Increased Platelet Activation in the Chronic Phase After Cerebral Ischemia and Intracerebral Hemorrhage

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Increased Platelet Activation in the Chronic Phase After Cerebral Ischemia and Intracerebral Hemorrhage

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Background and Purpose—Enhanced thromboxane (TX) biosynthesis has previously been reported in the acute phase after ischemic stroke. We investigated whether enhanced urinary excretion of 11-dehydro-TXB_2, a noninvasive index of platelet activation, was present in the chronic phase after a transient ischemic attack (TIA) or stroke, including intracerebral hemorrhage.

Methods—We obtained a single urinary sample from 92 patients between 3 and 9 months after onset of stroke or TIA. The urinary excretion of the major enzymatic metabolite of TXA_2, 11-dehydro-TXB_2, was measured by a previously validated radioimmunoassay. The excretion rates were compared with those of 20 control patients with nonvascular neurological diseases.

Results—Urinary 11-dehydro-TXB_2 averaged 294±139, 413±419, and 557±432 pmol/mmol creatinine for patients with TIA, ischemic stroke, and intracerebral hemorrhage, respectively; the values were higher in all subgroups ($P<0.01$) than that in control patients (119±66 pmol/mmol). Increased 11-dehydro-TXB_2 excretion was present in 59% of all patients, in 60% ($P<0.001$) of patients with TIA, in 56% ($P<0.001$) of patients with ischemic stroke, and in 73% ($P<0.001$) of patients with intracerebral hemorrhage. Atrial fibrillation, no aspirin use, and severity of symptoms at follow-up contributed independently to the level of 11-dehydro-TXB_2 excretion in a multiple linear regression analysis.

Conclusions—Platelet activation is often present in patients in the chronic phase after stroke, including those with intracerebral hemorrhage. Persistent platelet activation, which is associated with atrial fibrillation and poor stroke outcome, can be substantially suppressed by aspirin treatment. **(*Stroke*. 1999;30:546-549.)**

Key Words: cerebral ischemia ■ intracerebral hemorrhage ■ platelet activation ■ thromboxanes

In previous studies[1–3] we have reported enhanced thromboxane (TX) biosynthesis, as reflected by the urinary excretion of a major TXA_2 metabolite, 11-dehydro-TXB_2, in patients with acute ischemic stroke. Increased TX production was found to occur episodically during the first 2 to 3 days after the onset of ischemic stroke[1–3] and could be largely suppressed by low-dose aspirin,[1,3] thus suggesting its cyclooxygenase-dependent formation in platelets. Repeatedly increased 11-dehydro-TXB_2 excretion was independently related to stroke severity and atrial fibrillation. However, metabolite excretion was not a statistically significant independent prognostic factor for outcome when added to stroke syndrome or stroke severity in a multiple logistic regression model, probably because of the rather small size of that study.[3] Whether increased 11-dehydro-TXB_2 excretion is detectable in the chronic phase after stroke, and whether this is related to cardiovascular risk factors and stroke outcome, is unknown.

In the present study we performed measurements of urinary 11-dehydro-TXB_2 excretion between 3 and 9 months after stroke onset in patients with transient ischemic attack (TIA), ischemic stroke, or intracerebral hemorrhage to investigate whether enhanced platelet activation is present in the chronic phase after stroke, and if so, whether such persistent platelet activation is associated with stroke type, cardiovascular risk factors, and outcome.

Subjects and Methods

Study Patients

Patients were recruited from the hospital-based stroke cohort of the Dutch Vascular Factors in Dementia Study, of which the inclusion and exclusion criteria are detailed elsewhere.[4] The most important inclusion criteria for this study were that patients (1) had had ischemic stroke, intracerebral hemorrhage, or TIA with neurological deficit on admission to the hospital; (2) had a reasonable life expectancy and were alive at follow-up; (3) were ≥55 years of age at time of stroke onset; (4) were native Dutch speakers; and (5) were not aphasic or were only mildly aphasic (<3 on the Aphasia Severity Rating Scale of the Boston Diagnostic Aphasia Examination). Of the 300 patients who met these criteria, we used data from all nondemented patients for whom urinary samples were available. To achieve the demented/nondemented ratio of 1:4 that was found in the

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whole cohort,[4] a random sample of demented stroke patients was added to the study group. Detailed information about cardiovascular risk factors and stroke characteristics was obtained during hospital admission. In addition to a full neurological examination, ancillary investigations consisted of standardized blood tests; a chest radiograph; brain CT and/or MRI; duplex scanning of the carotid arteries; a cardiac analysis, including 12-lead ECG; and if indicated, 24-hour ECG monitoring and ECG. At follow-up, between 3 and 9 months after onset of stroke, blood pressure measurements were performed, urinary samples were collected, and details about medication used at the time of follow-up were obtained. Stroke severity was assessed by means of the modified Rankin Scale.[5]

Control Patients

We used the data from our previous study,[1] in which 11-dehydro-TXB_2 excretion was measured in 20 control patients (11 men and 9 women; mean age, 64.2 years; range, 41 to 85 years) with nonvascular neurological disorders, such as minor cerebral trauma, Parkinson's disease, epilepsy, or cervical spondylotic myelopathy, who were admitted to the same hospital. Urine was collected during the night as soon as possible after the patient's admission to the hospital.

Urine Measurements

Urine samples were collected 3 to 9 months after stroke. The creatinine concentration was measured, and samples of 50 mL were immediately frozen and stored at −20°C until extraction. Analytical measurements of 11-dehydro-TXB_2 excretion were performed by researchers blinded to clinical characteristics. Immunoreactive 11-dehydro-TXB_2 was extracted from 10-mL aliquots of each coded urine sample (the pH was adjusted to 4.0 with formic acid) on SEP-PAK C18 cartridges (Waters Associates) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). Immunoreactive 11-dehydro-TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:30 to 1:1000, as described previously.[6] The urinary excretion rate of 11-dehydro-TXB_2 was expressed as picomoles per millimole of creatinine.

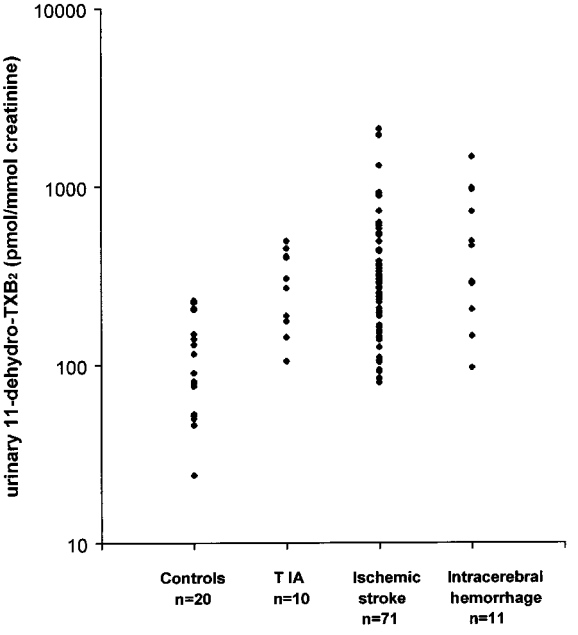
Statistical Analysis

Data were analyzed by means of Stata statistical software.[7] The Student *t* test was used to compare urinary 11-dehydro-TXB_2 excretion between groups. Multiple linear regression was used to assess the relationship between the level of 11-dehydro-TXB_2 excretion and other clinical characteristics. Values of $P<0.05$ were considered statistically significant.

Results

The present study group consisted of 92 patients (mean age, 73.8±8.2 years), of whom 53 were men and 39 women. Ten (11%) had had a TIA, 71 (77%) ischemic stroke, and 11 (12%) intracerebral hemorrhage.

The individual values of 11-dehydro-TXB_2 in all patients and controls are depicted in Figure 1. These values ranged from 105 to 496 (median, 287) pmol/mmol creatinine in patients with TIA, 80 to 2105 (median, 290) in patients with ischemic stroke, and 96 to 1467 (median, 466) in patients with intracerebral hemorrhage. Compared with control patients, 11-dehydro-TXB_2 excretion was significantly higher in patients with TIA ($P<0.001$), patients with ischemic stroke ($P=0.003$), and in patients with intracerebral hemorrhage ($P<0.001$). In 60% of the patients with TIA ($P=<0.001$), 56% of the patients with ischemic stroke ($P<0.001$), and 73% of the patients with intracerebral hemorrhage ($P<0.001$), the excretion rate exceeded 2 SDs of the mean value of control patients with nonvascular disorders (119±66 pmol/mmol creatinine). Overall, persistently increased urinary 11-dehydro-TXB_2 excretion was present in 59% of the patients. Table 1 shows the urinary 11-dehydro-TXB_2 excretion of the 92 patients on the basis of demographic characteristics, cardiovascular risk factors, use of antiplatelet and anticoagulant medication, and stroke characteristics. In the univariate analysis, urinary 11-dehydro-TXB_2 excretion was significantly higher in women ($P=0.006$) and in patients with atrial fibrillation ($P<0.001$) or congestive heart failure ($P<0.001$). Patients who used aspirin (n=56) had significantly lower excretion rates of 11-dehydro-TXB_2 than patients on oral anticoagulant treatment or patients without antiplatelet or anticoagulant treatment ($P=0.004$). Mean 11-dehydro-TXB_2 excretion in patients with TIA was significantly lower than that in patients with cerebral infarction or intracerebral hemorrhage ($P=0.04$). No association was found between the level of 11-dehydro-TXB_2 excretion and subtype of cerebral infarction. Poor stroke outcome, as measured by a Rankin Scale score of >3 at follow-up, was associated with increased 11-dehydro-TXB_2 excretion ($P<0.001$).



Individual measurements of urinary 11-dehydro-TXB_2, plotted on a log scale, for control patients (mean±SD, 119±66 pmol/mmol creatinine) and for patients with TIA (294±139), ischemic stroke (413±419), and intracerebral hemorrhage (557±432).

Five patients had a recurrent vascular event between their qualifying event and the time of urinary sampling during follow-up. Three of them had an ischemic stroke, 1 a TIA, and 1 an intracerebral hemorrhage. Urinary 11-dehydro-TXB_2 excretion averaged 716±693 pmol/mmol creatinine and was numerically higher ($P=0.09$) than in patients without early recurrence. The 3 patients with ischemic stroke recurrence had significantly enhanced metabolite excretion: 976±840 pmol/mmol creatinine ($P=0.01$).

In a multiple linear regression analysis, presence of atrial fibrillation and severe strokes (Rankin Scale score of >3 at follow-up) were independently associated with increased 11-dehydro-TXB_2 levels whereas treatment with aspirin was associated with reduced metabolite excretion (Table 2).

TABLE 1. 11-Dehydro-TXB_2 Excretion Values by Demographic Factors, Cardiovascular Risk Factors, Medication, and Stroke Characteristics

Characteristic	n	11-Dehydro-TXB_2	*P*
Age, y			
<75	44	385±368	0.46
≥75	48	447±433	
Gender			
Male	53	320±262	0.006
Female	39	549±512	
Cardiovascular risk factors			
Smoking	26	408±336	0.89
Hypertension	56	371±328	0.17
Diabetes mellitus	18	513±578	0.26
Hypercholesterolemia	30	326±188	0.13
Intermittent claudication	7	262±150	0.29
Angina pectoris	13	247±126	0.10
Atrial fibrillation	19	759±637	<0.001
Congestive heart failure	7	890±611	<0.001
History of cardiovascular disease			
Myocardial infarction	9	309±154	0.40
Stroke	22	365±243	0.49
Retinal infarction	2	517±584	0.72
Medication			
No oral anticoagulant and no aspirin	10	616±412	0.13†
Aspirin	56	294±294	0.004*
Oral anticoagulant	26	606±500	0.95*
Type of stroke			
TIA	10	294±139	0.04
Cerebral infarction	71	413±419	0.83
Intracerebral hemorrhage	11	557±432	0.27
Clinical subtype cerebral infarction			
TACS	7	462±332	0.70
PACS	32	374±364	0.48
LACS	25	434±520	0.78
POCS	7	465±395	0.72
Severity			
Rankin score at follow-up ≤3	76	320±229	<0.001
Rankin score at follow-up >3	16	877±667	

n is number of patients; values are mean±SD. TACS indicates total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar stroke; and POCS, posterior circulation stroke.

*vs no oral anticoagulant and no aspirin; †vs aspirin or oral anticoagulant.

TABLE 2. Coefficients With 95% CIs of Factors That Remained Independently Related to 11-Dehydro-TXB_2 Excretion in a Multiple Linear Regression Analysis

Factor	Coefficient	95% CI	*P*
Aspirin	−224	−355–−94	0.001
Atrial fibrillation	291	133–449	<0.001
Severe stroke	507	346–668	<0.001

Discussion

The main finding of the present study is that biochemical evidence of in vivo platelet activation is detectable in the chronic phase after stroke in approximately 60% of patients. In the present study, patients with intracerebral hemorrhage and TIA were also included. Of the ischemic stroke patients, 56% had increased urinary 11-dehydro-TXB_2 excretion in the chronic phase. Perhaps unexpectedly, in the vast majority (73%) of patients with intracerebral hemorrhage, we also found enhanced TX biosynthesis. This finding may suggest that increased platelet activation is a reflection of vascular risk factors, diffuse atherosclerotic lesions, or the extent of vascular damage due to the stroke. However, it is unlikely that platelet activation merely reflects a generalized vascular disease. A recent study in patients with peripheral arterial disease has clearly demonstrated that hypertension, diabetes mellitus, and hypercholesterolemia, but not peripheral vascular disease per se, are associated with enhanced TX biosynthesis.[8] In our study, we found no relationship between hypertension, diabetes mellitus, and hypercholesterolemia on the one hand and elevated levels of 11-dehydro-TXB_2 excretion on the other. However, two thirds of our patients were using aspirin at the time of sampling. Davì et al reported that a daily regimen of low-dose aspirin could largely suppress enhanced TX biosynthesis in patients with hypercholesterolemia[9] and diabetes mellitus.[10] The relatively small number of patients with one or more vascular risk factors in our study probably explains why adjustment for aspirin intake did not eliminate its confounding effect in the multivariate analysis.

As in our previous study,[3] presence of atrial fibrillation and absence of aspirin therapy were associated with increased TX production in the univariate analysis. We previously reported that poor stroke outcome tended to be associated with increased TX production in the acute phase. In the present study, we found a statistically significant higher rate of TX metabolite excretion in patients with a Rankin Scale score of >3 at follow-up. The association between increased TX production and atrial fibrillation may reflect, at least in part, the fact that atrial fibrillation is more likely to cause severe strokes. Moreover, patients with atrial fibrillation usually receive oral anticoagulant treatment rather than aspirin. However, in the multiple regression analysis, both atrial fibrillation and stroke outcome were independently related to the rate of urinary 11-dehydro-TXB_2 excretion.

The study of Davì et al[8] suggests that persistently increased platelet activation is a predictor of ischemic events in the setting of peripheral arterial disease, since patients who experienced vascular events (myocardial infarction, cardiac death, ischemic stroke) during 48 months of follow-up had significantly higher levels of 11-dehydro-TXB_2 excretion at baseline than patients who remained event free. Five of our patients (5.4%), all of whom had had an ischemic stroke, had a vascular event in the time between their qualifying stroke and follow-up at 3 to 9 months later. In the 3 patients with recurrent ischemic stroke, TX metabolite excretion was significantly higher than in patients with no recurrences. This could not be explained by acute episodes of platelet activation due to the vascular event, because all patients were tested at least 3 months after their last event. This procedure was part

of the protocol.[4] Although the number of patients is small and the samples were taken after the recurrent event, the findings are in line with those of Davì et al[8] in a different clinical setting. Whether persistent platelet activation is a risk factor for recurrent ischemic events in patients with ischemic and hemorrhagic stroke remains to be investigated in larger studies with longer follow-up.

We conclude that platelet activation is often present in patients in the chronic phase after stroke, including those with intracerebral hemorrhage. Persistent platelet activation, which is associated with atrial fibrillation and poor stroke outcome, can be substantially suppressed by aspirin treatment.

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