ONLINE SUPPLEMENT FOR

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

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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).[1] 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 Of the 54 patients randomized to enteric-coated aspirin, nine were withdrawn: three for unsuccessful phlebotomy, two for use of other antiplatelet agents mid-study, two for self-reported use of systemic anti-inflammatory medication, one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 Of the 52 patients randomized to immediate-release aspirin, five were withdrawn: two for laboratory abnormalities discovered on the day of recruitment but after enrollment, one for self-reported NSAID use, one for loss to follow-up, and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 A_2 biosynthesis by platelets to less than 5% of untreated levels or to equivalent levels of $sTxB_2$ 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 TxB_2 formation (radioimmunoassay) is required for maximal inhibition of platelet aggregation and serotonin release.[2] The studies with the TxA_2 antagonist presented herein also demonstrate that inhibition of TxA_2 dependent platelet aggregation is incomplete when $sTxB_2$ levels exceed 13 ng/ml. Biosynthesis of thromboxane A_2 in humans, measured as excretion of its metabolite, is sustained to a substantial degree until more than 95% inhibition of $sTxB_2$ (mass spectrometric analysis) is achieved.[3] Santilli *et al*. found that > 97% suppression of $sTxB_2$ (radioimmunoassay) was required for maximal inhibition of platelet function.[4] Frelinger *et al*. demonstrated that major adverse cardiovascular events were increased in patients with $sTxB_2$ (ELISA) greater than 3.1 ng/ml.[5] The levels of $sTxB_2$ measured in normal individuals by immunologic and mass spectrometric methods are similar, but data comparing the two analytical approaches at the low levels of $sTxB_2$ during aspirin treatment are lacking to our knowledge. All of this evidence, obtained with both methods of analysis, supports a conclusion that inhibition of

$sTxB_2$ by less than 95% represents suboptimal inhibition of TxA_2 dependent platelet activation. Because of the evidence that >95% inhibition may well be required for maximal inhibition of TxA_2 dependent platelet activation, log-transformed $sTxB_2$ was analyzed as a continuous variable in a linear regression model in which metabolic syndrome significantly associated with higher $sTxB_2$ (P=0.006).

References

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Table S1. Medication Use by Metabolic Syndrome Status

Medications	**MetSyn (n=83)**	**No MetSyn (n=52)**	**P**
Proton pump inhibitor	25 (30%)	12 (23%)	0.37
H_2 antagonist	4 (5%)	4 (8%)	0.48
Antacid	29 (35%)	16 (31%)	0.62
ACE inhibitors	41 (49%)	24 (46%)	0.71
Angiotensin-receptor blocker	20 (24%)	10 (19%)	0.51
Beta-blockers	58 (70%)	34 (65%)	0.59
Calcium-channel blocker	16 (19%)	11 (21%)	0.79
Statin	74 (89%)	46 (88%)	0.90
Other lipid-lowering therapy	20 (24%)	20 (38%)	0.075
Diuretic	38 (46%)	25 (48%)	0.80
Warfarin	8 (10%)	5 (10%)	0.99
Antidiabetic medication	29 (35%)	3 (6%)	<0.001
Insulin	8 (10%)	2 (4%)	0.21

Table S2. $sTxB_2$ by Medication Class

Medications	**sTxB2 > 13 ng/mL (n=12)**	**sTxB2 ≤ 13 ng/mL (n=123)**	**P**
Proton pump inhibitor	3 (25%)	34 (28%)	1.0
H_2 antagonist	0	8 (7%)	1.0
Antacid	3 (25%)	42 (34%)	0.75
ACE inhibitors	3 (25%)	62 (50%)	0.13
Angiotensin-receptor blocker	2 (17%)	37 (30%)	0.51
Beta-blockers	8 (67%)	84 (68%)	1.0
Calcium-channel blocker	3 (25%)	24 (20%)	0.71
Statin	10 (83%)	110 (89%)	0.62
Other lipid-lowering therapy	3 (25%)	37 (30%)	1.0
Diuretic	5 (42%)	58 (47%)	0.77
Warfarin	1 (8%)	12 (10%)	1.0
Antidiabetic medication	3 (25%)	29 (24%)	1.0
Insulin	0	10 (8%)	0.60

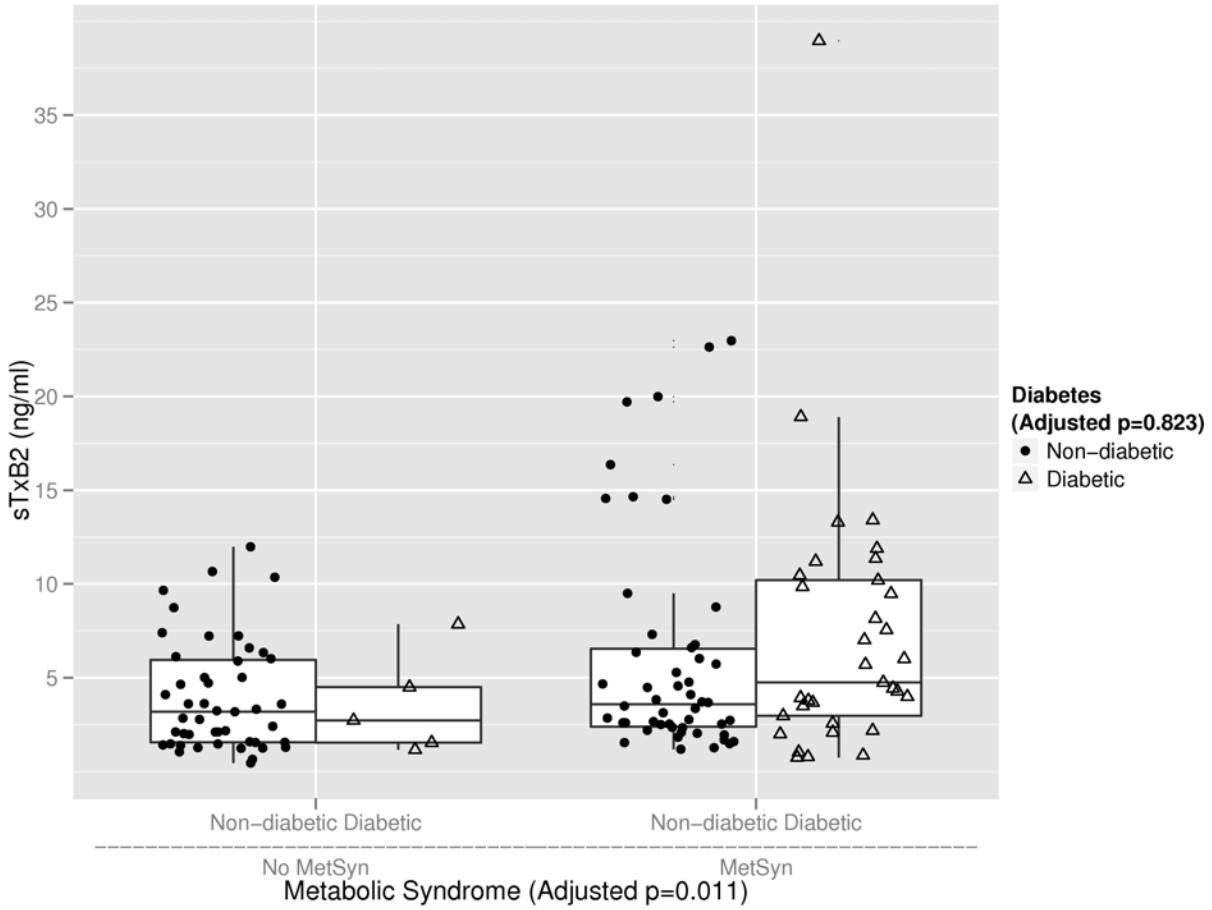

Figure S1. Serum TxB_2 levels stratified by presence of diabetes and metabolic syndrome. Metabolic syndrome, but not diabetes, significantly associated with higher levels of $sTxB_2$ in a linear regression model that included adjustment for age, sex, smoking status, platelet count, and aspirin formulation.

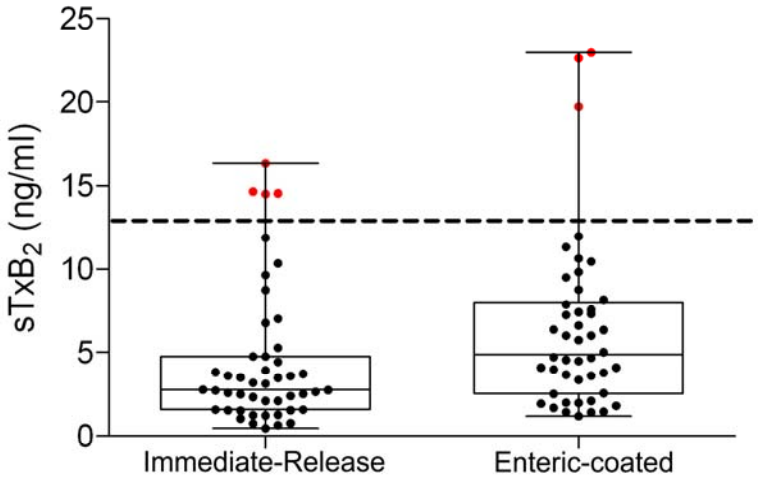

Figure S2. Serum TxB_2 levels were systematically higher and exhibited greater variability among patients randomized to enteric-coated aspirin compared with those randomized to immediate-release aspirin (P=0.005). The dashed line (13 ng/mL) represents an estimate of 95% inhibition of cyclooxygenase. The bounds of the boxes indicate the 1st and 3rd quartiles; the line within the box indicates the median.