Serial Urinary 11-Dehydrothromboxane B2, Aspirin Dose, and Vascular Events in Blacks After Recent Cerebral Infarction

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Background and Purpose—Incomplete platelet inhibition by aspirin (aspirin resistance) may be a reason for stroke recurrence in some patients. 11-Dehydrothromboxane B2 (11-DTB2) is a stable thromboxane A2 metabolite that reflects in vivo platelet activation. This pilot study was intended to evaluate the reproducibility of urinary 11-DTB2 over time and to look for evidence of aspirin resistance.

Methods—All subjects were screened for the African American Antiplatelet Stroke Prevention Study (AAASPS) 7 to 90 days after noncardioembolic cerebral infarction. Of 83 subjects with at least 1 urine sample, 52 were enrolled in AAASPS (randomized to blinded treatment with aspirin 650 mg/d or ticlopidine 500 mg/d), and 31 were enrolled in an open-label antiplatelet therapy cohort. Subjects were followed up for 2 years, with 11-DTB2 measurements scheduled at baseline and 6, 12, and 24 months. Vascular events were cerebral infarction, myocardial infarction, or vascular death.

Results—Despite considerable individual up or down fluctuations, the median 11-DTB2 change did not significantly differ from zero in any of the subgroups. However, in 6 subjects with a 4-fold decrease in aspirin dose from 1300 to 325 or 81 mg/d, the 11-DTB2 level increased from 611 to 1881 pg/mg creatinine ($P=0.06$). Vascular events occurred in 7 of 61 aspirin-treated subjects, and 11-DTB2 levels did not correlate with the events.

Conclusions—Fluctuations in urinary 11-DTB2 after cerebral infarction in blacks do not correlate with changes in aspirin doses, except perhaps when the dose changes by a factor of 4 or more. A larger study is needed to look further for aspirin resistance. (Stroke. 2004;35:727-730.)

Key Words: aspirin • cerebral infarction • platelets

Aspirin has a modest and well-established effectiveness in preventing recurrent cerebral infarction. However, in some patients, the platelet antiaggregating effect of aspirin may be suboptimal (aspirin resistance).1,2 Various criteria have been used to define aspirin resistance, including platelet aggregation studies, biochemical markers, and clinical outcomes. Multiple mechanisms have been proposed as potential reasons for aspirin resistance, including a dose-response effect of aspirin.

The principal mechanism proposed for the antithrombotic effect of aspirin is inhibition of the platelet cyclooxygenase enzyme, decreasing production of the potent stimulator of platelet aggregation thromboxane A2. The stable thromboxane metabolite 11-dehydrothromboxane B2 (11-DTB2) reflects in vivo platelet activation3 and can be measured in plasma or urine. This metabolite could prove useful in monitoring platelet activity in patients not taking antithrombotic medications or in testing for aspirin resistance. This hypothesis has rarely been tested with 11-DTB2 measurements.4–7

In previous studies, we have shown that urinary 11-DTB2 is elevated during acute stroke, particularly in patients not taking aspirin,8 and that black stroke patients taking aspirin have a significantly lower urinary 11-DTB2 than those not taking aspirin.9 In those studies, we did not detect a dose-response effect between aspirin and urinary 11-DTB2 at baseline. Having completed the 2-year follow-up in this pilot study, we now report on all the serial urinary 11-DTB2 measurements. We also look for biochemical and clinical evidence for aspirin resistance by analyzing the 11-DTB2 levels with respect to aspirin dose and vascular events.

Materials and Methods

Subjects and Methods

Institutional review committees at each site approved this study. All subjects gave informed consent, and procedures were in accordance with institutional guidelines. This study was conducted at 4 sites involved in the African American Antiplatelet Stroke Prevention Study (AAASPS). AAASPS was a randomized, double-blind trial comparing ticlopidine with aspirin treatments for recurrent stroke.
Patients screened for the AAASPS were also considered for this pilot study. Between June 1996 and December 1999, consecutive black patients 7 to 90 days after a noncardioembolic, nonincapacitating cerebral infarction were invited to participate. Patients needing treatment with anticoagulants were excluded.

Subjects who missed the deadline for AAASPS (90 days) by ≤30 days or who declined to participate in AAASPS were invited to enroll in an open-label antiplatelet therapy cohort. In AAASPS, subjects were evaluated in person at least every 4 months; in the open-label cohort, every 6 months. Sample collections were scheduled at baseline and 6, 12, and 24 months in both groups. If a vascular event occurred, samples were not collected after the event. Stroke subtype was determined by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.11 Black status was confirmed by patient self-report. Patient interview and medical record review determined the presence of vascular risk factors.

Subjects who enrolled in AAASPS were randomized to blinded treatment with aspirin 650 mg/d or ticlopidine 500 mg/d. Subjects who enrolled in the open-label antiplatelet therapy cohort remained on their baseline antiplatelet therapy for the duration of the study, unless clinical indications necessitated a change. Baseline antiplatelet therapy was decided by each subject’s attending physician and varied according to individual preferences. Subject interview determined current antiplatelet treatment and dose, defined as that used during the 7 days before urine sample collection. Antiplatelet treatments in AAASPS were unblinded after completion of the trial. 11-DTB2 was measured in random urine samples with labeled antibodies as previously reported.9,12 Briefly, samples were placed in aliquots and stored at −30°C within 60 minutes of collection, shipped to the Special Coagulation Laboratory at Indiana University Medical Center packed in dry ice, and analyzed in batches within 12 months. All subjects and clinical investigators were blinded to the 11-DTB2 assay results. The assay results were recorded in the Special Coagulation Laboratory by 1 technologist who performed the assays and were not revealed to investigators until after the follow-up examinations and vascular event determinations were finalized.

Vascular event was a cerebral infarction, myocardial infarction, or vascular death. Cerebral infarction was diagnosed by an investigator if the medical history, physical examination, and neuroimaging were consistent with cerebral infarction and symptoms or signs lasted ≥24 hours. Myocardial infarction was diagnosed if it was documented in the medical record on the basis of medical history, physical examination, and supporting tests such as ECG and cardiac enzymes. Vascular death was diagnosed if it occurred as a complication of stroke, myocardial infarction, or cardiac arrest or if it occurred suddenly without another explanation. We counted only the first vascular event in each subject. Cerebral and myocardial infarction included both fatal and nonfatal events.

We evaluated within-subject 11-DTB2 fluctuations in all 59 subjects with ≥2 measurements. We analyzed the reproducibility of the 11-DTB2 assay over time in 17 subjects taking the same aspirin dose when the samples were collected. In this subgroup, we chose the lowest and highest 11-DTB2 measurements in subjects with ≥2 measurements.

We analyzed for possible aspirin resistance in 27 subjects taking different aspirin doses when the samples were collected. The aspirin dose usually changed either between baseline and randomization in AAASPS or at any time during the study because of intolerance. In this subgroup, we used the mean 11-DTB2 level corresponding to each aspirin dose. In 2 subjects with 3 different aspirin doses and corresponding 11-DTB2 levels, we chose the highest and lowest aspirin doses and the corresponding 11-DTB2 levels.

Ticlopidine does not affect the cyclooxygenase enzyme and 11-DTB2 levels, and we present the measurements in ticlopidine-treated subjects primarily for validation of the assay. We analyzed within-subject 11-DTB2 fluctuations in 15 subjects taking ticlopidine when the samples were collected who were not included in the aspirin analyses. If there were ≥2 samples, we chose the lowest and highest measurements.

We analyzed for possible association between 11-DTB2 levels and vascular events in 61 subjects taking aspirin throughout this study who gave at least 1 sample. For this analysis only, we used the mean 11-DTB2 level in each subject. The nonparametric signed-rank test evaluated for within-subject 11-DTB2 fluctuations. Distribution of the mean 11-DTB2 levels among the subjects was not normal, so the Wilcoxon rank-sums test compared differences in 11-DTB2 between subjects with and without vascular events. Proportional-hazard survival analysis tested for an association between each subject’s mean 11-DTB2 and vascular events, controlling for the potential confounding factors listed in Table 1. The JMP statistical software package (SAS Institute Inc) was used.

Results

The Figure shows the flow of subjects in this study. Table 1 shows the baseline characteristics of all 83 subjects analyzed. Stroke subtypes among these subjects were as follows: lacunar in 61, undetermined in 17, and large-vessel atherothrombotic in 5. The entire cohort was followed up for a mean of 22 months. There were 13 end points in this study: 9 cerebral infarcts, 3 vascular deaths, and 1 myocardial infarction.

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![Flow diagram of study subjects and urine samples available for analysis.](image-url)
Urinary 11-DTB2 Levels and Aspirin Doses Over Time

Table 2 shows the within-subject fluctuations in 11-DTB2 levels in different subgroups of 59 subjects who had at least 2 measurements. Aspirin dose changed between 1300 and 650 mg/d in 13 subjects, between 650 and 325 mg/d in 8, and between 1300 and 325 or 81 mg/d in 6 (325 mg/d in 5, 81 mg/d in 1). Despite considerable individual up or down fluctuations, the median 11-DTB2 change did not significantly differ from zero in any of the subgroups. However, in the 6 subjects whose aspirin dose decreased from 1300 to 325 or 81 mg/d, 11-DTB2 increased from 611 to 1881 pg/mg creatinine (P = 0.06). As expected, 11-DTB2 was considerably higher among subjects taking ticlopidine than aspirin.

Urinary 11-DTB2 Levels and Clinical Outcomes

Among 61 subjects taking aspirin who gave at least 1 sample, the median 11-DTB2 in those without a vascular event (n = 54) was 813 pg/mg (interquartile range, 574 to 1291) creatinine and in those with an event (n = 7) was 783 pg/mg (interquartile range, 678 to 914) creatinine (P = 0.84 by Wilcoxon rank-sums test). The vascular events in these 7 subjects occurred at a mean of 14 months after entry (range, 4 to 23 months), and 6 of these subjects had taken the same dose since entry. In multivariate analysis, including the vascular risk factors listed in Table 1 and each subject’s mean 11-DTB2 level, only history of diabetes mellitus (P = 0.04) and cerebral infarction (P = 0.02) were associated with an event.

Discussion

In this study the fluctuations in urinary 11-DTB2 did not correlate with changes in aspirin dose. The reproducibility of this assay in stroke patients over time has rarely been reported. One study found similar reproducibility of urinary 11-DTB2 over 2 years in 14 subjects with peripheral artery disease.5

We did not detect a significant dose-response effect of aspirin on urinary 11-DTB2 levels. In 2 studies that found a dose-response effect of aspirin on 11-DTB2,4,7 aspirin doses varied by a factor of ≥4, and in our study, it varied by a factor of only 2 in most subjects (Table 2). In a study of 19 poststroke subjects, an increase in aspirin dose from 40 to 320 mg/d significantly decreased urinary 11-DTB2 by 58%, and an increase in aspirin dose from 320 to 1280 mg/d significantly decreased it by 59%.4 In another study of 48 subjects with vascular disease, an increase in aspirin dose from 325 to 1300 mg/d significantly decreased urinary 11-DTB2 by 29%, and a decrease in aspirin dose from 325 to 81 mg/d significantly increased it by 20%.7 In the 6 subjects in our study with a ≥4-fold change in aspirin dose, the higher aspirin dose was associated with a nonsignificant 46% lower 11-DTB2 level (Table 2).

We did not find an association between 11-DTB2 levels and vascular events. Analysis of the urinary 11-DTB2 with respect to vascular events has rarely been reported.5,6 In a study of 64 patients with peripheral artery disease, baseline urinary excretion of 11-DTB2 was significantly higher in the 8 subjects who had major vascular events than in the 56 subjects who did not during a median 48-month follow-up.5 In a larger case-control study within the Heart Outcomes Prevention Evaluation (HOPE) trial, 976 subjects at high risk for vascular events who were followed up for 5 years were analyzed.6 Higher baseline urinary 11-DTB2 levels were associated with significantly increased odds of vascular events.

Our pilot study was probably underpowered to detect differences in 11-DTB2 between the aspirin doses studied. Also, the dose-response effect between aspirin and urinary 11-DTB2 may be different in blacks than in other races. The 11-DTB2 assay in random urine samples could be used to evaluate aspirin resistance in a larger cohort.

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References


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