Increased Thromboxane Biosynthesis in Patients With Acute Cerebral Ischemia

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Background and Purpose: Clinical and experimental studies suggest that platelets have a major role in the pathogenesis of cerebral ischemia. However, ex vivo both platelet aggregation studies and measurements of platelet-derived products in patients with cerebral ischemia have shown inconsistent results. The present study was designed to resolve this inconsistency.

Methods: We have measured the urinary excretion of a thromboxane metabolite, 11-dehydro-thromboxane B₂, by a previously validated radioimmunoassay technique in 51 patients with acute cerebral ischemia who had experienced either a transient ischemic attack (14 patients) or an ischemic stroke (37 patients) and in 20 control patients with nonvascular neurological disorders. The median time between the onset of symptoms and urine sampling was 24 hours (range, from 2 hours to 8 days).

Results: The excretion rate of immunoreactive 11-dehydro-thromboxane B₂ ranged between 39 and 478 pmol/mmol creatinine in patients with a transient ischemic attack and between 23 and 5,916 pmol/mmol creatinine in stroke patients, with 29% (p=0.18) and 51% (p=0.004) of the urine samples, respectively, exceeding the upper limit of the control samples (251 pmol/mmol creatinine [mean±2 SD]) (p=0.01). In stroke patients, metabolite excretion was not related to the type (cortical or "lacunar") or site of cerebral infarction. Low-dose aspirin (50 mg per day for 7 days) reduced the urinary excretion by approximately 85% in 11 consecutive stroke patients.

Conclusions: We conclude that 1) episodes of enhanced thromboxane biosynthesis are detected infrequently in patients with a transient ischemic attack, 2) aspirin-suppressible episodes of increased thromboxane formation can be detected during the early phase of acute ischemic stroke, and 3) this finding may provide a rationale for testing the efficacy and safety of this drug in this setting. (Stroke 1993;24:219–223)

Key Words: • aspirin • cerebral ischemia, transient • platelet aggregation • thromboxanes

Since thromboembolism from extracranial arteries is widely regarded as one of the most common causes of transient ischemic attacks (TIAs) and ischemic strokes, platelet function in these conditions has received increasing attention in the past years. A few older studies have reported platelet hyperaggregability, particularly in young stroke victims, but the validity of these in vitro studies has been challenged. Others have studied in vivo platelet activation by measuring blood levels of a platelet-specific protein, β-thromboglobulin, which is secreted from α-granules of activated platelets. Some have found elevated levels of β-thromboglobulin in all types of transient or permanent cerebral ischemia or exclusively in some subtypes of stroke. A major drawback of many of these studies is that the measurements were not confined to the acute phase after the ischemic event but were performed up to 7 months later. Furthermore, β-thromboglobulin levels varied widely among these studies and showed a considerable overlap with those of control patients. These inconsistent results may be explained, at least in part, by ex vivo platelet activation during and after blood sampling; this artifact is a major disadvantage of testing platelet activation in peripheral blood.

To investigate the actual rate of thromboxane biosynthesis in vivo, we have studied the urinary excretion of a major enzymatic metabolite of thromboxane B₂, 11-dehydro-thromboxane B₂, in patients with acute cerebral ischemia and in appropriate control patients. Thromboxane A₂ represents an amplification mechanism of platelet activation and aggregation by virtue of its being synthesized and released in response to a variety of agonists (e.g., collagen, ADP, and thrombin) and in turn inducing platelet aggregation. Evidence for enhanced thromboxane biosynthesis, as reflected by increased urinary excretion of thromboxane metabo-
lies, has been reported in patients with unstable angina and acute myocardial infarction.\textsuperscript{14–16} In the present study, we sought to determine whether the formation of this potent platelet agonist is altered in vivo, in association with acute cerebral ischemia.

**Subjects and Methods**

**Study Patients**

Between June 1987 and November 1990 a total of 51 consecutive patients with acute cerebral ischemia were prospectively studied. Fourteen patients (11 men and three women; mean age, 62.5 years; range, 48–81 years) had experienced a TIA, defined as an episode of temporary and focal cerebral dysfunction, presumably of vascular origin, lasting less than 24 hours and leaving no persistent deficit. The 47 other patients (21 men and 16 women; mean age, 62.5 years; range, 19–93 years) had an ischemic stroke, with symptoms lasting longer than 24 hours. All patients were examined within 48 hours after the onset of neurological symptoms by two neurologists, including one of us (P.J.K.). The nature and time course of the symptoms were recorded by means of a detailed checklist.\textsuperscript{17} The symptoms and signs in patients with stroke were classified as related either to the carotid or the vertebrobasilar arterial territory. In patients with stroke in the carotid territory, the symptoms were further subdivided according to a cortical localization (aphasia, dysgraphia, dyslexia, or hemianopsia) or one of the following lacunar syndromes: pure motor hemiplegia, pure sensory stroke, or sensorimotor stroke.\textsuperscript{18} Apart from the neurological history, the following vascular risk factors were recorded: smoking habits, hypercholesterolemia (fasting level >8.0 mmol/l), hypertension (systolic blood pressure >160 mm Hg and/or diastolic pressure >90 mm Hg, treated or not), diabetes mellitus (type I or II, treated or not), and a history of intermittent claudication, stable angina pectoris, prior myocardial infarction, or previous vascular surgery (carotid, coronary, or peripheral vascular surgery).

All patients had routine laboratory investigations, including hemoglobin, hematocrit, leukocyte, erythrocyte, and platelet counts, erythrocyte sedimentation rate, blood urea, creatinine, fasting cholesterol and glucose, liver enzymes, and syphilis serology. Other ancillary investigations performed in all patients included computerized tomographic scanning of the brain, standard 12-lead electrocardiogram (ECG), and chest x-ray. The computerized tomographic scans were reviewed by two neurologists who had no knowledge of the clinical features or the results of the biochemical studies. Cerebral infarcts were defined as sharply defined hypodense lesions and were subdivided into lacunar infarcts (small deep lesions), cortical infarcts (radiolucent lesions in the territory of one or more cortical arteries), and border-zone infarcts (wedge-shaped hypodensities between the territories of two major cerebral arteries or between deep and superficial branches of the middle cerebral artery).

**Control Patients**

During the study period 20 control patients (11 men and nine women; mean age, 64.2 years; range, 41–85 years) with nonvascular neurological disorders, such as cerebral trauma, Parkinson’s disease, epilepsy, or cerebral spondylotic myopathy, were studied within 2 days after admission to the hospital. In addition, we included eight patients with atypical attacks, i.e., patients in whom the diagnosis of TIA was considered on referral but could not be confirmed, according to the independent judgment of two neurologists; these patients included three men and five women with a mean age of 50.8 years (range, 30–83 years). Their attacks consisted of one or more of the following symptoms: isolated dizziness or sensory symptoms; accompanying unconsciousness, amnesia, or confusion; focal symptoms associated with a previous history of migraine or scintillating scotomas; and isolated diplopia, dysphagia, or vertigo. Informed consent was obtained from each control subject. Vascular risk factors and vascular disease were similarly recorded as in the other study patients. Ancillary investigations included laboratory tests as specified above, ECG, and chest x-ray.

**Exclusions**

Patients were excluded if they had been taking aspirin or other nonsteroidal anti-inflammatory drugs during the preceding 10 days. All other drugs were continued during the study period. Also excluded were patients requiring invasive investigations (particularly angiography) within the next 48 hours, patients with possible vasculitis (erythrocyte sedimentation rate >40 mm in the first hour), renal disease (creatinine >150 μmol/l), or unstable angina pectoris (recent onset of class III–IV chest pain according to the Canadian Heart Association, in the absence of an increase in the muscle and/or brain fraction of plasma creatinine kinase).

Urine was collected during the night as soon as possible after admission to hospital in all patients. The median time between neurological symptoms and the first urine collection was 24 hours (range, from 2 hours to 8 days). In the first 25 of the patients with cerebral ischemia and in the first 10 control patients, urine was sampled again 24 hours later. The volume of each urine sample was recorded, and the creatinine concentration was measured. The samples were frozen immediately after voiding and stored at \(-20^\circ\text{C}\) until extraction. In 11 unselected and consecutive stroke patients, additional overnight urine samples were collected on the third, fifth, and seventh day of treatment with 50 mg aspirin per day.

Immunoreactive 11-dehydro-thromboxane B\(_2\) was extracted from 20-ml aliquots of each coded urine sample (the pH was adjusted to 4.0–4.5 with formic acid) on SEP-PAK C18 cartridges (Waters Associates, Milford, Mass.) and eluted with ethyl acetate. The eluate was subjected to silicic acid column chromatography and further eluted with benzene:ethyl acetate: methanol (60:40:30). The overall recovery, as determined by the addition of 11-dehydro-[\textsuperscript{3}H]thromboxane B\(_2\), averaged 80±6%. Immunoreactive 11-dehydro-thromboxane B\(_2\) eluted from silicic acid columns was assayed at a final dilution of 1:15–1:1,000, as described elsewhere.\textsuperscript{19} The urinary excretion rate of 11-dehydro-thromboxane B\(_2\) was expressed as picomoles per millimole creatinine.

The data were analyzed by means of the Statistical Package for the Social Sciences (SPSS). Comparisons between groups were made with the Wilcoxon rank-sum test for unpaired data. To account for differences in baseline data between study and control patients,
we performed a logistic regression analysis. All numerical data are expressed as mean ± 1 SD. A level of p < 0.05 (two-sided testing) was considered statistically significant.

**Results**

The individual values of 11-dehydro-thromboxane B₂ in all patients and controls are depicted in Figure 1. These values ranged between 39 and 478 (median, 127) pmol/mmol creatinine in TIA patients and between 23 and 5,916 (median, 264) pmol/mmol creatinine in stroke patients. In four of the 14 TIA patients (29%; 95% confidence interval, 8–58%; p = 0.18) and in 19 of the 37 stroke patients (51%; 95% confidence interval, 34–68%; p = 0.004), the excretion rate exceeded 2 SD of the mean value of the control patients with nonvascular disorders (119 ± 66 pmol/mmol creatinine). To account for minor differences in baseline data between the study and control patients, none of which reached statistical significance, we performed a logistic regression analysis. This showed that the difference in thromboxane production was independent of age and sex, vascular risk factors (in particular, diabetes), and the results of blood tests. Of all blood tests, only the platelet count moderately correlated with the 11-dehydro-thromboxane B₂ excretion rate (n = 79, r = 0.38, p = 0.01). The mean platelet count did not differ significantly between the four patient groups. No correlation was found between the level of metabolite excretion and the time interval between the onset of neurological symptoms and the collection of urine in patients with cerebral ischemia. The episodic nature of changes in metabolite excretion was supported by repeated measurements performed on 2 consecutive days in the first 25 patients with cerebral ischemia (eight with TIA and 17 with stroke). Of these 25 patients, two TIA patients and six stroke patients had increased excretion rates on the first day; however, one TIA patient and two stroke patients had normal levels on the second occasion. The mean excretion rate in all patients with cerebral ischemia fell from 275 ± 225 pmol/mmol creatinine on day 1 to 160 ± 132 pmol/mmol creatinine (p = 0.08). In 10 control patients in whom urine was sampled twice, the mean excretion rate was within the same range on both occasions (63 ± 21 pmol/mmol creatinine the first time and 65 ± 27 pmol/mmol creatinine the second time).

The rate of urinary excretion of 11-dehydro-thromboxane B₂ was not related to the nature, the duration, or the severity of clinical symptoms. In particular, an increased level was equally frequent in patients with and without headache during the attack. In stroke patients, metabolite excretion was not related to the type (cortical or "lacunar") or site (carotid versus vertebrobasilar territory) of cerebral infarction (Table 1).

In patients with atypical attacks, 11-dehydro-thromboxane B₂ averaged 99 ± 40 pmol/mmol creatinine. In one of the eight patients with atypical attacks, the metabolite excretion was abnormally elevated (Figure 1). This patient had been admitted with repeated dizzy spells, which were on one occasion followed by nonrotatory dizziness, nausea, and brief unconsciousness. Two days after admission this patient was found to have signs of myocardial ischemia on the ECG during a further attack.

To investigate whether the enhanced biosynthesis of thromboxane in patients with cerebral ischemia was of platelet or nonplatelet origin, we studied the short-term effects of a platelet-selective regimen of aspirin therapy (50 mg per day for 7 days) on metabolite excretion in 11 consecutive stroke patients. Before aspirin treatment, 11-dehydro-thromboxane B₂ excretion averaged 889 ± 734 pmol/mmol creatinine. After 3 days of aspirin administration, metabolite excretion was significantly (p = 0.002) reduced to 234 ± 224 pmol/mmol creatinine, and on the fifth and seventh days these values were further reduced to 164 ± 78 and 129 ± 63 pmol/mmol creatinine, respectively (Figure 2). This represented an average reduction of 85% in thromboxane biosynthesis in comparison with pretreatment measurements. This finding is consistent with the cumulative acetylation of platelet prosta glandin G/H synthase and inhibition of thromboxane production by low-dose aspirin, as previously found in healthy subjects.

**TABLE 1. Urinary 11-Dehydro-thromboxane B₂ Excretion Rates Among Patients With an Ischemic Stroke, According to Type of Cerebral Infarct**

<table>
<thead>
<tr>
<th>Type of cerebral infarct</th>
<th>Excretion rate (pmol/mmol creatinine)</th>
<th>Stroke patients with values above the upper limit of control patients</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacunar (n=10)</td>
<td>318</td>
<td>26–2,895</td>
<td>60</td>
<td>26–88</td>
</tr>
<tr>
<td>Cortical* (n=22)</td>
<td>306</td>
<td>23–5,916</td>
<td>55</td>
<td>32–76</td>
</tr>
<tr>
<td>Brain stem/cerebellum (n=5)</td>
<td>75</td>
<td>36–1,712</td>
<td>20</td>
<td>1–72</td>
</tr>
</tbody>
</table>

CI, confidence interval; n, number of patients.

Each type of cerebral infarct was defined either by the presence of a relevant infarct on computed tomographic scan or by clinical symptoms.

* Included one cortical border-zone infarct, with an excretion rate of 139 pmol/mmol creatinine.

FIGURE 1. Urinary excretion of 11-dehydro-thromboxane B₂ (TXB₂), expressed as a log scale, in two groups of patients with cerebral ischemia (transient ischemic attack [TIA] and ischemic stroke) and in two control groups (patients with nonvascular neurological disorders [controls] and patients with atypical attacks). Each dot indicates one individual. The broken line represents 2 SDs above the mean of control values.
Discussion

In the past, platelet aggregation studies\(^1\)\(^-\)\(^5\) as well as measurements of platelet-derived products\(^7\)\(^-\)\(^13\) have been performed in patients with cerebral ischemia. Limitations inherent to both approaches have been outlined previously.\(^9\) In view of the large body of evidence for the efficacy of aspirin in preventing thromboembolic complications in patients with cerebrovascular disease,\(^2\) we have focused our attention on the study of thromboxane biosynthesis during the first week after cerebral ischemia. Thromboxane A\(_2\) represents the major product of platelet arachidonate metabolism, and changes in the rate of its biosynthesis have been described in patients with unstable angina and acute myocardial infarction.\(^14\)\(^-\)\(^16\) The results of the present study demonstrate that a variable proportion of patients with clinical symptoms of a TIA or ischemic stroke provide biochemical evidence of enhanced thromboxane biosynthesis. Because there is uncertainty about the cellular source(s) of thromboxane synthesis and release under pathological circumstances,\(^23\) we have used low-dose aspirin as a pharmacological tool to confirm the platelet origin of enhanced thromboxane metabolite excretion in ischemic stroke. Thus, both the cumulative pattern of inhibition and the virtually complete suppression of thromboxane biosynthesis achieved after 1 week of single daily dosing with 50 mg aspirin are consistent with platelets representing the primary source of increased thromboxane production after cerebral ischemia.

Biochemical evidence of platelet activation was obtained in only four of 14 TIA patients. This finding does not support the commonly held notion that there is something inherently abnormal in the platelets of these patients (“hyperreactive” platelets). If platelet activation occurs in such patients, this is likely to be an episodic rather than a continuous process, similar to unstable coronary syndromes.\(^14\)\(^,\)\(^15\) Thus, patients with unstable angina have been characterized by episodic increases in thromboxane metabolite excretion in approximately 45\% of all urine samples (\(n=56\)) collected in aspirin-free periods.\(^13\) A more precise determination of the time relation between episodic platelet activation and the onset of cerebral ischemia would require earlier and more frequent urine sampling than carried out in the present study, given the 45-minute half-life of 11-dehydro-thromboxane B\(_2\) in the human circulation.\(^24\)

In contrast, enhanced thromboxane biosynthesis was a relatively more frequent finding in patients with an ischemic stroke. The fact that half of these patients had perfectly normal values of 11-dehydro-thromboxane B\(_2\) excretion within the first week after the onset of symptoms suggests that platelet activation is of episodic nature also in patients with stroke. This suggestion is reinforced by the finding of markedly elevated metabolite excretion beyond 24 hours after the event in some patients, which would not be expected from the half-life of this metabolite if thromboxane-dependent platelet activation occurred as a single episode, at the time of the stroke. Together, our findings indicate that platelet activation resulting in thromboxane biosynthesis does not occur in all TIA or stroke patients, a finding consistent with the limited effect of antplatelet therapy, which prevents only about one third of all strokes.\(^22\)

In patients with ischemic stroke, the excretion rate of 11-dehydro-thromboxane B\(_2\) was not related to the type (cortical or lacunar) or site (carotid versus vertebrobasilar) of cerebral infarction. This finding is at variance with the widely supported notion that the majority of lacunar infarcts are caused by local fibrinoid lipohyalinosis and subsequent occlusion of small perforating cerebral vessels and not by thromboembolism.\(^25\) It is also at odds with the lower \(\beta\)-thromboglobulin levels in patients with lacunar infarcts, reported in a previous study.\(^13\) In contrast, small deep infarcts may well have a more diverse pathogenesis than previously recognized.\(^26\) Our finding that 60\% of patients with a lacunar infarct have enhanced thromboxane biosynthesis suggests that aspirin may be a rational mode of secondary prevention in these patients as well.

An additional objective of our study was to investigate thromboxane biosynthesis in patients with atypical attacks that are not considered TIAS according to internationally accepted criteria.\(^27\) Many of these attacks represent global rather than focal cerebral ischemia and are therefore probably caused by hemodynamic changes, for instance, attacks secondary to cardiac arrhythmia rather than thromboembolism. In one of the eight patients in our study with such atypical symptoms, thromboxane metabolite excretion exceeded the upper limit of the control patients. This patient later showed ECG evidence of myocardial ischemia during an attack. This is consistent with a recent finding that patients with atypical attacks may be threatened by cardiac rather than cerebral events.\(^28\)

Finally, our finding that a daily dose of 50 mg aspirin can profoundly suppress enhanced thromboxane biosynthesis after acute cerebral ischemia should be viewed in the context of the apparent lack of a dose dependence in its prevention of strokes and myocardial infarction at 75–1,500 mg per day.\(^22\)\(^,\)\(^29\) This biochemical finding is consistent with the clinical observation recently made in the Dutch TIA trial that aspirin at 30 mg per day is at least as effective as 300 mg per day in reducing the risk of major vascular events (nonfatal stroke, nonfatal myocardial infarction, or vascular death) in patients with TIA or minor stroke.\(^30\)

We conclude that 1) episodes of enhanced thromboxane biosynthesis are detected infrequently in patients with a transient ischemic attack, 2) aspirin-suppressible
episodes of increased thromboxane formation can be detected during the early phase of acute ischemic stroke, and 3) this finding may provide a rationale for testing the efficacy and safety of this drug in this setting.

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