

Platelet Response to Low-Dose Enteric-Coated Aspirin in Patients With Stable Cardiovascular Disease

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OBJECTIVES	We investigated whether use of low-dose enteric-coated (EC) aspirin for secondary prevention of cardiovascular events has sufficient bioavailability to achieve complete platelet cyclooxygenase (COX) inhibition in all individuals.
BACKGROUND	Aspirin reduces cardiovascular morbidity and mortality in patients with pre-existing vascular disease; however, there is variability in the way individuals respond. Persistent normal platelet function despite therapy, referred to as “aspirin resistance,” is associated with an increased risk of major cardiovascular events.
METHODS	We studied 131 stable cardiovascular patients between March and September 2002 who were taking 75 mg EC aspirin. Serum thromboxane (TX) B ₂ levels were assayed as a measure of COX activity. Mean arachidonic acid (AA)-induced platelet aggregation $\geq 20\%$ was deemed evidence of persistent platelet activity and an incomplete aspirin response.
RESULTS	Patients of median age 63 years (61% men) were enrolled. Forty-four percent of patients had elevated serum TX B ₂ levels (>2.2 ng/ml). Arachidonic acid-induced platelet aggregation occurred more frequently in these patients (21% vs. 3%; $p = 0.004$). In all cases addition of exogenous aspirin during the assay abolished platelet aggregation. Patient weight and age were significant independent predictors of an incomplete response to EC aspirin ($p = 0.025$ and $p < 0.001$, respectively). These patients were also more likely to have a history of myocardial infarction (MI) ($p = 0.038$).
CONCLUSIONS	Many patients who are prescribed low-dose EC aspirin for secondary prevention of cardiovascular events have persistent uninhibited platelet COX activity. Younger and heavier patients and those with a previous MI are most likely to have an inadequate response to treatment. (J Am Coll Cardiol 2005;47:1258–63) © 2005 by the American College of Cardiology Foundation

Aspirin reduces cardiovascular morbidity and mortality in patients with pre-existing vascular disease and is therefore widely prescribed for secondary prevention of cardiovascular events (1). Aspirin acts by irreversibly inhibiting platelet cyclooxygenase (COX), the enzyme that catalyzes conversion of arachidonic acid (AA) to thromboxane (TX) A₂ (Fig. 1) (2). Thromboxane A₂, a short-lived platelet agonist, enhances aggregation in response to most platelet activators (3). Being anucleate, the platelet cannot regenerate COX once inhibited, and therefore recovery of TX A₂ biosynthesis depends on new platelet formation, which occurs at a rate of 10% daily. Thus, irreversible enzyme deactivation combined with slow recovery of platelet COX explains the profound inhibitory effect on platelet TX formation achieved with low-dose aspirin therapy (4,5).

Substantial inhibition of platelet COX is required to prevent TX formation and subsequent platelet aggregation.

Indeed, $>95\%$ inhibition of enzyme activity is necessary to suppress aggregation in healthy volunteers (6). Aspirin is the only COX inhibitor known to sustain such levels of inhibition with once-daily dosing. This is achieved with 80 to 160 mg of plain (non-coated) aspirin used for secondary prevention of cardiovascular disease (7). Despite the fact that this dose range is 2.5- to 10-fold greater than the 30 mg daily dose reported to fully inactivate platelet COX (5), response to aspirin therapy is variable. Continued platelet activity has been reported in 5% to 40% of patients, a phenomenon referred to as “aspirin resistance” (8,9). One recent study associated aspirin resistance, defined as persistent platelet aggregation in vitro, with a three-fold increased risk of major cardiovascular events (10).

In an earlier crossover study of healthy volunteers, we compared different preparations of aspirin at low doses and found that enteric-coated (EC) aspirin was less effective than plain aspirin. This difference was most obvious in heavier patients (11). On the basis of this finding, we hypothesized that low-dose EC aspirin preparations, frequently prescribed for secondary prevention of cardiovascular events, may provide inadequate drug bioavailability and incomplete inhibition of platelet COX and thus contribute to the phenomenon of aspirin resistance.

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Manuscript received February 11, 2005; revised manuscript received June 7, 2005, accepted June 13, 2005.

Abbreviations and Acronyms

AA	= arachidonic acid
COX	= cyclooxygenase
EC	= enteric-coated
MI	= myocardial infarction
NSAIDs	= non-steroidal anti-inflammatory drugs
TX	= thromboxane

METHODS

Patients. The ethics committees of Beaumont and St. James's Hospitals, Dublin, Ireland, approved the study protocol, and written informed consent was obtained from all patients before enrollment. Men and women age 21 years and older were recruited consecutively while attending cardiology outpatient clinics at one of two tertiary referral hospitals in Dublin, Ireland, between March and September 2002. Patients were identified by medical chart review in the outpatient clinic. Of the 412 charts reviewed, 208 patients had cardiovascular disease, which had been documented by cardiac catheterization or determined by medical history, electrocardiography, and exercise testing, and were taking aspirin daily. Those with a recent history (within 6 weeks) of myocardial infarction (MI), unstable angina, coronary angioplasty, coronary artery bypass grafting, or major surgical procedure were ineligible. Patients with myeloproliferative disease, personal or family history of bleeding disorders, a platelet count $<150,000/\mu\text{l}$ or $>450,000/\mu\text{l}$, or hemoglobin <8 g/dl were excluded. Recent ingestion (within 2 weeks) of non-steroidal anti-inflammatory drugs (NSAIDs), other COX inhibitors, alternative antiplatelet therapy, or antico-

agulants were also criteria for exclusion. Of these 208 patients, 158 were taking a 75-mg EC aspirin preparation (76%). These patients were then requested to participate in the study and 131 (83%) agreed to do so. The main reason for non-participation was an unwillingness to attend the clinic for further unscheduled visits.

Study design and main outcome measures. To optimize compliance, all patients were informed that the purpose of the study was to assess their response to aspirin. Demographic data and concomitant medication were recorded by direct interview and from the medical chart. Follow-on appointments were scheduled for assessment of platelet response to aspirin. Patients were re-contacted several days in advance and the day before to confirm the appointment and remind them to take their aspirin daily, including the morning of the test.

Serum TX B₂, the stable metabolite of TX A₂, was assayed as a measure of COX activity in blood (largely in platelets) (12). To further determine the presence of uninhibited platelet COX, the amount of TX B₂ produced when AA was added to platelet-rich plasma, referred to as "platelet TX," was determined in a subgroup. Because COX is a catalytic enzyme, if incomplete aspirin inhibition of platelet COX exists, addition of a high concentration of its substrate, AA, results in production of large amounts of its end product, TX, and thus provides a highly sensitive assay of aspirin response (Fig. 1).

Platelet aggregation to AA (1.6 mmol/l) was measured by light transmission in platelet-rich plasma as previously described (8). Arachidonic acid-induced platelet aggregation $\geq 20\%$ was deemed evidence of continued platelet activity and potential for thrombus formation (10). When significant platelet aggregation to AA was detected, the assay was repeated following *in vitro* addition of aspirin (2.5 to 50 $\mu\text{mol/l}$) (13).

Statistical analysis. Results are reported as median and 25th to 75th percentile range for continuous variables and percentages for discrete variables. The Student *t* test was used to compare continuous data and the Fisher exact test for discrete data. Non-parametric data were analyzed by the Mann-Whitney test and data correlation was determined using Spearman's rank-correlation coefficient. A two-tailed *p* value of <0.05 was considered statistically significant. Multiple-variable analysis was performed by analysis of variance (ANOVA). Predictors of treatment failure and failure to inhibit serum TX B₂ generation were examined using logistic regression, and predicted probabilities of non-inhibition were generated.

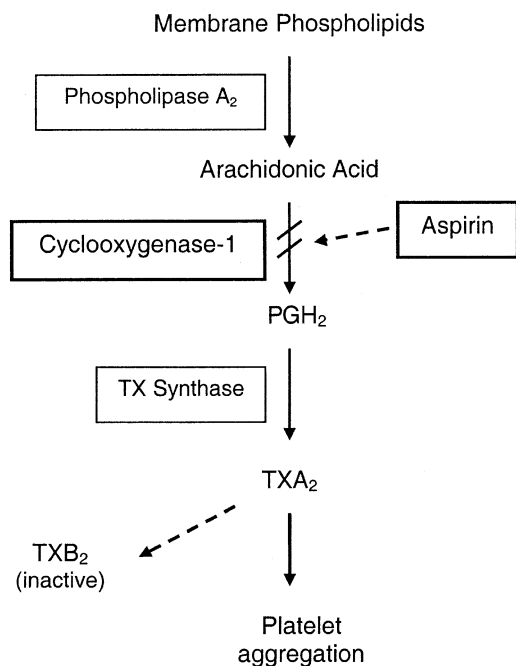


Figure 1. The role of cyclooxygenase-1 in thromboxane (TX) synthesis and the inhibitory action of aspirin. PGH₂ = prostaglandin H₂.

RESULTS

Patient characteristics. A total of 131 patients with median age 63 years (55 and 71.5, 25th and 75th centiles, respectively) were enrolled consecutively in the study. Sixty-one percent (n = 80) were men, 39 (n = 51) percent had a history of MI, 44% (n = 58) had previously undergone

Table 1. Comparative Analysis of Patients Stratified by Serum Thromboxane Value

	All Patients (n = 131)	TX ≤2.2 ng/ml (n = 74)	TX >2.2 ng/ml (n = 57)	p Value
Weight (kg)	77.0 (69.6, 86.0)	76.0 (64.5, 80.8)	83 (75.0, 89.0)	0.025
Age (yrs)	63.0 (55.0, 71.5)	67.0 (57.0, 73.0)	59.0 (54.0, 64.0)	0.0086
Body mass index (kg/m ²)	27.6 (25.4, 30.1)	27.1 (24.7, 29.4)	28.4 (26.0, 31.0)	0.04
Gender (male)	61	54.1	70.2	0.11
Smoking	18	18.9	17.9	0.92
Diabetes	10	10.8	8.9	0.85
Statin	66.2	66.2	66.1	0.99
Beta-blocker	63.1	67.6	57.1	0.3
ACE inhibitor	27.6	25.4	30.8	0.6
Previous revascularization	44	39.2	50.9	0.19
Previous myocardial infarction	39	31.1	49.1	0.038

ACE = angiotensin-converting enzyme; TX = thromboxane.

coronary artery revascularization, 52% (n = 68) had a history of hypertension, 10% (n = 13) were diabetic, 18% (n = 24) were current smokers, and 66% (n = 86) were taking statin therapy (Table 1).

Platelet and serum TX B₂. Serum TX B₂ was markedly suppressed in most patients. A plot of platelet TX B₂ versus serum TX B₂ revealed a sigmoid relationship (n = 45, R² = 0.67). Incomplete COX inhibition, reflected by platelet TX formation, was detected when serum TX B₂ values exceeded 2.2 ng/ml (Fig. 2). This reflects the concentration of serum thromboxane that is associated with 50% of the maximum platelet thromboxane levels in a log scale and is equivalent to an EC₅₀ value. Thus, levels of serum TX B₂ >2.2 ng/ml were deemed evidence of incomplete aspirin response. Platelet TX generation also correlated closely with AA-induced platelet aggregation (n = 45, R² = 0.9) (Fig. 3).

Forty-four percent of patients had a serum TX B₂ level >2.2 ng/ml indicating suboptimal inhibition of platelet COX and an incomplete aspirin response. Seventeen percent of the population had values exceeding 10 ng/ml. Patients with an incomplete aspirin response were more likely to demonstrate platelet aggregation to AA (21% vs.

3%; p = 0.004). Figure 4 shows the relationship between serum TX and AA-induced aggregation. At TX levels of approximately 2 ng/ml, the population divides into two groups: one group maintains a low percentage of aggregation, even with TX levels as high as 100 ng/ml; the second group shows maximal aggregation at TX levels as low as 3 ng/ml. However, in all cases when platelet aggregation occurred it was abolished by addition of aspirin in vitro.

Patient weight, age, and body mass index were significant predictors of incomplete suppression of serum TX B₂ formation by aspirin. Weight and age remained independent determinants of this incomplete treatment response on multiple regression analysis (p = 0.025 and 0.0086, respectively) (Table 1). Patients who showed an inadequate response to aspirin were heavier, younger (Fig. 5), and more likely to have had a previous MI (p = 0.038).

DISCUSSION

Many cardiovascular patients who take aspirin for secondary prevention will have further vascular events. Indeed, treat-

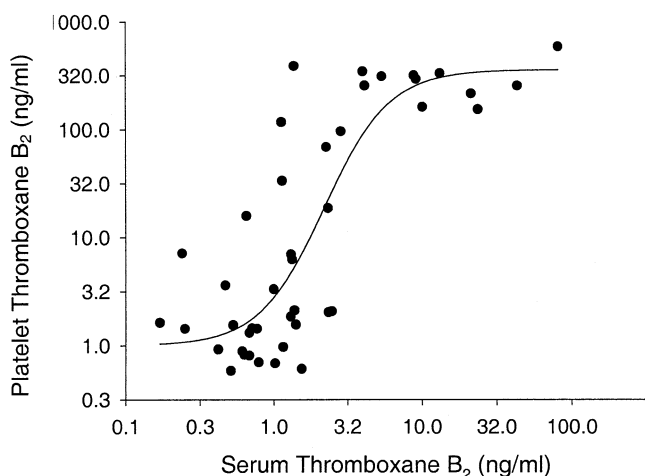


Figure 2. A sigmoid relationship between serum thromboxane (TX) (ng/ml) on the y-axis and platelet TX (ng/ml) (TX generated when exogenous arachidonic acid is added to platelets) on the x-axis is evident (n = 45, R² = 0.67). The point of inflection of the graph occurs at a serum TX level of 2.2 ng/ml.

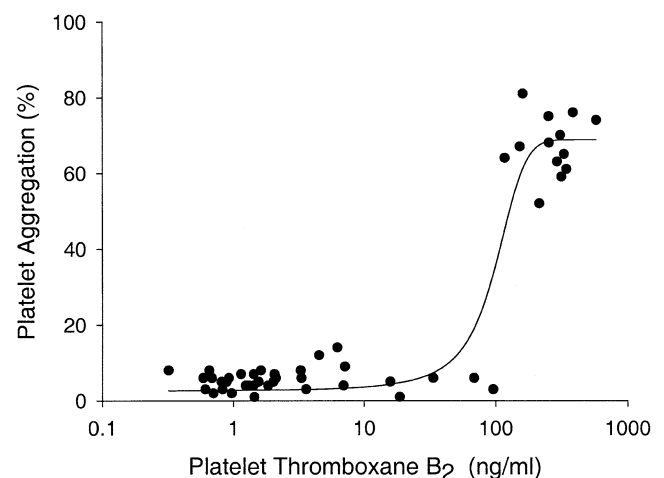


Figure 3. Platelet thromboxane generation (ng/ml) on the x-axis correlates with arachidonic acid-induced platelet aggregation (%) on the y-axis (n = 45, R² = 0.9). Both variables depend on cyclooxygenase (COX) activity. Two distinct populations are evident reflecting absence and presence of active platelet COX.

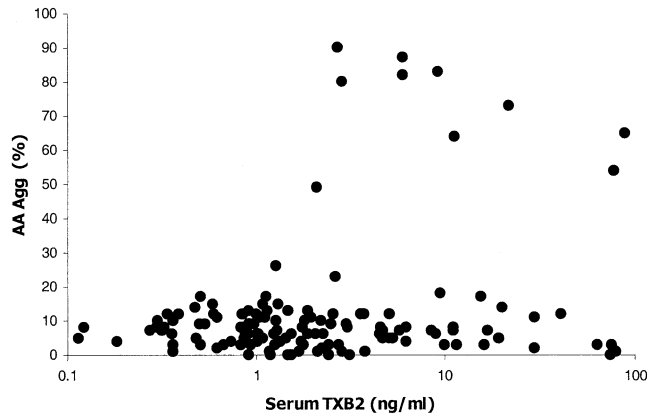


Figure 4. The relationship between serum thromboxane (TX) and arachidonic acid-induced platelet aggregation (AA Agg). At thromboxane levels above 2 ng/ml the population divides into two groups, one with full inhibition and the other with no inhibition of arachidonic acid-induced platelet aggregation.

ment with aspirin before presentation with an acute coronary syndrome indicates poor prognosis (14). However, this may reflect complex pathology rather than resistance to therapy. Failure to respond fully to aspirin, referred to as aspirin resistance, has been detected using a variety of platelet function assays. These include platelet aggregation to AA, thrombus formation on collagen membranes, and TX generation in vivo (10,13,15). Each assay, however, measures a distinct aspect of platelet function, which may explain variation in the reported prevalence of aspirin resistance.

To address what constitutes aspirin “resistance” or “non-response” it is important to define what response is expected and with what assay. We know that in vivo, erythrocytes and leukocytes modulate platelet reactivity (16), whereas collagen, ADP, TX A₂, thrombin, epinephrine, serotonin, and shear stress act synergistically on the platelet. Ex-vivo replication of this environment is impractical; however, assays of serum TX B₂ generation and AA-induced platelet aggregation depend on COX-1 function and thus reflect aspirin’s pharmacologic effect on platelets (17).

Reasons for failure to respond to aspirin may be pharmacokinetic (failure to achieve an adequate level of drug) or pharmacodynamic (failure to inhibit platelet function). Studies to date have largely overlooked this distinction because it is assumed that all preparations deliver an adequate aspirin dose. Interindividual pharmacodynamic variability occurs with most drugs, and aspirin is no different; thus, aspirin resistance assays may reflect, at least in part, pharmacodynamic heterogeneity. In addition, when contemplating causes of resistance to any drug, poor patient compliance, inadequate dosing and drug interaction must also be considered at the outset. We went to considerable lengths to confirm patient compliance and prevent drug interaction.

Dose-finding studies on which current regimens are based used plain aspirin, which differs significantly in its pharmacokinetic profile from EC preparations. Seminal

studies demonstrated that TX generation is highly susceptible to aspirin inhibition and that profound cumulative inhibition of platelet TX production occurred with chronic administration of low-dose plain aspirin (5,18). With such aspirin preparations, peak plasma levels occur 30 to 40 min after ingestion and platelet inhibition is apparent after 1 h. In contrast, it takes 3 to 4 h to achieve peak plasma levels with EC aspirin (19). In a healthy volunteer study, we detected lower efficacy with EC preparations compared with plain aspirin (11). This finding is of increasing importance because many patients who take aspirin as antiplatelet therapy for secondary prevention of cardiovascular events now receive low-dose EC preparations (76% of our population).

Aspirin is rapidly inactivated by enzymatic and non-enzymatic hydrolysis to salicylate, thus assays of plasma aspirin concentration provide an unreliable index of bio-availability. Therefore we used functional assays of aspirin’s effect and concentrated on inhibition of platelet COX, its target enzyme (19). Aspirin acetylates a serine residue in the substrate pocket of COX in an irreversible reaction that inactivates the enzyme. We assayed serum TX B₂, a measure of the enzyme’s capacity to generate the parent compound TX A₂ from endogenous AA stored in the platelet membrane (Fig. 1). Even a trivial level of enzyme activity can generate sufficient amounts of TX to support platelet activity. Thus, at least 95% suppression of serum TX B₂ is required to achieve an optimal antiplatelet effect (6).

In a subset of patients we also measured the ability of platelets to metabolize exogenous AA and form TX (platelet TX). This index of platelet COX activity correlated with platelet aggregation. Comparison of serum and platelet TX B₂ levels demonstrated that when serum levels exceeded 2.2 ng/ml (equivalent to the IC₅₀ value), platelets continued to generate TX from AA, indicating the presence of uninhibited enzyme (Fig. 2). This value is also close to mean serum thromboxane + 1 SD (1.5 ± 1.1 ng/ml, n = 25) for 75 mg aspirin in healthy volunteers (unpublished data). Consistent with this, platelets from these patients were more likely to

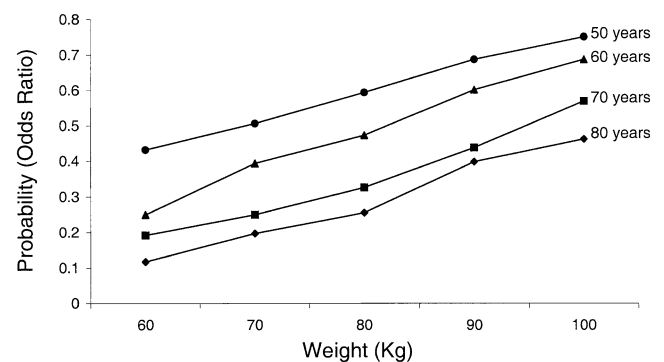


Figure 5. Probability of an incomplete response to aspirin (serum thromboxane >2.2 ng/ml) on the y-axis is related to patient weight on the x-axis and stratified by patient age. Weight and age are significant independent predictors of aspirin response on multivariate analysis (p = 0.025 and 0.0086, respectively).

exhibit aggregation to AA. In a previous study among healthy volunteers taking 75 mg plain aspirin, mean serum TX B₂ ± 1 SD was 1.5 ± 1.1 ng/ml, n = 25. In the same study, serum TX B₂ values seen in untreated subjects were 245 ± 111 ng/ml (± SD), n = 74 (unpublished data). Thus, our results indicate that profound inhibition (99%) of serum TX is necessary to achieve optimal platelet suppression. Even a more generous target serum TX level of 3.2 ng/ml, which corresponds to maximum levels of platelet TX generation in our population, is equivalent to 98.7% inhibition of serum TX levels (Fig. 2).

Of 131 patients taking 75 mg EC aspirin daily, 58 (44%) failed to attain optimal inhibition of serum TX (≤2.2 ng/ml) (Table 1). Heavier and younger patients were less likely to respond to aspirin. For example, if a 50-year-old patient weighed 90 kg (198 lb), the likelihood of an inadequate treatment response was 70% (Fig. 4). In every case where platelet aggregation to AA was detected, addition of aspirin overcame the platelet response. These results suggest that the dose of aspirin delivered by 75 mg EC preparations is not sufficient to prevent platelet activity in many cases and that larger subjects are most at risk of an inadequate response to treatment.

Bioavailability of plain aspirin given orally is approximately 50% (20). Deactivation of aspirin by hydrolysis to salicylate may occur at a number of sites, including the gut, liver, and blood. Aspirin (pKa = 3.5) is highly protonated and thus rapidly absorbed from the stomach where the pH is low and hydrolysis is minimal. In contrast, EC preparations release aspirin into the upper small intestine, where the pH of 6.5 exceeds the drug's pKa and thus aspirin is less protonated and absorbable. Here slower absorption and a more alkaline environment may facilitate hydrolysis to salicylic acid, and thus bioavailability of aspirin from these preparations may be lower (19,21). Plain aspirin, at doses as low as 40 mg, fully inhibits platelet aggregation in healthy volunteers. Low doses of EC aspirin may be less bioavailable and thus prove insufficient in heavier patients with a large volume of distribution (22).

The relationship between lower patient age and an incomplete response to aspirin is more difficult to explain; however, consistent with our findings, particular benefit of aspirin therapy to older stable cardiovascular patients has previously been described (23). Age-related changes in drug sensitivity are increasingly appreciated and both pharmacokinetic and pharmacodynamic factors appear to contribute. The COX gene is polymorphic and variants have been described, which modify response to aspirin (24). Although addition of aspirin overcame continued platelet aggregation when it occurred in our population, genetic variation in COX may alter aspirin sensitivity. Heritable factors predominate in younger patients; thus, genetic factors may contribute to the relationship with age. Indeed, family history is an independent risk factor for MI, and age of onset can be related to strength of association (25). The inability of aspirin to inhibit TX generation in younger

patients therefore may represent evidence of pharmacodynamic resistance.

Patients who demonstrated suboptimal COX inhibition were more likely to have had a previous MI. An association between previous MI and increased cardiovascular risk is well established (26). One possible explanation for our findings is that patients with previous MI may have a more aggressive disease process and may therefore be inherently less sensitive to aspirin. Our finding may represent functional evidence of an incomplete aspirin response. Coincidental administration of NSAIDs such as ibuprofen with aspirin may reduce its efficacy. Ibuprofen occupies the active site of COX and can prevent enzyme acetylation by aspirin; however, we excluded all patients taking NSAIDs (27).

The Antithrombotic Trialists' Collaborative meta-analysis of 287 antiplatelet trials and 137,000 subjects reported that 75 to 150 mg of aspirin daily is effective secondary prevention in patients with cardiovascular disease (7). Although their findings may appear to conflict with ours, many of the trials used plain rather than coated aspirin preparations, and, in fact, relatively few patients were taking the 75 mg aspirin dose. In a healthy volunteer study in which five different preparations containing low-dose aspirin were evaluated, we found that all volunteers receiving soluble aspirin had >95% inhibition of serum TX, whereas between 5% and 25% of volunteers taking EC aspirin (depending on brand) failed to achieve this (11). Consistent with our findings, a recent meta-analysis suggests that aspirin dose upon hospital discharge influences the clinical course after admission with unstable angina or acute MI (28).

Conclusions. Our results indicate that inhibition of platelet function with 75 mg EC aspirin daily may be incomplete in many patients with cardiovascular disease. Heavier and younger patients and those with a history of MI are most likely to demonstrate an inadequate treatment response.

Acknowledgment

The access to facilities at the Clinical Research Centre in Beaumont Hospital is gratefully acknowledged by the authors.

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