Enhanced In Vivo Platelet Activation In Subtypes Of Ischemic Stroke

Arun B. Shah, M.D., Nancy Beam, M.S., and Bruce M. Coull, M.D.

SUMMARY It remains uncertain whether platelet activation in ischemic stroke is contributory or secondary to brain ischemia. The efficacy of aspirin (ASA) in stroke prevention suggests that platelet activation contributes to the occurrence of stroke. On the other hand, platelet activation may be simply a generalized consequence of cerebral ischemic damage. To examine this issue, plasma levels of the platelet specific proteins β-thromboglobulin (β-TG) and platelet factor 4 (PF4) were measured in fifty-eight patients with various defined types of acute ischemic strokes.

β-TG was a broader indicator of platelet activation than PF4. Compared with an age-matched control group, thromboembolic and cardioembolic stroke patients had significantly elevated β-TG levels (p < 0.001). Also, β-TG levels in these stroke categories were significantly higher in samples drawn within the first week after the event than in those drawn later (p < 0.001). In contrast, β-TG levels in lacunar stroke patients and in most TIA patients were normal.

β-TG levels did not correlate with the volume of cerebral infarction as measured by planimetry from CT scans. Moreover, β-TG levels in patients on chronic ASA therapy at the time of stroke did not differ from those in patients of the same diagnostic categories not taking aspirin. These data indicate that platelet activation may be important in some, but not all, subtypes of ischemic stroke and that platelet activation can occur in stroke even though the platelet cyclooxygenase pathway is suppressed.

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control group, some of whom were hospitalized, had significant vascular disease or hypertension.

**Stroke Classification**

Standard clinical and laboratory criteria were used to classify acute strokes as thromboembolic (TE), cardioembolic (CE) or lacunar infarction. The thromboembolic group was considered to have primarily carotid artery disease, giving rise to arterial thromboembolism. All of the cardioembolic group had significant heart disease and appeared to have suffered stroke as a result of embolism of thrombus from within the heart. Lacunar infarction was diagnosed by the characteristic clinical syndrome, the absence of significant heart disease and the absence of significant carotid or vertebrobasilar atherosclerosis as determined by cerebral angiography. For diagnosis, CT scans performed in persons with lacunar infarction were either negative or showed a lucency characteristic of a lacune. The specifics of these diagnostic categories are shown in table 1.

When all diagnostic criteria were applied, there remained a group of patients in whom a definitive diagnosis which resolved thromboembolic from cardioembolic stroke could not be accomplished. None of these patients had lacunar syndrome and none were considered to have had lacunar infarction. Cerebral angiography was either negative or not performed and overt significant heart disease was clinically absent. Accordingly, these strokes were classified as infarctions of uncertain mechanism (UM) of either thrombo- or cardioembolic origin. Eleven patients experienced ischemic neurologic symptoms typical of TIAs. In most, symptoms were from minutes to hours in duration, and in all the neurologic deficit was resolved within 24 hours. Blood samples were obtained in all patients prior to cerebral angiography. Significant risk factors were noted for each patient, and table 2 shows the distribution and the number of patients in each stroke category.

**Measurement of β-TG, PF₄, and TXB₂**

β-TG and PF₄ were measured by radioimmunoassay using reagents supplied by Amersham and Abbott Laboratories, respectively. Blood was withdrawn without stasis from the antecubital vein with a 19-gauge butterfly needle and placed into chilled tubes containing anticoagulant and platelet inhibitors supplied by the manufacturers. The tubes were inverted, chilled on ice for 30 minutes, and spun at 4500 x g for 20 minutes at 4°C to obtain platelet poor plasma. Plasmas were frozen at −20°C and tested within one week.

The first 3cc sample of blood withdrawn to establish blood flow without stasis was used to measure serum TXB₂ production. The blood was allowed to clot at 37°C for 60 minutes in a nonsiliconized glass tube and then spun at 4°C for 20 minutes at 4500 x g. The serum was stored frozen at −20°C until analysis of TXB₂ by radioimmunoassay. Antibody and standard were obtained from Seragen; tritiated thromboxane from New England Nuclear. The TXB₂ assay was sensitive to 5 pg, with less than 5% interference from any of eight other prostaglandins tested. Serum from patients who had not received aspirin was diluted 1:50 in assay buffer prior to analysis; serum from patients taking aspirin was not diluted. Data were evaluated by Student’s t-test, two-tailed distribution.

**Results**

Table 3 summarizes β-TG and PF₄ values for each stroke category and controls. In general, β-TG values...
TABLE 3  Levels of Platelet Marker Proteins (mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (years)</th>
<th>β-TG (ng/ml)</th>
<th>PF4 (ng/ml)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young controls</td>
<td>26.7 ± 1.3*</td>
<td>3.7 ± 0.3*</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Elderly controls</td>
<td>63.2 ± 2.5</td>
<td>6.5 ± 1.4</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Thromboembolic</td>
<td>62.5 ± 4.1†</td>
<td>14.9 ± 3.5†</td>
<td></td>
<td>13(12)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>68.7 ± 5.2†</td>
<td>9.3 ± 1.8</td>
<td></td>
<td>10 (9)</td>
</tr>
<tr>
<td>Uncertain mech.</td>
<td>66.4 ± 6.8‡</td>
<td>10.0 ± 1.9</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Lacune</td>
<td>59.5 ± 3.9</td>
<td>11.5 ± 3.2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>TIA</td>
<td>60.9 ± 5.9</td>
<td>7.6 ± 1.9</td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

(†) indicates n for PF4 when different from β-TG.
*0.1 > p > 0.05, †0.05 > p > 0.025, ‡p < 0.001 vs. elderly controls.

exhibited less variability than PF4 values. β-TG was slightly higher in elderly controls than in young healthy individuals, but was significantly elevated in the thromboembolic, cardioembolic and uncertain mechanism stroke patients. Within these three diagnostic categories the levels were quite similar. On the other hand, β-TG values for the lacunar and TIA groups were within the normal range. In concert with β-TG, PF4 levels tended to be higher in the elderly compared with younger controls. However, the only stroke patients in whom PF4 values were significantly elevated were those in the thromboembolic group.

Figure 1 shows the relationship between the timing of the β-TG and PF4 determinations and the onset of infarction. The data are combined for TE, CE and UM groups and show significantly higher β-TG levels for samples drawn within the first week after the event (p < 0.001). The trend for PF4 was in the opposite direction, with later sampling tending to yield higher values, but this difference did not attain significance. For the lacunar group, most determinations were done within hours to days after the onset of symptoms; thus, meaningful comparisons could not be made.

Figure 2 shows the individual β-TG determinations for each of the diagnostic groups except lacune. Among those with TE, CE, and UM strokes, 15 patients were taking between 300 and 1200 mgs of aspirin daily when the stroke occurred. The mean (± SE) β-TG level in these individuals was 54.5 ± 4.3 compared with 57.8 ± 3.2 ng/ml in those of the same diagnostic types who were not on chronic aspirin (ASA) treatment. This lack of suppression of circulating β-TG levels occurred despite ASA inhibition of platelet cyclooxygenase activity as monitored by total TXB2 production determinations: TXB2 production in patients on ASA averaged 1.9 ± 2.0 vs. 182 ± 11.8 ng/ml in patients not on ASA (p < 0.001). Moreover, TXB2 production in nonaspirinated stroke patients did not differ from that of elderly controls (182 ± 18.6 ng/ml, n = 11).

We had also postulated that if platelet activation were a generalized consequence of ischemic damage, it might relate quantitatively to the volume of infarcted tissue. Since β-TG appeared to be the broader indicator of platelet involvement in stroke, we investigated the relationship of circulating levels of β-TG to the area of cerebral infarction as estimated by planimetry in those with positive CT scans. As shown in figure 3, there is no correlation between β-TG levels and infarct volume.

Discussion

Our normal ranges for both platelet proteins are in good agreement with other studies.7-9,10 Although levels of these proteins have been reported to increase with age,11 our data show the increase to be marginal, possibly because our elderly control group was free of chronic disease.

In agreement with others, we observed far more variability in PF4 values than in β-TG.12 Since the rate of release of both proteins into the circulation appears to be equivalent, the lower PF4 values in normal individuals is thought to be a consequence of its shorter half-life and concomitant binding to vascular endothelium.8 Accordingly, the greater variability of PF4 levels observed in healthy older individuals and in stroke patients may reflect changes in endothelial binding with age and/or vascular disease. It is unclear why PF4 was significantly elevated in the TE, but not CE or UM categories; but this may be related to altered vascular

Figure 1  In non-lacunar strokes the β-TG levels are significantly higher in those patients tested during the first week after stroke.
Plasma \( \beta \)-TG in Controls and Patients

**Figure 2.** Individual plasma levels of \( \beta \)-TG in non-lacunar stroke do not distinguish patients receiving aspirin at the time of stroke.

The presence of significantly elevated \( \beta \)-TG levels in TE, CE, and UM stroke categories strongly suggests that the role of platelets in these types of stroke is similar. That the \( \beta \)-TG values did not differ significantly among these three stroke categories strengthens this interpretation. We also noticed that \( \beta \)-TG levels in these categories of stroke were significantly lower in samples drawn more than a week after the event. This could be interpreted as evidence for a secondary, non-specific involvement of platelets in these strokes. Alternatively, this trend may simply reflect a diminution in the platelet activation which precipitated the acute stroke. In this regard, it should be recalled that no correlation between the \( \beta \)-TG level and the volume of infarcted tissue could be demonstrated.

The \( \beta \)-TG level was not elevated in most (9/10) lacunar stroke patients. In fact, four of these patients were low normal for their age group, with values ranging from 12 to 20 ng/ml. Others have recently reported that the ADP-induced platelet release reaction is reduced in persons with primary intracerebral hemorrhage — a disease strongly related to hypertension and involving the same vascular territory as lacunar infarction. Nearly normal \( \beta \)-TG values have also been noted in patients with hemorrhagic cerebrovascular disease. Thus, studies with larger numbers of lacunar and hemorrhagic stroke patients should be pursued to determine if, in fact, platelet hypo-function contributes to the occurrence of these types of strokes.

Although elevations of \( \beta \)-TG have been reported in patients with TIA, the majority (8/11) of our patients had normal values. The three TIA patients in our study with abnormally high \( \beta \)-TG values included two scheduled for carotid endarterectomy and one who had suffered a completed stroke prior to the occurrence of TIAs. Since TIAs arise from diverse processes, it would clearly be of value to characterize TIA patients as thoroughly as completed stroke patients when reporting platelet activation studies.

We also noted that \( \beta \)-TG was elevated in many patients who had experienced either TE or CE stroke despite receiving chronic ASA therapy sufficient to inhibit thromboxane production. These data tend to support earlier observations regarding the persistence of circulating platelet aggregates in stroke patients receiving ASA and imply that secondary platelet activation in vivo can occur even though cyclooxygenase activity is inhibited. Finally, in agreement with others, we found serum TXB production in nonaspirated stroke patients to be within the normal range for age-matched controls.

These results suggest that elevated \( \beta \)-TG is not simply a generalized consequence of cerebral ischemia, and that platelet activation may be important in the genesis of some, but not all, subtypes of ischemic stroke. Most noteworthy in this regard are the normal or even subnormal \( \beta \)-TG levels found in lacunar stroke patients. The pathophysiologic heterogeneity of stroke is also reflected in the finding of normal \( \beta \)-TG values in approximately one-fourth of all embolic stroke cases and in the majority (8/11) of patients with TIAs. Still unresolved, however, is the issue of whether enhanced platelet activation contributes to or is secondary to the stroke itself. To clarify this issue it will be necessary to refine the clinical diagnosis of stroke subtypes. For example, in roughly one-third of embolic cases we were unable to determine a source of embolism, nor

**Figure 3.** Plasma \( \beta \)-TG levels do not correlate with the volume of cerebral infarction as estimated by planimetry of the infarction seen on CT scans.
Hemodynamics In Hemorrhagic Infarction — An Experimental Study

HIROBUMI SEKI, M.D., TAKASHI YOSHIMOTO, M.D., AKIRA OGAWA, M.D., AND JIRO SUZUKI, M.D.

SUMMARY Using the canine thalamic infarction model, hemodynamics, CO₂ responses and thalamic EEG changes were studied in 7 dogs. Of the 7 animals, 4 showed hemorrhagic infarction and 3 did not, following recirculation after 6 hours of vascular occlusion. 1) The rCBF of the animals showing hemorrhagic infarction included hyperperfusion due to recirculation, and then fell to a level below the pre-occlusion level in a relatively short period. The CO₂ response became disturbed both during occlusion and after release of occlusion. Thalamic EEG was nearly flat during vascular occlusion and recovery was not seen following recirculation. 3) rCBF of the animals not showing hemorrhagic infarction recovered rapidly to the pre-occlusion level due to recirculation. The CO₂ response was somewhat disturbed during occlusion, but recovered following recirculation. Thalamic EEG was well preserved both during occlusion and after release.

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