Aspirin and Urinary 11-Dehydrothromboxane B₂ in African American Stroke Patients

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Background and Purpose—The aim of the study was to evaluate the relationship between daily aspirin use and urinary excretion of a stable thromboxane metabolite, 11-dehydrothromboxane B₂ (11-DTB₂), in African American stroke patients.

Methods—Subjects were a subgroup of those screened for the African American Antiplatelet Stroke Prevention Study. Subjects were within 4 months of noncardioembolic ischemic stroke and were not being treated with anticoagulants. Antithrombotic therapy at the time of urine collection varied according to the practice patterns of various attending physicians who treated the patients during their acute strokes. 11-DTB₂ was measured by enzyme immunoassay in random urine samples 1 to 4 months after the stroke.

Results—Eighty-seven of 92 patients enrolled were able to give a urine sample at the time of enrollment. There were 51 men and 36 women aged 36 to 87 (mean 62) years. On the basis of antithrombotic treatment before the sample collection, we divided patients into 4 groups: (1) 16 patients treated with no aspirin (no antithrombotic drugs [n=4] or ticlopidine [n=12]), (2) 21 patients treated with 81 to 325 mg aspirin per day (81 mg/d [n=2], 325 mg/d [n=19]), (3) 20 patients treated with 650 mg aspirin per day, and (4) 30 patients treated with 975 to 1300 mg aspirin per day (975 mg/d [n=2] and 1300 mg/d [n=28]). In patients taking daily aspirin at any dose, the median urinary 11-DTB₂ was 783 pg/mg creatinine compared with 1386 pg/mg creatinine in patients not taking daily aspirin (P=0.01 by Wilcoxon rank sum test). In multivariate regression analysis, aspirin use remained significantly associated with lower urinary 11-DTB₂ (P=0.008). There was no dose-response effect between the 3 aspirin dose groups and urinary 11-DTB₂ (P=0.70).

Conclusions—In African American stroke patients, aspirin use is associated with significantly lower urinary 11-DTB₂ independent of other vascular factors, and there does not appear to be a dose-response effect for aspirin doses of 325 to 1300 mg daily. The clinical significance of these finding remains to be determined. (Stroke. 2002;33:57-60.)

Key Words: aspirin • cerebral infarction • platelets • thrombosis

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spirin has a modest and well-established clinical anti-
thrombotic effect for secondary stroke prevention. The principal mechanism proposed for the antithrombotic effect of aspirin is inhibition of the platelet cyclooxygenase enzyme, thus decreasing production of the potent platelet aggregation promoter thromboxane. The stable thromboxane metabolite, 11-dehydrothromboxane B₂ (11-DTB₂) in urine, has been shown to reflect in vivo platelet activation.¹ This metabolite could prove useful in monitoring platelet activity in patients not taking aspirin or in testing for “aspirin resistance.” In a previous study, we have shown that urinary 11-DTB₂ is elevated during acute stroke, particularly in patients not taking aspirin.² Because there are little published data on the relationship between daily aspirin dose and urinary 11-DTB₂ levels in stroke patients,³ we undertook the present study to provide additional useful information for this potentially important issue.

Subjects and Methods

The present study was conducted at 4 centers involved in the African American Antiplatelet Stroke Prevention Study (AAASPS). The AAASPS is a randomized double-blind trial comparing ticlopidine with aspirin treatments for secondary stroke prevention after a noncardioembolic stroke in African Americans.⁴ Patients screened for the AAASPS were also screened for the present study. A stroke service identified patients as either inpatients with an acute stroke or outpatients who had experienced a stroke in the preceding 4 months. An institutional review committee approved the present study, all subjects gave informed consent, and the procedures were in accordance with institutional guidelines.

Between June 1996 and December 1999, consecutive African American patients 1 to 4 months after a noncardioembolic, noncapacitating ischemic stroke were invited to participate. Patients being treated with anticoagulants were excluded. Urinary 11-DTB₂ was measured at entry and at 6 months, 1 year, and 2 years after the stroke. Occurrence of vascular events (stroke, myocardial infarction, and vascular death), any death, and adverse events were monitored. Stroke subtype was determined by the criteria of the Trial of ORG

Received July 24, 2001; final revision received August 23, 2001; accepted October 4, 2001.
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10172 in Acute Stroke Treatment and with the use of all the available medical information. African American status was confirmed by patient self-reporting. Antiplatelet therapy was decided by the attending physician and varied according to individual preferences. Patient interview determined current antiplatelet treatment and dose, defined as that used during the 7 days before urine sample collection. Patient interview and medical record review determined the presence of vascular risk factors. The relationship between 11-DTB2 and clinical outcomes will be the focus of a future report after subject follow-ups are complete. In the present report, we focus on the comparison of daily aspirin dose with urinary 11-DTB2 excretion.

11-DTB2 was measured in random urine samples by enzyme immunoassay. Samples were divided into aliquots, stored at −30°C within 60 minutes of collection, and analyzed in batches within 12 months. Samples from collaborating centers were shipped in dry ice to the special coagulation laboratory at Indiana University Medical Center. The enzyme immunoassay for 11-DTB2 was set up at Indiana University Medical Center Special Coagulation Laboratory with the use of labeled antibodies, as previously reported. Calibrators were prepared by dilution of a 5 ng 11-DTB2 standard (Cayman Chemicals) in 0.2 mol/L glycine buffer, pH 9.5, containing 1 g/L BSA, 50 g/L mannitol, and 50 mg/L thimerosal to achieve concentrations of 2500, 1250, 625, 312, 156, 78, and 0 pg/mL immediately before analysis. This assay has been well characterized in terms of its analytical performance, and this was confirmed in our laboratory. Linearity was between 78 and 1250 pg/mL, and average between-run precision for 3 samples (1349, 1431, and 2710 pg/mg creatinine) was 15%.

Because the 11-DTB2 results were not normally distributed, the Wilcoxon rank sum test compared differences between groups. Stepwise regression analysis tested multiple factors for a possible effect on the 11-DTB2 levels. A JMP statistical software package (SAS Institute Inc) was used.

**Results**

A total of 92 patients were enrolled in the present study, and 5 could not give a urine sample during enrollment, leaving 87 patients with data for analysis. The 87 patients consisted of 51 men and 36 women aged 36 to 87 (mean 62) years. Stroke subtypes among these 87 subjects were as follows: lacunar in 60, undetermined in 18, and large-vessel disease in 9.

On the basis of the antithrombotic therapy at the time of sample collection, we divided the patients into 4 groups.

The control group (16 patients) consisted of 4 patients not taking any antithrombotic drug and 12 patients taking ticlopidine (500 mg daily). Although ticlopidine inhibits platelet aggregation, studies in patients with vascular disease did not find a significant reduction in urinary 11-DTB2 by ticlopidine. Also, in healthy volunteers and patients with vascular disease, ticlopidine use did not significantly decrease platelet thromboxane production. The remaining 71 patients were divided into 3 aspirin groups based on their daily aspirin dose: (1) low dose (81 to 325 mg/d), 21 patients (19 were taking 325 mg/d, and 2 were taking 81 mg/d), (2) medium dose (650 mg/d), 20 patients, and (3) high dose (975 to 1300 mg/d), 30 patients (28 were taking 1300 mg/d, and 2 were taking 975 mg/d). None of the patients varied their daily aspirin dose during the 7 days before sample collection.

The Figure shows the median 11-DTB2 levels in the 4 aspirin dose groups. Compared with the no-aspirin group (1386 [range 176 to 3844] pg/mg creatinine), the 3 aspirin groups combined had a significantly lower urinary 11-DTB2 (783 [range 149 to 7415] pg/mg creatinine, 56% of control; P = 0.01). There was no significant difference in urinary 11-DTB2 between the 3 aspirin groups: 812 (range 149 to 4856) pg/mg creatinine, or 59% of control, in the low-dose group; 774 (range 276 to 3588) pg/mg creatinine, or 56% of control, in the medium-dose group; and 794 (range 283 to 7415) pg/mg creatinine, or 57% of control, in the high-dose group (P = 0.70).

The Table compares vascular factors between patients not taking aspirin and those taking aspirin. Among those not taking aspirin, the main differences were a higher prevalence of hypercholesterolemia (56% versus 30% for those taking aspirin, P = 0.04) and prior stroke (44% versus 21% for those taking aspirin, P = 0.06). In stepwise regression analysis with aspirin use, age, sex, hypertension, diabetes mellitus, current smoking, hypercholesterolemia, prior stroke, ischemic heart disease, and current ticlopidine treatment as potential confounders, aspirin use remained significantly associated with...
lower urinary 11-DTB2 ($P = 0.008$). Also, hypercholesterolemia was significantly associated with higher urinary 11-DTB2 ($P = 0.04$).

**Discussion**

In our novel study on the relationship between daily aspirin use and thromboxane production in African American stroke patients, daily aspirin use was associated with a significant (44%) lower urinary 11-DTB2 independent of other vascular factors, without a dose-response effect for aspirin doses of 325 to 1300 mg daily. Because only 2 patients took 81 mg aspirin daily, we could not properly evaluate the dose-response effect of aspirin doses at 81 to 325 mg daily. Studies in healthy subjects and stroke patients have shown a variable decrease in urinary 11-DTB2 after aspirin use. In healthy subjects, 30 to 650 mg aspirin reduces urinary 11-DTB2 by 70% to 77%.1,13,14 These reports do not state the ethnicity or race of the subjects.

One study of 19 ischemic stroke patients in Japan showed a dose-response effect of aspirin on urinary 11-DTB2.3 In that study, aspirin progressively reduced urinary 11-DTB2 excretion from 994 pg/mL by 42%, 78%, and 91% after doses of 40, 320, and 1280 mg daily, respectively. In that study, measurements were not adjusted for fluctuations in urine concentration (picograms per milligram creatinine). In a study of 11 patients with acute ischemic stroke in the Netherlands, aspirin at 50 mg/d reduced urinary 11-DTB2 by 85% (from 3181 to 462 pg/mg creatinine).15

Investigators in the Dutch Vascular Factors in Dementia Study measured urinary 11-DTB2 in 92 patients 3 to 9 months after transient ischemic attack (10 patients), ischemic stroke (71 patients), or intracerebral hemorrhage (11 patients), of whom 56 were taking aspirin and 36 were not (26 were taking oral anticoagulants, and 10 were not receiving either treatment).16 The aspirin dose was not stated, and aspirin use was associated with a significant (52%) lower urinary 11-DTB2 (2204 versus 1052 pg/mg creatinine). In a study of 9 patients with retinal artery or vein occlusion in Japan, aspirin treatment at 40 mg/d reduced urinary 11-DTB2 by 63% (from =800 to 300 pg/mg creatinine).17

**Acknowledgments**

This project was supported by a grant from Roche Laboratories Inc. We thank Annette S. Lynn, MT (ASCP), from the Special Coagulation Laboratory at Indiana University School of Medicine for expert assistance with the 11-dehydrothromboxane assay and Carol Kempf, RN, for subject follow-ups and sample collections.

**References**


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Stroke. 2002;33:57-60
doi: 10.1161/hs0102.102010
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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