Metabolic Syndrome

Suboptimal Inhibition of Platelet Cyclooxygenase-1 by Aspirin in Metabolic Syndrome

James P. Smith, Elias V. Haddad, Mary B. Taylor, Denise Oram, Dana Blakemore, Qingxia Chen, Olivier Boutaud, John A. Oates

Abstract—Interindividual variation in the ability of aspirin to inhibit platelet cyclooxygenase-1 (COX-1) could account for some on-treatment cardiovascular events. Here, we sought to determine whether there are clinical phenotypes that are associated with a suboptimal pharmacological effect of aspirin. In a prospective, 2-week study, we evaluated the effect of aspirin (81 mg) on platelet COX-1 in 135 patients with stable coronary artery disease by measuring serum thromboxane B2 (sTxB2) as an indicator of inhibition of platelet COX-1. A nested randomized study compared enteric-coated with immediate-release formulations of aspirin. We found that sTxB2 was systematically higher among the 83 patients with metabolic syndrome than among the 52 patients without (median: 4.0 versus 3.02 ng/mL; \( P=0.013 \)). Twelve patients (14%) with metabolic syndrome, but none without metabolic syndrome, had sTxB2 levels consistent with inadequate inhibition of COX (sTxB2 ≥13 ng/mL). In linear regression models, metabolic syndrome (but none of its individual components) significantly associated with higher levels of log-transformed sTxB2 (\( P=0.006 \)). Higher levels of sTxB2 associated with greater residual platelet function measured by aggregometry-based methods. Among the randomized subset, sTxB2 levels were systematically higher among patients receiving enteric-coated aspirin. Last, urinary 11-dehydrothromboxane B2 did not correlate with sTxB2, suggesting that the former should not be used to quantitate aspirin’s pharmacological effect on platelets. In conclusion, metabolic syndrome, which places patients at high risk for thrombotic cardiovascular events, strongly and uniquely associates with less effective inhibition of platelet COX-1 by aspirin. (Hypertension. 2012;59:719-725.)

Key Words: aspirin  thromboxane  platelets  coronary disease  metabolic syndrome

Therapy with low-dose aspirin for secondary prevention of cardiovascular disease reduces the risk of major coronary events by 20% to 25%.1,2 Despite treatment with aspirin, however, ≈10% to 20% of patients experience recurrent arterial thrombotic events.3 This led to the consideration of whether interindividual variation in the effect of aspirin could contribute to those recurrent thrombotic events.

Aspirin exerts its antiplatelet effect by acetylating serine 529 of platelet cyclooxygenase (COX) 1, which inhibits the enzyme and prevents formation of the platelet activator thromboxane (Tx) A2.4 We found that acetylation of the COXs by aspirin is inhibited by the redox cycling of the enzyme that occurs when hydroperoxides are reduced in the peroxidase active site of the COXs.5 Accordingly, the response to aspirin varies considerably among different cell types, based on the hydroperoxide concentration of the cells. This suggested that the extent to which aspirin inhibits platelet COX-1 could vary among patients. Because activation of platelets and/or lipid peroxidation could lead to formation of several peroxides, platelet activation might attenuate aspirin’s effect by this mechanism, as well as by increased platelet turnover.

Although low-dose aspirin is almost uniformly effective in inhibiting platelet COX-1 among healthy, young individuals,6 there could be pathophysiologic states that impair the effect of aspirin. Here, we investigated the relationship of the pharmacological effect of low-dose (81 mg) aspirin to a number of clinical phenotypic characteristics in a group of patients with documented coronary artery disease (CAD). Both obesity and diabetes mellitus have been associated with increased platelet activation ex vivo and in vivo7–12 and with increased oxidative stress,9,13,14 and the efficacy of aspirin in preventing cardiovascular events is marginal among diabetics compared with nondiabetics.15 Body weight has been inversely correlated with suppression of sTxB2.16–18 Metabolic syndrome constitutes a subset of both the diabetic and obese...
populations that confers a substantial increase in the risk of thrombotic cardiovascular events compared with both the general population and patients with diabetes mellitus.\textsuperscript{19,20} Accordingly, metabolic syndrome was a predetermined phenotype of interest in this study.

Drug formulation could also contribute to interindividual variation in response to aspirin. A summary of bioequivalence studies demonstrated that the enteric-coated formulations investigated were less potent in inhibiting sTxB\textsubscript{2} than immediate-release formulations among healthy volunteers.\textsuperscript{16} Because these studies evaluated neither the McNeil formulation of enteric-coated aspirin nor the extent to which enteric coating affects platelet COX-1 inhibition among patients with CAD, we nested a randomized substudy within our cohort to study the effects of formulation in this population.

Furthermore, we evaluated the validity of measuring the urinary metabolite of TxA\textsubscript{2}, 11-dehydrothromboxane B\textsubscript{2} (Tx-M),\textsuperscript{21} to assess the efficacy of aspirin. Higher levels of Tx-M have been associated with adverse cardiovascular events in high-risk patients,\textsuperscript{22} but we found previously that this biomarker reflects TxA\textsubscript{2} derived from both platelet and nonplatelet (eg, COX-2) sources in cigarette smokers.\textsuperscript{23}

Methods

Study Population

This study was approved by the Vanderbilt University Institutional Review Board. Participants provided written informed consent. Recruitment occurred between June 2006 and May 2009. Patients with known CAD were approached if they appeared to satisfy inclusion and exclusion criteria based on review of their medical chart. Inclusion criteria included \( \geq \) 40-year-old men or postmenopausal women who were receiving aspirin 81 to 325 mg as part of their outpatient regimen. Exclusion criteria included concurrent use of other antiplatelet drugs, NSAIDs, or COX-2 inhibitors, coronary artery bypass grafting, or percutaneous coronary intervention within 6 months of enrollment; uncontrolled hypertension (systolic blood pressure \( \geq \) 180 mm Hg); decompensated congestive heart failure; acute coronary syndrome within 6 months; significant gastrointestinal bleeding; creatinine \( \geq 1.78 \) mg/dL (10 \( \mu \text{mol/L} \)), hemoglobin \( < 10 \) g/dL; or platelet count \( < 135,000 \) \( \mu \text{L} \). Approximately 25% of patients approached declined participation, the majority citing an unacceptable travel distance to complete the study.

Study Design

A prospective observational study was conducted to evaluate the phenotypic characteristics of patients with stable CAD in whom inhibition of platelet COX-1 by aspirin was suboptimal. Patients received a blister pack containing a 2-week supply of a daily dose of aspirin (81 mg; McNeil Pharmaceuticals) administered in the evening. The importance of strict adherence to therapy was emphasized, and participants were contacted by the research coordinator midstudy to assess and encourage continued compliance. A pill count was performed at the conclusion of the study. After 2 weeks, patients returned for phlebotomy and provided a first-morning urine specimen.

We enrolled 181 patients with CAD in the observational study. Of these, 135 fulfilled the criteria for inclusion in the cohort for analysis (see the online-only Data Supplement).

From the above 181 patients, 106 consecutive subjects were enrolled in a nested, randomized, controlled investigation of enteric-coated aspirin. Of the 54 patients randomized to enteric-coated aspirin, 9 were withdrawn, 3 for unsuccessful phlebotomy, 2 for use of other antiplatelet agents midstudy, 2 for self-reported use of systemic anti-inflammatory medication, 1 for percutaneous coronary intervention with stent placement during the study, and 1 for an error in enrollment (coronary artery bypass graft within 6 months). Of the 52 patients randomized to immediate-release aspirin, 5 were withdrawn, 2 for laboratory abnormalities discovered on the day of recruitment but after enrollment, 1 for self-reported NSAID use, 1 for loss to follow-up, and 1 for withdrawal for personal reasons. Therefore, the final analytic cohort of 135 patients in the observational study included 45 randomized to enteric-coated aspirin and 47 randomized to immediate-release aspirin. We assigned the metabolic syndrome phenotype in accord with the American Heart Association/National Heart, Lung, and Blood Institute criteria.\textsuperscript{24} Additional prospectively selected phenotypic characteristics of interest were body mass index (BMI), diabetes mellitus, smoking status, and age.

Laboratory Measurements

**Serum TxB\textsubscript{2}**

Serum TxB\textsubscript{2} was measured as an indicator of inhibition of platelet COX activity. Nonanticoagulated blood was incubated at 37°C for 45 minutes immediately after phlebotomy.\textsuperscript{25} Serum was separated by centrifugation and stored at \( \leq 80°C \) until analysis. Serum TxB\textsubscript{2} was assayed by stable isotope dilution gas chromatography/mass spectrometry with selective ion monitoring.\textsuperscript{26} Suboptimal inhibition of platelet COX, the primary endpoint of the study, was defined prospectively as failure to reduce sTxB\textsubscript{2} to \( < 5\% \) of the mean level obtained in normal individuals taking no antiplatelet drugs; using the analytic techniques described herein, this equated to \( \leq 13 \) mg/mL. The rationale and supporting evidence for this criterion for a suboptimal effect of aspirin are presented in the online-only Data Supplement.

**Urinary Tx-M**

Urine was stored at \( \leq 80°C \) until analysis. Urinary Tx-M was assayed by stable isotope dilution gas chromatography/mass spectrometry.\textsuperscript{27}

**Platelet Aggregation Studies**

Citrated platelet-rich plasma, adjusted to 2.5 \( \times 10^\text{10} \) cells per milliliter with autologous platelet-poor plasma, was used to assess turbidimetric platelet aggregation induced by 2 \( \mu \text{g/mL} \) of collagen (Chronolog Corp, Havertown, PA) in a dual-channel aggregometer (model 460VJS, Chronolog Corp), as described previously.\textsuperscript{28} As a functional measure of residual platelet COX-1 activity in these patients treated with aspirin, we compared the extent of collagen-induced aggregation in platelet-rich plasma with or without preincubation with the highly selective thromboxane receptor antagonist SQ 29 548 (SQ; 10 \( \mu \text{mol/L} \), final concentration; Cayman Chemical, Ann Arbor, MI). These measurements were added to the protocol midstudy but then were performed on all of the subsequent patients, except when difficulties with phlebotomy precluded aggregation studies.

**Statistical Analysis**

Data are expressed as median (interquartile range [IQR]) or frequency (percentage). For bivariate group comparisons, Wilcoxon rank-sum tests were used for continuous data, and \( \chi^2 \) or Fisher exact tests were used for categorical data. Spearman rank correlations were used to study relationships between pairs of continuous variables. Linear models were used to study the relationships between log-sTxB\textsubscript{2} and aspirin formulation, age, sex, smoking status, platelet count, BMI, and metabolic syndrome (or each of its component criteria). In addition, a linear model was used to study the association between sTxB\textsubscript{2} and residual platelet function, as measured by the difference in collagen-induced aggregation with or without the addition of SQ. Analyses were conducted by using R \( 2.10.1 \) (r-project.org). Two-sided \( P \) values \( < 0.05 \) were considered statistically significant.

Results

**Study Population**

Clinical characteristics for the patients included in the analysis are shown in Table 1. Twenty-eight percent had type 2 diabetes mellitus, and all but 1 patient carried a diagnosis of hypertension. Metabolic syndrome was present in 83 (61%)
of the 135 patients. These patients were younger, had higher blood pressure at baseline, were more likely to have diabetes mellitus, and had larger median BMI and waist circumference. In addition, lower high-density lipoprotein, higher triglycerides, higher fasting glucose, and higher platelet count accompanied the metabolic syndrome (Table 1). Patients with metabolic syndrome were more likely to be taking antidiabetic drugs, but otherwise the use of medications was similar between those with and without metabolic syndrome (Table S1 in the online-only Data Supplement). There were no significant differences in clinical characteristics between patients in the randomized and nonrandomized cohorts.

### Metabolic Syndrome Associates With Inadequate Inhibition of Platelet Cyclooxygenase

To determine whether metabolic syndrome associates with apparent biochemical resistance of cyclooxygenase to aspirin, sTxB$_2$ was compared across metabolic syndrome status. Serum TxB$_2$ was systematically higher among the 83 patients with metabolic syndrome than among the 53 patients without (median: 3.98 ng/mL [IQR: 2.52–8.46 ng/mL] versus 3.02 ng/mL [IQR: 1.55–5.92 ng/mL]; $P=0.013$; Figure 1).

It is generally accepted that ≥95% inhibition of platelet cyclooxygenase is required for aspirin to confer a significant antiplatelet effect.$^{6,29,30}$ Because we could not ethically discontinue aspirin in these patients with known CAD for this study, we estimated that a serum TxB$_2$ level of 13 ng/mL represented 95% inhibition of platelet COX based on the mean sTxB$_2$ in 83 healthy volunteers. Biochemical resistance to aspirin (sTxB$_2$ ≥13 ng/mL) was significantly more common among patients with metabolic syndrome than without. In the absence of the metabolic syndrome, no patient had an sTxB$_2$ level ≥13 ng/mL after 2 weeks of monitored therapy.

![Figure 1. Comparison of serum thromboxane B$_2$ (TxB$_2$) levels between patients with and without metabolic syndrome ($P=0.013$), all of whom had known coronary artery disease. The dashed line (13 ng/mL) represents an estimate of 95% inhibition of cyclooxygenase.](image-url)
whereas 12 patients (14%) with metabolic syndrome had sTxB2 levels above this threshold (P=0.003).

Because setting an absolute sTxB2 threshold to define biochemical resistance to aspirin could be considered arbitrary, log-transformed sTxB2 was analyzed as a continuous variable in a linear regression model. Metabolic syndrome significantly associated with higher sTxB2 even after adjusting for age, sex, smoking history, platelet count, and aspirin formulation (enteric–coated vs immediate–release). The P value corresponds with the listed variable in the model.

Table 2. Comparison of Metabolic Syndrome With Its Components in Separate Multivariable Linear Models of Log–Transformed Serum Thromboxane B2

<table>
<thead>
<tr>
<th>Model*</th>
<th>Estimate</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: metabolic syndrome†</td>
<td>0.441</td>
<td>0.006</td>
</tr>
<tr>
<td>Model 2: low high-density lipoprotein‡</td>
<td>0.172</td>
<td>0.26</td>
</tr>
<tr>
<td>Model 3: elevated triglycerides§</td>
<td>0.118</td>
<td>0.52</td>
</tr>
<tr>
<td>Model 4: elevated fasting glucose¶</td>
<td>0.244</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 5: elevated waist circumference¶</td>
<td>0.282</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*In addition to the variable listed, all of the models adjust for age, sex, smoking status (current/former vs never), platelet count, and aspirin formulation (enteric–coated vs immediate–release). The P value corresponds with the listed variable in the model.

†Data use American Heart Association/National Heart, Lung, and Blood Institute criteria.24
‡HDL was <1.03 mmol/L (40 mg/dL) for men and <1.29 mmol/L (50 mg/dL) for women.
§Triglycerides were ≥1.69 mmol/L (150 mg/dL).
¶Fasting glucose was ≥5.56 mmol/L (100 mg/dL) or diagnosis of diabetes mellitus.
¶Waist circumference was ≥102 cm (40 inches) for men and ≥88 cm (35 inches) for women.

This resistance is even more common among those who have metabolic syndrome and are also overweight/obese.

Because higher gastric pH could reduce the bioavailability of aspirin, we examined whether concomitant use of proton pump inhibitors, H2 antagonists, or antacids was associated with higher levels of sTxB2. Of the 37 patients taking proton pump inhibitors, 3 (8%) had sTxB2 ≥13 ng/mL compared with 9 (9%) of the 98 patients not taking proton pump inhibitors. Similarly, the use of neither H2 antagonists nor antacids was associated with sTxB2 ≥13 ng/mL. Additional data regarding concomitant medications are summarized in Table S2.

Functional Consequence of Incomplete sTxB2 Inhibition

The functional consequence of incomplete inhibition of sTxB2 was evaluated with light-transmission aggregometry. The extent of collagen-induced aggregation was compared with and without preincubation of the platelet-rich plasma with the highly selective thromboxane receptor antagonist SQ. In the setting of complete inhibition of platelet COX-1 by aspirin, preincubation with SQ does not attenuate collagen-induced aggregation further, producing a “without SQ – with SQ” difference of 0. In the setting of incomplete inhibition of platelet COX-1 by aspirin, however, preincubation with SQ attenuates collagen-induced aggregation, producing a “without SQ – with SQ” difference >0. By this functional measure, patients with sTxB2 ≥13 ng/mL had significantly greater residual platelet function (P=0.02; Figure 3). Furthermore, simple linear regression suggested that a 1-ng/mL increase in sTxB2 is associated with a 1.14 absolute percentage point increase in difference between collagen-induced aggregation without and with SQ preincubation, on average (P<0.0001).

Enteric-Coated Aspirin Affects Suppression of sTxB2 by Aspirin

The effect of enteric-coating on the inhibition of sTxB2 by aspirin was studied in a randomized subgroup of the patients.
Serum TxB₂ levels were systematically higher and exhibited greater variability among patients randomized to enteric-coated aspirin compared with those randomized to immediate-release aspirin (median: 5.02 ng/mL [IQR: 3.36–7.86 ng/mL] versus 2.78 ng/mL [IQR: 1.60–4.76]; P=0.005; Figure S2). Despite randomization, the median age was slightly lower and the median waist circumference was higher in the group receiving enteric-coated aspirin. Adjusting for these potential confounders in a linear model of log-transformed sTxB₂, enteric-coated aspirin remained associated with higher sTxB₂ levels (P=0.030).

**Urinary Tx-M Does Not Correlate With sTxB₂**

Because sTxB₂ is the most direct measure of the pharmacological effect of aspirin, we compared each patient’s sTxB₂ level with their urinary Tx-M to determine the extent to which Tx-M predicted inhibition of platelet COX-1. Urinary Tx-M did not correlate with sTxB₂ in this population (Spearman ρ=0.04; P=0.63; Figure 4).

**Discussion**

The effect of aspirin on platelet COX-1 is substantially and significantly diminished in patients with the metabolic syndrome. Twelve patients (14%) with metabolic syndrome, but none without metabolic syndrome, had sTxB₂ levels consistent with inadequate inhibition of COX. These 12 poorest responders also were overweight/obese, constituting 16% of that BMI subgroup.

Several abnormalities in ex vivo platelet function have been demonstrated in patients with metabolic syndrome who are not on aspirin. Closure time in the flow-based clotting system, PFA-100, is prolonged; platelets exhibit increased P-selectin expression in response to ADP; and aggregation in response to ADP, collagen, and arachidonic acid is modestly but significantly increased. Increased platelet surface expression of P-selectin and GP IIb/IIIa has been demonstrated, as well as elevated levels of circulating soluble P-selectin, soluble CD40 ligand, and conjugates of leukocytes with platelets and/or platelet microparticles. Increased numbers of reticulated platelets suggest accelerated platelet turnover. Furthermore, platelet count is higher in metabolic syndrome, as demonstrated by both Jesri et al and the present study.

Increased in vivo platelet activation in metabolic syndrome can be hypothesized based on several observations. Platelet activation could be a basis for impaired acetylation of platelet COX-1 by aspirin. The biosynthesis of peroxides (eg, 12-hydroperoxyeicosatetraenoic acid [12-HPETE], prostaglandin G₂ [PGG₂], and peroxynitrite) during platelet activation would lead to redox cycling of COX-1, which we have shown to inhibit acetylation of COX-1 by aspirin. Moreover, increased platelet turnover resulting from abnormal platelet activation in vivo would generate more young platelets with active COX-1 between the aspirin-dosing interval, thus impairing acetylation. It can be shown that increased platelet activation and suboptimal effect of aspirin are associated in the same population, this would mean that aspirin’s therapeutic effect is impaired in the patients who need it most. However, it is of note that aspirin produces a slight and nonsignificant reduction in thrombotic cardiovascular events in patients with diabetes mellitus, many of whom have metabolic syndrome.

Although a pharmacokinetic effect of obesity is a conceivable basis for increased sTxB₂ levels in aspirin-treated patients with metabolic syndrome, direct evidence is elusive given the substantial acetylation of platelet COX-1 in the portal circulation and the extent of first-pass metabolism. Moreover, our data indicate that waist circumference, the parameter that measures central obesity, does not alone explain the relation of metabolic syndrome to elevated sTxB₂ (Table 2).

This represents the first evidence that the effect of aspirin on its pharmacological target, platelet COX-1, is reduced in metabolic syndrome. Previously, investigations have found that inhibition of the function of platelets by aspirin is diminished in metabolic syndrome. However, platelet function assays (eg, ADP- and collagen-induced aggregation, PFA-100, and VerifyNow) indirectly measure the net effects of both thromboxane-dependent and thromboxane-independent
pathways. Although these tests may provide information about platelet reactivity, they cannot determine the success or failure of aspirin to inhibit platelet COX-1 in an individual patient. Indeed, a major predictor of hyperfunction of platelets during aspirin treatment is elevated function in the absence of aspirin, which is clearly the case in metabolic syndrome; therefore, it is not surprising that multiple studies have suggested that platelet reactivity in patients receiving aspirin is a harbinger of increased cardiovascular risk. It cannot be inferred that failure of aspirin to acetylate platelet COX-1 is the principal cause of this increased risk, but COX-1-dependent residual platelet activity is certainly a contributor.

The most effective treatment strategy for patients with metabolic syndrome who have an impaired response to low-dose aspirin remains to be determined. Certainly, an increase in the dose of aspirin is a consideration, and evidence supports an advantage of twice-daily dosing to compensate for the accelerated rate of entry of platelets with unacetylated platelet COX that results from the increased platelet turnover in diabetes mellitus and metabolic syndrome. Higher doses, however, also would further inhibit prostacyclin biosynthesis, which may be deleterious and could increase risk for gastrointestinal bleeding. Further research must identify the optimal antiplatelet therapy for patients with metabolic syndrome in whom the pharmacological effect of aspirin is suboptimal.

Enteric-coated formulations have several potential pharmacokinetic disadvantages: there is significant variability in the pill-coating process, possibly affecting bioavailability; delivery of aspirin to the more alkaline small bowel increases the likelihood of intraintestinal deacetylation; and slower absorption allows for more efficient first-pass hepatic clearance. In healthy volunteers, Cox et al demonstrated that 75-mg enteric-coated preparations had higher median sTxB2 than a 75-mg immediate-release preparation. The reduction in aspirin effect also varied between the different enteric-coated formulations, prompting us to study the McNeil 81-mg enteric-coated formulation in a target population for the drug, patients with CAD. We found that this formulation also has a diminished and more variable effect on platelet COX-1 compared with an immediate-release formulation. The cumulative evidence indicating a reduced pharmacological effect of enteric-coated formulations is cogent in light of the fact that the basis for the use of low-dose aspirin for prevention of thrombotic cardiovascular disease largely derives from a meta-analysis that was composed of studies using immediate-release formulations with the exception of 2 studies that used a 100-mg enteric-coated preparation. Thus, no evidence exists to support the use of 81-mg enteric-coated aspirin to prevent cardiovascular events.

Interest in the possibility that urinary Tx-M could mark suboptimal suppression of platelet TXA2 biosynthesis by aspirin followed the demonstration that higher levels of urinary Tx-M associate with an increased risk for cardiovascular events in a high-risk population. Multiple subsequent studies used urinary Tx-M as an indicator of “aspirin resistance,” although urinary Tx-M could indicate resistance to the pharmacological effect of aspirin, noncompliance, or nonplatelet sources of thromboxane. For example, we found that 22% of Tx-M derives from COX-2 among smokers. In healthy volunteers, Tx-M has not been found to correlate with sTxB2. In the present study of patients with CAD, we did not detect a correlation between these 2 measures, indicating that Tx-M is not a reliable biomarker of suboptimal inhibition of platelet COX-1 by aspirin. The concerted evidence, therefore, indicates that urinary Tx-M should not be used to interpret whether a patient is resistant to the pharmacological effects of aspirin.

In conclusion, metabolic syndrome strongly and uniquely associates with suboptimal inhibition of platelet COX-1 by aspirin. The increasing prevalence of the metabolic syndrome and its association with greater risk for cardiovascular events highlight the importance of optimizing antiplatelet therapy to reduce cardiovascular risk for these patients.

**Perspectives**

In patients with metabolic syndrome, platelet hyperactivity likely contributes to the risk for acute coronary syndrome. This emphasizes the need for effective antiplatelet therapy in the very patients in whom we found the effect of low-dose aspirin to be suboptimal. Inhibition of platelet cyclooxygenase by aspirin may be substantially inadequate in patients with metabolic syndrome and CAD. Because a growing proportion of the 18-million patients in the United States with CAD have metabolic syndrome, this suggests that hundreds of thousands of patients may be receiving inadequate treatment for their hyperactive platelets.

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**Disclosures**

J.A.O. was an ad hoc consultant to McNeil Pharmaceuticals before 2007.

**References**


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SUBOPTIMAL INHIBITION OF PLATELET CYCLOOXYGENASE-1 BY ASPIRIN IN METABOLIC SYNDROME

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* Authors contributed equally.
Supplemental Methods

We enrolled 181 patients with CAD in the observational study. Of these, 135 fulfilled the criteria for inclusion in the cohort for analysis. Eight of the enrolled patients were excluded because of laboratory abnormalities that returned after initiation of aspirin therapy. In five, phlebotomy was inadequate for platelet function studies. Ten patients admitted use of systemic anti-inflammatory medications and one patient admitted non-compliance. A change in aspirin dose or initiation of other anti-platelet agents by a non-study physician occurred in three patients. Inclusion/exclusion errors were discovered in two patients, and the presence of metabolic syndrome could not be determined for 11 patients because of missing data (American Heart Association/National Heart, Lung and Blood Institute [AHA/NHLBI] criteria). One underwent percutaneous coronary intervention, four were lost to follow-up, and one withdrew for personal reasons.

From the above 181 patients, 106 consecutive subjects were enrolled in a nested randomized controlled investigation of enteric-coated aspirin. Of the 54 patients randomized to enteric-coated aspirin, nine were withdrawn: three for unsuccessful phlebotomy, two for use of other antiplatelet agents mid-study, two for self-reported use of systemic anti-inflammatory medication, one for percutaneous coronary intervention with stent placement during the study, and one for an error in enrollment (CABG within 6 months). Of the 52 patients randomized to immediate-release aspirin, five were withdrawn: two for laboratory abnormalities discovered on the day of recruitment but after enrollment, one for self-reported NSAID use, one for loss to follow-up, and one for withdrawal for personal reasons. Therefore, the final analytic cohort of 135 patients in the observational study included 45 randomized to enteric-coated aspirin and 47 randomized to immediate-release aspirin.

Supplemental Information

Maximal inhibition of platelet activation by aspirin requires almost complete inhibition of platelet cyclooxygenase. This conclusion is drawn from a number of investigations which, although employing different analytical methods and endpoints, all conclude that inhibition of thromboxane A2 biosynthesis by platelets to less than 5% of untreated levels or to equivalent levels of sTxB2 is required to achieve the maximal effect of aspirin on platelet function or cardiovascular events. Data from our laboratory indicated that more than 95% inhibition of thrombin stimulated TxB2 formation (radioimmunoassay) is required for maximal inhibition of platelet aggregation and serotonin release. The studies with the TxA2 antagonist presented herein also demonstrate that inhibition of TxA2 dependent platelet aggregation is incomplete when sTxB2 levels exceed 13 ng/ml. Biosynthesis of thromboxane A2 in humans, measured as excretion of its metabolite, is sustained to a substantial degree until more than 95% inhibition of sTxB2 (mass spectrometric analysis) is achieved. Santilli et al. found that > 97% suppression of sTxB2 (radioimmunoassay) was required for maximal inhibition of platelet function. Frelinger et al. demonstrated that major adverse cardiovascular events were increased in patients with sTxB2 (ELISA) greater than 3.1 ng/ml. The levels of sTxB2 measured in normal individuals by immunologic and mass spectrometric methods are similar, but data comparing the two analytical approaches at the low levels of sTxB2 during aspirin treatment are lacking to our knowledge. All
of this evidence, obtained with both methods of analysis, supports a conclusion that inhibition of sTxB2 by less than 95% represents suboptimal inhibition of TxA2 dependent platelet activation. Because of the evidence that >95% inhibition may well be required for maximal inhibition of TxA2 dependent platelet activation, log-transformed sTxB2 was analyzed as a continuous variable in a linear regression model in which metabolic syndrome significantly associated with higher sTxB2 (P=0.006).

References


Table S1. Medication Use by Metabolic Syndrome Status

<table>
<thead>
<tr>
<th>Medications</th>
<th>MetSyn (n=83)</th>
<th>No MetSyn (n=52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton pump inhibitor</td>
<td>25 (30%)</td>
<td>12 (23%)</td>
<td>0.37</td>
</tr>
<tr>
<td>H₂ antagonist</td>
<td>4 (5%)</td>
<td>4 (8%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Antacid</td>
<td>29 (35%)</td>
<td>16 (31%)</td>
<td>0.62</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>41 (49%)</td>
<td>24 (46%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Angiotensin-receptor blocker</td>
<td>20 (24%)</td>
<td>10 (19%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>58 (70%)</td>
<td>34 (65%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>16 (19%)</td>
<td>11 (21%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Statin</td>
<td>74 (89%)</td>
<td>46 (88%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Other lipid-lowering therapy</td>
<td>20 (24%)</td>
<td>20 (38%)</td>
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</tr>
<tr>
<td>Diuretic</td>
<td>38 (46%)</td>
<td>25 (48%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Warfarin</td>
<td>8 (10%)</td>
<td>5 (10%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Antidiabetic medication</td>
<td>29 (35%)</td>
<td>3 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>8 (10%)</td>
<td>2 (4%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Medications</td>
<td>sTxB2 ≥ 13 ng/mL (n=12)</td>
<td>sTxB2 &lt; 13 ng/mL (n=123)</td>
<td>P</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>--------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>3 (25%)</td>
<td>34 (28%)</td>
<td>1.0</td>
</tr>
<tr>
<td>H₂ antagonist</td>
<td>0</td>
<td>8 (7%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Antacid</td>
<td>3 (25%)</td>
<td>42 (34%)</td>
<td>0.75</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>3 (25%)</td>
<td>62 (50%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Angiotensin-receptor blocker</td>
<td>2 (17%)</td>
<td>37 (30%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>8 (67%)</td>
<td>84 (68%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>3 (25%)</td>
<td>24 (20%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Statin</td>
<td>10 (83%)</td>
<td>110 (89%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Other lipid-lowering therapy</td>
<td>3 (25%)</td>
<td>37 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diuretic</td>
<td>5 (42%)</td>
<td>58 (47%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Warfarin</td>
<td>1 (8%)</td>
<td>12 (10%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Antidiabetic medication</td>
<td>3 (25%)</td>
<td>29 (24%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>0</td>
<td>10 (8%)</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Figure S1. Serum TxB₂ levels stratified by presence of diabetes and metabolic syndrome. Metabolic syndrome, but not diabetes, significantly associated with higher levels of sTxB₂ in a linear regression model that included adjustment for age, sex, smoking status, platelet count, and aspirin formulation.
Figure S2. Serum TxB$_2$ levels were systematically higher and exhibited greater variability among patients randomized to enteric-coated aspirin compared with those randomized to immediate-release aspirin ($P=0.005$). The dashed line (13 ng/mL) represents an estimate of 95% inhibition of cyclooxygenase. The bounds of the boxes indicate the 1$^{st}$ and 3$^{rd}$ quartiles; the line within the box indicates the median.