Prolongation of Bleeding Time and Inhibition of Platelet Aggregation by Low-Dose Acetylsalicylic Acid in Patients With Cerebrovascular Disease

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SUMMARY Platelet aggregation and bleeding time was measured in 43 cerebrovascular patients participating in a controlled, double-blind study of low-dose acetylsalicylic acid. In 19 patients with satisfactory inhibition of the platelet aggregation obtained by 50 to 70 mg acetylsalicylic acid per day the bleeding time averaged 11.2 minutes in contrast to 7.0 minutes in the placebo group, \( p < 0.001 \).

This study confirms our previous findings of platelet inhibition by low-dose acetylsalicylic acid in patients with cerebrovascular disease. The prolongation of the bleeding time demonstrates that we are dealing not merely with an in vitro phenomenon but with a significant in vivo effect. The study provides the rationale for clinical evaluations of low-dose acetylsalicylic acid in stroke prophylaxis.

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ACETYLSALICYLIC ACID (ASA) is still the most widely used pharmaceutical agent in platelet inhibition. In stroke prophylaxis two large studies, the Canadian\(^1\) and the French\(^2\) have shown a significant reduction of stroke risk by giving 1.3 g and 1 g daily respectively. Other studies\(^3\)\^-\(^6\) as well as the recent Danish study\(^7\) failed to show a stroke preventing effect of 1 g of ASA daily. The latter study was thoroughly discussed in an accompanying editorial,\(^8\) in which it was pointed out that the material was too small to rule out a type II error. However, if it holds true that ASA of 1 g doses lower the stroke risk there is still room for an improvement of the therapeutic results and the possibility exists that a lower dose might be more effective.

The dose of ASA needed to inhibit the cyclooxygenase of the platelets is much less than 1 gram.\(^2\)\^-\(^10\) The optimal dose, however, has not been settled. The evidence of a differential effect of small doses of ASA, which inhibit the platelet cyclooxygenase without affecting that of the endothelial cell in the vessel wall speaks in favour of a low-dose regimen in order to preserve the formation of prostacyclin.\(^11\)

We have previously shown that ASA doses of 25–75 mg a day during long-term treatment is sufficient to inhibit platelet aggregation in patients with cerebrovascular disease.\(^12\)\^-\(^13\) In the present study we evaluated the effect on bleeding time of small ASA doses in order to demonstrate an "in vivo" effect as a supplement to the "in vitro" aggregation test.

Patient Material

Forty-three patients who participate in a controlled, randomized double-blind study of the effect of low-
dose ASA on recurrent cerebrovascular events, were selected for this study. These patients had all had a carotid endarterectomy for TIA or completed stroke and had been randomized to either placebo or 50 mg ASA per day. The platelet aggregation was monitored in every patient on each visit and ASA was adjusted individually to achieve at least 80% inhibition of platelet aggregation. The clinicians following the patients were blind to the actual treatment regimen, which was run by a laboratory technician. The bleeding time was measured when the patients came for follow-up visits after 3 months of treatment. There were 15 women and 28 men with a median age of 61 (range 47–76).

Methods

Bleeding Time

The bleeding time was measured by two of us (AHB and NV) who as mentioned above were blind as to the treatment regimen. Ivy's method\(^{14,15}\) was used with a slight venous stasis on the arm achieved by a blood pressure cuff of 40 mm Hg on the upper arm. The incision was made by a sterile, disposable double lancet (Simplate II, General Diagnostics, Morris Plains, New Jersey) with 2 small spring knives which, when the spring is released, gives 2 cuts 1 mm deep and 5 mm long. The incisions were placed in the longitudinal direction on the volar surface on the lower arm. The bleeding time was taken as the time from incision until blood ceased to appear on a filter-paper applied to the edge of the incision every 30 seconds.

Platelet Aggregation

To 1 ml stirred platelet-rich plasma containing approximately \(4 \times 10^{10}\) thrombocytes was added 1.64 micromol sodium arachidonate. Change in light transmission was recorded with a Born aggregometer. If the platelets were not fully aggregated within 5 minutes, 5 nanomol of ADP was added as a control, since ADP aggregates cyclooxygenase inhibited as well as normal thrombocytes. The full height of the aggregation curve after ADP was taken as 100% aggregation, at least 80% inhibition of aggregation was required as a sufficient ASA response.

Results

In the placebo group the platelet aggregation was normal in all cases and the bleeding time averaged 7.0 minutes, S.D. ± 1.44 (table 1). In the treatment group the dose of ASA ranged from 50 to 70 mg day. In 19 patients with a satisfactory inhibition of platelet aggregation the bleeding time averaged 11.2 minutes, S.D. ± 3.02. This difference in bleeding time between the placebo group and the treatment group was biologically and statistically significant. In 4 patients with insufficient platelet inhibition the bleeding time ranged from 4 to 9 minutes. The response to ASA was equal in women and men, and there was no age related variation within the examined group.

There was no dyspeptic complaints in either group.

Discussion

In the sixties ASA was shown to prolong the bleeding time\(^{14,16,17}\) and in the seventies a paradoxical effect of ASA on bleeding time was described.\(^{7,18}\) In experimental animals as in normal volunteers the bleeding time was found more prolonged by small than by large ASA doses. Further, it was shown that 2 hours after ingestion of a single high ASA dose (3.9 g) there was no change in bleeding time although platelet inhibition was marked. Twenty-four to 72 hours later, however, the bleeding time became prolonged when recovery of the endothelial cyclooxygenase and prostacyclin formation had taken place.\(^{18}\)

Since then it has been shown repeatedly, that the cyclooxygenase of the vessel wall is less sensitive to ASA than that of the platelets, and the use of small ASA doses has been recommended.\(^{7,9,11,19}\) The optimal dose, however, has not been clearly defined. Jørgensen et al\(^{20}\) showed that a dose as low as 1 mg/kg bodyweight increased the bleeding time significantly, yet the authors favoured a dose of 3.5 mg/kg.

In the present study we have demonstrated that a daily ASA dose about 1 mg/kg (range 50–70 mg per day) results in inhibition of platelet aggregation and in a significant prolongation of the bleeding time. The two tests supplement each other, the latter showing a significant in vivo effect of the truly low-dose ASA regimen.

The bleeding time in the control group was 7 minutes in contrast to the 5 minutes usually seen in normals. This may reflect the well-known interindividual variation between investigators but does not invalidate the difference found between the treatment group and the control. Contributing to the long bleeding times was the placing of the incisions in the longitudinal direction of the arm transversely to the skin creases, whereas in most studies the incision is placed in the same direction as the creases.

An effect of ASA on thromboembolism independent of its effect on cyclooxygenase is a theoretical possibility which, however, lies outside the scope of this study. If platelet inhibition has any effect at all on atherothrombotic disease, a low-dose regimen might be preferable to a high-dose due to the preservation of prostacyclin formation and to the reduction in dyspeptic side-effects. Whether there will be a clinical benefit of such treatment remains to be shown. Our on-going controlled double-blind randomized study on low-dose ASA from which the patients in this study were drawn will not be concluded until 1986. The on-going United

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**Table 1** Bleeding Time in Patients Treated with Low-dose Acetylsalicylic Acid or Placebo

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>ASA daily dose</th>
<th>Bleeding time min.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group</td>
<td>20</td>
<td>0</td>
<td>7.0 S.D. 1.44</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Treatment group</td>
<td>19</td>
<td>50–70 mg</td>
<td>11.2 S.D. 3.02</td>
<td></td>
</tr>
</tbody>
</table>

ASA = acetylsalicylic acid.
Kingdom TIA trial\textsuperscript{21} compares two doses of 300 mg and 1200 mg ASA daily with placebo. It might be anticipated that even the 300 mg dose is on the high side\textsuperscript{22, 23} although both doses were shown to prolong the bleeding time.\textsuperscript{24}

Addendum:

Since the completion of the above study we have conducted an identical study in 19 patients except that the incisions for the bleeding time determinations were placed in the transversal in contrast to the longitudinal direction of the arm. By using the cutting technique commonly employed we have obtained a normalization of the bleeding time in the placebo group. The difference in bleeding time between placebo and ASA treated patients persists (table 2).

Acknowledgment

We thank laboratory technician Britta Randlev for carrying out the platelet aggregation tests.

References

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15. Stoffersen E: Determination of bleeding time by Ivy’s method. Ugeskr Laeg \textbf{144}: 1389-1390, 1982

Table 2. Bleeding Time in Patients Utilizing Transverse Incision

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group</td>
<td>7</td>
<td>5.0</td>
<td>(3-7)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Treatment group</td>
<td>12</td>
<td>7.2</td>
<td>(5-11)</td>
<td>Mann Whitney’s test</td>
</tr>
</tbody>
</table>

Mann Whitney’s test
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