

Note: AspirinWorks is reported in pg
11-dehydro thromboxane B₂/mg creatinine
(instead of ng/mmol)

Aspirin-Resistant Thromboxane Biosynthesis and the Risk of Myocardial Infarction, Stroke, or Cardiovascular Death in Patients at High Risk for Cardiovascular Events

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Background—We studied whether aspirin resistance, defined as failure of suppression of thromboxane generation, increases the risk of cardiovascular events in a high-risk population.

Methods and Results—Baseline urine samples were obtained from 5529 Canadian patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study. Using a nested case-control design, we measured urinary 11-dehydro thromboxane B₂ levels, a marker of in vivo thromboxane generation, in 488 cases treated with aspirin who had myocardial infarction, stroke, or cardiovascular death during 5 years of follow-up and in 488 sex- and age-matched control subjects also receiving aspirin who did not have an event. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of 11-dehydro thromboxane B₂, with patients in the upper quartile having a 1.8-times-higher risk than those in the lower quartile (OR, 1.8; 95% CI, 1.2 to 2.7; *P*=0.009). Those in the upper quartile had a 2-times-higher risk of myocardial infarction (OR, 2.0; 95% CI, 1.2 to 3.4; *P*=0.006) and a 3.5-times-higher risk of cardiovascular death (OR, 3.5; 95% CI, 1.7 to 7.4; *P*<0.001) than those in the lower quartile.

Conclusions—In aspirin-treated patients, urinary concentrations of 11-dehydro thromboxane B₂ predict the future risk of myocardial infarction or cardiovascular death. These findings raise the possibility that elevated urinary 11-dehydro thromboxane B₂ levels identify patients who are relatively resistant to aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block in vivo thromboxane production or activity. (*Circulation*. 2002;105:1650-1655.)

Key Words: aspirin ■ thromboxane ■ atherosclerosis ■ myocardial infarction ■ stroke

Aspirin reduces the risk of cardiovascular events by ≈25% in a broad category of patients with arterial vascular disease.¹ However, its effectiveness is limited because 10% to 20% of patients with arterial thrombosis who are treated with aspirin have a recurrent vascular event during long-term follow-up.² Aspirin exerts its major antithrombotic effect by irreversibly acetylating platelet cyclo-oxygenase-1, thereby inhibiting thromboxane A₂ synthesis. Although other poorly defined effects of aspirin on platelet function have been described, their contribution to the antithrombotic effect of aspirin is uncertain.^{2,3}

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There are several possible explanations for the limited efficacy of aspirin. First, it is well recognized that platelets can be activated by pathways that are not blocked by aspirin.⁴⁻⁷ Second, it has been suggested that higher doses of

aspirin than are currently used (75 to 325 mg/d) may be required in some patients to achieve the optimal antithrombotic effect of aspirin.^{8,9} However, low-dose aspirin blocks > 95% of platelet cyclooxygenase-1 activity,^{2,10} and there is no convincing evidence that the antithrombotic effect of aspirin is dose-related.¹¹⁻¹³ Third, some patients may be able to generate thromboxane A₂ despite usual therapeutic doses of aspirin and therefore fail to benefit from aspirin treatment.¹⁰ The clinical importance of this third mechanism is unclear.

All three potential causes of aspirin failure have been designated as aspirin resistance. For the purpose of the present study, we use the term to describe the third potential mechanism, namely, incomplete suppression of thromboxane generation with the usual dose of aspirin. The extent of inhibition of thromboxane A₂ generation can be determined by measuring urinary levels of 11-dehydro thromboxane B₂, a stable metabolite of thromboxane A₂.¹⁴ Accordingly, base-

Received December 27, 2001; revision received January 25, 2002; accepted January 31, 2002.

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Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000013777.21160.07

line urinary 11-dehydro thromboxane B₂ concentrations were measured in 976 aspirin-treated patients at high risk of cardiovascular events from Canadian centers who were enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study to determine whether incomplete suppression of thromboxane generation is associated with an increased risk of recurrent cardiovascular events.

Methods

Patients

The HOPE study^{15,16} was an international, randomized, placebo-controlled, 2×2 factorial trial of ramipril and vitamin E for the secondary prevention of cardiovascular disease. The institutional review committee at each participating center approved the study, and all subjects gave informed consent. A total of 9541 patients ≥55 years of age at the time of randomization who had a history of coronary artery disease, stroke, peripheral vascular disease, or diabetes plus at least one other cardiovascular risk factor were assigned to one of four treatments: ramipril titrated up to 10 mg daily, 400 IU vitamin E daily, both, or neither. The study commenced in December 1993 and was terminated prematurely on March 22, 1999, because of clear evidence of a benefit of ramipril.

Urine Sample Collection

All study participants were asked to provide a first morning urine specimen at the time of randomization. Of the 9541 patients in the HOPE study, 9282 (97%) provided baseline urine samples.¹⁷ Samples (n=5529) from the 129 Canadian centers participating in this study were sent to the central laboratory in Hamilton, Canada, where they were stored at -80°C until analysis. Only samples from Canadian centers were used for the present study.

Follow-Up and Ascertainment of Clinical Outcomes

All patients in the HOPE study were followed at 1 month, 6 months, and 6-month intervals thereafter until completion of the study. At each follow-up, clinical outcomes were recorded and medication use, including aspirin, was documented. The primary outcome was the composite of myocardial infarction, stroke, and death from cardiovascular causes, as previously defined.^{15,16}

Selection of Cases and Control Subjects

Of patients with available urine samples (n=5529), only those who were taking aspirin at the time of commencement of the run-in phase (before randomization), at randomization (coinciding with the time of urine collection), and at each follow-up visit were eligible for inclusion. Aspirin-treated patients who provided an adequate baseline sample of urine and had a confirmed myocardial infarction, stroke, or cardiovascular death after randomization were defined as cases. Control subjects were randomly selected from aspirin-treated patients who provided an adequate baseline urine sample but did not have myocardial infarction, stroke, or cardiovascular death after randomization. Control subjects were matched according to sex and age (±5 years) in a 1:1 ratio with cases.

Laboratory Analysis

For each case and control subject, urine collected and stored at baseline was thawed and assayed for 11-dehydro thromboxane B₂ levels with a commercially available enzyme immunoassay (Cayman Chemical) that has interassay and intra-assay coefficients of variation of 12.1% and 10%, respectively. Assays were performed by laboratory staff blinded to patient status as case or control subject. In addition, case and control specimens were assayed in random order, thereby reducing the possibility of systematic bias.

Statistical Analysis

Means or proportions for baseline demographics and risk factors were calculated for cases and control subjects. The significance of any difference between cases and control subjects was tested by means of Student's paired *t* test for means and McNemar χ^2 test for proportions, which takes into account the matching between cases and control subjects. Because 11-dehydro thromboxane B₂ values are skewed, geometric means were calculated after log transformation of the raw data; the significance of any differences in geometric mean values between cases and control subjects was tested by means of Student's paired *t* test. Median concentrations also were calculated, and levels in cases and control subjects were compared by means of Wilcoxon's rank-sum test.

Tests for trend were used to assess any association between increasing baseline urinary 11-dehydro thromboxane B₂ concentrations and risk of myocardial infarction, stroke, or cardiovascular death after dividing the samples into quartiles defined by the distribution of the complete cohort. Adjusted estimates of the association between increasing baseline urinary 11-dehydro thromboxane B₂ concentrations and risk of myocardial infarction, stroke, or cardiovascular death were obtained by means of conditional logistic regression modeling that accounted for the matching variables and controlled for the random treatment assignment and baseline differences between cases and control subjects. A separate multivariable regression model was used to examine the association between baseline patient characteristics, including age, sex, heart rate, blood pressure, body mass index, past history of vascular disease, conventional vascular risk factors, lipid-lowering therapy, β -blockers, diuretics, and randomized treatment allocation (ramipril or vitamin E) and urinary 11-dehydro thromboxane B₂ concentrations in the urine.

All probability values are 2-sided; confidence intervals were calculated at the 95% level.

Results

Baseline characteristics of cases and control subjects are shown in Table 1. As expected, patients in whom myocardial infarction, stroke, or cardiovascular death subsequently developed had a higher mean body mass index and baseline blood pressure and were more likely than those who remained free of these events to be current smokers or have a history of hypertension, diabetes, myocardial infarction, or peripheral vascular disease. Cases also were more often treated with diuretics or calcium channel blockers at baseline and less often treated with lipid-lowering drugs or randomized to ramipril therapy. Because of the matching, the age and sex of cases and control subjects were similar.

Geometric mean and median urinary concentrations of 11-dehydro thromboxane B₂ at baseline were significantly higher among patients who had subsequent development of the composite outcome of myocardial infarction, stroke, or cardiovascular death compared with those who remained free of these events (Table 2). The difference between cases and control subjects was greatest in those who had a myocardial infarction (24.5 versus 20.9 ng/mmol creatinine, *P*=0.003) or died of a cardiovascular cause (25.6 versus 20.4 ng/mmol creatinine, *P*<0.001). Baseline urinary concentrations of 11-dehydro thromboxane B₂ were not significantly different between cases who had subsequent development of stroke and their matched control group (25.0 versus 27.4 ng/mmol creatinine, *P*=0.47).

The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of baseline urinary 11-dehydro thromboxane

TABLE 1. Baseline Characteristics of Study Participants*

Characteristic	Cases (n=488)	Controls (n=488)	P
Age, y	67.3±7.2	67.4±7.2	0.78
Female sex, n (%)	77 (15.8)	77 (15.8)	...
Body mass index, kg/m ²	27.8±4.1	26.9±3.7	<0.001
Heart rate, bpm	66.2±10.3	65.6±10.9	0.41
SBP, mm Hg	137.1±20.6	133.5±18.0	0.002
DBP, mm Hg	76.6±9.8	75.6±9.4	0.08
History of coronary disease, n (%)			
Any	469 (96.1)	464 (95.1)	0.54
MI	364 (74.6)	309 (63.4)	<0.001
Stable angina	355 (72.7)	336 (68.9)	0.19
Unstable angina	184 (37.7)	176 (36.1)	0.65
CABG	176 (36.1)	154 (31.6)	0.15
PCI	87 (17.8)	104 (21.3)	0.22
Stroke or TIA, n (%)	59 (12.1)	40 (8.2)	0.06
Peripheral vascular disease, n (%)	240 (49.2)	173 (35.5)	<0.001
Hypertension, n (%)	219 (44.9)	154 (31.6)	<0.001
Diabetes, n (%)	159 (32.6)	105 (21.5)	<0.001
Elevated total cholesterol, n (%)	279 (57.2)	310 (63.5)	0.38
Current cigarette smoking, n (%)	81 (16.6)	57 (11.7)	0.03
Medications, n (%)			
Aspirin	488 (100)	488 (100)	...
β-Blocker	241 (49.4)	235 (48.2)	0.76
Lipid-lowering agent	121 (24.8)	166 (34.0)	0.002
Diuretics	73 (15.0)	34 (7.0)	<0.001
Calcium channel blockers	289 (59.2)	238 (48.8)	0.002
Ramipril	227 (46.5)	274 (56.1)	0.003
Vitamin E	246 (50.4)	252 (51.6)	0.74

Values are mean±SD or n (%). CABG indicates coronary artery bypass graft surgery; CV, cardiovascular; DBP, diastolic blood pressure; MI, myocardial infarction; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; and TIA, transient ischemic attack.

B₂ concentration (*P* for trend across quartiles, 0.01), with patients in the highest quartile having a risk 1.8-fold higher than those in the lowest quartile (OR, 1.8; 95% CI, 1.2 to 2.9; *P*=0.009) (Figure). A similar association was seen with myocardial infarction (*P* for trend across quartiles, 0.005) and cardiovascular death (*P* for trend across quartiles, 0.001) but not for stroke (*P* for trend across quartiles, 0.20) (Table 3). Results were similar with or without adjustment for baseline differences between cases and control subjects, including conventional vascular risk factors, cointerventions, and randomized treatment allocation.

To evaluate whether increased baseline urinary 11-dehydro thromboxane B₂ concentrations were associated with early rather than late cardiovascular events, we performed separate analyses in patients who had an event within the first 12 months of study entry and those whose event occurred >12 months after study entry. The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death that was associated with the highest quartile of urinary 11-dehydro thromboxane B₂ as compared with the lowest quartile was 2.9 (95% CI, 0.9 to 9.1) for events occurring with the first 12 months and 1.7 (95% CI, 1.0 to 2.7) for events occurring after the first 12 months.

Using linear multivariable regression modeling, variables that were found to be independently associated with baseline urinary 11-dehydro thromboxane B₂ concentrations in the urine were female sex (*P*=0.004), body mass index (*P*=0.001), history of peripheral vascular disease (*P*=0.01), current cigarette smoking (*P*=0.09), use of calcium channel blockers (*P*=0.08), and randomization to vitamin E (*P*=0.04). However, these variables combined were able to predict <5% of the variation in urinary 11-dehydro thromboxane B₂ concentrations (*R*²=0.045).

Discussion

This is the first study to demonstrate an association between aspirin resistance, defined as failure of suppression of thromboxane generation, and cardiovascular risk. In a well-defined cohort of aspirin-treated patients at high risk of cardiovascular events, increasing baseline urinary concentrations of 11-dehydro thromboxane B₂ were associated with an increasing risk of cardiovascular events, particularly myocardial infarction and cardiovascular death. This association was strong, graded, and independent of conventional vascular risk factors, including elevated body mass index, blood pressure,

TABLE 2. Baseline Urinary Concentrations of Urinary 11-Dehydro Thromboxane B₂ in Cases and Controls

Outcome	11-Dehydro Thromboxane B ₂ Concentration, ng/mmol Creatinine		P
	Cases	Controls	
MI, stroke, or CV death (n=488)			
Geometric mean	24.5	21.5	0.01
Median	22.7	21.0	0.01
MI (n=378)			
Geometric mean	24.5	20.9	0.003
Median	22.8	20.3	0.001
Stroke (n=80)			
Geometric mean	25.0	27.4	0.47
Median	21.3	25.9	0.40
CV death (n=244)			
Geometric mean	25.6	20.4	<0.001
Median	24.0	19.9	<0.001

MI indicates myocardial infarction; CV, cardiovascular.

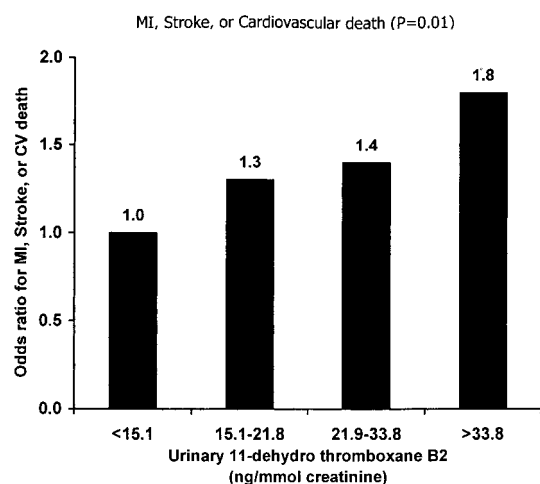
hypertension, diabetes, smoking, and previous history of vascular disease. Moreover, the strength of the association was not modified by differences between cases and control subjects in the proportion of patients receiving lipid-lowering or antihypertensive therapy or by randomization to vitamin E or ACE inhibitors.

Several mechanisms can be proposed to account for the incomplete suppression of thromboxane generation by aspirin.² First, polymorphisms or mutations of the cyclooxygenase-1 gene that make it relatively resistant to inhibition by aspirin may provide a molecular basis for aspirin resistance. However, to our knowledge, such a mutation has not been identified. Second, nucleated cells such as monocytes or

vascular endothelial cells can provide prostaglandin H₂ to platelets to bypass platelet cyclooxygenase-1 or can use prostaglandin H₂ to synthesize their own thromboxane A₂ because they are endowed with substantial amounts of thromboxane synthase.^{4,18–20} Arachidonate conversion to prostaglandin H₂ is catalyzed by cyclooxygenase-1 or -2. Although low-dose aspirin permanently and completely blocks cyclooxygenase-1 in platelets, nucleated cells can regenerate the enzyme. Consequently, these cells can produce prostaglandin H₂ even in the face of aspirin treatment. In addition to cyclooxygenase-1-mediated prostaglandin H₂ generation, nucleated cells can also produce prostaglandin H₂ through cyclooxygenase-2.^{20,21} Whereas cyclooxygenase-1 is blocked by 80 to 325 mg of aspirin, doses similar to that used in the HOPE trial, inhibition of cyclooxygenase-2 requires doses of aspirin in excess of 500 mg daily.² Unlike cyclooxygenase-1, which is constitutively expressed, cyclooxygenase-2 expression in nucleated cells is augmented 10- to 20-fold by inflammatory stimuli. Augmented cyclooxygenase-2 expression may contribute to aspirin resistance in patients with ischemic heart disease because atherosclerosis is an inflammatory disease.^{21–23}

Our finding of an independent albeit weak association between history of peripheral vascular disease and urinary 11-dehydro thromboxane B₂ levels in the urine is consistent with prior reports suggesting that the severity of atherosclerosis is an important determinant of thromboxane generation.^{14,24} In patients being treated with aspirin, differences in the extent or severity of atherosclerosis are unlikely to affect de novo platelet thromboxane production because even very low doses of aspirin completely and irreversibly block platelet cyclooxygenase-1.²⁵ Upregulation of cyclooxygenase-2 has been demonstrated in atherosclerotic tissue²⁴ and may be associated with greater synthesis and transfer of prostaglandin H₂ to platelets, thereby bypassing platelet cyclooxygenase-1 and leading to aspirin-insensitive thromboxane biosynthesis in these patients. However, our study cannot distinguish between failure of suppression of platelet cyclooxygenase-1 and upregulation of COX-2 expression as the cause for the observed differences in 11-dehydro thromboxane B₂ excretion between cases and control subjects.

The reason for the lack of an association between urinary 11-dehydro thromboxane B₂ and risk of stroke is unclear. Aspirin reduces the risk of stroke in a broad category of high-risk patients^{1,2}; elevated urinary concentrations of 11-dehydro thromboxane B₂ have been reported in patients after stroke,²⁶ and failure of aspirin to suppress “platelet reactivity” or inhibit platelet aggregation in response to various platelet agonists also has been documented in patients after stroke.^{27–29} In our study, the mean urinary concentration of 11-dehydro thromboxane B₂ in cases who had a stroke was similar to that in all cases (25.0 versus 24.5 ng/mmol creatinine), but the corresponding urinary concentration in matched stroke control subjects with was higher than the concentration in all control subjects (27.4 versus 21.5 ng/mmol creatinine). However, the number of stroke cases and matched control subjects was relatively small (n=80). Given the clear and graded association between urinary 11-dehydro



Association between quartiles of 11-dehydro thromboxane B₂ and composite of myocardial infarction (MI), stroke, or cardiovascular (CV) death after adjustment for baseline differences between cases and control subjects (P value is for trend of association).

TABLE 3. Adjusted Odds* of Future Cardiovascular Death, Myocardial Infarction, and Stroke According to Baseline Urinary Concentrations of 11-Dehydro Thromboxane B₂

Outcome	Quartiles of 11-Dehydro Thromboxane B ₂ Concentration, ng/mmol Creatinine				P
	<15.1	15.1–21.8	21.9–33.7	>33.7	
MI/stroke/CV death (n=488)					
Odds ratio (95 CI)	1.0	1.3 (0.9–2.0)	1.4 (0.9–2.2)	1.8 (1.2–2.7)	0.01
P	...	0.13	0.09	0.009	
MI (n=378)					
Odds ratio (95 CI)	1.0	1.3 (0.8–2.1)	1.5 (1.0–2.5)	2.0 (1.2–3.4)	0.005
P	...	0.26	0.07	0.006	
Stroke (n=80)					
Odds ratio (95 CI)	1.0	2.5 (0.6–10.0)	0.6 (0.2–2.2)	0.6 (0.2–1.8)	0.20
P	...	0.18	0.45	0.34	
CV death (n=244)					
Odds ratio (95 CI)	1.0	2.0 (1.0–3.9)	2.5 (1.3–4.9)	3.5 (1.7–7.4)	0.001
P	...	0.06	0.006	<0.001	

*Adjusted for baseline differences between cases and controls.

MI indicates myocardial infarction; CV, cardiovascular.

thromboxane B₂ concentration and the composite outcome of myocardial infarction, stroke, or cardiovascular death, as well as other individual components of this outcome, the absence of a demonstrable association between stroke risk and urinary concentration of 11-dehydro thromboxane B₂ probably reflects a play of chance.

Our study has several potential limitations. First, there were important differences between cases and control subjects with regard to potentially important confounders, including body mass index, systolic blood pressure, hypertension, diabetes, smoking, history of vascular disease, and cointerventions. However, even after adjustment for these differences, a clear association between urinary 11-dehydro thromboxane B₂ concentrations and risk of death, myocardial infarction, and stroke was demonstrated. The weak association between baseline patient characteristics and urinary 11-dehydro thromboxane B₂ concentrations further supports the conclusion that confounding did not account for our results. Second, urinary 11-dehydro thromboxane B₂ concentrations may have been influenced by recent acute thrombotic events, such as myocardial infarction or stroke, processes that are known to be associated with platelet activation and enhanced urinary excretion of thromboxane metabolites. However, patients who had a myocardial infarction or stroke within the previous 7 weeks were not randomized into the HOPE study, making this explanation less likely. Third, single baseline determinations of urinary 11-dehydro thromboxane B₂ concentrations may not accurately reflect the extent of platelet activation over long periods of time. However, the association between elevated urinary 11-dehydro thromboxane B₂ concentrations at baseline and subsequent risk of cardiovascular events was evident both during the first 12 months after randomization and beyond 12 months, indicating a stable association over an extended period of time. Fourth, we did not confirm patient compliance with aspirin therapy by measuring salicylate levels in the

blood or urine. However, we specifically assessed compliance with aspirin therapy at each follow-up visit and only considered patients for inclusion who were taking aspirin before randomization and at 6-month follow-up visits. Patients who discontinued aspirin at any time during the study were not included. Finally, the extent of biological variation in urinary 11-dehydro thromboxane B₂ levels is unknown but could potentially limit the value of this marker to predict the risk of future cardiovascular events in an individual patient.

We conclude that among aspirin-treated patients at high risk of cardiovascular events, persistent thromboxane generation predicts the risk of the composite outcome of myocardial infarction, stroke, or cardiovascular death, independent of other cardiovascular risk factors. These data raise the possibility that high urinary levels of 11-dehydro thromboxane B₂ can prospectively identify patients who are relatively resistant to conventional antithrombotic doses of aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block thromboxane production or activity.

Acknowledgments

Professor Weitz is the recipient of a Career Investigator Award from the Heart and Stroke Foundation of Canada and holds the Heart and Stroke Foundation of Ontario/J. Fraser Mustard Chair in Cardiovascular Research and the Canada Research Chair in Thrombosis. Professor Yusuf is the recipient of a Canadian Institute of Health Research Senior Scientist Award and holds a Heart and Stroke Foundation of Ontario Research Chair.

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