have similar inhibitory effects on platelets of patients with vascular disease or acute episodes of ischemia as on platelets of healthy normal individuals. Thus, larger doses could conceivably be required to inhibit platelet activation in patients with active atherosclerotic disease.

The results reported here indicate that patients with atherosclerotic cerebrovascular disease, initially evaluated within a week of onset of acute cerebral ischemia, demonstrate inhibition of platelet aggregation, thromboxane formation and serotonin release after 3–7 days of therapy with 40 mg of aspirin/day. These findings suggest that platelets from atherosclerotic subjects, even when tested at a time which may represent heightened platelet activation (7/23 patients showed spontaneous platelet aggregation in this study), are responsive to low dose aspirin in the same manner as platelets from normal subjects without vascular disease. A similar response to 50 mg/d aspirin has been reported for patients with acute myocardial infarction. In the present study, platelet aggregation was tested with four agents which induce responses which are cyclooxygenase-dependent, and these were compared to an agent which is independent of cyclooxygenase (U44619). The changes in aggregation pattern for all stimuli tested were of the same extent following low dose aspirin therapy as changes observed in other patients given much higher dosage. Inhibition of serum thromboxane formation after the 40 mg/day regimen, was, however, slightly less than that observed after higher dose aspirin, although thromboxane was more than 90% inhibited after one week of therapy. It is likely that following a "loading dose" of 100 mg of aspirin, daily maintenance with 20–40 mg will suffice to maintain more than 95% inhibition of TXA2. Serotonin release was also > 95% inhibited by the low dose aspirin, and doubling the dose did not result in further inhibition.

In this series of patients, all subjects responded to the daily administration of 40 mg aspirin with inhibition of aggregation responses and marked inhibition of thromboxane formation during blood clotting. Several patients retested after weeks to months on this dosage of aspirin demonstrated continued antiplatelet effects (data not shown). Inhibition of serum thromboxane for individual patients ranged from 70–100%, suggesting 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. Boyden 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. 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. 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. 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, 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.
B B Weksler, J L Kent, D Rudolph, P B Scherer and D E Levy

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