Platelet Thromboxane Release After Subarachnoid Hemorrhage and Surgery

Seppo Juvela, MD, Markku Kaste, MD, and Matti Hillbom, MD

We studied adenosine diphosphate–induced platelet aggregation and the associated release of thromboxane B₂ in platelet-rich plasma from 88 patients with subarachnoid hemorrhage and 26 healthy controls. During the first 3 days after subarachnoid hemorrhage, the patients showed significantly decreased (p<0.05) platelet aggregability and thromboxane release relative to the controls, but these effects disappeared in a few days. Platelet count increased for 3 weeks after subarachnoid hemorrhage. Surgery in 67 patients was followed by significant increases in platelet aggregability (p<0.05) and thromboxane release (p<0.001). Greatest thromboxane release was found in the eight patients showing delayed (postoperative) ischemic deterioration. Although platelet hyperaggregability and increased thromboxane release were particularly prominent in these eight patients, the role of these hematologic parameters in the pathogenesis of delayed ischemic deterioration remains unclear.

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Delayed ischemic deterioration with a fixed neurologic deficit frequently complicates aneurysmal subarachnoid hemorrhage (SAH).1,2 Thromboxane produced by platelets and prostacyclin released from vascular endothelium may contribute to the development of delayed cerebral ischemia and vasospasm, which frequently occurs after SAH, as suggested by recent animal experiments.3–6 Thromboxane A₂ is a potent vasoconstrictor and platelet aggregating agent, while prostacyclin exerts opposite effects.7 Although reduced platelet aggregation has been observed soon after the onset of SAH,8 the release of thromboxane from platelets has not been studied.

We evaluated platelet count, adenosine diphosphate (ADP)-induced platelet aggregation, and the associated release of thromboxane in relation to time after SAH, surgery, the development of postoperative ischemic symptoms, and outcome.

Subjects and Methods

Our prospective study comprised 88 patients with SAH (49 men and 39 women aged 21–65 [median 44.2] years) admitted ≤96 hours after onset to the Department of Neurosurgery, Helsinki University Central Hospital and 26 healthy controls (10 men and 16 women aged 22–55 [median 40.1] years).

Blood was sampled repeatedly on weekdays from the patients; the number of samples per patient varied from one to 10. The patients and their relatives were asked about the patient’s use of steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), other drugs, and alcohol, and the patients’ urine was screened qualitatively for salicylates. After admission, the patients were given neither aspirin nor other NSAIDs. Headache was treated with narcotic analgesics and sedatives. One blood sample was taken from each control, all of whom denied having used any drugs or alcohol for 1 week before sampling.

Patients were considered to have hypertension if they used antihypertensive medications or if their blood pressure readings before the SAH had repeatedly exceeded 160/95 mm Hg. Twenty-eight patients (32%) had hypertension, and 11 of the 28 used antihypertensive medications, mostly vasodilators or diuretics. Only severe hypertension was treated after admission. No hypertensive patient had used β-adrenergic blocking agents before or during blood sampling.

SAH was verified with computed tomography (CT) (85 patients) or lumbar puncture and surgery (three patients). Each patient’s clinical status was graded on admission (all 88 patients) and before surgery (67 patients) according to Hunt and Hess (Table 1). Eighteen patients (20%) had an intracerebral hematoma at least 1 cm in diameter, and 18 (20%) had intraventricular extension of the bleeding.

Location of the ruptured aneurysm in all 88 patients and timing of the surgery in 49 patients (dictated by another study) are also shown in Table 1. The median
time from SAH (day 0) to surgery in the 49 randomized patients was 5.0 (range 1–23) days, which provided an opportunity to study platelet function at different times relative to surgery. Time to surgery was not randomized in 18 patients because they had emergency surgery necessitated by an intracerebral space-occupying hematoma (n=4), were admitted too late for randomization (n=6), were not in clinical grades I–III on admission but later recovered (n=3), or had an aneurysm in the vertebrobasilar region (n=5).

CT was obtained on admission, after clinical deterioration, and at discharge. Delayed ischemic deterioration with fixed neurologic deficit was considered to be the gradual development of totally or partially irreversible focal signs of cerebral ischemia and/or deterioration in the level of consciousness not due to any other known reason. These other reasons were excluded by CT, routine laboratory investigations, postoperative angiography, and/or autopsy.

Outcome was assessed at 1 year according to the three-point Glasgow Outcome Scale as independence (good recovery or moderate disability), dependence (severe disability or persistent vegetative state), or death.

To study the release of thromboxane from platelets (measured as the concentration of the stable metabolite thromboxane B2 [TXB2]), we applied the method of ADP-induced platelet aggregation. Briefly, after an overnight fast blood samples were collected from each subject with minimal stasis from an antecubital vein into plastic tubes containing 3.8% citrate, giving a final volume ratio of 1:10. The amount of anticoagulant was adjusted according to the packed cell volume to be the same in every case. Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 330g for 10 minutes, and platelet-poor plasma (PPP) was prepared by centrifuging the blood at 1,500g for 10 minutes. The platelet count of PRP was adjusted to 200×10^3/μl with autologous PPP. All procedures were performed at room temperature, but aggregation did not begin until the samples were warmed to 37° C.

Two hours after blood sampling, platelet aggregation was measured as the maximal percentage change in light transmittance within 5 minutes using a dual-channel aggregometer (Chrono-Log Corp., Havertown, Pennsylvania). Aggregation was induced in PRP by adding ADP to a final concentration of 8 μM with continuous stirring by a magnetic stirrer (1,200 rpm) and was allowed to proceed for 5 minutes. Aggregation was then stopped by adding 1N HCl to a final concentration of 0.1 mol/L, and the tubes were immediately transferred to an icebath. The amount of TXB2 released during aggregation was measured directly in the plasma using a double-antibody radioimmunoassay technique and a standardized kit (New England Nuclear, Boston, Massachusetts). The results of TXB2 release were subjected to logarithmic transformation because the distribution was skewed. TXB2 values and times after SAH onset are expressed as median±standard error of the median (SE). Platelet aggregation and platelet count are expressed as mean±standard error of the mean (SEM).

Because samples from different patients were collected on different post-SAH days, using BMDP (data manager program and statistical package, 1988 version) for each patient the results of samples taken on the day closest to days 2 (range 1–3), 5 (4–6), 8 (7–9), 12 (10–14), 18 (15–20), and 25 (22–28) were analyzed. Data for all samples, for preoperative samples, and for postoperative samples were analyzed separately. Data were also analyzed separately for samples showing irreversible aggregation because TXB2 is released from platelets during the secondary phase of aggregation.
Table 2. Reversibility of Platelet Aggregation Induced by 8 μM Adenosine Diphosphate in Patients With Subarachnoid Hemorrhage by Days After Onset

<table>
<thead>
<tr>
<th>Days after onset</th>
<th>Yes</th>
<th>No</th>
<th>%</th>
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<tr>
<td>All patients</td>
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<td>25</td>
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<td>13</td>
<td>48</td>
<td>21.3</td>
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<td>48</td>
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<td>33</td>
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<td>24</td>
<td>33.3</td>
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<td>34</td>
<td>5.6</td>
<td>1</td>
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<td>20</td>
<td>9.1</td>
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<td>13</td>
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<td>0</td>
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<td>1</td>
<td>16</td>
<td>5.9</td>
<td>2</td>
<td>8</td>
<td>20.0</td>
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</table>

\*, \% percent with reversible aggregation. For control, 13 yes and 13 no, 50% reversible aggregations. No control had used NSAIDs for 1 week before blood sampling. NSAIDs, nonsteroidal anti-inflammatory drugs.

*p<0.05, 0.001, 0.01, respectively, different from control by Fisher's exact two-tailed test.

Results

Platelet aggregability increased after the onset of SAH, as shown by a decrease over time in the percentage of patients with reversible aggregations (Table 2). Irreversible (secondary-phase) platelet aggregation was verified from the aggregation curves. The effect was observable for 3 weeks after the end of the first week. The use of NSAIDs before admission increased the proportion of patients with reversible aggregations observed during the first week after SAH. The differences between preoperative and postoperative values were composed using the paired \( t \) test or the Wilcoxon signed rank test.

After excluding all patients with reversible aggregations and all patients who had used NSAIDs within the 2 weeks before admission, platelet aggregation of the patients was significantly \((p<0.05)\) lower than control shortly after the onset of SAH (Figure 1). Platelet count in patients with irreversible aggregations who used no NSAIDs increased significantly \((p<0.01)\) relative to control after the first week from the onset of SAH (Figure 1).

Intake of NSAIDs before admission significantly decreased TXB\(_2\) release (data not shown). However, patients with irreversible aggregations not using NSAIDs had significantly lower TXB\(_2\) release 2 days after the onset of SAH than the controls (Figure 1). TXB\(_2\) release per 10\(^7\) platelets multiplied by the platelet count per liter of whole blood gives TXB\(_2\) release per liter of blood. If the capacity of blood to form TXB\(_2\) is calculated this way, the patients with irreversible aggregations not using NSAIDs had a significantly reduced capacity \((p<0.01)\) approximately 2 days after the onset of SAH. One to three weeks after onset, the capacity increased (particularly in patients treated surgically [data not shown]), an
effect that can at least partly be explained by the observed increase in platelet count (Figure 1).

Irreversible aggregations for TXB₂ analyses both before and after surgery were available for only 29 patients who had not taken NSAIDs. The preoperative sample was collected on the day of or the day before surgery, and the postoperative sample was collected 2–5 days after the operation. Preoperative samples were taken 5.0±0.6 (range 1–23) days and postoperative samples 8.0±0.9 (range 4–31) days after the onset of SAH. Platelet aggregation and both TXB₂ parameters increased significantly after surgery, while platelet count did not (Table 3). A significant increase in platelet count occurred somewhat later, during the second and third weeks after SAH.

Twelve patients (nine of whom had not used NSAIDs) had delayed ischemic deterioration with fixed neurologic deficit. Eight of the nine patients had both preoperative and postoperative blood samples with irreversible aggregation. Three of the eight had ischemic symptoms before surgery, but the symptoms were clearly enhanced after surgery. Increases in platelet count and TXB₂ release were more pronounced in these eight patients than in the 21 who did not develop ischemia (Table 4). Preoperative TXB₂ release in the two groups were 1,918.8±843.5 and 1,371.0±472.7 fmol/10⁷ platelets, respectively. The corresponding postoperative values were 4,170.5±1,394.2 and 2,320.5±589.2 fmol/10⁷ platelets. The capacity of blood to form TXB₂ rose in the two groups from 36.7±19.6 to 126.8±52.1 nmol/l and from 51.1±10.1 to 78.1±13.8 nmol/l, respectively. The postoperative capacity of blood to form TXB₂ was significantly greater in patients developing ischemia (p<0.05).

Postoperative TXB₂ release and formation capacity in patients developing ischemia were significantly higher (p<0.05) in samples taken approximately 12 (range 10–14) days after the onset of SAH than in either patients not developing ischemia or controls. At that time, TXB₂ release in nonischemic patients was 2,323.5±637.7 fmol/10⁷ platelets, while that in ischemic patients was 6,524.0±2,648.0 fmol/10⁷ platelets. The corresponding values for TXB₂ formation capacity were 77.2±25.6 and 235.9±65.8 nmol/l.

Timing of surgery in the 49 randomized patients was not significantly associated with delayed ischemic deterioration. The differences between preoperative and postoperative values for platelet function variables did not differ significantly among groups operated on 0–3, 4–7, and ≥8 days after SAH onset. However, the group that underwent surgery early (0–3 days) had postoperative values not significantly different from those of controls, while the groups that were operated on ≥4 days after the onset of SAH had significantly greater TXB₂ release (p<0.05) and TXB₂ formation capacity (p<0.01) 10–14 days after onset than controls. TXB₂ release was similar in those operated on 4–7 days after onset (3,731.5±1,186.9 fmol/10⁷ platelets) and those operated on ≥8 days after onset (3,629.3±2,031.3 fmol/10⁷ platelets). The corresponding values for TXB₂ formation capacity were 135.6±49.3 and 119.5±52.4 nmol/l.

We found no significant associations between outcome, clinical grade on admission, clinical grade before surgery, sex, age, presence of intraventricular or intracerebral hemorrhage, location of the aneurysm, or blood loss or transfusion due to surgery and the measured hemostatic parameters.

Thirty-five patients (40%) reported that they had used NSAIDs before admission, and 14 of the 35 had salicylates in their urine on admission. Although intake of NSAIDs before admission decreased platelet aggregability and TXB₂ release during the first week after SAH, three of these 35 patients developed ischemia. The corresponding figures for those who had not used NSAIDs were nine of 53. The hemostatic parameters were almost identical in the two groups after the first week from the onset of SAH.

### Table 3. Effects of Surgery on Irreversible Platelet Aggregation, Platelet Count, TXB₂ Release, and Capacity of Blood to Form TXB₂ in 29 Patients With Subarachnoid Hemorrhage Not Using Nonsteroidal Anti-inflammatory Drugs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet aggregation (%)</td>
<td>66.8±3.1</td>
<td>74.3±3.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Platelet count (10⁹/l)</td>
<td>262.3±18.0</td>
<td>288.4±17.9</td>
<td>NS</td>
</tr>
<tr>
<td>TXB₂ release (fmol/10⁷ platelets)</td>
<td>1,568.0±362.0</td>
<td>3,050.5±719.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TXB₂ formation (nmol/l)</td>
<td>49.1±8.8</td>
<td>84.1±17.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

TXB₂, thromboxane B₂. Data are mean±SEM for platelet variables (p by paired t test) and median±SE for TXB₂ variables (p by Wilcoxon's signed rank test). p, different from before.

### Table 4. Effects of Postoperative Ischemic Deterioration on Differences Between Postoperative and Preoperative Irreversible Platelet Aggregation, Platelet Count, TXB₂ Release, and Capacity of Blood to Form TXB₂ in Patients With Subarachnoid Hemorrhage Not Using Nonsteroidal Anti-inflammatory Drugs

| Parameter                 | Difference | p   |
|---------------------------|           |-----|
| Platelet aggregation (%)  | No ischemia: 5.2±3.9 | NS  |
|                           | Ischemia:  11.5±6.6  |     |
| Platelet count (10⁹/l)    | No ischemia: −0.2±15.8 | <0.01 |
|                           | Ischemia:   95.0±13.4 |     |
| TXB₂ release (fmol/10⁷ platelets) | No ischemia: 697.2±303.7 | <0.01 |
|                           | Ischemia:   2,692.3±849.1 |     |
| TXB₂ formation (nmol/l)   | No ischemia: 17.3±8.8 | <0.01 |
|                           | Ischemia:   99.0±22.8  |     |

TXB₂, thromboxane B₂. Data are mean±SD, p by Mann-Whitney U test. n=21 for no ischemia, n=8 for ischemia.
Discussion

In spite of extensive investigation, both the etiology and pathogenesis of delayed cerebral ischemia associated with SAH are still obscure and therefore no effective treatment is available.\(^1,2\) The release of and the capacity for formation of TXB\(_2\) in human platelets after SAH have not been studied.

We demonstrate that ADP-induced platelet aggregation and the associated release of TXB\(_2\) decreased after the onset of SAH, then increased. Platelet function was activated irrespective of surgery, but surgery clearly enhanced the effect, which was particularly prominent if the patient developed permanent symptoms of cerebral ischemia.

Several factors can be considered to influence platelet function after SAH. Among them are drug effects, the effects of stress hormones, nutritional factors, infections, acute platelet consumption leading to qualitative changes in the platelet population, and desensitization or activation of platelets by other factors. It can be assumed that disturbances in platelet function influence the patient's outcome by predisposing to more profound bleeding, recurrent bleeding, or the development of ischemic complications during the first few weeks after the onset or after surgery.

We tried to exclude the confounding effects of drug treatment by asking the patients (and/or their relatives) about drug intake before admission and by screening their urine for salicylates. Many patients had used antiplatelet drugs for severe headaches due to the SAH, and most of these patients showed absence of the secondary or irreversible phase of ADP-induced platelet aggregation and absence of marked TXB\(_2\) release, as expected. As a rule, such findings disappeared within 1 week after admission since NSAIDs were not administered. We could not observe any significant effect of previous drug intake on outcome, including the development of ischemic complications.

Adrenaline has been reported to stimulate platelet aggregation and TXB\(_2\) release.\(^3,16,17\) Other stress hormones may also have an effect.\(^16\) However, in one study adrenaline infusion did not stimulate ADP-induced platelet aggregation and associated TXB\(_2\) release.\(^18\) We cannot exclude the possibility that stress hormones play some role in platelet activation after the onset of SAH and surgery.

TXB\(_2\) release increased after surgery, and peaked during the second week after SAH. High values were found in patients operated on \(\geq 4\) days after SAH onset, and the highest values were found in those who developed ischemic deterioration with fixed neurologic deficits. The postoperative increase in TXB\(_2\) release in patients developing cerebral ischemia cannot be explained by their deteriorated clinical condition because those whose conditions worsened due to postoperative hematoma, clipping of the arterial branch containing the aneurysm, hydrocephalus, or rebleeding did not have such an increase.

Platelet count may temporarily increase after severe hemorrhage, surgery, and splenectomy.\(^19\) The reason patients with delayed ischemic deterioration had more obvious increases in platelet count soon after surgery (average 3 days) than the other patients remains unknown.

Intimal damage in cerebral arteries around the ruptured aneurysm exposes subendothelial structures to circulating platelets. This may lead to adherence of circulating platelets and hence aggregation. TXB\(_2\) released from aggregating platelets further promotes platelet activation and local vasoconstriction.\(^2\) Surgery, with its accompanying stress, might also activate this process.

We observed reduced TXB\(_2\) release on admission, just after the onset of SAH, even in patients who had not used NSAIDs. An experimental study\(^20\) demonstrated that platelets accumulate on the intimal surface of cerebral arteries after SAH. Probably the most active platelets are trapped on the intimal surface. A population of platelets releasing less TXB\(_2\) may remain in circulation, and this may explain the reduced TXB\(_2\) release just after the onset of SAH. However, we have no evidence to suggest that our findings are associated with rupture of an aneurysm. A peak incidence of rebleeding has been observed during the first 24 hours after SAH.\(^21,22\) Whether a causal relation exists between the peak incidence of rebleeding and decreased platelet function is unclear.

In conclusion, the release of TXB\(_2\) associated with ADP-induced platelet aggregation was low 1–3 days after the onset of SAH. Thereafter, TXB\(_2\) release normalized or even increased relative to that in controls. TXB\(_2\) release increased after surgery. The highest values were associated with the development of permanent cerebral ischemic deficits. The role of TXB\(_2\) in the pathogenesis of SAH and its complications remains unknown.

References


KEY WORDS • platelet aggregation • subarachnoid hemorrhage • thromboxane B₂
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